U.S. FOOD & DRUG ADMINISTRATION

## Listing of Abstracts

# 2023 Student Scientific Research Day

**August 10, 2023**

https://www.fda.gov/science-research/fda-stem-outreach-education-and-engagement/

## FDA Annual Student Scientific Research Day

### *2023 Listing of FDA Student Abstracts*

**Background**

Every year, FDA gives high school, college, and graduate students from different backgrounds and scientific disciplines the opportunity to train with mentors from across FDA on regulatory science research projects. Students are exposed to the broad expanse of regulatory science activities underway across the Agency as well as the range of scientific disciplines they call on. Students also learn first-hand about the Agency's domestic and global impact. After completing their FDA training, students are encouraged to explore careers in public health and STEM.

FDA is committed to recognizing the importance of mentor-led student research in STEM related fields. Annually, FDA holds Scientific Research Day to recognize and highlight the importance of FDA student programs and the direct impact their research projects have on advancing regulatory science at FDA. The FDA Office of Scientific Professional Development (OSPD) works with an Agency-wide planning committee to coordinate the FDA student research recognition activities annually.

In addition to the recognition program, FDA showcases the abstracts submitted by our students for the public on www.FDA.gov.

This book contains the abstracts from the 2023 FDA summer students. Among these participants, OSPD received 9 abstract from the Center for Biologics Evaluation and Research, 38 submissions from the Center for Drug Evaluation and Research (CDER), 25 from the Center for Device Evaluation and Research (CDRH), 12 from the Center for Food Safety and Applied Nutrition (CFSAN), 2 from the Center for Veterinary Medicine (CVM), 10 from the National Center for Toxicological Research (NCTR), and 1 from the Office of Regulatory Affairs (ORA).

There were 97 total abstract submissions.

**Program Goals**

1. Recognize FDA student research and contributions to FDA.
2. Present FDA student research on a public website annually.
3. Support STEM education for students in FDA scientific priority areas.

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***2023 Listing of FDA Student Abstract Titles listed by FDA Center/Office***

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## 2023 FDA Student Abstracts (listed by FDA Center/Office)

### Center for Biologics Evaluation and Research (CBER)

1) **Abstract title:** *Evaluating the Potential for Eosinophils as a PD biomarker to Demonstrate Biosimilarity in Patients with Eosinophilic Asthma*
**Authors:** Arao, Rob FDA/CDER (Student); Gershuny, Victoria, FDA/CDER (Mentor); Florian, Jeffry, FDA/CDER; Wang, Yow-Ming , FDA/CDER
**FDA Strategic Initiative:** Stimulate Innovation in Clinical Evaluations and Personalized Medicine to Improve Product Development and Patient Outcomes
**Abstract:**
    - **Synopsis**
        - The purpose of this study is to evaluate the relationship between the PD biomarker circulating eosinophils, and clinical endpoint, exacerbations, for mepolizumab and reslizumab, two interluekein-5 (IL-5) antagonists indicated for treatment of severe asthma with an eosinophilic phenotype. We used univariate cox proportional hazard modelling to identify baseline factors that are prognostic for exacerbations 1,176 placebo, 489 reslizumab and 846 mepolizumab-treated subjects. Prognostic factors for untreated eosinophilic asthma included higher baseline eosinophil counts, lower forced expiratory volume in one second, one or more hospitalizations in the prior year, and oral corticosteroid use in the past year. Although high baseline eosinophil counts are prognostic of increased exacerbation risk in untreated patients, this relationship is diminished when treated with IL-5 antagonists.

- **Purpose**
    - To date, most biosimilar approvals have included pharmacokinetic (PK) similarity data and a comparative clinical study, which are time-consuming, costly, and need many participants. A more streamlined approach may rely on PK and pharmacodynamic (PD) similarity alongside comparative safety and immunogenicity data. While a direct association between a PD biomarker and clinical outcome is not necessary for use in biosimilar development, it can increase confidence in its use.

      One PD biomarker is circulating eosinophils for mepolizumab and reslizumab, two interluekein-5 (IL-5) antagonists indicated for treatment of severe asthma with an eosinophilic phenotype. The purpose of this study is to evaluate the relationship between changes in eosinophils and the clinical endpoint, exacerbations.
- **Methods**
    - Individual-patient data from four clinical trials submitted to the FDA for the mepolizumab (75, 250, and 750 mg intravenous; 100 mg subcutaneous) and reslizumab (3.0 mg/kg intravenous) to support the treatment of severe eosinophilic asthma indication was considered. Data across all mepolizumab doses were pooled after determining that exacerbation outcomes were similar. Univariate Cox proportional hazard modeling was used to determine prognostic baseline factors. Demographics and patient history (emergency room visits, oral corticosteroid use, etc.) were considered as covariates. Continuous variables were dichotomized based on medians for mepolizumab trial data and reslizumab trial data separately.
- **Results**
    - A total of 1,176 placebo, 489 reslizumab and 846 mepolizumab-treated subjects were included in the analysis. For placebo, prognostic factors included higher baseline eosinophils (hazard ratio (HR)-1.50 95%-confidence interval (CI)-[1.22,1.86], lower baseline forced expiratory volume in one second (HR- 0.77 CI-[0.62,0.95]), one or more hospitalizations in the prior year (HR-1.41 CI-[1.06, 1.86]), and oral corticosteroid use over the past year (HR-1.66 CI-[1.32,2.09]). After treatment, subjects with higher baseline eosinophil counts had a slightly lower event rate than those with lower baseline eosinophil counts.
- **Implications**
    - High baseline eosinophil counts are prognostic of increased exacerbation risk in untreated patients with eosinophilic asthma. However, the relationship between baseline eosinophil counts and exacerbations is attenuated following treatment with mepolizumab or reslizumab. Further

investigations to include on-treatment eosinophil counts may improve understanding of the association between circulating eosinophils and exacerbations in patients with eosinophilic asthma.

2) **Abstract title:** *Evaluation of long-term stability of lyophilized antivenom products beyond their expiration date*
**Authors:** Chen, Devon, FDA/CBER (Student); Scott, Dorothy, FDA/CBER (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
  - Alarming global shortages of many antivenom (AV) products prompt an urgent need to seek new ways to ensure availability of these exclusive and sole specific therapies for potentially fatal or debilitating envenomation particularly in sub-Saharan Africa and parts of Asia. However, there is also a critical need to use these often-unavailable products in the USA to treat envenomation by nonnative snake species. The immunoglobulin fraction manufactured from the plasma of animals immunized by specific venoms is known to be well preserved in the lyophilized form. Due to the documented stability of some AV products even several years after their expiration, the shelf life of some AVs has been extended to assure adequate supply. Emergency use of expired AVs in multiple countries has been reported in literature. Typically, expired AVs remain in stock, providing a potential source for emergency clinical use if no in date product is available. Although AV potency is known to decrease with time, more studies are needed, as well as further physical/chemical evaluation of expired AVs. To characterize these expired AVs beyond their official dating period, we assessed the stability of lyophilized non-FDA licensed AV products indicated for envenomation by various Asian snakes, nonnative to the USA, that are popular among private herpetologists and in Zoo collections. Prior to more extensive planned studies including evaluation of neutralization activity and other efficacy testing, we performed the initial screening of 2 lots of Russel's Viper Antivenin, 2 lots of Malayan Pit Viper Antivenin, and 1 lot Neuro Polyvalent Antivenin (all manufactured by Queen Saovabha Memorial Institute; 2 vials per each lot). The initial testing (n=10) revealed varying protein concentration and uniform pH in all products across the lots. The acquired high levels of endotoxins in most of the lots will require further testing. Two Russel's Viper Antivenin and 2 Malayan Pit Viper Antivenin vials with the highest endotoxin levels were excluded from further AV assessment. Based on the initial testing of visual inspection, sterility, pH and protein content, 6 (2 Neuro Polyvalent Antivenin, 2 Russel's Viper Antivenin,

and 2 Malayan Pit Viper Antivenin) out of 10 vials qualified for further AV evaluation. Our results warrant further studies of additional lots and AV products. Qualified specimens will be further assessed by in vitro potency assays to evaluate their potential utility for emergency clinical use.

- **Purpose**
  - Antivenoms (AV), immunoglobulin fractions from venom immunized animals, are the only proven effective and potentially lifesaving treatment for envenomation by venomous snake species. The extreme shortage of AVs has affected their availability in many parts of the world, including the USA. AVs are crucial products needed to treat patients bitten by local venomous species (WHO categorizes snakebite envenomation as a neglected tropical disease) as well as globally to treat bites by exotic snakes kept in Zoos or for research. While there are FDA licensed AVs indicated to treat envenomation by native North American snake species, specific non-licensed AVs against exotic snake bites need to be imported through special processes. These products are needed to keep professionals (Zoos, aquariums, research centers staff, military personnel) and the public (private herpetologists safe. Over 60 exotic venomous species have been reported to envenomate patients including pediatric within 7 years in the USA. Often, only expired AVs are available in many countries including the USA. Thus, detailed information on AV stability, including physiochemical properties and neutralizing potency, is essential to assess their potential emergency use if no unexpired product is available.
- **Methods**
  - Expired exotic AVs were obtained from the Viper Institute collection (Tucson, AZ). Expired vials were donated by Zoos to the Viper Institute for research purposes. Appearance of reconstituted Avs was evaluated by visual inspection (visual inspection station, 2000 – 3750 lux) assessing degree of opalescence (EU Pharm, hydrazine sulfate solution & hexamethylenetetramine solution reference suspensions OS 0, I, II, III, IV), color (Sigma Color Reference Solutions – Brown/Yellow, Brown, and Yellow standards – BY, B, and Y were used) and particulate matter content (foreign particles; intrinsic/proteinaceous particles – category 0: no particles, category I: <1mm, <10 particles/vial, category II: <1mm, ≥10 particles/vial, category III: 1-10 mm, category IV: ≥10 mm). The endotoxin concentration was quantified by LAL (Limulus Amebocyte Lysate) assay (1:200 dilution) employing Endosafe Nexgen-PTS Reader/cartridge with lower limit of detection of 0.005 – 0.1 EU/ml (Charles River Laboratories). Protein concentration (NanoDrop 2000 spectrophotometer, Thermo Scientific, absorbance at 280 nm), and pH (Mettler Toledo) were assessed as well.

- **Results**
    - Four vials from 2 lots of Russell's Viper Antivenin, 4 vials from 2 lots of Malayan Pit Viper Antivenin, and 2 vials from 1 lot of Neuro Polyvalent Snake Antivenin (all manufactured by Queen Saovabha Memorial Institute) up to 156 months after their expiration were tested. The initial testing (n=10) revealed varying protein concentrations for certain AVs ranging from 27.38 mg/ml to 49.5 mg/ml for the Russell's Viper Antivenin, 19.35 mg/ml to 26.884 mg/ml for the Malayan Pit Viper Antivenin, and uniform protein concentrations for the Neuro Polyvalent Snake Antivenin, with a range of 60.472 mg/ml to 61.098 mg/ml. Endotoxin values varied greatly, ranging from 4.16 EU/mL to >100 EU/mL. Color of all samples were similar to what was described in the package insert (PI) as clear colorless to pale yellow. Two Russel's Viper Antivenin samples had an opalescence of OSIII, and two were slightly opalescent (≤OSII). All of the Malayan Pit Viper Antivenin samples were slightly opalescent (OSII). One Neuro Polyvalent Snake Antivenin had an opalescence of OSII, while the other had OSIII.
- **Implications**
    - The initial evaluation of expired AV products shows some promise. pH suggests the products are stable over time beyond their expiration date (shelf life of all tested lyophilized products is 60 months). Based on limited information about handling and storage of these lots prior to shipment to the FDA, all evaluated products were exposed to temperature deviations. It is possible that more provided AV vials that were properly stored will qualify for further evaluation. It will include in-vitro potency assays to assess the potential of expired AVs for emergency clinical use. During the initial testing, 6 out of 10 expired lyophilized AVs were prescreened for the further assessment.

3) **Abstract title:** *Dynamic Light Scattering: Can It Have A Role In Understanding Product Quality*
**Authors:** De Silva, Minoli, FDA/CBER (Student); Eller, Nancy, FDA/CBER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - Protein aggregation has the potential to affect product safety and efficacy. This includes, but not limited to, immunogenicity, anti-drug antibodies, and a reduction in drug effectiveness. Orthogonal methods for measuring particles of various sizes should be included during the various stages of product development to understand where protein aggregation occurs in the manufacturing process and to minimize the level of particles when possible. Dynamic Light Scattering (DLS) is a qualitative method for

determining the presence particles in the sample, requires no sample preparation, and is relatively simple to run. Two different DLS instruments were used to test multiple lots from various, purchased IGIV products which had been unstressed or stress. For the unstressed material, reproducible DLS patterns were observed for the IGIV products. Stressing the products highlights the various parameters than can be affected which are not readily seen in the results. This information can assist in setting appropriate acceptance criteria for DLS results.

- **Purpose**
  - Protein aggregation has the potential to affect product safety and efficacy. This includes, but not limited to, immunogenicity, anti-drug antibodies, and a reduction in drug effectiveness. Orthogonal methods for measuring particles of various sizes should be included during the various stages of product development to understand where protein aggregation occurs in the manufacturing process and to minimize the For the unstressed material, reproducible DLS patterns were observed for the IGIV products. Stressing the products highlights the various parameters than can be affected which are not always obvious. The results for vortexing appeared to be similar to the unstressed samples, except for the measurement distance. The algorithm for the backscatter angle can adjust the depth of the measurement. When the measurement distance is significantly reduced, the results appear similar to the unstressed samples. method for determining the presence particles in the sample, requires no sample preparation, and is relatively simple to run. The purpose of this project is to determine the appropriateness of using DLS as one component of a plan in understanding product quality during development, clinical studies, process validation, and manufacturing modifications.
- **Methods**
  - Multiple lots from various Immune Globulin, Intravenous (IGIV) products were purchased for testing. To understand the variability in the test method, five separate samples from each vial will be tested. Two different instruments by Malvern Panalytics, Zetasizer Nano ZS (software version 8.01) and the Zetasizer Ultra Red (software ZS XPLORER version 3), will be used. The Nano uses backscatter ($173^{o}$) only for measuring. The Ultra has the capability to use Multi-Angle Dynamic Light Scattering (MADLS) which includes backscatter, or backscatter alone. Samples were tested unstressed and stressed (agitation and heat) using both instruments.
- **Results**
  - For the unstressed material, reproducible DLS patterns were observed for the IGIV products. Stressing the products highlights the various parameters

than can be affected which are not always obvious. The results for vortexing appeared to be similar to the unstressed samples, except for the measurement distance. The algorithm for the backscatter angle can adjust the depth of the measurement. When the measurement distance is significantly reduced, the results appear similar to the unstressed samples.

- **Implications**
    - Stressing the products highlights the limitations of the method. This information can assist in setting appropriate acceptance criteria for DLS results. With the ease of use, DLS has the potential to be the first of the orthogonal methods used to understand the level of particles present in the sample. If the results are atypical, an investigation can be undertaken to determine what may have caused these differences.
      "My comments are an informal communication and represent my own best judgment. These comments do not bind or obligate FDA."

4) **Abstract title:** *Exploring the Role of Lysophosphatidylcholine on Neutrophil Function in a Live-Attenuated Leishmania Parasite Vaccine*
**Authors:** Hobson, Caroline, FDA/CBER (Student); Kitthanawong, Grace FDA/CBER (Student); Bhattacharya, Parna FDA/CBER (Mentor); Pacheco-Fernandez, Thalia FDA/CBER (Mentor); Sun, Jinchun FDA/NCTR (Mentor); Azodi, Nazli FDA/CBER (Mentor); Markle, Hannah FDA/CBER (Mentor); Beger, Richard FDA/NCTR (Mentor); Gannavaram, Sreenivas FDA/CBER (Mentor); Nakhasi, Hira FDA/CBER (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Leishmaniasis is a parasitic infection found in tropical countries that is caused by *Leishmania*, a blood transfusion-transmissible parasite, which is spread by sand fly vectors. Towards discovering a safe and effective human vaccine against leishmaniasis, a *centrin* gene deleted mutant of the *Leishmania major* parasite (*LmCen*$^{-/-}$) was developed. The metabolite lysophosphatidylcholine (LysoPC) is implicated in the functions of neutrophils, which are recruited to the site of *Leishmania* infections. By studying the role of Lyso-PC in neutrophil recruitment and activity, the immune mechanism of protection incued by *LmCen*$^{-/-}$ parasites can be elucidated.
- **Purpose**
    - Leishmaniasis is a parasitic infection found in tropical countries that is caused by *Leishmania* parasites, which are spread through the bites of sand fly vectors. Clinical manifestations of *Leishmania* infections include cutaneous leishmaniasis (CL) which presents as skin lesions, mucocutaneous

leishmaniasis (MCL) that affects mucosal tissues, and visceral leishmaniasis (VL) which affects spleen and liver. *Leishmania* is a blood transfusion-transmissible parasite, so there is a pressing need to develop effective tools to ensure the safety of the national blood supply and help curb the spread of *Leishmania* infections. Towards developing a safe and efficacious vaccine against *Leishmania*, we developed a *centrin* gene deleted mutant of *Leishmania major* parasites (*LmCen*$^{-/-}$). Preclinical studies have shown that immunization with *LmCen*$^{-/-}$ parasites induces protection from CL and VL, however, the mechanism of protection behind *LmCen*$^{-/-}$ immunization has not been fully detailed. Towards this goal, untargeted metabolomic analysis of the neutrophils isolated from infected mice was performed. Results of the early metabolic immune regulation following immunization with *LmCen*$^{-/-}$ parasites will be discussed.

- **Methods**
  - *Sample preparation for mass spectrometry:*
    - C57Bl/6 were mice inoculated with *LmWT* or *LmCen*$^{-/-}$ parasites intradermally. At 48h post-infection, 2.5-3x$10^6$ parasitized and non-parasitized neutrophils were sorted from ear draining lymph nodes (dLN) by flowcytometry and quenched immediately. Untargeted metabolomic analyses were performed on the neutrophil populations using mass spectrometry.
  - *Antigen-presenting activity:*
    - To investigate the role of lysophosphatidylcholine (LysoPC) in neutrophil activities, neutrophils isolated from mouse bone marrow were infected with *LmWT* or *LmCen*$^{-/-}$ parasites *in vitro* and injected intradermally into mice that were pretreated with BrdU. Three days post inoculation, mice were sacrificed, and the draining lymph nodes were harvested to study the activation and proliferation of T cells.
  - *Cytokine production:*
    - To investigate the role of LysoPC in the production of inflammatory cytokines, neutrophils were isolated from mice infected with *LmWT* or *LmCen*$^{-/-}$ parasites three days post inoculation. Expression of inflammatory cytokines was assessed by qRT-PCR.
- **Results**
  - Untargeted metabolomic analysis revealed that LysoPC was enriched in neutrophils isolated from mice infected with *LmCen*$^{-/-}$ parasites compared to *LmWT* or naïve controls. Several other bioactive lipids were also found enriched in *LmCen*$^{-/-}$ infection. Flow cytometry analysis showed that significantly more antigen experienced T cells ($CD3^+CD4^+CD44^+$ $BrdU^+$ T cells)

were significantly found in mice that received *LmCen*$^{-/-}$ infected neutrophils compared to the naïve and *LmWT* controls.

- **Implications**
    - The enrichment of LysoPC in neutrophils isolated from *LmCen*$^{-/-}$ infected mice in comparison to *LmWT* and naïve controls, suggests that it could play important immunoregulatory roles in vaccine immunity. Recruitment of neutrophils to the site of inflammation is known to be mediated by leukotrienB4-CXCL12 signaling. The enrichment of LysoPC in neutrophils and enhanced migration of neutrophils in LysoPC supplementation experiments points to a potentially new signaling mechanism utilized by *LmCen*$^{-/-}$ parasites. Additionally, elevated LysoPC seems to affect the proliferation of antigen experienced $CD4^+$ but not $CD8^+$ T cells that mediate protective immunity. Future studies will explore if depletion of LysoPC alters the CD4 T cell response in vaccinated animals thus showing a definitive role of LysoPC in vaccine immunity.

5) **Abstract title:** *Antibodies To the GII.4 Norovirus Capsid Reveal Complex Relationships Between Distinct Variable Antigenic Sites*
**Authors:** Landivar, Michael GMU/FDA/CBER (Student); Pilewski, Kelsey FDA/CBER (Mentor); Tohma, Kentaro FDA/CBER (Staff); Ford-Siltz, Lauren FDA/CBER (Staff); Parra, Gabriel FDA/CBER (Mentor).
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**

    - Norovirus is a rapidly evolving RNA virus that causes acute non-bacterial gastroenteritis, leading to significant morbidity and mortality in vulnerable populations. The diversity of norovirus, with around 40 genotypes capable of infecting humans, hinders the development of preventative and protective therapeutics. GII.4 norovirus has predominated globally for over three decades, causing six known pandemics. The persistence of this genotype can be attributed to the emergence and replacement of phylogenetic variants, which can be mapped to several antigenic sites on the major capsid protein, VP1. Our study investigated the relationship between antibody recognition at each antigenic site and its effect on antibody accessibility, neutralization, and viral escape. Our results revealed new patterns of antibody recognition both within and between distinct antigenic sites on the GII.4 capsid. Antibodies recognizing antigenic site A demonstrated three footprints, creating variable levels of accessibility to sites C, G, and A/G. We also found that antibodies recognizing site G displayed three distinct competition profiles, with enhanced binding to antigenic site G in the presence of excess site C mAbs. We found mixed binding of multiple antibodies recognizing A/G sites leading to two groups based on competition profile. Together, our results demonstrate the

complex relationships between recognition of antigenic sites. A better understanding of the antigenic topology of human noroviruses can facilitate the design of broad and potent vaccines.

- **Purpose**
    - Norovirus is a rapidly evolving RNA virus and the leading cause of acute non-bacterial gastroenteritis, resulting in significant morbidity and mortality in vulnerable populations. This burden is driven by insufficient natural immunity, and lack of approved vaccines, or antiviral therapies. An important impediment to the development of preventative and protective therapeutics is the extraordinary diversity of norovirus, with ~40 genotypes capable of infecting humans. Despite this diversity, GII.4 norovirus has predominated globally for over three decades, causing six known pandemics. The persistence of this genotype can be attributed to the emergence and replacement of phylogenetic variants in which major differences can be mapped to the several antigenic sites presented on the major capsid protein, VP1. Previous studies have shown that the antibody response targets different antigenic sites on VP1 over the course of the chronological evolution of the GII.4 genotype, likely contributing to immune escape. Here, we sought to investigate the relationship between antibody recognition at each antigenic site and its effect on antibody accessibility, neutralization, and viral escape.
- **Methods**
    - We used a panel of previously isolated mouse monoclonal antibodies (mAbs) raised against GII.4 norovirus to investigate the relationship between recognition of variable antigenic sites. From this panel, we selected 24 neutralizing (HBGA-Blocking) mAbs mapping to the variable antigenic sites A, C, G, I, and sites overlapping A and G (A/G). Utilizing virus like particles (VLPs), which replicate the conformationally-native GII.4 capsid we measured the steric relationship between recognition of these sites by competition ELISA.
- **Results**
    - Our results reveal new patterns of antibody recognition both within and between distinct antigenic sites on the GII.4 capsid. We found that antibodies recognizing antigenic site A demonstrated three footprints that create variable levels of accessibility to sites C, G and A/G. When we measured binding of site G antibodies in the presence of competitors, we found that antibodies recognizing this site also displayed three distinct competition profiles. We observed enhanced binding to antigenic site G in the presence excess site C mAbs. One site C mAb also increased binding of multiple antibodies recognizing A/G sites. Finally, we found that competition with site A/G antibodies displays two groups based on competition profile.

- **Implications**
    - GII.4 noroviruses have predominated globally for decades enabled by the emergence and replacement of antigenically distinct strains. A better understanding of the antigenic topology of human noroviruses can facilitate the design of broad and potent vaccines.

6) **Abstract title:** *Bioinformatic pipelines for characterizing blood-borne pathogens using nanopore long read technology*
**Authors:** Aadarsh Govada, FDA/CBER (Student); Kamila Abdukadirova, FDA/CBER (Student); Indira Hewlett, FDA/CBER; Viswanath Ragupathy, FDA/CBER (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - This project resulted in the development of a bioinformatic pipeline that enables the comprehensive characterization of blood-borne pathogens using long read sequence data from which host and spiked sequences have been removed. Initially developed for an HCV reference genome, the pipeline can be extended to analyze other blood-borne pathogens. The pipeline was tested using human HCV samples, including those with coinfections of HTLV, SYPHILIS/CMV, HBc, or no coinfection. Fastq inputs were directly processed from the CLC Genomics Workbench v21.0 following the removal of host and spiked sequences. The pipeline incorporated data quality checks, de novo assembly to generate contigs, blast analysis to identify the closest genetic relative, reference mapping, genotyping, and visualization. The long read pipeline successfully characterized each sample, providing a comprehensive and detailed summary of the analysis. This pipeline offers a robust alternative for optimal analysis and summarization compared to other long-read pipelines.
- **Purpose**
    - Despite the existence of several well-established short read analysis pipelines, the field lacks comprehensive tools for the analysis and characterization of long reads. This project aims to address this gap by developing a metagenomic pipeline that enables a thorough analysis of long reads from fastq input, facilitating the determination of the genetic profile of the sample. The primary objective is to equip the FDA HIVE with a bioinformatic pipeline capable of accepting fastq input, generating contigs, identifying the sample's closest genetic relative, mapping the consensus sequence to a reference genome, and performing quality checks and data visualization.

- **Methods**
    - The long read characterization pipeline was initially designed for analyzing a reference genome of the hepatitis C virus (HCV) and was tested using four plasma samples obtained from individuals naturally infected with HCV. Three of the samples exhibited coinfections with HTLV, SYP/CMV, or HBc, while the fourth sample had no coinfection. The samples underwent DNA/RNA extraction, nanopore sequencing, and filtration to eliminate host and spiked reads using the CLC Genomics Workbench v21.0. The resulting fastq files were processed through the pipeline, and the sample data underwent quality checks using fastqc. The raven assembler package was utilized for de novo assembly, generating a set of fasta contigs for each sample. The contig series were subjected to blast analysis to identify the closest genetic relative, followed by reference mapping using Minimap2 and genotyping through nucleotide blast and the hepatitis C virus phylogenetic genotyping tool. Results from each step were visually represented and displayed.
- **Results**
    - The long read pipeline successfully identified and characterized the samples' closest genetic relatives, providing a comprehensive analysis of the consensus sequence generated from the contigs. Ongoing work includes expanding the pipeline to encompass HIV and other viral sample reference genomes, as well as optimizing the pipeline to enhance output quality.
- **Implications**
    - By employing quality checks, consensus sequencing, reference mapping, and genotyping strategies implemented in our long read characterization pipeline, we offer an effective method for accurate and comprehensive analysis of raw sequencing reads after removing host and spiked sequences.

7) **Abstract title:** *Metagenomic characterization of Blood Borne Pathogens using Nanopore sequencing*
**Authors:** Kamila Abdukadirova, FDA/CBER (Student); Aadarsh Govada, FDA/CBER (Student); Indira Hewlett, FDA/CBER; Viswanath Ragupathy, FDA/CBER (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**
    - **Synopsis**
        - This study explores the utility of nanopore sequencing for metagenomic characterization of blood-borne pathogens. Fourteen plasma samples from individuals infected with hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and West Nile virus (WNV) were analyzed. Nanopore sequencing allowed comprehensive sequencing and

identification of viral genomes. High viral load samples exhibited full-length genome coverage, while low viral load samples had fewer hits. Taxonomic profiling accurately identified WNV, HIV, and HCV, but HBV detection was limited due to circular viral DNA. Phylogenetic analysis revealed HCV genotype 4a and HIV genotype B. The study also identified co-evolution of Human pegivirus (HPgV-1) with HCV infection. This approach enhances our understanding of blood-borne pathogens, improves diagnostics, and aids in prevention and treatment strategies.

- **Purpose**
    - Blood-borne pathogens pose significant threats to human health, causing a wide range of infectious diseases and posing challenges for effective diagnosis and treatment. Metagenomic characterization, utilizing Nanopore sequencing technologies, has emerged as a powerful approach to comprehensively study the genetic diversity, interactions, and host-pathogen dynamics of blood-borne pathogens. Nanopore sequencing offers advantages over traditional sequencing methods, such as portability (point-of-care setting), long-read capabilities, and real-time data generation. This study provides an overview of the applications and implications of metagenomic characterization in the context of blood-borne pathogens.
- **Methods**
    - In this study, we characterized 14 plasma samples obtained from individuals naturally infected with hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and West Nile virus (WNV). The samples underwent DNA/RNA extraction (Cat No: D7020) and unbiased sample preparation, including double-strand cDNA synthesis (Cat No: K2561), followed by barcoded library preparation using nanopore sequencing protocols (Cat No: SQK-NBD114.24). The libraries were loaded onto a nanopore sequencer, and base calling was performed using state-of-the-art algorithms. Core bioinformatics tools, algorithms, and microbial databases were employed for data analysis. Targeted sequence analysis was conducted using CLC Genomic Workbench v21.0.
- **Results**
    - The metagenomic datasets were subjected to a series of bioinformatics analyses to explore the microbial composition of the plasma samples. Barcoding enabled multiplexing of samples (n=14) and comprehensive sequencing. Over 100,000 reads were obtained for each sample, with approximately 97% of reads originating from the host or spiked run control, and the remaining 3% representing virus-specific reads with read lengths ranging from 1610 to 2950 bp. Taxonomic profiling accurately identified WNV, HIV, and HCV. However, due to limitations associated with the

circular nature of viral DNA, HBV was not detected. High viral load samples from HIV and HCV exhibited near full-length genome coverage, while low viral load samples had fewer hits to the viral genome. Additional phylogenetic analysis revealed HCV genotype 4a and HIV genotype B. Despite fewer hits, blast analysis indicated that the WNV sequences had the highest homology to WNV Lineage 1A. Metagenomic profiling also identified the co-evolution of Human pegivirus (HPgV-1) with HCV-infected individuals. HPgV-1 was formerly known as GB virus type C (GBV-C) or hepatitis G virus (HGV).

- **Implications**
    - Overall, this study demonstrates the feasibility and utility of nanopore sequencing for metagenomic characterization of plasma samples for pathogen detection and characterization. The comprehensive analysis of microbial composition and functional potential in plasma provides a foundation for further investigations into the role of plasma microbiota in transfusion safety. By leveraging this approach, we can advance our understanding of these pathogens, improve diagnostics, and develop more effective strategies for prevention and treatment.

8) **Abstract title:** *Evaluation of long-term stability of liquid antivenom products beyond their expiration date*
**Authors:** Simak, Joseph, FDA/CBER (Student); Scott, Dorothy, FDA/CBER (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Category:** Rare Diseases
**Abstract:**

- **Synopsis**
    - Alarming global shortages of many antivenom (AV) products cause an urgent need to seek new ways how to ensure availability of this only specific therapy for potentially fatal or debilitating envenomation particularly in sub-Saharan Africa and parts of Asia. However, there is also a critical need to use these often-unavailable product in the US to treat envenomation by nonnative snake species. The Immunoglobulin fraction manufactured from plasma of animal immunized by specific venom(s) is known to be well preserved in the liquid form. Due to the documented stability of some AV products even several years after their expiration, the shelf life of some AVs was extended to assure adequate supply. Emergency use of expired AVs in multiple countries was reported in literature. Regularly, expired AVs remain in stock, providing potential source for emergency clinical use if no in date product is available. Although AV potency is known to decrease with time, more studies are needed, as well as further physical/chemical evaluation of

expired AVs. To characterize these expired AVs beyond their expiry, we assessed the stability of liquid non-FDA licensed AV products indicated for envenomation by various African snakes, nonnative in the USA, however, popular among private herpetologists and in Zoo collections. Prior further AVs qualification evaluating their neutralization activity and other efficacy testing, we performed the initial screening of 2 lots (6 vials each) of 2 liquid AVs – monovalent Echis carinatus (SAIMR) and polyvalent Central Africa Anti-Snake Venom Serum (Behring) 175 to 322 months after their expiration. The initial testing (n=12) revealed uniform protein concentration and pH in both products across the lots. One vial of SAIMR had endotoxin level above 5 EU/ml and based on visual inspection, 7 additional vials were found unacceptable due to either high opalescence, visible particulate content or out of range color. Based on the initial testing of visual inspection, sterility, pH and protein content, 4 (2 SAIMR, 2Behring) out of 12 vials qualified for further AV evaluation. Our results warrant further studies of additional lots and AV products. The qualified specimen will be further assessed by in vitro potency assays to evaluate their usefulness for emergency clinical use.

- **Purpose**
    - Antivenoms (AV), immunoglobulin fractions from venom immunized animals, are the only proven effective and potentially lifesaving treatment for envenomation by venomous snake species. AVs extreme shortages have affected their availability in many parts of the world, including the USA. Antivenoms are crucial products needed to treat patients bitten by local venomous species (WHO categorizes snakebite envenomation as the priority neglected tropical disease) as well as globally to treat bites by e.g. exotic pets, snakes kept in Zoo's collections or for research. While there are FDA licensed AV products indicated to treat specific envenomation by native North American snake species, specific non-licensed AVs against exotic snake bites (considered experimental drugs) need to be imported through special process. These products are needed to keep professionals (Zoo, amusement parks, research institutions employees, emergency responders, deployed personnel) and public (private keepers, pet traders) safe. Envenomation of 60+ venomous species over 7 years was reported in the USA. Often, only expired specific AVs are available in many countries including the USA. Detailed information on AVs stability, including physiochemical properties and neutralizing potency, is essential to assess their potential emergency use if no in date product is available.

- **Methods**
    - Appearance of liquid antivenoms was evaluated by visual inspection (visual inspection station, 2000 – 3750 lux) assessing degree of opalescence (EU Pharm, hydrazine sulfate solution & hexamethylenetetramine solution reference suspensions OS 0, I, II, III, IV), color (Sigma Color Reference Solutions – Brown/Yellow standards - BY were used) and particulate matter content (foreign particles; intrinsic/proteinaceous particles – category 0: no particles, category I: <1mm, <10 particles/vial, category II: <1mm, ≥10 particles/vial category III: 1-10 mm, category IV: ≥10 mm). The endotoxin concentration was quantified by LAL (Limulus Amebocyte Lysate) assay (1:200 dilution) employing Endosafe Nexgen-PTS Reader/cartridge with lower limit of detection of 0.005 EU/ml (Charles River). Also, protein concentration (NanoDrop UV-Vis spectrophotometer 2000, absorbance at 280 nm), pH (Accumet) and Dynamic Light Scattering (DLS, Malvern) were assessed.
- **Results**
    - Six vials from 2 lots of Echis carinatus (SAIMR) and 6 vials from 2 lots of Central Africa (Behring) up to 26 years after their expiration (collected from US Zoos) were tested. The initial testing (N=12) revealed uniform protein concentration of 248.2 – 263.9 mg/ml for SAIMR and 61.4 to 72.3 mg/ml for Behring. pH showed a minor fluctuation only, in the range of 6.247 to 6.378 for SAIMR and 6.153 to 6.246 for Behring. Endotoxin was <5 EU/mL for all but one tested vial, which contained >12 EU/ml. Color of all SAIMR vials was much darker than described in the Package Insert (PI) as light yellowish to light brown – 2 vials were dark brown/yellow, and 4 vials were out of ranges (darker than any standard). All 6 Behring vials were also darker than described in the PI as clear, colorless to pale yellow. Two SAIMR vials were slightly opalescent (comparable to OSII) and 2 SAIMR and 2 Behring vials had opalescence of OSIII, all other samples were highly opalescent (OSIV) with 2 SAIMR vials OOR (>OSIV). After the initial testing, only 2 SAIMR and 2 Behring vials passed visual inspection and particle analysis while other 8 expired specimens were disqualified from further assessment.
- **Implications**
    - The initial evaluation of limited number of expired AV products appears promising. Protein content and pH suggest the products are stable over time way beyond their expiration (shelf life of both SAIMR and Behring is X and 41 months, respectively). Four SAIMR and 4 Behring expired vials were disqualified from further evaluation after the initial testing due to poor appearance (8 vials) or high endotoxin content (1 vial). Based on limited information about handling and storage of these vials prior their shipment

to the FDA, 6 out of 8 disqualified vials experienced temperature excursions out of the recommended temperature storage range of 2-8$^{o}$C. Since all 4 vials qualified for further product evaluation were stored withing the recommended temperature range, it is possible that more vials properly stored will qualify during additional planned testing. Further evaluation, at least in-vitro potency of neutralization of prothrombin-activating activity, is needed to assess the potential of expired AVs for emergency clinical use.

9) **Abstract title:** *A 24-Color Flow Cytometric Method to Analyze Immune Responses against Francisella tularensis LVS in Fischer 344 Rats*
**Authors:** Toney, Mykia, FDA/CBER (Student); De Pascalis, Roberto, FDA/CBER (Mentor); Elkins, Karen, FDA/CBER (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**


- **<u>Synopsis</u>**
    - Among small animals, the Fischer 344 rat is a useful research model to evaluate immune responses against Francisella tularensis, due to infection and immune response similarities to human outcomes. However, compared to human and mouse research, much less characterization of rat immune cell populations and subsets has been conducted. Here, we developed a comprehensive methodology to measure immune responses in rats by flow cytometry. We designed a 24-color flow cytometric panel to evaluate both innate or the adaptive immune responses against Francisella tularensis LVS vaccination. Antibodies were selected for immune cell and subset characterization based on available literature. In addition, we included detection of activation markers that may be overexpressed in response to infection or vaccination in the panel. Antibody label colors were selected based on the availability from the manufacturers and based on the similarity index calculation, which allows the identification and exclusion of colors whose laser excitations overlap in a Cytek Aurora analyzer. The antibodies were titrated using spleen and PBL preparations, and the panel was tested and adjusted as needed for optimal fluorescence of each antibody. We then validated this method in a vaccination model. Rats were injected with PBS or with LVS and analyzed within few days after vaccination. We developed gating strategies to evaluate the frequency and activation of B cells, T cells and subpopulations, NK and NK T cells, neutrophils, monocytes, and dendritic cells. This 24-color panel now allows a better evaluation of rat leukocyte markers and their overlap among cell subsets and facilitates the analyses of samples with a minimal number of cells which can then be applied to evaluating immune correlates of protection against Francisella tularensis infection.

- **Purpose**
  - A comprehensive methodology was developed to measure immune responses in rats by flow cytometry. A 24-color flow cytometric panel was designed to evaluate both innate or adaptive immune responses against Francisella tularensis LVS vaccination.
- **Methods**
  - Antibodies were selected for immune cell and subset characterization based on available literature. In addition, we included detection of activation markers that may be overexpressed in response to infection or vaccination in the panel. Antibody label colors were selected based on the availability from the manufacturers and based on the similarity index calculation, which allows the identification and exclusion of colors whose laser excitations overlap in a Cytek Aurora analyzer. The antibodies were titrated using spleen and PBL preparations, and the panel was tested and adjusted as needed for optimal fluorescence of each antibody. We then validated this method in a vaccination model. Rats were injected with PBS or with LVS and analyzed within few days after vaccination.
- **Results**
  - Gating strategies were developed to evaluate the frequency and activation of B cells, T cells and subpopulations, NK and NK T cells, neutrophils, monocytes, and dendritic cells.
- **Implications**
  - This 24-color panel now allows a better evaluation of rat leukocyte markers and their overlap among cell subsets and facilitates the analyses of samples with a minimal number of cells which can then be applied to evaluating immune correlates of protection against Francisella tularensis infection.

## Center for Drug Evaluation and Research (CDER)

1) **Abstract title**: *Manufacturing Deficiencies in Liquid Drug Products: Data Extraction and Analytics*
**Authors**: Bataille Backer, Perpetue, FDA/CDER (Student); Mayer-Bacon, Christopher, FDA/CDER (Mentor); Aldridge, Allison, FDA/CDER (Mentor); Sathigari, Sateesh, FDA/CDER (Mentor); Pai, Vidya, FDA/CDER (Mentor)
**FDA Strategic Initiative**: Unleashing the Power of Data
**Abstract**:

   - **Synopsis**
     - The Office of Pharmaceutical Manufacturing Assessment (OPMA) plays a vital role in ensuring the availability of quality medicines for the American public. Their primary responsibility involves evaluating manufacturing processes and facilities to ensure a consistent supply of high-quality drug products that meet the necessary quality standards for approval. OPMA assessors thoroughly review application data, such as process development and batch information, to assess critical quality attributes (CQAs) of each unit operation in drug

manufacturing. These attributes encompass factors like product appearance, active ingredient content, pH, sterility, dose uniformity, and more. Whenever deficiencies related to manufacturing processes, controls, and facilities are identified, OPMA communicates them to the applicant/sponsor through information requests (IRs) and complete response letters (CRLs) during the review process.

The objective of our study is to analyze and categorize the deficiencies found in liquid dosage form development, based on the IRs issued to both generic and new drug product applicants. To collect data, we utilized the Panorama database, covering the period from January 2019 to June 2023, and employed a Visual Basic for Application (VBA) script to extract relevant information from documents obtained from Panorama. By categorizing these deficiencies according to unit operations like compounding and filling/sealing, we aim to provide statistical insights into common manufacturing issues in liquid drug product applications. Our focus will also extend to injectables and suspension dosage forms to identify trends and offer valuable insights to sponsors, thereby enhancing assessor training and harmonizing review assessments. This study intends to furnish sponsors with meaningful information to help reduce the occurrence of common deficiencies and clarify FDA expectations for liquid unit operations, ultimately leading to an improvement in review practices for OPMA assessors and faster approval of drug products.

- **Purpose**
    - The objective of our study is to analyze and categorize the deficiencies found in liquid dosage form development, based on the IRs issued to both generic and new drug product applicants. To collect data, we utilized the Panorama database, covering the period from January 2019 to June 2023, and employed a Visual Basic for Application (VBA) script to extract relevant information from the documents. By categorizing these deficiencies according to unit operations like compounding and filling/sealing, we aim to provide statistical insights into common manufacturing issues in liquid drug product applications. Our focus will also extend to injectables and suspension dosage forms to identify trends and offer valuable insights to sponsors, thereby enhancing assessor training and harmonizing review assessments. This study intends to furnish sponsors with meaningful information to help reduce the occurrence of common deficiencies and clarify FDA expectations for liquid unit operations, ultimately leading to an improvement in review practices for OPMA assessors and faster approval of drug products.

- **Methods**
    - We downloaded Integrated Quality Assessment (IQA) documents from the Panorama database and used a (VBA) script to extract relevant data, including IRs, from these documents. The collected IQAs were filtered using another VBA script to include those published between January 2019 and June 2023 as Microsoft Word documents (.doc, .docx, or .docm filetypes) and excluded facilities evaluations and biologics license applications (BLAs). IQAs meeting these criteria were then extracted for further analysis to generate statistical information about specific deficiencies mentioned in the IRs.
      To organize the data, we categorized the IRs based on the primary unit operations involved, such as compounding/blending, filtration, filling/sealing, lyophilization, and packaging. Additionally, we categorized deficiencies by other key concern(s) (hold time, flush volume, process diagram, certification, yield, extractables/leachables, drug substance attributes, and batch records). We performed this categorization via string matching in Excel, categorizing deficiencies based on the presence of specific substrings within the IR text.

- **Results**
    - We downloaded 2,324 documents from Panorama followed by data extraction with a VBA macro to compile IRs and other data contained within the IQAs (application number, formulation type, etc.) into a single Excel workbook. Out of these 2,324 documents, we successfully extracted data from 1,850 IQA forms to yield a collection of 6,548 IRs. Out of the 1,850 IQA reviews compatible with the Microsoft macro and automation, 1,353 were successfully processed. The remaining 497 documents could not be processed due to various reasons, including alterations in the IQA document, reviews predating the creation of the current IQA form (before 1st January 2019), or other formatting issues within the form. For these 497 failed forms, manual extraction of the IR was carried out by individually reviewing each form and manually copying and pasting the relevant information. Subsequently, all IRs were compiled into an Excel document, and the data was manually categorized and filtered using 'Key Terms.' Deficiencies were then summated based on the keyword match.

      Initially, the data was analyzed by the categories/sections of the IQA which contains unit operations (Compounding, Filling/Sealing, Filtration, Packaging) and process controls (Process Diagram, Yield, Hold Time Drug Substance Attributes and Batch Record). Over 50% of the deficiencies were found in the Unit Operations, with Filling/Sealing having the most deficiencies (23%), followed by Batch Record (15%).

      The data was further analyzed to obtain a more in-depth understanding of the deficiencies in each category. When analyzed by Unit Operations, Filling (41%), Compounding/Blending (23%) and Filtration (22%) contained the most

deficiencies by keyword search. To gain a more in depth understanding of the common deficiencies within the Filling and Compounding/Blending Unit Operations, we further examined the data based on other critical aspects such as in-process controls (IPCs), process parameters, Extractables/Leachables (E/L), Certification and parameters specific to the unit operation.

The aim of this study is to provide meaningful information to sponsors that can help reduce the occurrence of these common deficiencies and clarify FDA expectations for liquid unit operations. Analyzing and rectifying deficiencies in manufacturing processes is of paramount importance for both the FDA and the pharmaceutical industry to ensure safe and effective drug products for consumers. This study utilized an extraction tool to obtain IRs for IQA reviews from January 2019 to June 2023, examining deficiencies based on keyword matches for unit operations and process controls. The findings revealed that 'Filling' exhibited the highest number of deficiencies due to its critical role, complexity, precision, and reliance on specialized equipment, making addressing these issues vital for product safety and efficacy. Similarly, deficiencies in 'Batch Records' were prevalent due to their comprehensive nature, human errors, and frequent revisions, necessitating stringent reviews and adherence to standard operating procedures. We further examined the deficiencies within the 'Filling' and 'Compounding' unit operations as well as 'Drug Substance Attributes'. In both unit operations, the highest number of deficiencies were seen in 'other', meaning they did not easily fall into any of the categories described thus far. As such, we will further analyze and validate IRs in of these categories to identify primary deficiencies in each parameter, thereby providing valuable insights into unit operations in liquid dosage forms, injectables, and suspensions.

- **<u>Implications</u>**
    - This study will provide meaningful information to applicants that can help reduce the occurrence of common deficiencies and clarify FDA expectations for liquid unit operations and to enhance the quality review practices for assessors and faster approval of drug products.

2) **Abstract title:** *A Strategy for Biologics Optimization using Machine Learning: Leveraging Random Forest Regression with Large Experimental Data*
**Authors:** Ali, Muhammad, FDA/CDER (Student); Madhavarao, Chikkathur, FDA/CDER (Mentor); Roper, Thomas, VCU/M4ALL Institute (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**
    - **<u>Synopsis</u>**

- Cell culture formulation plays a crucial role in many different fields of science, from biopharmaceutical production and biomedical research. However, achieving optimal cell growth and production requires careful management of environmental factors (such as humidity, pH, and appropriate gas levels) and addition of cell culture media (with supplements). This abstract presents a machine learning model programmed to optimize and model Chinese Hamster Ovary (CHO) cell growth by integrating experimental data for input parameters of cell medium volume, O2, N2, and, CO2 levels, dissolved oxygen (DO), pH, and addition of supplements for cell growth. The machine learning program utilizes supervised machine learning to analyze datasets collected from several cell growth experiments, and batch runs conducted under a differing subset of environmental conditions. The model incorporates a random forest regressor to identify relationships between the features and overall impact on cell growth dynamics and production, characterized by monoclonal antibody count. By leveraging a comprehensive dataset, the program can generate predictive models capable of forecasting cell growth patterns and optimizing conditions for maximum productivity. The program offers insights into the most effective combination of nutrients, pH ranges, DO, and gas compositions for promoting optimal cell growth. Likewise, the algorithm facilitates an ease-of-use approach to streamline bioprocess development; by accurately predicting cell growth outcomes and optimizing environmental conditions, time, resources, and driving costs for each campaign can be significantly reduced. Ultimately, this project seeks to contribute to advancements in biopharmaceutical manufacturing by making more reliable and efficient cell culture processes.

- **Purpose**
  - The development of biologic drugs has revolutionized the field of medicine, offering targeted therapies for various diseases. However, the production of biologics requires meticulous optimization of complex manufacturing processes to ensure consistent quality and yield. These techniques are often found from countless years of experience from trial and error and experimentation or through costly analysis equipment that take a considerable effort to prepare and utilize, as well as risking contamination through repeated sampling. Machine learning (ML) and artificial intelligence (AI) has been an ever-growing field in pharmaceuticals as a solution to compile and analyze the large chemical and biological data being produced, accelerating the drug discovery and drug development process. Machine learning programs can accurately determine relationships and patterns within datasets and experiments that are otherwise difficult to perceive.

Supervised machine learning algorithms specifically are able to be trained on labeled data to build predictive models to optimize productivity, reduce impurity formation, and increase overall quality on the drug produced. By utilizing the patterns found within these datasets, machine learning programs can streamline the drug development timeline, reduce costs and waste, and overall improve the overall efficacy and quality of these biologics.

- **Methods**
    - The machine learning program utilizes supervised machine learning to train and validate from previous Chinese Hamster Ovary (CHO) cell growth and production experiments and batch runs conducted under a differing subset of environmental conditions. The model incorporates a random forest regressor from an ensemble of decision trees consisting of various input features (including gas levels, dissolved oxygen content (DO), pH, cell medium contents and volume, and cell media supplements) within Scikit-Learn's Machine Learning package in Python to identify relationships between the variables and overall impact on cell growth dynamics and production. The model's hyperparameters will be tuned to optimize performance and prevent overfitting using K-fold cross-validation. By leveraging a comprehensive dataset, the program can generate predictive models capable of forecasting cell growth patterns and optimizing conditions for maximum productivity. The model will be validated against a real-world scenario, in which the model will be tested to determine its accuracy.
- **Results**
    - Results from the model will be characterized by evaluating the overall performance of the model using mean square error (MSE) and R2 on growth of CHO cells and production of monoclonal from the CHO cells. During training, the training set and validation set will be divided 70:30. The model's hyperparameters, including forest depth and number of trees, were obtained through a grid search to achieve convergence with an MSE of less than 0.5 and an R2 value of greater than 0.85. These values provide a reliable threshold at which the model can be considered valid for the purposes of optimization of biologics. To perform a final validation of the model's generalization ability, a batch run will be conducted using the optimized input parameters given by the model on a separate test set and will be allowed to run for a full duration of ≥10 days before harvesting. The monoclonal antibody production levels will then be compared to previous experimental results to determine the validity of the model.

- **Implications**
    - The program offers insights into the most effective combination of nutrients, pH ranges, DO, and gas compositions for promoting optimal cell growth. This knowledge empowers researchers and bioengineers to fine-tune the process leading to enhanced cell culture productivity, improved process efficiency, and improved product quality. The extent of accuracy achieved by the algorithm to predict cell growth outcomes and optimize environmental conditions can add notable benefits in terms of time, resources, and total cost. The streamlined process development will reduce the trial-and-error approach often required for these types of experiments. Ultimately, the addition of machine learning in bioprocess development will not only accelerate the manufacturing timeline but also reduce the driving costs and financial burdens associated with each batch run. Additionally, the ease-of-use approach offered by the machine learning algorithm can allow for more widespread adoption and implementation in industry. Overall, this project's implications span from improved process efficiency and product quality to reduced costs and accelerated development timelines. By harnessing the power of machine learning, the goal is to contribute to the advancement of biopharmaceutical manufacturing, making cell culture processes and long-term bioreactor batch runs more reliable, efficient, and economically viable.

3) **Abstract title:** *Application of Exposure-Response Analyses in Oncology Drug Development*
**Authors:** Anna Dong, FDA/CDER (Student); Youwei BI, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**
    - **Synopsis**
        - Exposure-Response analyses has been increasingly applied in the drug development in recent years for efficacy and safety assessment. To better understand its application in oncology drug development, we aim to take an extensive look at scope, utility, methods, and outcome of exposure-response analyses for the new molecular entities and new therapeutic biological products for oncology indications approved in the past 5 years.
    - **Purpose**
        - Specifically, this research will focus on:
        - survey of oncology drugs and biologics approved in past 5 years for mechanism of action and indications
        - summarizing matrices and endpoints used for the exposure-response analysis for efficacy and safety

- overviewing the results of the exposure-response analysis for efficacy and safety
- current status of the exposure-response analysis information in the product labeling

- **Methods**
  - For this research, the oncology new molecular entities submitted in the past 5 years were extracted from New Drugs at FDA: CDER's New Molecular Entities and New Therapeutic Biological Products. The exposure-response analysis information from these New Molecular Entities and New Therapeutic Biological Products were captured from clinical pharmacology review from integrated review, multidisciplinary review or clinical pharmacology review at drugs@fda. Specifically, the following information were summarized: exposure metrics, endpoints, results of the exposure-response analysis for efficacy and safety. Further, how these findings were described in the USPI label were also summarized. The summary statistics was conducted in the Excel.
- **Results**
  - A total of 61 New Molecular Entities and New Therapeutic Biological Products approved in the past 5 years for the treatment of oncology diseases were summarized in this research, including the treatment of various cancers and solid tumors, including breast cancer, prostate cancer, etc. These drugs and biologics have at least 10 MOA's being the kinase inhibitor. Among these 61 drugs and biologics, the exposure-response relationship for efficacy varies: 3% with an inverse relationship, 31% with a flat or no significant relationship, 39% with a positive relationship, and 26% were inconclusive, insufficient, or not conducted. For exposure-response relationship for safety, 39% had a flat or no significant relationship, 30% had a positive relationship, and 28% were inconclusive, insufficient, or not conducted. The metrics used for exposure-efficacy response were primarily AUC and Cmin. The metrics for exposure-safety response used were primarily Cavg and Cmax. The endpoints used for exposure-efficacy response were mostly ORR and PFS, while the endpoints for exposure-safety response were mostly the adverse events of significant interest, varying based on treatment and disease. In addition, a total of 27 of the exposure-response results are stated in the labelling.
- **Implications**
  - In conclusion, exposure-response analyses are extensively applied in oncology drug development and regulatory review, providing supportive evidence of effectiveness in oncology treatment. Among the drugs surveyed, positive relationships were most prevalent for exposure-efficacy

response, while a flat or no significant relationship were more common for exposure-safety response. These results may be limited due to the narrow exposure range from a single dose level tested in oncology clinical trials.

4) **Abstract Title:** *Rare Disease Patient Experience Use in Clinical Trial Design and Endpoint Selection for Novel Orphan Drug Development*
**Authors:** Arnette, Caroline, FDA/CDER (student); Nugent, Bridget, FDA/CDER (mentor); Welsh, Cynthia, FDA/CDER; Rothblum-Oviatt, Cynthia, FDA/CDER; Lee, Kerry Jo, FDA/CDER
**FDA Strategic Initiative:** Empowering Patients and Consumers
**Abstract:**

- **<u>Purpose</u>**
  - Rare diseases affect approximately 1-in-10 people, amounting to over 30 million rare disease patients in the US. Of the more than 7,000 known rare diseases, less than 10% have an FDA approved treatment. Rare disease drug development remains challenging for a variety of reasons. Many rare diseases have poorly understood natural histories, with phenotypically and genotypically diverse patient populations. These diseases are often progressive, serious, and life-limiting creating an urgent need for therapeutics. Small populations limit study design options. Patient experience data could be useful when designing clinical trials as it may increase the likelihood that drugs developed for rare diseases patients will address their unmet needs. Drug developers and FDA use various sources of patient/caregiver experience data including Patient Focused Drug Development (PFDD) and Patient Listening Session (PLS) meetings to help inform the design and assessment of clinical trials. Patients and caregivers are experts in their disease, and it is important to consider their most meaningful symptoms and burdens when designing the trials that assess the safety and efficacy of novel drugs.
- **<u>Methods</u>**
  - We utilized publicly available PFDD or PLS meeting reports to extract patient experience data (PED) for novel drug approvals from 2018-2023. We reviewed publicly available regulatory review documents to obtain information about the clinical trial design and whether and how PED was incorporated into the drug development process or considered in regulatory decisions. We extracted data elements from PFDD or PLS meeting that are important to patients including trial endpoints, study design, inclusion/exclusion factors, burdens of trial participation, and preferences for novel medications (e.g. route of drug administration). We compared these data to information about the novel drug and the design of the pivotal trial used to support its approval to determine how closely these factors

align. For approvals that occurred before the PFDD or PLS meeting was held or prior to publication of the meeting report, we analyzed alignment of the newly approved drug with patient needs. Further, we compared differences in endpoint alignment between approvals that considered PED from a PFDD or PLS and those that did not have a PFDD or PLS meeting prior to the approval.

- **Results**
    - We identified thirty orphan drug approvals from 2018-2023 that had publicly available PFDD or PLS meeting reports. Twenty-one had PLS or PFDD meeting that occurred prior to the novel drug's approval and nine had PLS or PFDD meetings that occurred after the drug's approval. Data collection and analysis from these PFDD and PLS meeting reports and regulatory review documents is ongoing.

- **Implications**
    - Evaluating the alignment of approved orphan drug trial design, clinical endpoints, and drug characteristics (e.g. route of administration) with patient input will provide a clearer understanding of whether and how the needs of patients and caregivers are currently being considered during drug development and regulatory review. Ultimately, we hope these findings will highlight patient needs and enable prioritization of patient preferences during the development of rare disease therapies.
- **Synopsis**
    - Development of orphan drugs can be challenging, especially due to inherently small patient populations and limited knowledge of the pathophysiology of many rare diseases. Since there are relatively few treatments approved for rare diseases, it is important to incorporate patient input to facilitate efficient rare disease drug development. Additionally, patient/caregiver perspectives are critical for rare disease drug development because patients have unique expertise regarding their disease. We investigated how patient experience data (PED) from PLS and PFDD meetings is used when defining clinical trial design and endpoints for orphan drugs. We extracted and compared information from 21 publicly available PFDD and PLS meeting reports and 30 regulatory review documents.

5) **Abstract title:** *Exploration of Food Conditions and Study Populations in Bioequivalence Studies with Pharmacokinetic Endpoints for Antineoplastic Drugs in Generic Drug Development*
**Authors:** Bae, Jihyun, FDA/CDER (Student); Tran, Tony, FDA/CDER (ORISE Fellow); Shon, Jihong, FDA/CDER (Mentor); Kim, Myong-Jin, FDA/CDER (Mentor); Li, Karen, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**

- **Synopsis**
  - Product-specific guidances (PSGs) describe FDA's current thinking and expectations on how to develop generic drug products therapeutically equivalent to specific reference listed drugs (RLDs). This project retrospectively evaluates the food condition of oral antineoplastic agents for which the currently published PSGs recommend PK BE studies in patients. PSG recommendations, dosing instructions regarding food, and Biopharmaceutics Classification System (BCS) data for each RLD along with individual fasting/fed PK BE studies in abbreviated new drug applications (ANDAs) submitted from 2013 to 2023 were collected to identify trends and potential inconsistencies among RLD labeling, BE studies, and PSG recommendations. Lastly, key components from the available corresponding European Medicines Agency (EMA) PSGs for the respective RLDs were captured to identify differences from the FDA PSGs.
    Of a total of 41 RLDs, 38 unique antineoplastic drug products were included in the analysis. Nineteen of the 41 PSGs specified a food condition, while 22 were silent. Sixteen RLDs with 81 corresponding ANDAs met the specified criteria for analysis, among which 44 ANDAs conducted 55 in vivo studies. Twenty-two studies were conducted under fasting condition, while 19 with high-fat meals, 12 with low- or moderate-fat meals, and 2 with unspecified meals. The definitions for high-fat meals were in accordance with the FDA guidance. However, the specifications for low-fat meals were heterogeneous and overlapped with moderate-fat meals. Of the 55 BE studies, 27 were conducted in healthy subjects with the food conditions specified in their PSGs, and 28 were conducted in patients. Only 8 of the 41 RLDs had PSGs available from EMA. All specified food conditions, and five recommended healthy subjects for BE studies.
    The current survey provided insights into the level of agreement on study population and food conditions between FDA PSG recommendations and submitted BE studies by ANDAs, and between FDA and EMA PSG recommendations. This collective information coupled with recommendations in the general guidances could serve as a resource for developing a standardized framework for PSG development on the selection of food condition in PK BE studies enrolling patients.
- **Purpose**
  - Food can change the bioavailability (BA) of a drug and affect the bioequivalence (BE) between test and reference listed drug (RLD) products if there are differences in formulation-food interactions. Product-specific guidance's (PSGs) describe FDA's current thinking and expectations on how to develop generic drug products therapeutically equivalent to specific RLDs. Major considerations in the development of PSGs recommending pharmacokinetic (PK) BE studies include the selection of study population,

study design, and food conditions from safety and/or PK perspectives. BE studies are generally conducted in healthy subjects or patients. Fed BE studies are recommended along with fasting BE studies, except when the RLD labeling recommends that the product should be taken on an empty stomach or serious adverse events are anticipated under the fed conditions. The Biopharmaceutics Classification System (BCS)-based biowaiver option may be used as an alternative approach for BCS Class I or III drug products (FDA Guidance: M9 Biopharmaceutics Classification System-Based Biowaiver). This project aimed to retrospectively analyze the food condition of antineoplastic agents for which the currently published PSGs recommend PK BE studies in patients and to identify common factors and potential inconsistencies among the RLD labeling, BE studies in abbreviated new drug applications (ANDAs), and PSG recommendations.

- **Methods**
    - FDA PSG databases were searched to compile a list of oral drug product PSGs recommending PK BE studies in patients. The list was filtered by USP Therapeutic Categories to include only antineoplastics to be used for analysis. PSG recommendations, dosing instructions regarding food administration, and BCS class data were collected for each RLD. Internal FDA search engines were utilized to identify ANDAs submitted for each RLD meeting the criteria of being submitted from 2013 to 2023 with the following statuses: approved, pending, complete response, or tentative approval. The following information from individual fasting and/or fed PK BE studies submitted for each ANDA were collected and analyzed: study design, meal type (e.g., fat content), and study population. Lastly, key components from the available corresponding European Medicines Agency (EMA) PSGs for the respective RLDs were captured: food condition (fasting or fed or both) and study population (healthy subjects or patients).
- **Results**
    - Forty-one PSGs (38 unique active pharmaceutical ingredients) for oral antineoplastic drug products recommended patients in PK BE studies (as of May 2023). Of the 41 PSGs, 19 specified a food condition, while 22 were silent. Six PSGs had food condition recommendations that were different from their RLD labeling. Their PSGs recommended fasting, but labeling was either silent or recommended intake with food. Sixteen RLDs with 81 corresponding ANDAs met the specified criteria, among which 44 ANDAs conducted 55 in vivo BE studies. Twenty-two studies were conducted under fasting condition, while 19 with high-fat meals, 12 with low- or moderate-fat meals, and 2 with unspecified meals. The calorie count and percentage of calories from fat for high-fat meals were in accordance with the FDA guidance. However, the specifications for low-fat meals were heterogeneous and overlapped with moderate-fat meals. Of the 55 BE studies, 27 were conducted in healthy subjects with the food conditions specified in their PSGs, and 28 were conducted in patients. Only 8 of the 41 RLDs had PSGs available from EMA. All specified a food condition, and five recommended healthy subjects for BE studies.

- **Implications**
    - Understanding the effect of food on the BE of a drug product is important to consider in the process of generic drug development. The current survey provided insights into the level of agreement on study population and food conditions between FDA PSG recommendations and submitted BE studies by ANDAs, and between FDA and EMA PSG recommendations. This data-driven information coupled with recommendations in the general guidances could serve as a resource for developing a standardized framework for PSG development on the selection of food condition in PK BE studies enrolling patients. A standardized decision framework will contribute to the efficiency of PSG development and promote consistency among PSGs.

6) **Abstract title:** *Stable Isotopic Labeling of Glucose for Tracing CHO Cell Metabolism and Mass Spectrometry-based Metabolic Flux Analysis*
**Authors:** Xin Bush, FDA/CDER (Student); Erica Berilla, FDA/CDER (Mentor); David Powers, FDA/CDER (Mentor); Casey Kohnhorst, FDA/CDER (Mentor); Nicholas Trunfio, FDA/CDER (Mentor); Nicole Azer, FDA/CDER (Mentor); Roberta King, URI/COP (Mentor), Cyrus Agarabi, FDA/CDER (Mentor).
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
    - Approximately 80% of therapeutic monoclonal antibodies are produced by Chinese Hamster Ovary (CHO) cell lines which have been optimized in studies involving nutrient profiling and genomic modification to maximize performance in culture. However, changes in metabolites between culture states and how they are related to product quality and quantity have not been well characterized. CHO cell lines can rapidly proliferate utilizing glucose as a main source of energy. This metabolic state is known as the Warburg effect and leads to high levels of lactate production by the cell. The high accumulation of lactate and cellular debris in culture can hinder cell growth in upstream production and negatively impact downstream protein purification by clogging filters. Previously, our team achieved high protein yield through a design-of-experiment (DoE) approach by studying feed strategies and process parameters, but a fundamental understanding of the inner metabolic state of the cell could lead to more accurate predictions on how the desired cell state can be achieved and maintained. Therefore, it is essential to trace the distribution and flux of downstream metabolites. This project proposes a model study using stable isotopes 1,2 13C2-Glucose and 1,6 13C2-Glucose to trace our in-house VRC01 cell line for metabolic flux analysis (MFA) by utilizing LC-MS. This linkage of intracellular data with product quality information may be used to understand and develop potential intracellular control strategies to improve and maintain product

quality attributes of monoclonal antibodies during commercial manufacturing.

- **Purpose**
  - Approximately 80% of therapeutic monoclonal antibodies are produced by Chinese Hamster Ovary (CHO) cell lines which have been optimized in studies involving nutrient profiling and genomic modification to maximize performance in culture. However, changes in metabolites between culture states and how they are related to product quality and quantity have not been well characterized. Previously, our team achieved high protein yield through a design-of-experiment (DoE) approach by studying feed strategies and process parameters, but a fundamental understanding of the inner metabolic state of the cell could lead to more accurate predictions on how the desired cell state can be achieved and maintained. Therefore, it is essential to trace the distribution and flux of downstream metabolites. This project proposes a model study using stable isotopes 1,2 13C2-Glucose and 1,6 13C2-Glucose to trace our in-house VRC01 cell line for metabolic flux analysis (MFA) by utilizing LC-MS.
- **Methods**
  - An in-house CHO K1 cell line expressing an IgG1 for anti-HIV-1 was cultured in ActiPro media for production. The cell cultures were inoculated at a density of 0.3 x 10^6 cells/mL in a Sartorius Ambr® 15 Cell Culture system. The three-day unfed batch phase was followed by a seven-day fed-batch phase. Cell Boost 7a and 7b supplements were added daily once glucose level drops below 3.0 g/L to a target of 6.0 g/L. The DO was maintained at 50% and the pH was maintained at 7.2 throughout all cultures. Normal operation of the cell culture process temperature was maintained at 37°C. However, in order to intentionally disrupt normal metabolic behavior, some batches had their temperature reduced to 33°C at the same time of the first glucose feed and held at this temperature for the remainder of the seven-day process. A set of 24 batches were run for model training: 12 were operated at 37°C for the whole batch and 12 had their temperature reduced to 33°C on day 3 at their first glucose feed. A daily sample was analyzed with a nutrient analyzer to measure the VCD, glucose and lactate concentration and additional samples were analyzed after feed additions.
- **Results**
  - After three successful Ambr15 bioreactor runs, the viability of all culture vessels was maintained above 90%. Interestingly, the glucose uptake rate for reduced temperature groups was significantly slower compared to the control groups. This suggested that CHO cell lines can rapidly proliferate utilizing glucose as a main source of energy, but the cellular metabolism was

significant slower due to the temperature change, which indicates the differences of intracellular metabolite states between two culture conditions.

- **Implications**
    - This linkage of intracellular data with product quality information may be used to understand and develop potential intracellular control strategies to improve and maintain product quality attributes of monoclonal antibodies during commercial manufacturing.

7) **Abstract Title:** *The Effect of Excess Hydrogen Sulfide and Oxidants on Particle Formation in Therapeutic Protein Formulations as Measured by Micro Fluidic Imaging*
**Authors:** Embretsen, Mattias, FDA/CDER(Student), Pritts, Jordan, FDA/CDER (Mentor), Lehtimaki, Mari FDA/CDER (Mentor), Ashutosh, Rao, FDA/CDER(Mentor).
**FDA Strategic Initiative**: Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
    - Protein based drug products can experience degradation due to environmental and manufacturing or storage conditions which can have drastic effects on product quality and to the health and wellbeing of patients. The objective of this study is to quantify the relative rates of degradation under specific stress conditions known to occur with protein drugs. Analysis of degradation will be accomplished using MFI which can track particle formation, concentration and size. Stress conditions utilized include oxidation, and exposure to H2S under pharmaceutically relevant conditions in the context of protein drugs.
- **Purpose**
    - This study aims to analyze protein particle formation in erythropoietin (EPO), growth hormone, rituximab, and trastuzumab under pharmaceutically-relevant stress conditions. Stress conditions used in this study are selected based on previously reported stresses and degradation events for large proteins. These include oxidation and exposure to hydrogen sulfide ($H_2S$) during manufacture or storage. Quantification of protein degradation under stress conditions in these selected drugs is important to determine product quality and safety prior to patient use. Since these products are designed to be injected into the body, it is essential that the protein is stable to avoid introducing abnormal particles, which could lead to adverse health outcomes like an immune response or toxicity. Additionally, this study will further investigate the effect of trisulfide bridge formation on protein due to $H_2S$ exposure. The results can be used to

determine the relationship between protein particle formation rates and trisulfide bridge formation or oxidation.

- **Methods**
    - Analysis of protein particle formation in EPO (innovator drug and biosimilar), growth hormones (innovator and biosimilar drugs), rituximab, and trastuzumab started with the introduction of different stress conditions. EPO and growth hormone products were treated with either untreated (control), or exposed to 2.5mM, or 10mM $H_2S$. All samples where then incubated at 37 °C for 3 hours with shaking at 300rpm to aid trisulfide bond formation. Finally, prior to Micro Fluidic Imaging (MFI), 1ml of Phosphate Buffered Saline (PBS) was added to each sample before each run. Rituximab and trastuzumab received oxidative stress via Metal Catalyzed Oxidization (MCO) or 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH). To accomplish this, MCO samples were treated with 0.1mM L-ascorbic acid and 10uM copper sulfate while AAPH samples were treated with 1mM AAPH. Control samples were diluted with equivalent PBS volume only. These steps were repeated to make two groups, one incubated for 3 hours and the other incubated for 24 hours. Each sample was then run through MFI following established protocols from prior publications.
- **Results**
    - Preliminary results of MFI on EPO, rituximab and 1 biosimilar show increases in particles/ml in relation to increasing severity of stress conditions. For increasing $H_2S$ concentration samples, a linear increase in particle counts per ml was observed based on the average data points for either EPO. Negative Controls for EPO showed around 998 and 601 small particles (≥ 1.00 < 5.00) per ml respectively. For the 2.5 mM $H_2S$ treated samples, there was an increase in particle concentration per ml to 1152 (EPO) and 955 (EPO biosimilar). The 10mM $H_2S$ samples of EPO showed the highest amounts of small particle sizes at 1417 for EPO and 1284 for EPO biosimilar. Interestingly, the number of larger particle sizes (≥ 5.00 < 150.00 µm) increased logarithmically with higher concentrations of $H_2S$. Rituximab exposed to MCO and AAPH showed increases in particle concentrations as time progressed, however, not by the same rates. In general, AAPH seems to follow a similar linear trend to $H_2S$ treated samples while MCO followed a logarithmic trend. Rituximab showed a significantly more pronounced increase after 24 hours of MCO with the highest concentrations reaching ~428,916 particles per ml.
- **Implications**
    - Results from this study can be used to further our understanding on the effects of trisulfide bridges and oxidative stress on these therapeutic protein

products. Specifically, data from this study shows a recurring trend of trisulfide bridge formation and oxidative stress inducing higher particle concentrations overall. This suggests that higher $H_2S$ and oxidative stress conditions will cause greater amounts of protein degradation and loss in product quality. Trends from this study can be used to advise risk assessment strategies if necessary.

8) **Abstract title:** *Non-destructive water activity measurement: An alternative to traditional moisture analysis and an at-line PAT tool for assessing homogeneity in quality attributes of lyophilized products*

**Authors:** Frimpong, Esther, FDA/CDER (Student); Xiangyi,Dong;Victor, Ng; Korang-Yeboah, Maxwell, FDA/CDER (Mentor)

**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation

**Abstract:**


- **Synopsis**
  - Lyophilization is a process used for stabilizing pharmaceuticals in which water is sublimated and further desorbed from the resulting dry layer. The moisture content of lyophilized products is a critical quality and determines the rate and mechanism of degradation. Traditional moisture analytical techniques, like Karl Fischer titration, cannot differentiate between water states, are time-consuming, require chemical reagents, and destructive. This limits the number of products that can be analyzed. To this end we studied the feasibility of replacing traditional moisture analysis with nondestructive water activity measurement with Frequency Modulated Spectroscopy. Further we studied the impact of lyophilization process parameters and formulation variables on homogeneity in water activity of lyophilized biologics. Our study showed that frequency modulated head space water activity analysis is a viable alternate to traditional Karl fisher analysis of moisture content of lyophilized products.
- **Purpose**
  - Lyophilization is a process used for stabilizing pharmaceuticals in which water is sublimated and further desorbed from the resulting dry layer. The moisture content of lyophilized products is a critical quality and determines the rate and mechanism of degradation. However, the state of water, i.e., free or bound determines the impact of moisture on product degradation. Free water, not bound water, is responsible for product degradation. Further there is an inherent intra and inter-batch heterogeneity in moisture content of lyophilized products. Traditional moisture analytical techniques, like Karl Fischer titration, cannot differentiate between water states, are time-consuming, require chemical reagents, and destructive. This limits the

number of products that can be analyzed. To this end we studied the feasibility of replacing traditional moisture analysis with nondestructive water activity measurement with Frequency Modulated Spectroscopy. Further we studied the impact of lyophilization process parameters and formulation variables on homogeneity in water activity of lyophilized biologics. The aim of the study is to determine the utility of frequency Modulation Spectroscopy (FMS) as a non-destructive alternate to Karl Fischer titration for moisture content analysis of lyophilized products.

- **Methods**
    - Sample solutions were prepared using a) Bovine serum albumin (BSA) 25 mg/ml and Sucrose 75 mg/ml. b) Bovine serum albumin (BSA) 50 mg/ml, Trehalose 50 mg/ml, and c) 5% Bovine IgG, Sucrose, and Mannitol (1.5:1:7.5) d) 5% Lactose Monohydrate e) 5% Dextran, 5% Sucrose, and f) 5% Mannitol in 10 mM phosphate buffer at a pH of 7.4. Three batches of sample solutions were lyophilized with a fixed primary during cycle but different secondary drying hold times. For each lyophilization cycle, the primary drying time was determined using the difference in pressure between the capacitance manometer and Pirani pressure gauge. The duration of the secondary drying step was however varied to yield products with different moisture contents. We explored two technologies for their capability to measure the water activity and moisture content in the lyophilized products: a) Frequency Modulation Spectroscopy (Headspace moisture analysis, HMA) and b) Karl Fischer Titration. HMA measured the absorption of a laser light with a wavelength of 1,400 nm that passed through the gaseous headspace of the vial containing the freeze-dried drug product. The water content in the headspace was determined by using a calibration curve from standards of known water vapor concentrations. The amount of water in a sample was determined directly by titration with Karl Fischer titration in a V30 Titrator (Mettler Toledo, Ohio, USA).
- **Results**
    - The lyophilization process resulted in intact lyophilized products showed signs of macroscopic collapse. Thus, the primary drying time determined for all products by an in-line pressure differential technique was adequate to ensure complete sublimation of all ice. There was variation in moisture content and water activity for different products with similar lyophilization process parameters. However, there was a correlation between the water activity and moisture content of the lyophilized products. In addition, we observed a correlation between the lyophilization process parameters used, specifically secondary drying time, product's moisture content and water activity. No intra-day variation was observed in the water activity or total

head space pressure of the lyophilized products, an indicator of properly sealed vials and rapid equilibration between the unbound water and head space water vapor pressure Thus water activity may be a good indicator of the seal integrity of lyophilized products. Further, water activity measurement was successfully used to assess the homogeneity in water activity in an entire batch of lyophilized products manufactured with a pre-clinical/clinical stage lyophilizer.

- **Implications**
    - Frequency modulated head space water activity analysis is a viable alternate to traditional Karl fisher analysis of moisture content of lyophilized products. The rapid, and nondestructive nature of the technique may allow testing of each individual product vial and for assessing the intra and inter-batch homogeneity in product quality of lyophilized products.

9) **Abstract title:** *Development of A Celigo-Based ADCC Assay to Assess the Critical Quality Attributes of Biosimilar Drugs*
**Authors:** Garg, Ria, FDA/CDER (Student); Zhao, Madison, FDA/CDER (Student); Gorospe, Jordan, FDA/CDER (Student); Simhadri, Venkateswara, FDA/CDER (Mentor); Bacot, Silvia, FDA/CDER (Mentor); Wang, Tao, FDA/CDER (Mentor); Feldman, Gerald, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition Through Innovation
**Abstract:**

- **Synopsis**
    - The development and release of biosimilar drugs on the market necessitates reproducible validation of analytical assays to demonstrate biosimilarity between the proposed biosimilar and its reference product. One of the most important effector cell functions that characterize and define many biosimilar drugs is antibody-dependent cellular cytotoxicity (ADCC). Currently, there are many assays used to detect ADCC, but they have several limitations including but not limited to: high background readouts, low degree of standardization, use of radioactive materials, inability to fully recapture ADCC occurrence, etc. To overcome these limits, we are proposing a novel ADCC assay based on the Celigo Image Cytometry System. This fluorescence-based assay can quantitively assess live and dead cells using AOPI dye to provide a viability percentage as well as distinguish between target cells and effector cells based on their cell size. In this study, we first isolated human NK cells from frozen PBMCs and then treated them with 500 units/mL of IL-2 for 24 hours. A CD20 positive human lymphoblastoid cell line, Raji cells, was used as the target cell. Various concentrations of Rituximab (an FDA approved drug primarily used to treat CD20 positive Non-Hodgkin's Lymphoma) were added to the samples. Our

preliminary results demonstrate that increasing concentrations of Rituximab induced the killing of the Raji cells in a dose-dependent manner. The Celigo assay generates comparable sensitivity compared to commercial ADCC reporter assays, responding to antibody concentrations at least as low as 0.5 ng/mL. In summary, we have developed an ADCC assay that is highly sensitive, easy to handle and scale-up, and highly reproducible.

- **Purpose**
    - The development and release of biosimilar drugs on the market necessitates reproducible validation of analytical assays to demonstrate biosimilarity between the proposed biosimilar and its reference product. This validation is crucial as it allows more biologics to be available on the market for the same treatment indications and lowers the cost of those treatments. It is estimated that the number of biosimilars being developed will increase dramatically, thus requiring efficient assays that can reliably compare biosimilars to their reference drugs. The analysis of effector function (a critical quality attribute of biosimilars) is necessary because biosimilar drugs may show unexpected effector functions that are not shown by their reference products. One of the most important effector cell functions that characterize and define many biosimilar drugs is antibody-dependent cellular cytotoxicity (ADCC). Analyzing the occurrence and frequency of ADCC by biosimilar drugs in comparison to reference drugs allows us to assess the similarity between the two. Currently, there are many assays used to detect ADCC, but they have several limitations including but not limited to: high background readouts, low degree of standardization, use of radioactive materials, inability to fully recapture ADCC occurrence, etc.
- **Methods**
    - To overcome these limits, we are proposing a novel ADCC assay based on the Celigo Image Cytometry System. This fluorescence-based assay can quantitively assess live and dead cells using AOPI dye to provide a viability percentage as well as distinguish between target cells and effector cells based on their cell size. In this study, we first isolated human NK cells from frozen PBMCs and then treated them with 500 units/mL of IL-2 for 24 hours. A CD20 positive human lymphoblastoid cell line, Raji cells, was used as the target cell. Various concentrations of Rituximab (an FDA approved drug primarily used to treat CD20 positive Non-Hodgkin's Lymphoma) were added to the samples.
- **Results**
    - Our preliminary results demonstrate that increasing concentrations of Rituximab induced the killing of the Raji cells in a dose-dependent manner. The Celigo assay generates comparable sensitivity compared to commercial

ADCC reporter assays, responding to antibody concentrations at least as low as 0.5 ng/mL.

- **Implications**
    - As part of the validation of this assay, we will conduct comparability studies of the ADCC activity of three different biosimilars compared with the reference drug. We will also use an orthogonal flow cytometry-based assay to support the validation of this assy. In summary, we have developed an ADCC assay that is highly sensitive, easy to handle and scale-up, and highly reproducible. This new assay is an effective and efficient method to assess ADCC, a critical quality attribute of biosimilars needed to facilitate the development of biosimilar drugs.

10) **Abstract title:** *Signature Peak and Stability of Therapeutic Proteins Using High-Throughput Dynamic Light Scattering*
**Authors:** Harish, Siri FDA/CDER (Student); Bhirde, Dr. Ashwinkumar FDA/CDER (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
    - The physiochemical stability of therapeutic proteins (TPs) is a critical quality attribute of drug products (DPs). Changes in physiochemical properties like size due to changes in temperature during manufacturing, shipping, administration, and throughout the DP's life cycle can impact the quality of TP drugs. Our research uses High- Throughput Dynamic Light Scattering (HT-DLS), a routinely-used analytical tool to study the signature peak and stability of monoclonal antibody (mAb) and insulin DPs under high-temperature stress conditions. Our results indicate that each DP behaves differently under the same stress conditions, indicating the importance of understanding the failure modes that can impact product quality. Though HT-DLS is a commonly-used technique during manufacturing by the biotech industry, our study shows the significance of using appropriate data processing methods to accurately interpret the results collected for submission. Our experimental results indicate that Z-average hydrodynamic size parameters may not be the best indicator of size stability during thermal stress stability studies of TPs.
- **Purpose**
    - Therapeutic proteins (TPs) are biological drug products (DPs) that are used to prevent, diagnose, treat, and cure different medical conditions. Monoclonal antibodies (mAbs), for instance, are an example of TPs which target a specific protein to regulate its function, deliver a drug, or help diagnose a patient. Changes in the physiochemical properties like size can

impact the quality of a TP DP. Knowing the stability-indicating parameters like signature peak and failure modes like thermal stress helps keep a check on the quality of the DP. High-Throughput Dynamic Light Scattering (HT-DLS) is a routinely-used technique in the biotechnology industry that can measure the size and stability of TP DPs. While running HT-DLS experiments are not complicated, it can be challenging to meaningfully interpret data due to the nature of the samples and the methods used to process the data. Our study aims to help drug manufacturers accurately interpret HT-DLS data collected for submission, which in turn can help FDA reviewers in making sound, science-based regulatory decisions during the review of an application.

- **Methods**
    - Dynamic Light Scattering (DLS) is a technique used to analyze particle size distributions of a sample based on the principle of Brownian motion. DLS is especially helpful for investigating the size and stability of particles. High-Throughput Dynamic Light Scattering (HT-DLS) takes this technique one step further by running multiple samples at the same time using a plate reader detection system. Using HT-DLS, users can remove outliers without compromising the overall integrity of the data due to the vast volume of data points. In our study, we evaluated six marketed drug products (DPs) including three monoclonal antibodies (Cetuximab, Rituxan, and NIST mAb) and three insulin analogs (Humalog, Novolog, and Apidra). The DLS experiments were performed using Wyatt Technology's DynaPro Plate Reader III and DYNAMICS software. We used a 384-well Corning plate. Each well contained 30 µL of DP sample with 10 µL of paraffin oil. After pipetting the samples, we centrifuged the plate to remove air bubbles that could potentially impact data collection. For our experiments, the laser power was set to 100%. First, we ran control experiments to assess the signature peaks. Later, we ran a thermal ramp from 25 to 82 °C to assess stability under high-temperature stress. For the thermal ramp experiment, both the cumulants and regularization diameter were recorded for all temperatures.
- **Results**
    - Hydrodynamic size diameters for the therapeutic protein (TPs) samples were collected with cumulants (Z-average) and regularization data processing methods. The cumulants method assumes the sample to be monomodal. That is, all the particles in the sample are one population. Our data shows that cumulants diameter is acceptable at temperatures around room temperature for monomodal samples with low polydispersity index. However, at higher temperatures, once TP drug products (DPs) begin to degrade or generate aggregates, and for multimodal samples, it is better to

consider regularization diameter. The regularization method assumes the sample to be multimodal, differentiates between particles three to five times different in size, and reports size distribution based on % intensity, % mass, and % number. When running the control experiment at room temperature, monoclonal antibody (mAb) DP samples showed a signature peak at 11 +/- 2 nm while insulin DP samples showed their signature peak at 6 +/- 2 nm. For the mAbs, Novolog, and Apidra (control, non-stressed samples), the signature peak was the same for both the cumulants and regularization. However, for Humalog's signature peak, size data were evaluated from both cumulants and regularization methods due to the multimodal nature of the sample. Thermal stress data showed multiple size peaks for all the DPs tested at higher (> 60 °C) temperatures as indicated by the regularization method. Our high-temperature experiments provide information on the thermal stress stability temperature range and unfolding temperature of the specific TP DP.

- **<u>Implications</u>**
    - With therapeutic protein (TP) drug products (DPs) becoming more popular, it is sensible to include High-Throughput Dynamic Light Scattering (HT-DLS) as an analytical tool to screen for size stability during upstream processing, downstream processing, shipping, handling, and throughout the DP's life cycle. Furthermore, HT-DLS allows scientists to run hundreds of samples at a time, boosting the confidence levels of the collected data. Our research shows that HT-DLS is a useful method for assessing the signature peak and stability of TPs like monoclonal antibody (mAb) DPs. While DLS is already a commonly-used technique, interpreting the data can be challenging. Pharmaceutical companies can learn from our research on how to evaluate their DLS data correctly, which in turn will help FDA reviewers assess submitted data with confidence. When investigating signature peaks of monomodal samples, both cumulant and regularized diameters in control experiments can be evaluated. However, for multimodal samples, as well as all samples under forced degradation stability studies at higher temperatures where protein unfolding may occur, it is better to consider data using the regularization method along with the samples' polydispersity index.

11) **Abstract title:** *Review of PAT for Crystallinity Control in Advanced Pharmaceutical Manufacturing (Part 2)*
**Authors:** Harms, Caroline, FDA/CDER (Student); Ma, Chaoying, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition Through Innovation
**Abstract:**

- **Synopsis**
    - Advanced manufacturing is considered the future of pharmaceuticals due to its ability to process challenging active pharmaceutical ingredients (APIs) into commercially viable forms and its potential to expand production efficiency and scope. However, the novelty of such techniques requires the identification and implementation of analytical strategies to monitor the physicochemical properties of an API – particularly its crystallinity – that may be susceptible to change during production or storage. These changes, if unmonitored, could significantly impact the bioavailability, efficacy, and safety of the desired pharmaceutical product. Further, such qualities can be increasingly difficult to assess as industry begins to incorporate continuous manufacturing techniques that require greater in-line process analytical technology (PAT). In response to these anticipated challenges, this investigation assesses current and exploratory crystallinity monitoring practices in pharmaceutical production, considering literature reports and industrial practices from new drug application (NDA) and abbreviated new drug application (ANDA) case studies. Building on previous work, specific focus is given to advanced manufacturing techniques and API formulation strategies that are susceptible to changes in API crystallinity, including amorphous spray-dried dispersions (SDDs), continuous manufacturing (CM), and 3D printing or additive manufacturing. Case studies confirm PXRD's popularity as a highly sensitive technique for crystallinity monitoring, while new advances using in-line methods such as Raman spectroscopy indicate the potential for the efficient assessment of multiple drug attributes during continuous manufacturing processes. The conclusions drawn from this analysis serve to guide the incorporation of new analytical tools in future industrial processes that will improve product quality, stability, and health outcomes.
- **Purpose**
    - Advanced manufacturing is considered the future of pharmaceuticals due to its potential to process previously challenging APIs into commercially viable forms and expand production efficiency and scope. However, the novelty of such techniques requires the identification and implementation of analytical strategies to monitor product attributes, such as water content, impurity content, assay, and crystallinity, that are susceptible to change during production or storage. Focusing specifically on crystallinity, poorly soluble BCS Class II and IV APIs often require sophisticated processing or phase transformations for their feasible manufacture and overall performance. Any change in the desired physical state of these APIs (i.e., transitions from amorphous to crystalline form) could compromise the safety and efficacy of pharmaceutical products. To address these challenges, this investigation

assesses current and exploratory PAT practices for crystallinity monitoring in advanced pharmaceutical manufacturing.

- **Methods**
  - The merits and disadvantages of conventional and emerging crystallinity monitoring PAT techniques reported in literature are reviewed and compared with state-of-the-art commercial and regulatory practices. For this assessment, the crystallinity monitoring procedures for 11 commercially approved NDAs and ANDAs produced via advanced manufacturing techniques are investigated, specifically including five products that utilize amorphous spray-dried dispersions (SDDs), three products produced via continuous manufacturing (CM), and three investigational new drug (IND) applications for 3D-printed products. Relevant information was extracted from submitted documents in the FDA docuBridge and DARRTS databases. The detection limit, in-line capability, sampling procedure, operating parameters, and conclusions drawn from PAT methods are identified for each case study to draw broad conclusions regarding the current state of crystallinity monitoring in industrial pharmaceutical production. Alternative assessments of critical quality attributes (CQAs) relevant to changes in the API or final product's physical form are also considered.
- **Results**
  - PXRD is established as the industry standard for monitoring pharmaceutical crystallinity, used in 100%, 36%, and 55% of case studies during formulation development, manufacture, and stability testing, respectively. SDD and 3D printing case studies use PXRD or XRD to confirm amorphous API content after spray drying or hot melt extrusion, reporting detection limits between 0.375-2.47% w/w of crystalline API in final drug products. While PXRD is used for product validation in CM processes, at-line PAT methods are employed in 33% of CM examples for crystallinity monitoring during manufacture. Despite literature reports highlighting its capabilities, NIR is not used for in-line crystallinity assessment, while Raman spectroscopy exhibits at-line sensitivity comparable to PXRD, capable of detecting 1.4% API crystallinity in drug products sampled for real time release testing (RTRT). DSC or ssNMR is employed in 73% of case studies during formulation development to predict stability behavior from characteristic thermal transitions and crystallization kinetics. Likewise, water content or dissolution studies are employed in 73% of case studies as a substitute for crystallinity monitoring PAT or to confirm analytical results. However, their correlation with crystallinity is often insufficiently justified, with 36% of case studies requiring clarifying documentation or further testing to meet regulatory specifications.

- **Implications**
    - As the pharmaceutical industry continues to adopt novel manufacturing techniques to expand production capabilities, it is critical to incorporate analytical tools to ensure product quality. This investigation provides insight regarding the general state of crystallinity monitoring in advanced manufacturing processes, with PXRD and Raman spectroscopy being the most prevalent and sensitive techniques implemented to date. Beyond assessing emerging PAT methods like electron microscopy and isothermal microcalorimetry for their potential utility and feasible integration into advanced manufacturing schemes, further investigation of PAT strengths and limitations is required to understand why techniques commonly reported in literature, such as NIR and FT-IR, are not commonly used for crystallinity assessment in industry. Additionally, the potential use of these analytical methods for establishing a more robust correlation between a drug product's dissolution, water content, and crystallinity should be considered for the time-effective and reliable assessment of multiple drug product CQAs using a singular PAT method. These future pursuits, guided by the results of this study, will benefit both production and regulation spaces when development technologies to improve pharmaceutical manufacturing output, treatment options, and product attributes.

12) **Abstract title:** *Evaluation of Immunogenicity using Population Pharmacokinetic Modeling for Approved Biologics: A Review of Varied Approaches and Implications for Best Practices*
**Authors:** Hashimoto, Sora, FDA/CDER (Student); Florian, Jeffry, FDA/CDER (Mentor); Wang, Yow-Ming, FDA/CDER (Mentor); Liu, Jiang, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**
    - **Synopsis**
        - The evaluation of immunogenicity in population pharmacokinetic (popPK) modeling for approved biologics has witnessed variable approaches among sponsors. Different data analysis approaches can lead to different conclusions regarding the impact of immunogenicity on key PK parameters such as clearance. This study examines and summarizes the approaches used by sponsors in evaluating immunogenicity within PopPK modeling. By analyzing the existing practices, the research aims to provide insights that can inform the development of best practices for analyzing immunogenicity data and enhance the understanding of its impact on key PK parameters. The analysis reveals the diverse approaches used, highlighting the need for standardized practices for evaluating immunogenicity in popPK analyses and

further understanding sponsors' decision-making process when evaluating the impact of immunogenicity during in popPK modeling.

- **Purpose**
    - This study is to examine and summarize approaches used by sponsors in evaluating the impact of immunogenicity on key pharmacokinetic (PK) parameters during population pharmacokinetic (popPK) modeling for approved biologics. The approaches utilized by sponsors when evaluating impact of immunogenicity vary between submissions. The lack of consistent approaches makes it challenging to establish standardized guidelines for how the impact of immunogenicity on pharmacokinetic should be evaluated during popPK modeling. By summarizing how analyses were conducted and the conclusion across different sponsor submissions, this research seeks to provide valuable insights that can inform the development of best practices for the analysis of immunogenicity data.
- **Methods**
    - PopPK reports for approved biologics were collected and organized by the year of approval. PopPK reports in submissions were analyzed to determine if the impact of immunogenicity on PK was evaluated and to assess the handling of data points below the limit of quantification (BLQ) in analyses. The evaluation of anti-drug antibodies (ADA) in the popPK reports was examined and categorized based on whether ADA was implemented based on the ADA status of positive or negative, either a fixed value or a time-varying covariate during the study or based on a continuous titer measure. FDA review documents, including Clinical Pharmacology Reviews and Multi-Discipline Reviews, were pulled from Drugs@FDA and analyzed for insights regarding FDA's recommendations to sponsors regarding immunogenicity evaluation and conclusions regarding immunogenicity analyses conducted by the sponsor. Drug labels from DailyMed were reviewed to extract information on immunogenicity from different sections of labeling.
- **Results**
    - The analysis of PopPK reports revealed that among different approaches used for evaluating effects of ADA on PK, the most common method employed was incorporating ADA status (positive or negative) as a binary fixed covariate effect on clearance. This was followed by utilizing a time-varying covariate of ADA status and titer-based evaluation for the ADA effect on clearance, respectively. However, it was noted that in a subset of PopPK reports, an evaluation of immunogenicity was not conducted. Reasons for this omission varied and included low ADA incidence observed in the clinical studies, lack of any difference in exposure between patients with and without ADAs upon visual inspection, immunogenicity sampling

being too limited from clinical studies to support analyses, or too limited of a patient sample size to support ADA analyses (observed in some popPK analyses for rare disease). A subset of submissions did not have any popPK reports submitted. The most common reason for a submission not having a popPK analysis was limited systemic exposure due to route of administration.

- **Implications**
    - This project is the first comprehensive analysis conducted by the FDA on how immunogenicity is evaluated in PopPK modeling of approved biologics. The findings provide for understanding the methods employed by sponsors and shed light on the important considerations surrounding the assessment of clinical impact of ADA. While this analysis highlighted that some PopPK reports did not evaluate ADA as a time-varying covariate, further investigation will be beneficial to gain a deeper understanding of the sponsor's decision-making process in choosing covariate implementation approaches (using ADA status as a fixed value or a time-varying covariate). Potential reasons could include challenges in obtaining longitudinal ADA data or a lack of correlations between ADA evaluated as fixed or a time-varying covariate and PK parameters.

13) **Abstract title:** *Assessment of The Adequacy of Drug Tolerance of Anti-drug Antibody Assays in CDER Approved Biological License Applications*
**Authors:** Huang, Peirung, FDA/CDER (Student); Gharazi, Salimeh, FDA/CDER (ORISE Fellow); Wang, Yow-Ming, FDA/CDER (Co-Mentor); Rajabiabhari, Mohsen, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - Biotherapeutics are inherently immunogenic, i.e., they can trigger an unwanted immune response leading to the formation of anti-drug antibodies (ADA). The negative impact of ADAs on therapeutic outcomes, ranging from altered efficacy (including loss of efficacy or heightened effect) to serious adverse events (including serious hypersensitivity reactions), has been extensively documented in the literature.1-5 Therefore, sponsors develop and validate immunogenicity assay for ADA (ADA assay) to evaluate the clinical relevance of immune responses. The FDA recommends assessing levels of therapeutic protein in the study sample to evaluate the potential interference with detection of ADAs. We surveyed the biologics approved by Center for Drug Evaluation and Research (CDER) from 2019 to July 7, 2023 to assess the adequacy of the ADA assays. We compared drug's mean steady-state trough concentrations with the validated assay drug tolerance to evaluate the potential drug interferences. We found that about 74% of the ADA assays had adequate drug tolerance. Our observation demonstrates an improvement in the immunogenicity assays developed by the sponsor since our earlier study published in 20126 in which we found

about 41% of biologics approved by the FDA between 2005 to 2011 had sufficient drug tolerance.

- **Purpose**
  - Immunogenicity assays developed to assess the presence of ADAs in patient samples are ligand-binding assay (LBA). A common assay format is using the study drug as the capture reagent and the detection reagent that bind to the ADA. High concentration of drugs in the matrix can interfere with the detection of ADAs leading to false-negative results; therefore, ADA samples are generally collected at the time when the drug concentration is at the trough level. Drug tolerance is the maximum amount of drug that would allow detection of ADAs with the assay. The goal of this project is to compare the mean steady-state trough concentration to the drug tolerance to identify the prevalence of drug interference in immunogenicity assays by reviewing the ADA assays for the biologics approved by CDER from 2019 to 2023.
- **Methods**
  - We obtained a list of CDER approved biological license applications (BLAs) from the FDA's Purple Book Database of License Biological Products. From the list, we selected products with the license type of 351(a) and the original approval dates from 2019 to 2023. From the selected 351(a) BLAs, we extracted information about the ADA assay, including drug tolerance, low positive control (LPC) used, and assay sensitivity from integrated summary of immunogenicity (ISI) and bioanalytical method validation reports in the regulatory submission (internal database). The mean steady-state trough concentrations were obtained either from ISI, the Clinical Pharmacology Biopharmaceutics Review, or Multi-Discipline Review documents at the Drugs@FDA website which are available publicly. Then, we compared mean steady-state trough concentration to drug tolerance concentration of each of the products. When the drug tolerance is greater than the mean steady-state trough concentration, the assay drug tolerance is considered adequate for the purpose of this analysis.
- **Results**
  - Based on our survey of CDER-approved biological products from 2019 to July 7, 2023, we identified 33 products. Among these products, there were 21 monoclonal antibodies, three enzyme products, three fusion protein, two protein conjugate, and two botulinum toxins. Drug trough concentrations were not found for two products. Drug tolerance concentrations ranged from 4000 ug/mL to 10 ng/mL and drug trough concentrations range from 1516 ug/mL to 0.65 ng/mL. About 74% (23/31) of the evaluated biological products have steady-state trough concentrations below the drug tolerance level (i.e., the assay is adequate), and about 26% (8/31) of the biological products have steady-state trough concentrations above the drug tolerance level.
- **Implications**
  - The risk of immunogenicity (e.g., the incidence of ADA) and the clinical impact of immunogenicity are communicated to healthcare providers and

patients through the product labeling. Therefore, these assessments are expected in regulatory submissions. Adequately designed ADA assays, including with adequate drug tolerance, are critical to reliably determine the formation of ADA after treatment, which is necessary to evaluate the clinical impact of immunogenicity on biological products' pharmacokinetic, pharmacodynamic, efficacy, and safety. We found that BLAs approved in 2019-2023 have improved ADA assay drug tolerance when compared to those approved in 2005-2011 (described in our publication of 2012). However, further improvement would be desirable because only 74% of products in the current survey had adequate drug tolerance for ADA assays.

14) **Abstract title**: *Defining the operating space for continuous manufacturing ER tablets by twin screw granulator*
**Authors:** Humayra Iqbal, Nobel SierraVega, Muhammad Ashraf, Ahmed Zidan
**FDA Strategic Initiative:** Increasing Choice and Competition Through Innovation
**Abstract:**

- **<u>Synopsis</u>**
  - This study aims to understand process parameters of the twin screw wet granulation (TSWG) process, and its effects on granule characteristics. The study investigates effects of screw configuration and operating conditions by characterizing resulting dried granules. Continuous manufacturing using TSWG may be used to address drug shortages, and to reduce costs of manufacturing.
- **<u>Purpose</u>**
  - Twin-screw wet granulation (TSWG) process is an important continuous manufacturing (CM) technology for solid dosage forms that have gained interest in the pharmaceutical industry to improve drug quality, address drug shortages, and improve manufacturing and development time. TSWG process is used to improve the flow and compaction properties of the formulation and enhance the uniformity of the Active Pharmaceutical Ingredient (API) in the final product. TSWG enlarges particles from a fine powder blend with the help of a liquid binder solution. The properties of the granules depend on screw configuration (number of kneading (KEs), sizing (SEs), and conveying elements (CEs), number of kneading blocks, staggering angle of KEs) and operating conditions (screw speed, powder feeding rate, and liquid to solid (L/S) ratio). the relationship between these variables for manufacturing immediate release formulation has been investigated in different studies, but there is a lack of information on the effect of these screw design parameters and operating parameters for processing extended-release formulations. Therefore, the aim of this study is to determine the effects of the number of kneading elements (KE), sizing elements (SE), screw speed and powder feeding rate on the granule properties for an extended released formulation.
- **<u>Methods</u>**
  - The extended-release formulation included metoprolol succinate as model API, and HPMC K100M, DCP dihydrate, and HPC 100K as excipients. Each

raw material was first passed through sieve No. 60 to ensure uniform particle size distribution. The API and excipients were mixed for 40 minutes in a 24-quart stainless-steel V-blender at 20 RPM. After blending, the powder blend was added to loss-in-weight feeder of ConsiGmaTM 1 twin-screw granulator. The powder blend is fed to the feed section, which conveyed it to kneading elements where the powder is mixed with the liquid binder. The liquid binder is fed just before the first kneading zone using a peristaltic pump. The number of KEs and SEs, screw speed, and powder flow rate were changed according to the design of experiments to study its effect on granules properties. Granules obtained from each run were collected in a glass tray and dried to an LOD < 3 %w/w in a convection oven at 30ºC for 12 h. The dry granules were characterized in terms of conditioned bulk density (CBD), compressibility (CPS), permeability, cohesion, and flow factor using the Ft4 powder rheometer, and particle size distribution using the sieving method.

- **Results**
  - The results showed that the number of KEs, screw speed and powder feeding rate significantly affected the properties of granules, while the effect of number of SEs was not significant. The larger number of KEs was found to significantly increase PSD, cohesion, and compressibility and decrease permeability. Higher screw speed was found to increase the PSD and decrease the CPS. Higher powder feeding rate was found to decrease PSD, increase CPS and cohesion, while decreasing permeability.
- **Implications**
  - The screw speed, powder feeding rate, and the number of KEs and SEs are critical operating parameters to define the design space for manufacturing ER formulation in a TSWG process. TSWG may by effectively improve production, operation, and environmental costs. It may also address other challenges such as drug shortages, and quality issues.
- **Disclaimer**
  - This abstract represents the view of the authors and should not be construed to represent FDA's views or policies.

15) **Abstract title:** *A Probe Based Flow Cytometry Assay for Rapid and Quantitative Detection of BCC*
**Authors:** Tang, Linli, FDA/CDER (Student); McCabe, Megan, FDA/CDER (Student); Collins, Sylva, FDA/CDER (Mentor); Nie, Lei, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
  - The pharmaceutical industry has recently placed a strong emphasis on quality by design (QbD) and the adoption of continuous manufacturing (CM) to enhance process efficiency, reduce costs, and improve product quality. As expressed by ICH Q13, process models are a crucial tool for improving our understanding of how critical process parameters affect product critical quality attributes. In this work, we present a modeling approach for the

continuous synthesis and crystallization of carbamazepine (CBZ), an essential medicine used in the treatment of various neurological and psychiatric disorders. By comparing the kinetic parameters processes of each reaction obtained in a batch kinetic study with the experimental results obtained in continuous reactions, we realized that the continuous system underperformed the batch system. In the continuous system, the yield was lower and the impurity formation higher than was expected based on the batch study kinetic parameters. Adjustments were thus made to the continuous system to bring it closer to the kinetics of the batch system. This study demonstrates the value of comparative mechanistic modeling when developing CM as a tool towards affecting QbD.

- **Purpose**
    - The pharmaceutical industry has recently placed a strong emphasis on quality by design (QbD) and the adoption of continuous manufacturing (CM) to enhance process efficiency, reduce costs, and improve product quality. A critical aspect of QbD is understanding the relationships between critical process parameters, intermediate quality attributes (IQAs), and product critical quality attributes accurately. Moreover, because CM processes involve the integration of multiple unit operations, it is essential to understand the impact of each process outlet's IQAs on the downstream processes. As expressed by ICH Q13, process models are a crucial tool for improving our understanding of these relationships.
- **Methods**
    - In this work, we present a modeling approach for the continuous synthesis and crystallization of carbamazepine (CBZ), an essential medicine used in the treatment of various neurological and psychiatric disorders. The system incorporated a synthetic reactor unit followed by anti-solvent crystallization. A kinetic model was developed in a batch reaction study that revealed a four-reaction reaction network.
- **Results**
    - These reactions included the desired reaction, a reactant degradation reaction, and two impurity formation reactions. By comparing the kinetic parameters processes of each reaction obtained in the batch study with the experimental results obtained in continuous reactions, we realized that the continuous system had higher rates of reactant degradation and impurity formation. This realization enabled us to reduce the degradation and impurity formation rates back down to similar rates to those seen in the batch system by changing the reactant addition method, the feedstock dissolution, and the reactant split ratio (between the two CSTRs in series).

- **Implications**
    - This study demonstrates the value of comparative mechanistic modeling when developing CM as a tool towards affecting QbD. Without such comparisons, various ways in which a continuous system might under perform a batch system could go undiscovered. By considering the differences between these two systems, the limitations of the continuous system can be overcome.

16) **Abstract title:** *Evaluation of O-glycoprotein CD99 in Ewing Sarcoma Cell Lines as a Potential Biomarker for Ewing Sarcoma*

**Authors:** Li, Erin, FDA/CDER (Student); Kim, Su-Ryun, FDA/CDER (Mentor); Zhang, Yaqin, FDA/CDER (Mentor); Ju, Tongzhong, FDA/CDER (Mentor); Rao, Ashutosh, FDA/CDER, Donoghue, Martha, FDA/OND (Mentor); Summers, Jeff, FDA/OND (Mentor); Reaman, Gregory H, NIH/NIC (Mentor)

**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation

**Abstract:**

- **Synopsis**
    - Ewing's Sarcoma (EWS) is a rare and aggressive neuroectodermal tumor occurring in children and young adults. Although multiple potential biomarkers have been previously suggested for EWS, there is no investigation on the glycome of EWS as many tumor biomarkers are tumor-associated carbohydrate antigens (TACAs). Particularly, CD99, an O-glycosylated protein was reportedly overexpressed in EWS cells. CD99 is a highly glycosylated membrane protein with ten potential O-glycans without N-glycosylation. Besides suggested regulation of immunity by interactions with receptors on B-cells, cell differentiation and adhesion of tumor cells, the biological function of CD99 is largely unknown. Protein O-glycosylation is one of the major post-translational modifications that play many pivotal roles in biological processes, such as cell-cell and cell-matrix interactions, signaling, development, immunoregulation, and others. In tumor biology, altered O-glycosylation on proteins, termed TACAs, are involved in tumorigenesis, progression, and metastasis. CD99 overexpressed in EWS cells has potential tumor promoting roles, but the details on its glycosites and O-glycan structures, as well as that from their counterpart, mesenchymal stem cells (MSCs) remain elusive. This project aims to analyze the expression of CD99 in 16 EWS cell lines in comparison to control cells, MCSs to evaluate it as a potential EWS-specific glycan epitope. The expression of CD99 was assessed with RT-PCR for its mRNA, western blotting (WB) for the total protein, and flow cytometry for its cell surface presentation. Immunoprecipitation was performed to isolate CD99 for its O-

glycoproteomic analysis. The WB results indicated that 15 of the 16 EWS cell lines highly expressed CD99 while one cell line had very low signal. Interestingly, flow cytometry analysis showed expression of CD99 in all 16 cell lines. The expression of CD99 will be compared to it in MSC which is recognized as normal control cell for EWS. Six CD99-high expression cell lines and MSCs will be processed for CD99 immunoprecipitation to O-glycoproteomics. The available results confirm that CD99 is predominantly expressed in EWS cells. The goal is to evaluate if a novel glycoform(s) of CD99 exists in EWS as a potential EWS tumor-specific glycan.

- **Purpose**
    - Ewing's Sarcoma (EWS) is a rare and aggressive neuroectodermal tumor with high relapse and fatality rates in children and young adults. EWS cells are hypothesized to be derived from mesenchymal stem cells with a translocation at t(ll;22) (q24;q12), leading to the expression of the oncogenic EWS-FLI1 fusion protein that drives EWS oncogenic properties through uncontrolled cell growth, differentiation, and impaired apoptosis. Ewing's Sarcomas have a wide spectrum of clinical manifestations that are relatively unexplored. Currently, there is no specific immunotherapy to treat EWS. Despite the identification of the EWS translocation, there is minimal understanding of the molecular biomarkers that delineate clinical subgroups of the disease. This research focuses on characterizing EWS tumor-specific glycan epitopes, specifically the O-glycoprotein CD99, as CD99 is reportedly overexpressed in EWS cells in previous studies. CD99 is known to be highly glycosylated with ten potential O-glycans, but its O-glycoproteomics, namely O-glycosites and O-glycan structures in EWS cells are unknown. Herein, this project aims to analyze the expression of CD99 in 16 EWS cell lines and their control cells, mesenchymal stem cells (MSCs), characterize its O-glycosites and glycan structures, and evaluate it as a potential EWS-specific glyco-epitope.
- **Methods**
    - Four approaches were taken to study the expression of CD99 in 16 EWS cell lines and MSCs. 1) RT-PCR to examine the mRNA transcription of CD99 gene: the total RNA was isolated from cultured cells, the cDNA was synthesized using reverse-transcriptase (RT), and then PCR was performed to examine the mRNA of CD99 using its specific primers with β-actin as the housekeeping gene control. 2) The Western blotting (WB) to analyze total CD99 protein: the total CD99 protein in the EWS cells and MSCs extracts was examined using WB with anti-CD99 antibodies, and β-actin was a loading control. 3) Flow cytometry to analyze the cell surface expression of CD99: cells were stained with rabbit anti-CD99 antibodies, flow cytometry analysis

was conducted to quantify the surface expression of CD99 on all EWS cell lines and MSCs. 4) O-glycoproteomics of CD99: immunoprecipitation was conducted on the CD99-highly expressing cell lines and MSCs to isolate CD99 for O-glycosites and O-glycan structure analysis using mass spectrometry.

- **Results**
    - Sixteen EWS cell lines were evaluated for CD99 expression. The RT-PCR results showed that CD99 gene was transcribed in all 16 EWS cell lines. The WB results demonstrated that 15 of the 16 cell lines had highly expressed CD99 protein, and only CHLA57 showed very low level of CD99. The flowcytometry analysis confirmed the cell surface presentation of CD99 in all 16 cell lines. The CD99 in highly expressed EWS cells and MSCs will be immunoprecipitated for O-glycosite and glycan structure analyses.
- **Implications**
    - Protein O-glycosylation is one of the major post-translational modifications and plays an important role in biology. Tumor cells often synthesize altered glycans on proteins, termed tumor-associated carbohydrate antigens (TACAs) which are involved in tumorigenesis, progression, and metastasis. Some TACAs are tumor biomarkers for diagnosis, prognosis, and even immune therapeutic targets. Although many biomarkers have been identified for EWS, their glycan profiles remain unknown. Particularly, CD99, known to be heavily O-glycosylated, is a highly expressed protein in EWS cell lines, but its O-glycosites and O-glycan structures are not investigated. Our results confirm the high expression of CD99 in EWS cell lines. Further O-glycoproteomic analysis will determine if novel glycoforms of CD99 are confined to EWS cells. The data could evaluate CD99 as a potential EWS-specific glyco-epitope for potential therapeutic target. The implication of the project is that these findings may facilitate the identification of a better EWS biomarker and development of better targeted immunotherapies for EWS.

17) **Abstract title:** *Determination of Penicillamine in Plasma by UHPLC-MS/MS*
**Authors:** Li, Sabrina, OTR/OPQ/CDER/FDA (ORISE Fellow); Gu, Jianghong, OTR/OPQ/CDER/FDA (Mentor); Shakleya, Diaa, OTR/OPQ/CDER/FDA (Mentor); Faustino, Parick, OTR/OPQ/CDER/FDA (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
    - D-penicillamine is a compound used to treat a variety of diseases including Wilson's disease, cystinuria, rheumatoid arthritis, and various heavy metal poisonings. However, there are many difficulties in assaying D-penicillamine in plasma due to its instability, the presence of endogenous compounds,

and the many chemical forms it exists in. Thus, the objective of this project is to develop and validate an analytical method to monitor D-penicillamine in plasma using UHPLC-MS/MS. A future regulatory goal is to develop a standardized procedure to assist sponsors to accurately measure the bioavailability of D-penicillamine in human plasma. A Sciex QTRAP 6500+ tandem mass spectrometer equipped with a TurboIonSpray source and an ExionLC system was utilized to perform the analysis. Deuterium labeled internal standard penicillamine-d3 was used for the isotope dilution analysis. The assay was validated according to the current FDA guidance for Bioanalytical Method Validation. The intra- and inter-day coefficients of variation (CVs) were below 6.2% for penicillamine at all concentrations tested. Accuracy ranged between 94% and 105%. Linearity was established over the analytical range of 15-10,000 ng/mL for penicillamine ($R2 > 0.998$). The recovery ranged from 90% to 101% for penicillamine and no significant matrix effect or carryover was observed for both the analyte and IS. Penicillamine was found to be stable under the experimental conditions. From this work, a sensitive and specific UHPLC-MS/MS method was developed and validated for the determination of penicillamine in plasma. The method will aid regulators and industrial scientists to standardize an approach to generate accurate data across the industry for D-penicillamine bioavailability study design.

- **Purpose**
    - Penicillamine is an amino acid derived from the hydrolytic degradation product of penicillin. It was originally discovered from the urine of patients with liver disease receiving penicillin antibiotics by John Walshe in 1956. It is structurally similar to cysteine and exists in 2 stereoisomers, D- and L-penicillamine. Of the two enantiomers, only D-penicillamine is used therapeutically as L-penicillamine causes optic neuritis. Due to the copper-chelating properties, penicillamine is primarily used for the treatment of Wilson's disease. Additionally, it is used for the treatment of cystinuria by binding with cysteine to form cysteine-penicillamine disulfide, which is more soluble than cystine itself. It is also used to treat rheumatoid arthritis. However, there are many difficulties in assaying D-penicillamine in plasma due to its instability, the presence of endogenous compounds, and the many chemical forms it exists in. The objective of this project is to develop and validate an analytical method to detect D-penicillamine in plasma using UHPLC-MS/MS. A future regulatory goal is to develop a standardized procedure to assist sponsors to accurately measure the bioavailability of D-penicillamine in human plasma.
- **Methods**
    - A Sciex QTRAP 6500+ tandem mass spectrometer equipped with a TurboIonSpray source and an ExionLC system was utilized to perform the analysis. Deuterium labeled internal standard (IS) penicillamine-d3 was used for the isotope dilution analysis. Protein precipitation was used to extract the D-penicillamine from a 50 µL sample. 10 µL aliquot was injected onto a BEH Z-HILIC column (2.1 x 100mm, 1.7µm). D-penicillamine and IS were

eluted from the column with a water/acetonitrile gradient containing 10 mM ammonium formate and 0.125% formic acid at a flow rate of 0.8 mL/min and finally introduced into the QTRAP. Quantitation by multiple reaction-monitoring analysis (MRM) was performed in the positive mode. The transitions to monitor were selected at mass-to-charge (m/z) 150.0 to 115.0 for D-penicillamine and 153.0 to 118.0 for IS. Nitrogen served as auxiliary, curtain, and collision gas. The main working parameters of the mass spectrometer were: medium collision gas 10, curtain gas 25, nebulizer gas 60, turbo gas 15, ionspray voltage 5500 V, probe temperature 500 °C.

- **Results**
  - The assay was validated according to the current FDA guidance for Bioanalytical Method Validation. Method validation was accomplished by analyzing 6 replicates of four concentration levels of in-house quality controls. The intra- and inter-day coefficients of variation (CVs) were below 6.2% for penicillamine at all concentrations tested. Accuracy ranged between 94% and 105%. Linearity was established over the analytical range of 15-10,000 ng/mL for penicillamine ($R2 > 0.998$). The recovery ranged from 90% to 101% for penicillamine and no significant matrix effect or carryover was observed for both the analyte and IS. Penicillamine was found to be stable under the experimental conditions.
- **Implications**
  - Currently there is no FDA guidance available for pharmaceuticals to conduct bioavailability measurements of D-penicillamine. Sponsors are using different approaches to stabilize D-penicillamine for its accurate measurement in biological matrices, leading to different results. The goal is to identify a standard approach that is the best at stabilizing D-penicillamine to provide the most accurate bioanalytical measurements for clinical studies. From this work, a sensitive and specific UHPLC-MS/MS method was developed and validated for the determination of penicillamine in plasma. The method will aid in developing analytical approaches to help standardize a penicillamine preparation and analytical procedures for researchers and industry.

18) **Abstract title:** *Characterization of nanomaterial-containing complex drugs using asymmetrical flow field flow fractionation (AF4): How does sample recovery affect particle size distribution?*
**Authors:** Liu, Joanne, FDA/CDER (Student); Qu, Haiou, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
  - For complex drug products containing nanomaterials, particle size distribution is often evaluated in demonstrating comparable physicochemical properties to the reference list drug. Dynamic Light Scattering (DLS) is one of the most applied techniques for sizing nanomaterials but has limited resolution in analyzing highly polydispersed samples. Asymmetrical flow field flow fractionation (AF4) is a high-

resolution separation technique that has gained popularity in characterizing nanomaterial particle size. It circumvents the DLS issue by separating the sample mixture into many relatively monodispersed fractions and measuring the size of each individual fraction but is a new technique that still lacks standardization. Sample recovery is a parameter commonly used to assess the performance of a chromatography type method. High recovery indicates that the results obtained are representative of the particle population in a sample. In AF4, recovery is typically determined by measuring the peak area from a concentration detector with and without the application of crossflow. Many complex drug products contain small excipient components that are filtered out through the AF4 channel membrane in the presence of crossflow. The loss of components may result in a decreased response from the concentration detector, leading to lower recovery values. However, such reduction in recovery may not necessarily affect the particle size distribution. The goal of this project is to examine how recovery affects the particle size distribution determination of different nanoparticle drug products. Model drug product samples were treated either by diluting with water or various concentrations of excipient-containing solutions, or with spin filtration or dialysis to remove small excipients. Average hydrodynamic size and offline DLS Z-average size, PDI, and mean count rates were comparable regardless of which diluent was used, while recovery was calculated from RI peak area and found to decrease with increasing concentrations of excipient-containing diluent. This indicates that target nanoparticles are not the only contributor to RI peak area, and the current recovery method is not reflective of actual nanoparticle recovery or size distribution. Further studies are needed to determine standards for calculating more accurate recovery values, potentially in tandem with offline analysis like HPLC.

- **Purpose**
    - For complex drug products containing nanomaterials, particle size distribution is often evaluated in demonstrating comparable physicochemical properties to the reference list drug. Dynamic Light Scattering (DLS) is one of the most applied techniques for sizing nanomaterials in complex drug products. However, it has limited resolution in analyzing highly polydispersed samples. Asymmetrical flow field flow fractionation (AF4) is a high-resolution separation technique that has gained popularity in characterizing nanomaterial particle size. It circumvents the DLS issue by separating the sample mixture into relatively monodispersed fractions and measuring the size of each individual fraction. As a new technique, it still lacks standardization, and proper method development

and validation strategies are needed to ensure repeatability and reproducibility. Sample recovery is a parameter commonly used to assess the performance of a chromatography type separation method. High recovery values indicate that the results obtained are representative of the particle population in a sample. In AF4, recovery is typically determined by measuring the peak area from a concentration detector with and without the application of crossflow. The ISO standard (ISO/TS 21362:2018) recommends a minimum 70% recovery for an AF4 method. Many complex drug products contain small excipient components like glycerin that are filtered out through the AF4 channel membrane in the presence of crossflow. The loss of components may result in a decreased response from the concentration detector, leading to lower recovery values. However, such reduction in recovery may not necessarily affect particle size distribution. The goal of this project is to examine how recovery affects particle size distribution determination for drug products containing nanomaterials.

- **<u>Methods</u>**
    - Difluprednate (DFP) ophthalmic emulsion and doxorubicin hydrochloride liposome were selected as model drug products. Samples were diluted either with water or an aqueous solution containing glycerin, Tween 80, and sucrose at varying concentrations. These compounds were commonly used as tonicity agent and emulsifier in the formulation. Several water-diluted samples were further subjected to spin filtration or dialysis to remove small excipient components. Offline DLS measurements were first performed and properties including Z-average size, polydispersity index (PDI), laser attenuation, and mean count rate were monitored for any significant change in particle size between samples with different diluents. All samples were then characterized by AF4, and the sample recovery was determined by following the procedure described in the ISO standard. The recovery percentage was given by R (%) = $A_S/A_D$ x 100. $A_S$ was the sample peak area from an online concentration detector in the presence of crossflow where liquid flows exited from both crossflow port and channel outlet port. $A_D$ was the peak area obtained under conditions without applying any crossflow where all liquid flows exited the channel from the outlet port which was then directly connected to the concentration detector. Peak areas, size distribution, and recovery values were compared across samples. Recovery values will also be determined by an offline method where samples fractions were collected and analyzed with reverse-phase liquid chromatography.

- **Results**
    - For the DFP emulsion, Z-average size, PDI and mean count rates were comparable across samples in offline DLS, suggesting no obvious difference among samples diluted with different diluents. AF4 confirmed the offline DLS results by showing comparable $A_S$ values and average hydrodynamic sizes for samples with same dilution factor regardless of what diluent was used. However, compared to samples diluted with water, the $A_D$ values were not only higher for samples diluted with diluent containing excipient component (e.g. glycerin), they also exhibited gradual increase with increasing excipient concentration in the diluent. Consequently, the calculated recovery values were much lower for samples diluted with excipient-containing diluents. In samples diluted with diluents containing Tween 80, a second peak can be observed, which is attributed to the elution of Tween 80 micelle particles. These results indicate that target nanoparticles are not the only contributor to $A_S$ and $A_D$, and the current recovery determination method is not reflective of the actual nanoparticle recovery.
- **Implications**
    - The obtained results suggested that the current practice of measuring AF4 recovery has its limitations when working with complex drug products. The excipient components which do not necessarily affect the particle size in the drug product have a strong impact on the recovery values. Our investigation will provide valuable information for the improvement of recovery determination and establish guidelines for how to properly set AF4 method validation criteria. The project will assist the standardization and harmonization process for the AF4 technique and promote its utilization in pharmaceutical development.

19) **Abstract title:** *Cross-Study Analysis through R Shiny Application*
**Authors:** Mallampalli, Girija FDA/CDER (Student), Snyder, Kevin FDA/CDER (Mentor), Ahmed, Sabbir FDA/CDER, Ali, Yousif, MD, FDA/CDER, Butler, Susan FDA/CDER, L. Quinn, Stephanie FDA/CDER
**FDA Strategic Initiative**: Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - The objective of this work was to create an R Shiny application that allows users to perform cross-study analyses of CDISC-SEND formatted electronic standardized toxicology study data. The application generates interactive visualizations that enable users to easily compare and contrast the results of multiple repeat dose toxicity studies. In order for the application to produce

visualizations incorporating numerical and categorical data from multiple studies, data normalization and scoring procedures were developed and implemented. An interactive user interface dashboard was then developed to allow users to select and group test studies to make customizable toxicology profiles based on user specified criteria. This application is aimed at comparing various factors simultaneously, specifically body weight and kidney toxicity. In addition, users can visualize relationships across multiple toxicology studies, such as trends in body weight and increases in liver enzymes, indicators of toxicity in groupings. The application, also consists of a scoring method, highlighting the toxicity in each organ system. During development, the application was designed to load and analyze a small selection of datasets, and it is currently in the process of being optimized for compatibility with additional datasets. The application allows flexibility as it enables users to customize and define selections of data and their thresholds. Using cross-study analysis, users can efficiently produce specific findings relating to their target. This application enables the user to produce comparisons of toxicity of drugs across multiple studies, providing an integrated understanding of toxicological profile of a given compound under various testing conditions, e.g. species, route of administration, dosing duration.

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    - In order for the application to produce visualizations incorporating numerical and categorical data from multiple studies, data normalization and scoring procedures were developed and implemented. An interactive user interface dashboard was then developed to allow users to select and group test studies to make customizable toxicology profiles based on user specified criteria.
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    - This application is aimed at comparing various factors simultaneously, specifically body weight and kidney toxicity. In addition, users can visualize relationships across multiple toxicology studies, such as trends in body weight and increases in liver enzymes, indicators of toxicity in groupings. The application, also consists of a scoring method, highlighting the toxicity in each organ system. During development, the application was designed to

load and analyze a small selection of datasets, and it is currently in the process of being optimized for compatibility with additional datasets.

- **Implications**
    - The application allows flexibility as it enables users to customize and define selections of data and their thresholds. Using cross-study analysis, users can efficiently produce specific findings relating to their target. This application enables the user to produce comparisons of toxicity of drugs across multiple studies, providing an integrated understanding of toxicological profile of a given compound under various testing conditions, e.g. species, route of administration, dosing duration.

20) **Abstract title:** *Role of Pharmacodynamic Biomarkers in the Development and Regulatory Approval of Enzyme Replacement Therapies for Lysosomal Storage Diseases*
**Authors:** Miner, Kristin, FDA/CDER (Student); Hossain, Nayeem, FDA/CDER (Mentor); Hon, Christine, FDA/CDER (Mentor); Wang, Jack, FDA/CDER (Mentor); Li, Ruojing, FDA/CDER (Mentor); Schuck, Robert, FDA/CDER (Mentor); Pacanowski, Mike, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
    - This study explores the important role of pharmacodynamic (PD) biomarkers in the development and regulatory approval of Enzyme Replacement Therapy (ERT) drugs for lysosomal storage diseases. Biomarker data from 18 Biologics License Applications (BLAs) were reviewed. The results showed that PD biomarkers play crucial roles throughout the drug development process, including dose selection, interpretation of the efficacy and safety results, and disease modeling. The research emphasizes the need for proper bioanalytical method validation and an early comprehensive biomarker assessment plan. It also underscores the role of PD biomarkers in streamlined regulatory approvals and the overall success of drug development.
- **Purpose**
    - This research focused on the important roles of pharmacodynamic (PD) biomarker in the development and regulatory approval of enzyme replacement therapy (ERT) drugs for lysosomal storage diseases (LSDs). The outcome of this research may benefit other drug development programs especially those for rare diseases using PD biomarkers to facilitate dose selection and optimization, to serve as confirmatory evidence of effectiveness, and to support other regulatory decision making.

- **Methods**
    - We surveyed for PD biomarker data in clinical and nonclinical studies in the BLA for each approved ERT product. A total of 18 BLAs were included in the survey. The survey relied on information from the product labeling and FDA's review documents that are publicly available at the Drugs@FDA. Data from published literatures are also considered as needed.
- **Results**
    - The results of our survey showed that most drug development programs for ERT have evaluated pharmacodynamic (PD) effect of the drug. PD biomarkers were frequently used to guide dose selection in clinical trials including first-in-human (FIH) studies. The dose-response and exposure-response for PD biomarkers were also used to justify the recommended dosing regimens and in some cases to support dosing in pediatric patients. In one BLA, PD biomarkers were used in a Quantitative Systems Pharmacology (QSP) model to understand disease similarity between pediatric and adult populations. The validity of the bioanalytical methods used for PD biomarker assessment played an important role in the determination of the regulatory utility of the PD biomarker data. Inadequate validation of the PD biomarker assay in some applications resulted in recommendations to further improve or validate the assay as postmarketing commitments. The validation or qualification of the biomarker as a surrogate or reasonably likely surrogate endpoint affected whether the application received traditional vs. accelerated approval. Because ERTs are usually approved for the treatment of rare disease conditions, PD biomarkers served as confirmatory evidence of effectiveness in several ERT marketing applications when only one adequate and well-controlled trial was conducted during the drug development process.
- **Implications**
    - Overall, our findings highlighted the immense potential and importance of PD biomarkers in facilitating drug development for ERTs. Because PD biomarker data are increasingly used in supporting new drug applications and regulatory reviews, it is critical for the sponsors to work with the FDA to establish a comprehensive biomarker assessment plan in the early phases of drug development.

21) **Abstract title:** *The development and validation of ion chromatography methods for the evaluation of nitrosamine precursors (nitrite, nitrate, and dimethylamine) in metformin drug products*
**Authors:** Mokbel, Alaa FDA/CDER (Student); Mohammad, Adil FDA/CDER, Abrigo, Nicolas FDA/CDER, Faustino, Patrick FDA/CDER (Mentor), and Diaa Shakleya FDA/CDER (Mentor)

**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response

**Abstract:**

- **Synopsis**
  - *The aim of this project was to support the evaluation of nitrosamine mitigation strategies in formulated metformin drug products by applying validated ion chromatography methods to monitor the concentrations of nitrogen-based precursors (nitrite, nitrate, and dimethylamine) following the addition of antioxidants as nitrite scavengers.*
- **Purpose**
  - Nitrosamines are carcinogenic impurities that have been found in drug products containing variable amounts of precursor ions of nitrite and nitrate. The project goal was to support the evaluation of nitrosamine mitigation strategies in formulated metformin drug products by implementing validated ion chromatography methods to monitor the concentrations of nitrogen-based precursors (nitrite, nitrate, and dimethylamine) following the addition of antioxidants as nitrite scavengers.
- **Methods**
  - Ion chromatography (IC) methods were developed and validated according to ICH Q2 (R1) guidelines to evaluate nitrite, nitrate, and dimethylamine in metformin drug products using UV and conductivity as detection methods. Nitrite and nitrate were evaluated using an AS19 column with a potassium hydroxide eluent generator cartridge with a concentration gradient. Dimethylamine was evaluated using a CS19 column with a methane sulfonic acid eluent generator cartridge with a concentration gradient.
    The methods were validated according to ICH Q2 (R1) for linearity, range, accuracy, precision, selectivity, specificity, limit of detection, and robustness. Stability studies were also conducted. The analytes were also evaluated for spike recovery in the drug products at different quality control concentrations over the analytical range. Marketed metformin drug products were purchased from Lupin Pharmaceuticals, Inc. Model metformin drug products were manufactured at CDER's Division of Product Quality Research formulation branch with and without nitrogen-based precursors ions.
- **Results**
  - The methods were successfully validated according to the ICH Q2 (R1) guidelines. The linearity of the nitrite, nitrate, and dimethylamine methods passed with $r2 \geq 0.99$. The accuracy over four QC concentrations tested ranged from 88-105% for nitrite, 98-107% for nitrate, and 95-108% for dimethylamine. The precision over four QC concentrations was ≤ 5%RSD for nitrite, ≤ 3%RSD for nitrate, and ≤ 4%RSD for dimethylamine. The methods

were applied to test nitrite, nitrate, and dimethylamine levels in marketed and FDA formulated metformin drug products. Multiple formulations of the products were manufactured in-house with some containing 100 ppm of nitrite, 100 ppm of dimethylamine, 0.1-1% of antioxidant, or a combination of the three. The results over a 6-month stability study at elevated temperatures and humidity demonstrated that certain antioxidants could provide better mitigation of nitrosamine formation relative to the control metformin formulations.

- **Implications**
    - The validated ion chromatography analytical methods were successfully applied for the evaluation of nitrite, nitrate, and dimethylamine in metformin drug products. Monitoring nitrite, nitrate, and dimethylamine during the manufacturing of drug products can provide valuable information to assess nitrosamine mitigation approaches.

22) **Abstract title:** *Characterization of Placebo Response in Migraine Drug Clinical Trials*
**Authors:** Rohan Nigam, FDA/CDER (Student); Anantha Ram Nookala, FDA/CDER; Gopichand Gottipati, FDA/CDER; Ramana Uppoor, FDA/CDER; Sreedharan Sabarinath, FDA/CDER (Mentor); Mehul Mehta, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - Migraine is a serious and debilitating condition that is accompanied with symptoms such as severe headache, photophobia, phonophobia, nausea, and vomiting. Migraines are typically characterized as episodic or chronic in nature and clinical trials are conducted in these two separate patient populations. This study explores how various disease characteristics, e.g., days with migraine, headache, photophobia, phonophobia, nausea, vomiting, and prior prophylactic medication use, and demographic factors, e.g., age, sex, and race affect trial outcomes in placebo arms from two phase 3 trials conducted to support the Food and Drug Administration (FDA) approval of a migraine drug. The preliminary analysis seems to suggest that there are differences in placebo response in the chronic migraine trials based on demographic factors such as sex and race as well as differences based on prior prophylactic medication use. Addressing the variability in placebo response in clinical trial design is crucial to optimizing trial design and better elucidating the net treatment effect.
- **Purpose**
    - Migraine is a prevalent neurological condition characterized by recurring headaches among other criteria according to the International Classification

of Headache Disorders. The headache phase may be accompanied with various symptoms such as nausea, vomiting, nasal congestion, depression, attention deficit, and heightened sensitivity to light and sound.
Placebo response in clinical trials in episodic and chronic migraine population has been reported in recently approved FDA therapies. This investigation aims to explore the potential impact of prophylactic medication uses or specific demographic factors on the response to clinical trials, particularly focusing on average monthly migraine or headache days, days of nausea, days of vomiting, days of photophobia, and days of phonophobia. By examining the variability in placebo response within migraine trials, we can identify potential differences and patient recruitment strategies to help inform optimal design of future migraine trials.

- **Methods**
  - The current work analyzed the data from the placebo-arm in the drug development program of a single FDA approved migraine drug, specifically focusing on two placebo-controlled phase 3 studies, one each in chronic migraine and episodic migraine population. These studies consisted of a baseline period followed by a double-blind period where clinical outcomes and endpoints of interest were measured. Relevant data such as disease characteristics (chronic or episodic migraine and prophylactic treatment), demographic characteristics (gender, race, and age) at baseline, and trial outcomes (average monthly migraine and headache days, days with sensitivity to light and/or sound, and days with nausea and/or vomiting) were extracted from electronic diary records of patients enrolled in placebo-arm during the double-blind period.
    The demographic and disease characteristics for patients enrolled in placebo arms were summarized numerically and graphically. Next, longitudinal placebo response was analyzed by stratifying the data by migraine type (chronic vs. episodic), as well as, based on age, sex, and race to assess the potential influence of these patient characteristics.
    Data management, calculations, graphic representations, and exploratory statistical analyses were performed using the R statistical software (version 4.1.2). An alpha value of 0.05 was used to determine statistical significance.
- **Results**
  - In phase 3 episodic and chronic migraine studies, 86.9% of the patients were female, 85.4% of patients were White, 11.6% of patients were Black or African American, and 81.3% of patients were between the ages of 18 and 50.

The average change from baseline in monthly migraine days in females seems to be higher than in males (P=0.0132) while such sex differences for other clinical outcomes were not apparent in the chronic migraine trial. The average change from baseline in monthly headache days seemed to be higher in Black or African American patients than in White patients (P=0.0156) in the chronic migraine trial. No differences in trial outcome based on sex or race were apparent in the episodic migraine trial, and no differences based on age were apparent in either the episodic or chronic migraine trial outcomes.

There appeared to be a higher average change from baseline in monthly migraine days in females than in males for patients who did not receive prior prophylactic medication in the chronic migraine trial (P<0.0001). A difference in placebo response in patients who received prior prophylactic treatment and those who didn't was not apparent in the overall patient population in chronic migraine trial.

- **Implications**
  - In the recent years, several migraine therapies that target calcitonin gene-related peptide (CGRP) have been approved. Current work aims at conducting a systematic evaluation of individual-level patient data from placebo-arms of phase 3 chronic migraine and episodic migraine trials for a recently approved product. Adequate characterization of placebo-response could offer insights into identifying important disease and demographic characteristics, which may be used to inform clinical trial enrichment strategies. Such enrichment strategies could aid in reducing patient variability when assessing the net treatment effect. This could help with improving efficiencies in the design and conduct of future clinical trials, e.g., smaller sample size to detect net treatment effects.

23) **Abstract title:** *Overview of Formulations Used in the Biosimilar Products*
**Authors:** Nikolov, Lillian, FDA/CDER (Student); Ji, Ping (Mentor), Suresh, Doddapaneni, Chandrahas, Sahajwalla, Chen, Jianmeng
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
  - Excipients are added to increase product stability, maintain tonicity, and facilitate drug delivery. The potential implications of these additive substances merit clinical consideration.
- **Purpose**
  - The purpose of this research is to survey the excipients used in the FDA-approved biosimilar products and assess the potential impact of different excipients on the pharmacokinetics (PK) of these biosimilar products.

- **Methods**
    - The formulation information of FDA-approved biosimilars and corresponding reference biologics was obtained from Package Insert at "Drugs@FDA" database. The excipients of each formulation of biosimilar products were categorized by their function and levels, which were compared with those of their reference biologics. The biosimilar pharmacokinetics was also compared with their reference products. Summary statistics of excipients were conducted with R.
- **Results**
    - A total of 52 biological products (41 biosimilars and 11 reference products) were identified and summarized in this research, 24 are IV, 22 are SC, and 6 are both IV and SC. The 6 biological products which are administered through IV as well as SC route have the same excipients for the same moiety. Formulation generally includes the following excipient categories: tonicity agent, buffer, preservative, stabilizer, solubilizing agents, and water. Among 41 biosimilars, 20 have same category of excipients, and 11 have same excipients as their reference biological products. Remicade (reference products) has most consistent excipients for biosimilar products. Among all the excipients other than water, solubilizing agent is the mostly used, and preservative is the least used in the formulation. None of these difference in excipients was found to negatively affect the pharmacokinetics of the biosimilar products as compared to their reference products.
- **Implications**
    - The category for biosimilar formulation is not necessarily consistent with reference products. However, no impact of these difference on pharmacokinetics has been observed.

24) **Abstract title:** *Evaluation of Cytochrome P450 Substrate Exposure in Patients with Chronic Inflammatory Diseases*
**Authors:** Pakala, Mayukha, FDA/CDER (Student); Al-Khouja, Amer, FDA/CDER (Mentor); Ji, Ping, FDA/CDER (Mentor); Kim, Insook, FDA/CDER (Mentor); Doddapaneni, Suresh, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - Disease-drug interactions occur in some inflammatory diseases due to changes in pro-inflammatory cytokines which can decrease the expression of drug metabolizing enzymes such as the cytochrome P450s (CYPs). The FDA generally recommends that sponsors determine the need for drug-drug interaction (DDI) studies during development. It is unclear whether results from a DDI study can be extrapolated to a different disease due to differences in inflammatory burden. Data was collected from the literature and publicly available FDA reviews identified by searching PubMed and

Drugs@FDA, respectively. From each study, pharmacokinetic (PK) parameters in healthy individuals and patients with inflammatory diseases were collected for commonly used CYP substrate drugs. In patients with psoriasis, atopic dermatitis, and multiple sclerosis baseline PK parameters of all CYP substrates were generally consistent with those of healthy individuals. Patients with rheumatoid arthritis (RA) had 1.8- to 4.0-fold increased exposure, based on $C_{max}$ and $AUC_{inf}$, for midazolam, warfarin, omeprazole, and simvastatin, but not for caffeine or dextromethorphan The data suggest that inflammatory burden is greater in patients with RA and is sufficient to observe changes in the PK of CYP substrates, whereas such changes were not observed in patients with psoriasis. This suggests that DDI studies conducted in patients with RA is likely to overestimate the DDI potential of cytokine modulator TPs in conditions with lower inflammatory burden, such as psoriasis or atopic dermatitis.

- **Purpose**
    - Disease-drug interactions occur in some inflammatory diseases due to changes in pro-inflammatory cytokines which can decrease the expression of drug metabolizing enzymes such as the cytochrome P450s (CYPs). The effect of the disease on CYPs is known to vary based on disease severity and between different disease states. Many chronic inflammatory disease states are treated with therapeutic proteins (TP) cytokine modulators, which are typically approved for multiple indications. These drugs may decrease cytokine signaling and normalize CYP expression. As a result, the FDA generally recommends that sponsors determine the need for drug-drug interaction (DDI) studies during development. It is unclear whether results from a DDI study can be extrapolated to a different disease due to differences in inflammatory burden. The FDA Guidance "Drug-Drug Interaction Assessment for Therapeutic Proteins" indicates that if sponsors prefer not to include language indicating the potential for DDIs in drug product labeling, the sponsor may provide justification, such as evaluating differences in CYP substrate exposure between healthy subjects and the indicated population. This study aims to look at the translatability of TP DDI studies across disease states by examining pharmacokinetic (PK) parameters of known CYP substrates in healthy individuals and patients with inflammatory diseases.

- **Methods**
    - Data was collected from the literature and publicly available FDA reviews identified by searching PubMed and Drugs@FDA, respectively. From each study, PK parameters in healthy individuals and patients with inflammatory diseases were collected for commonly used CYP substrate drugs such as midazolam (CYP3A4), warfarin (CYP2C9), dextromethorphan (CYP2D6), omeprazole (CYP2C19), caffeine (CYP1A2), and simvastatin (CYP3A4). Collected PK parameters included $C_{max}$, $AUC_{last}$, $AUC_{inf}$, CL, $t_{1/2}$, and $T_{max}$. Data were not collected for patients with acute states of inflammation (e.g., infection).

- **Results**
    - A total of 29 studies were found, 13 of which described TP DDI studies in patients with chronic inflammatory diseases, while 16 described PK parameters of CYP substrates in healthy individuals for comparison. The 13 TP DDI studies represent various patient populations, including 6 with psoriasis, 5 with rheumatoid arthritis (RA), 1 with atopic dermatitis, and 1 with multiple sclerosis. Four studies enrolled treatment-naïve and -experienced patients, while the remaining 9 did not describe the prior biologic treatment status.

      In patients with psoriasis, atopic dermatitis, and multiple sclerosis baseline PK parameters of all CYP substrates were generally consistent with those of healthy individuals. Data show that patients with RA had increased $C_{max}$ and $AUC_{inf}$, for midazolam, warfarin, omeprazole, and simvastatin, but not for caffeine or dextromethorphan. When compared to data in healthy individuals at the same dose, midazolam $C_{max}$ and $AUC_{inf}$ were increased 1.8-fold and 2.0-fold, respectively, while omeprazole $C_{max}$ and $AUC_{inf}$ were increased 2.7-fold and 3.0-fold, respectively, and simvastatin $C_{max}$ and $AUC_{inf}$ were increased 2.4- to 4.0-fold and 2.3- to 2.9-fold, respectively. Warfarin exposure in patients with RA receiving a 5 mg dose was similar to that in healthy individuals receiving a 10 mg dose.

- **Implications**
    - These PK observations in patients with psoriasis and RA are consistent with what is included in the labeling for TPs approved to treat those conditions. The data suggest that inflammatory burden is greater in patients with RA and is sufficient to observe changes in the PK of CYP substrates, whereas such changes were not observed in patients with psoriasis. This suggests that DDI studies conducted in patients with RA is likely to overestimate the DDI potential of cytokine modulator TPs in conditions with lower inflammatory burden, such as psoriasis or atopic dermatitis. On the other hand, studies conducted in patients with psoriasis may underestimate the DDI potential in patients with RA. Due to the limited number of studies identified, other chronic inflammatory diseases such as Crohn's disease or ulcerative colitis were not represented. Psoriasis and RA are examples of diseases with low and high inflammatory burden, respectively. Additional data is needed, especially in diseases with intermediate inflammatory burden, to determine whether DDI study results can be extrapolated across different chronic inflammatory conditions.

25) **Abstract title:** *Exploration for Exclusion of Males of Reproductive Potential as a Bioequivalence Study Population in Product-Specific Guidance's for Generic Drug Development*
**Authors:** Park, Se Jin, FDA/CDER (Student); Nguyen, Duyen, FDA/CDER (ORISE Fellow); Tran, Tony, FDA/CDER (ORISE Fellow); Li, Karen, FDA/CDER (Mentor); Kim, Myong-Jin, FDA/CDER (Mentor); Shon, Jihong, FDA/CDER (Mentor)

**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**

- **Synopsis**
  - Product-Specific Guidances (PSGs) outline the Agency's current thinking and expectations on the most appropriate methods for establishing therapeutic equivalence between generic drug products and their reference listed drugs (RLDs). When developing PSGs, safety assessment for selection of study population for pharmacokinetic bioequivalence (PK BE) studies also considers reproductive toxicity. To identify the characteristics of the RLDs that recommend the exclusion of males of reproductive potential (MRP) due to fertility impairment in the PSGs, this research retrospectively analyzed the toxicological information on male fertility impairment (levels of reproductive toxicity, reversibility, and contraception requirement), genotoxicity, and carcinogenicity along with the previous evidence on healthy subject recruitment. Based on the analysis, nine oral drug product PSGs out of ~2000 PSGs recommend the exclusion of MRP in their PSGs, while approved in both sex patient populations. These RLDs were classified based on the United States Pharmacopeia (USP) Therapeutic Categories (e.g., antineoplastic (N=2), antiparasitic (N=2), antiviral (N=1), genetic, enzyme, or protein disorder: replacement, modifiers, treatment (N=2), and unclassified (N=2) per 2023 internal database). All nine RLDs caused male fertility impairment with four having irreversible impact. Five and four RLDs had genotoxic and carcinogenic potential, respectively. These risk categories were addressed on one or more labeling sections (e.g., warnings and precautions, patient information, or the specific population). In addition, four RLDs recommended male contraception. Eight included studies enrolling healthy male subjects with four not allowing the MRP enrollment. The current analysis provided insights into the factors that support the exclusion of MRP for PK BE studies in healthy subjects. This information could be utilized for the future development of the standardized decision framework for deciding the subject population for the PSG development of the generic drugs.
- **Purpose**
  - PSGs outline the Agency's current thinking and expectations on the most appropriate methods for establishing therapeutic equivalence between generic drug products and their RLDs. A specific study population for PK BE studies is selected based on safety considerations during PSG development. PSGs generally recommend the inclusion of male subjects if the RLD is indicated in males. Exclusion of MRP has been recommended in PSGs for drugs associated with male fertility impairment. This project aimed to

retrospectively analyze the toxicological and clinical evidence on recruiting healthy subjects supporting the exclusion of MRP for PK BE studies in generic drug development.

- **Methods**
  - A list of PSGs that recommend the exclusion of MRP or entire male population in PK BE studies was obtained from multiple databases using keywords such as birth control, condom, contraception, fertility, reproductive, sterile, and surgical. The list of drugs was filtered to only include oral products (buccal, intramuscular oral, sublingual, tablet, capsules, and other oral products). PSGs for RLDs with approved indications only in females were excluded. In addition, PSGs that recommended excluding a specific subset of males (e.g., males with pregnant female sexual partners; males planning to donate sperm; males wishing to father a child) or only requiring effective contraception in males were excluded. The RLDs were classified based on the USP Therapeutic Categories. Nonclinical toxicology (e.g., male fertility impairment, genotoxicity, and carcinogenicity), contraception requirement for male patients, and enrollment of healthy subjects during the NDA program were collected from the labeling and regulatory submissions for each RLD. Labeling sections including warnings and precautions, patient information, and specific population were reviewed to evaluate how the RLD labelings addressed three toxicology risk categories. The type and level of fertility impairment were further categorized, such as sperm count, male reproductive organ weight, and spermatogenesis.
- **Results**
  - Nine oral drug product (eight active pharmaceutical ingredients) PSGs recommend the exclusion of all males or MRP. These PSGs have subjects including males of nonreproductive potential (MNRP) with females of nonreproductive potential (FNRP), females only, FNRP only, and MNRP with females. These RLDs were part of the USP Therapeutic Categories including antineoplastic (N=2), antiparasitic (N=2), antiviral (N=1), genetic, enzyme, or protein disorder: replacement, modifiers, treatment (N=2), and unclassified (N=2) per 2023 internal database. Nine RLDs resulted in male fertility impairment with no-observed-adverse-effect levels less than or equal to maximum recommended human doses. All RLDs addressed this risk and associated safety warnings on one or more RLD labeling sections (e.g., warnings and precautions, patient information, or the specific population). These RLDs significantly impacted spermatogenesis following chronic dosing (e.g., hypospermia, oligospermia, and/or aspermia) within the human therapeutic levels, with four having an irreversible impact. Five and four

RLDs resulted in genotoxicity and carcinogenicity, respectively. Furthermore, four RLDs required contraception for one week to four months after the last dose. Eight RLDs, except for one without information, included healthy male subjects. Four RLDs did not allow MRPs, four did not have information, and one included MRP.

- **Implications**
    - The current analysis provided insights into the risk factors (male fertility impairment, genotoxicity, and carcinogenicity) supporting the exclusion of MRP from PK BE study population in current PSGs. Ultimately, this collective information will be utilized to develop a standardized decision framework to inform study population selection in PSG development and to ensure subject safety in PK BE studies. Such decision framework could further improve consistency of the PSG development process and ensure subject safety in PK BE studies for oral generic drug development. Furthermore, the current analysis could be utilized to re-evaluate PSGs that share the same therapeutics class as the nine drugs to consider the exclusion of MRPs.

26) **Abstract title:** *Effect of agitation and polysorbate degradation on monoclonal antibody higher-order structure*
**Authors:** Rajabi Abhari, Ali, FDA/CDER (Student); Lehtimaki, Mari, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - Monoclonal antibody (mAb) drugs like trastuzumab can be affected by manufacturing, storage, and transportation conditions, impacting their stability. Polysorbate 20 is used in trastuzumab formulation to prevent surface-induced damage, but its interaction with proteins can have varied effects such as aggregation. This study used circular dichroism (CD) to examine how agitation affects trastuzumab's secondary structure, with and without polysorbate. Different agitation speeds, temperatures, and durations were tested to study formulation conditions. Results showed that the presence of polysorbate preserved trastuzumab's anti-parallel beta-strands despite temperature or agitation changes. Without polysorbate, the 4°C samples had more stable beta-strand content than the 25°C samples. Additionally, the 4°C samples agitated at 300 rpm saw decreased stability in anti-parallel beta-strand content over time, while the 25°C samples agitated at 24 rpm showed increased stability. Formulations lacking polysorbate also had higher parallel beta-strand content. Removing detergent made proteins more prone to losing secondary structure stability. Polysorbate potentially helped to maintain trastuzumab's stability by preventing intermolecular

interactions. Without it, formulations stored at 4°C and agitated at 300 rpm likely experienced increased intermolecular interactions, leading to loss of secondary structure stability. Conversely, formulations without polysorbate stored at 25°C and agitated at 24 rpm reduced intermolecular interactions, enhancing secondary structure stability. Agitation studies are critical for evaluating formulation stability during manufacturing, storage, and transportation. Polysorbates protect proteins, minimizing damage and aggregation in agitation stress. By studying agitation effects, scientists can identify stability issues, identify risk factors, and optimize formulations, ensuring the development of stable and effective mAb drugs.

- **Purpose**
    - Monoclonal antibody (mAb) drugs encounter multiple stressors throughout manufacturing, storage, and transportation which can lead to changes in the higher-order structure, affecting the stability and quality of the antibody. Trastuzumab is formulated with polysorbate 20 to prevent oxidation, aggregation, peroxide formation, and flocculation. However, binding of intact or degraded polysorbates to proteins can result in either stabilization against aggregation or lead to instability through unfolding. Through circular dichroism (CD), we investigated the unfolding of these proteins by analyzing changes in their secondary structure content after inducing stress conditions. Agitation was induced in formulations at different temperatures to simulate storage and manufacturing conditions. By examining changes to the secondary structure, we aim to investigate the impact of polysorbates as they protect or destabilize trastuzumab at various interfaces (air-liquid, liquid-liquid, liquid-solid). Comparing the effects of different stress conditions such as agitation speeds, temperatures, and durations on trastuzumab's structure gives insight into optimal formulation conditions and risk factors that drive drug quality and stability.
- **Methods**
    - We examined how agitation affects the higher-order structure of trastuzumab in the presence and absence of intact polysorbate, using CD. The anti-cancer biotherapeutic trastuzumab was used for this study. Polysorbate was removed from samples using DetergentOUT Tween columns, and a Bradford assay was conducted to provide 0.5 mg/mL of trastuzumab solution for each sample. Samples were either agitated at 4°C at 300 rounds per minute (rpm) or at 25°C at 24 rpm. Controls were used for both temperature conditions and agitation was induced for either 3h or 24h. Samples were prepared for CD by dialyzing them into sodium phosphate buffer. The instrument parameters were set to measure from 185-260 nm, 5 scans per sample, and 50 nm/min scanning speed. Sodium

phosphate buffer served as blanks and each stress condition was measured at 20°C, 35°C, 50°C, 65°C, and 75°C to examine secondary structure stability. The CD data was further analyzed by an algorithm for secondary structure determination and fold recognition from protein CD spectra. The algorithm provided an estimated percentage of secondary structure content for each sample and takes into consideration that mAbs are comprised of mainly anti-parallel beta-strands, which is difficult to accurately determine using other programs.

- **Results**
    - In formulations with polysorbate, the anti-parallel beta-strand stability was not affected by changes in temperature or agitation conditions. Detergent removal made proteins under stress conditions more likely to lose the secondary structure components. In polysorbate depleted formulations, the anti-parallel beta-strand content was more stable in the 4°C control than the 25°C control. Moreover, the 4°C samples agitated at 300 rpm showed less stable anti-parallel beta-strand content over time. Interestingly, the 25°C samples agitated at 24 rpm preserved anti-parallel beta-strand content in polysorbate depleted samples. Formulations without polysorbate had higher parallel beta-strand content than those with polysorbate for each condition, indicating intermolecular interactions between the anti-parallel beta-strand dominated mAbs. In the 4°C samples without polysorbate, parallel beta-strand content increased with agitation over time, whereas in the 25°C samples parallel beta-strand content decreased. As indicated by the higher parallel beta-strand content and less stable anti-parallel beta-strand content in the 4°C polysorbate depleted samples agitated at 300 rpm, increased interaction of proteins with the air-liquid interface and container surfaces may lead to increased intermolecular interactions. Less stable anti-parallel beta-strand content and higher parallel beta-strand content in the 25°C polysorbate depleted samples agitated at 24 rpm indicate the prevention of these intermolecular interactions.
- **Implications**
    - Agitation stress studies play a critical role in drug development and formulation studies used to evaluate stability in manufacturing, storage, and transportation of mAb drugs. By understanding the impact of agitation on the formulation, scientists can develop high-quality products with consistent performance and optimize manufacturing processes for efficiency and cost-effectiveness. Polysorbates contribute to the stability of formulations by minimizing surface-induced damage by competing with proteins for adsorption sites. These nonionic surfactants have favorable properties like emulsifying immiscible components, reducing surface

tension, preventing aggregation, and solubilizing hydrophobic substances. In the absence of these excipients, formulations stored at 4°C and agitated at 300 rpm may lose the stability of their secondary structure which leads to increased intermolecular interactions. In contrast, in formulations without polysorbate that are stored at 25°C, agitation at 24 rpm may reduce intermolecular interactions which leads to increased stability of their secondary structure. In the case of agitation stress, polysorbate protects the secondary structure stability. The objective of stability evaluations during formulation development is to establish conditions that inhibit aggregation. By studying the effects of agitation, scientists can identify potential stability issues and optimize the formulation to prevent or minimize these problems.

27) **Abstract title:** *Prophylactic effect of anti-EBOV-GP antibody on Ebola virus infection in pregnancy*
**Authors:** Iara Rattner, FDA/CDER (Student); Ha-Na Lee, FDA/CDER (Mentor); Aaron P Lewkowicz, FDA/CDER; Daniela Verthelyi, FDA/CDER
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Ebola virus (EBOV) infection causes fever, multi-organ failure, hemorrhage and death. EBOV infections during pregnancy are associated with preterm labor and perinatal death, and this virus has been detected in placental tissues, suggesting a potential risk of trans-placental infection. The scarcity of BSL4 containment animal facilities severely hinders development and assessment of therapeutics for Ebola. In this study, we developed a pregnancy mouse model of EBOV infection compatible with BSL2 containment by inoculating pregnant dams with replicating EBOV glycoprotein (EBOV-GP) pseudotyped vesicular stomatitis virus (rVSVΔG-EBOV-GP or VSV-EBOV) at embryonic day 7.5 (E7.5). A minor increase in fetal resorption and early postpartum loss was observed in infected groups compared to uninfected groups however, virus RNA was not detected in the pregnant dams, their offspring or placenta. While immunocompetent dams were resistant to VSV-EBOV infection, a transient low-grade immunosuppression using anti-IFNAR1 antibody treatment rendered pregnant dams susceptible to infection. These mice showed an increased rate of reabsorbed fetuses, reduced fetal weight and delayed fetus development, together with high virus titers in the ovary, uterus and spleen as well as infection of the fetuses, placenta and amniotic fluid. Using this model we evaluated the therapeutic effect of anti-EBOV-GP therapeutic (SAB-139) and showed that treatment prior to infection significantly reduced the fetal resorption and viral titers in dams and fetus. Together, our

findings indicate that this model can be used to test the safety and efficacy of anti-EBOV-GP therapeutics against EBOV infection in pregnant women.

- **Purpose**
  - Ebola virus (EBOV) infection causes fever, multi-organ failure, hemorrhage and death. EBOV infections during pregnancy are associated with preterm labor and perinatal death, and this virus has been detected in placental tissues, suggesting a potential risk of trans-placental infection. The scarcity of BSL4 containment animal facilities severely hinders development and assessment of therapeutics for Ebola. In this study, we aim to develop a pregnancy mouse model of EBOV infection compatible with BSL2 containment. Also, using this model, we will test the safety and efficacy of therapeutics against EBOV infection during pregnancy.
- **Methods**
  - We developed a pregnancy mouse model of EBOV infection compatible with BSL2 containment by inoculating pregnant dams with replicating EBOV glycoprotein (EBOV-GP) pseudotyped vesicular stomatitis virus (rVSVΔG-EBOV-GP or VSV-EBOV) at embryonic day 7.5 (E7.5).
- **Results**
  - A minor increase in fetal resorption and early postpartum loss was observed in infected groups compared to uninfected groups however, virus RNA was not detected in the pregnant dams, their offspring or placenta. While immunocompetent dams were resistant to VSV-EBOV infection, a transient low-grade immunosuppression using anti-IFNAR1 antibody treatment rendered pregnant dams susceptible to infection. These mice showed an increased rate of reabsorbed fetuses, reduced fetal weight and delayed fetus development, together with high virus titers in the ovary, uterus and spleen as well as infection of the fetuses, placenta and amniotic fluid. Using this model we evaluated the therapeutic effect of anti-EBOV-GP therapeutic (SAB-139) and showed that treatment prior to infection significantly reduced the fetal resorption and viral titers in dams and fetus.
- **Implications**
  - Our findings indicate that this model can be used to test the safety and efficacy of anti-EBOV-GP therapeutics against EBOV infection in pregnant women.

28) **Abstract title:** *Dose Determining Biomarkers for Nitrogen Binding Agents and Cystine Depleting Agents Indicated for Rare Diseases*
**Authors:** Reddy, Sanil, FDA/CDER (ORISE Fellow); Bandukwala, Abbas, FDA/CDER (Mentor); York, Tomita, FDA/CDER (Mentor); Siegel, Jeffrey, FDA/CDER (Mentor)

**FDA Strategic Initiative:** Empowering Patients and Consumers

**Abstract:**

- **Synopsis**
  - Biomarkers enhance clinical trials by helping assess disease severity, select an appropriate study population, and develop dosage regimens. The Biomarkers, EndpointS and other Tools (BEST) glossary was used to categorize biomarkers used for dose selection of cystine binding agents (CDAs) and nitrogen binding agents (NDAs) indicated for rare diseases. Furthermore, the dose determining pharmacodynamic biomarkers were classified as target engagement, pathway modulation, or disease related. Disease related pharmacodynamic biomarkers were mostly used for dose selection within both pharmacologic classes. Study populations included mostly healthy volunteers and all patients for NDA and CDA clinical trials, respectively. Exploring dose selection biomarkers and study populations across other pharmacologic classes can help inform future clinical trial design for rare diseases.
- **Purpose**
  - Challenges in (1) understanding disease pathophysiology, (2) designing and conducting clinical trials, and (3) forming a consensus on clinical outcome measures underpin the complexities of therapeutic development for rare diseases. Biomarker identification addresses these challenges by encouraging efficiency and innovation during all stages of drug development, thereby advancing public health. We categorized their use in establishing dosage regimens for CDAs (cystine depleting agents) and NBAs (nitrogen binding agents), two established pharmacologic classes that have successfully expanded treatment for rare diseases in the last two decades. CDAs reduce the buildup of cystine deposition in tissues by converting cystine to cysteine and cysteine-cysteamine mixed disulfides for excretion. NBAs conjugate excess nitrogen to amino acid derivatives for excretion. Both CDAs and NBAs directly target the pathophysiological cascade. However, the role of biomarkers that determined their doses and ultimately approval may differ.

    We aim to categorize the (1) biomarkers and (2) study populations involved in establishing dosage regimens for NBAs and CDAs that are indicated for rare diseases and were approved from 2010 onward. This retrospective analysis will contribute to understanding the application of biomarkers during regulatory review and reveal disease characteristics that can inform future clinical trial design.

- **Methods**
    - Regulatory review databases of the FDA were used to identify NBAs and CDAs approved from 2010 onward to treat rare diseases. Clinical study reports were reviewed to identify the (1) biomarkers used for dose selection, (3) the associated phase of development, and (3) the study populations involved. The Biomarkers, EndpointS and other Tools (BEST) glossary, developed by the FDA-NIH Biomarker Working Group, was used to categorize the dose selection biomarkers for ERTs and siRNAs into following: susceptibility/risk, diagnostic, monitoring, prognostic, predictive, pharmacodynamic (PD)/response, and safety. The PD biomarkers were then classified as target engagement, pathway modulation, or disease related, which are not included in the BEST glossary. These occur early, middle, and late, respectively, during the pathophysiological cascade. Target engagement biomarkers present proximal to the mechanism of action. At a molecular level, they reveal the biological and physical interactions with the target (e.g. receptor). Pathway modulation biomarkers illustrate if the drug is activating or antagonizing the progression of the disease. While pathway modulation biomarkers may not necessarily predict a clinical benefit, disease related biomarkers are intended to do so. Classifying PD biomarkers serves to understand the different aspects of drug response as drug development progresses.
- **Results**
    - Four of the five NBAs are indicated for urea cycle disorders and related conditions, and all five CDAs are indicated for lysosomal transport diseases. All but one dose establishing study among all NBAs and CDAs occurred during phases 1 or 2 of drug development. Biomarkers used to determine doses for both pharmacologic classes were mostly pharmacodynamic, and specifically disease related. For all nine products, only one PD biomarker each was used for dose selection. Amongst all PD biomarkers used for both CDA and NBA development, all were disease related except one, which served as pathway modulation for an NBA. While the volunteer participants for CDA clinical trials were all patients, three dose determining clinical studies for NBAs involved healthy volunteers and explored treatment for urea cycle disorders and related conditions. Only one clinical study for NBAs included patient volunteers, for which two different biomarkers were used for dose selection: (1) prognostic and (2) PD that served as pathway modulation. There were no prognostic biomarkers used for dose selection of CDAs. Although PD biomarkers understandably support dose selection the most, the incorporation of other biomarkers can help guide dose selection for rare disease therapies.

- **Implications**
    - Biomarker identification and classification advance public health by encouraging efficiency and innovation in expanding therapies. Furthermore, different types of PD biomarkers are crucial to understanding different aspects of drug response as drug development progresses. Although only CDAs and NBAs were explored in this study, assessing the landscape of biomarkers used for dose selection among other pharmacological classes and the associated study populations will inform future clinical trial design and ultimately, optimize therapy for an underserved population.

29) **Abstract title:** *Evaluation of B-cell receptor profiling platforms and analysis tools using next-generation sequencing data*
**Authors:** Emily Shang, Lily Zheng, Wenming Xiao
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Purpose**
    - B cell receptor (BCR) sequencing is a technique used to examine the diversity of various clonotypes in B cells within the immune system. This is a critical process for researchers to study individual B cell clones and characterize their antibody repertoires. Next generation sequencing platforms have revolutionized this process by allowing for high-throughput sequencing of many RNA fragments simultaneously and thus provide comprehensive profiles of the B cell antibody repertoires and their clonal diversity. However, there is very little data available comparing the various BCR analysis tools using corresponding NGS techniques. Furthermore, there are no reference studies available for analytical validation or variation controls. In this study, we analyze the single-cell transcriptome sequencing produced by Parse Biosciences in combination with the BCR adaptive immunity profiling software, MiXCR as part of a larger experiment comparing various BCR profiling tools using data obtained via NGS methods.
- **Methods**
    - Parse Evercode single-cell technology enables whole transcriptome sequencing and allows for comprehensive gene expression profiling. We utilized Parse Biosciences' transcriptome sequencing on cells in three combinations: DAUDI only, CA46 only, or a mix of DAUDI and CA46. We then utilized MiXCR, a software that takes in raw sequencing data and uses read alignment to align sequences to reference V,D, J, and C gene sequences with high accuracy, even in sequences with hypermutation. Thus, we used MiXCR to develop adaptive immunity profiles for each of the single cell RNA sequencing data obtained from Parse Biosciences for each cell type.

- **Results**
    - Our pilot study shows that given single cell transcriptome sequencing, MiXCR performs well and yields precise gene alignments and clone identifications for the various cell types. DAUDI and CA46 cells are monoclonal in their BCR, and the MiXCR output reflects these cell properties as well as the proper cell mixing ratios for all three conditions when identifying BCR clonotypes.
- **Implications**
    - The results of our study show that MiXCR performs well and can align raw single-cell transcriptome data and properly determine cell sample and BCR clonotype. This finding will improve the NGS and BCR profiling softwares and platforms available to researchers and help to provide a reference standard for accuracy and precision in BCR immunity profiling.

30) **Abstract title:** *Ensuring Therapeutic Equivalence for Drugs to be Used in Pregnant Patients: A Literature Review and Modeling Exercise to Extrapolate BE Results from Non-pregnant Individuals to Pregnant Individuals*

**Authors:** Xin, Ellen, FDA/CDER (Student); Babiskin, Andrew, FDA/CDER; Shoyaib, Abdullah Al (Mentor)

**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**

- **Synopsis**
    - This study aims to ensure therapeutic equivalence of drugs during pregnancy. It investigates the potential impact of pregnancy associated physiological and pharmacokinetic changes on the bioequivalence (BE) outcomes of generic drugs in pregnant individuals compared to non-pregnant individuals. Mechanisms behind these physiological and pharmacokinetic changes and utility of different modeling approaches (e.g. popPK & PBPK) for extrapolating BE results from non-pregnant to pregnant individuals are being investigated in this study. Our preliminary findings highlight the need for further research to explore the mechanisms underlying these pharmacokinetic changes and their impact on generic drug development for specific drug classes used by pregnant individuals.
- **Purpose**
    - This study aims to investigate the potential impact of physiological and pharmacokinetic changes during pregnancy on the bioequivalence (BE) outcomes of generic drugs in pregnant individuals compared to non-pregnant individuals. Use of high-quality generic medication during pregnancy is crucial to ensure the health of mother and the fetus. Typically, BE studies are conducted in healthy subjects and results are extrapolated to

support therapeutic equivalence in various patient populations following the labeling. The overarching goal of this work is to explore the potential of modeling and simulation approach for extrapolating BE results from non-pregnant individuals to pregnant individuals in order to understand potential risk factors that may impact therapeutic equivalence of generic drugs in pregnant individuals.

- **Method**
  - A comprehensive literature review was conducted to identify relevant data related to physiological changes and PK alteration during pregnancy considering different routes of drug administration and various elimination pathways that may impact drug clearance under pregnancy. A list of potential drug candidates with altered PK during pregnancy were prepared. Currently, we are comparing PK profiles of these drugs between non-pregnant and pregnant individuals. We also plan to develop population PK (popPK) & physiologically based pharmacokinetic model (PBPK) models to extrapolate BE results from non-pregnant individuals to pregnant individuals for our modeling and simulation exercise.
- **Results**
  - Our current literature search reveals that PK profiles of some drugs administered through oral and pulmonary route of administration are impacted by the physiological changes during pregnancy. Additionally, variability in PK parameters were higher in pregnant individuals compared to non-pregnant individuals. Currently, we are in the process of understanding the mechanisms behind these changes and exploring different modeling approaches (popPK & PBPK) for extrapolating BE results from non-pregnant to pregnant individuals.
- **Implication**
  - Understanding the physiological changes during pregnancy and their potential interactions with generic formulations coupled with modeling and simulation approach could help us to understand potential risk factors that may impact therapeutic equivalence of generic drugs in pregnant individuals. Moreover, our preliminary findings highlight the need for further research to explore the mechanisms underlying these pharmacokinetic changes and their impact on generic drug development for specific drug classes used by pregnant individuals.

31) **Abstract title:** *Data-driven Models for Predicting Site Surveillance Inspection Outcomes*
**Authors:** Ravichandran, Arun, FDA/CDER (Student); Tan, Kai, FDA/CDER (Student); Yang, Yidan, FDA/CDER (Student), Wan, John, FDA/CDER (Mentor); Meng, Xiandong, FDA/CDER (Mentor)

**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**

- **Synopsis**
  - In this research, we develop and test a data-driven predictive model that predicts compliance risk for drug manufacturing facilities. This data-driven model uses FDA inspection records and other diverse data sources to predict the public health risks for medical gas (MG) and non-medical gas (non-MG) facilities. Machine learning algorithms are applied to train the model and resampling method, such as cross-validation, are used to evaluate model performance (accuracy). The experimental outcomes indicate accurate predictions and satisfactory performance for this machine learning model.
- **Purpose**
  - This research developed an innovative science-based tool for evaluating and predicting future compliance inspection outcomes for drug manufacturing facilities. This enhanced understanding of public health risk can improve how medical gas (MG) and non-medical gas (non-MG) sites are prioritized for surveillance inspections. It leverages FDA inspection records and diverse data sources with machine learning models (tree-based, neural networks, etc.) to identify the facilities that may show unsatisfactory compliance with cGMP regulations thus to impact public health.
- **Methods**
  - This predictive model is based on diverse data sources, including FDA's inspection records (accessed via ORADSS). The most recent inspection outcome is considered as the target (response variable) for each facility. The training data contain 25 features that characterize the inspection history, manufacturing status, and other inspection-related information. Statistical methods were incorporated to accommodate the highly imbalanced nature of the risk levels (OAI/VAI). These methods, including subsampling, oversampling, and cost-based learning, improved the predictive accuracy of the machine learning models. Resampling by cross-validation was used to evaluate the model performance (accuracy) to allow for generalization of the results.
- **Results**
  - This data-driven model demonstrated satisfactory performance with high predictive accuracy. The decision-tree model has been tested with two versions of the ordinal target (response variable): with four levels (OAI, OAI/VAI, VAI and NAI) and binary (OAI and Others). The results indicate that both predictions are accurate, though modeling the binary target leads to a relatively high accuracy. Additionally, we achieve more accurate models by

training with multiple methods for the above different objectives and combining through voting-based mechanisms.

- **Implications**
    - This predictive model provides a science-based way to evaluate perceived risks for medical gas and non-medical gas facilities. As a complement to the current risk evaluation process, this prediction can identify and prioritize using a 2x2 grid with current SSM scores on one axis and the predicted risk class (OAI/VAI) on the other axis. Our model gives a new dimension to quantifying associated risk that is currently not fully captured in the SSM scores, thus improving the efficiency and quality of the overall process.

32) **Abstract title:** *Characterizing the Clinical Pharmacology Studies in Biosimilar Biologics License Applications*

**Authors:** Eshaghi, Anahita, FDA/CDER (student), Taur, Jan-Shiang FDA/CDER (Mentor), Wang, Yow-Ming FDA/CDER (Mentor)

**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**

- **Synopsis**
    - Biosimilars are highly similar to and displaying no clinically meaningful differences from an FDA-approved reference product. They are rapidly emerging and will provide affordable alternative which can reduce prescription drug cost. The objective of this study is to comprehensively examine and analyze the clinical pharmacology studies collected from 14 biologics license applications (BLAs) of biosimilar products currently seeking FDA approval. The information regarding study design (e.g., sample size, study population, dose, route of administration, sampling time, statistical method), and summary results of PK similarity studies, pharmacodynamic studies, and comparative clinical studies, including immunogenicity, are collected and analyzed. The results of this project help to develop the evidence-based approach in designing clinical pharmacology studies and evaluating the study results.
- **Purpose**
    - The approval of biosimilar products is essential to contain increasing healthcare cost and provide more affordable choices for patients. The results of PK/PD similarity studies and comparative clinical studies are critical in demonstrating the similarity between the biosimilar and reference products. The purpose of this study is to expand the collection of clinical studies in the biosimilar BLA submissions. This work would facilitate evidence-based approaches to designing the clinical program for

establishing biosimilarity and FDA's assessment of clinical data supporting biosimilarity.

- **Methods**
  - We evaluated the 14 biosimilar BLAs submitted in the past eight months. The study information on study design, sample size, study population, dose, route of administration, sampling time, statistical method, and immunogenicity were extracted from the study report of clinical pharmacology studies. Study findings on the geometric mean and the variability of PK and PD endpoints of drug products as well as the 90% confidence interval (CI) of geometric mean ratio between biosimilar and reference drugs were also collected. The analysis was conducted using descriptive statistics.
- **Results**
  - Our collection of biosimilar BLAs includes 14 different applications for 10 different reference products, comprising approximately 16% of the overall biosimilar database. There are 23 individual clinical pharmacology studies found in the surveyed BLA submissions. Of these studies, 16 and 7 studies are PK or PK/PD similarity and comparative clinical studies, respectively. For the PK similarity studies, all the 90% CIs of the geometric mean ratios of PK parameters are within the acceptance interval of 80% to 125%. In addition, variabilities of PK parameters are generally comparable between biosimilar drugs and the corresponding reference products.
- **Implications**
  - This project enhances our knowledge on the clinical pharmacology studies in the biosimilar BLA submission. Establishing a comprehensive resource that is up to date with incoming biosimilars is imperative to help facilitate Public Health efforts in reducing healthcare burdens.

33) **Abstract title:** *A Survey of First-In-Human Study Design for Bispecific T-Cell Engagers*
**Authors:** Wu, Shunwen, FDA/CDER (Student); Qi, Timothy, FDA/CDER (Contractor); Okusanya, Olanrewaju, FDA/CDER (Mentor); Liu, Jiang, FDA/CDER (Mentor); Jiang, Xiling, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Empowering Patients and Consumers
**Abstract:**

- **Synopsis**
  - Bispecific T-cell engagers (TCEs) are an expanding class of antibody-based therapies primarily employed in cancer treatment. Their development continues be challenging due to their unique PK/PD characteristics, acute toxicities during treatment initiation, and narrow therapeutic windows. This has resulted in novel and non-standardized approaches for first-in-human

(FIH) study design, including methods for determining initial doses for human use, dose escalation, and dose optimization for initial step-up and treatment doses. This survey aims to stimulate fresh perspectives and novel approaches that could advance the field of TCEs. We reviewed FIH studies for 111 novel TCE INDs submitted to the FDA between January 1, 2015, and December 31, 2022, to identify emerging trends in FIH clinical study design. Based on the current analysis, 56% of protocols were designed for hematological malignancies, while 44% were designed for solid tumor related indications. The most targeted tumor antigens included BCMA, CD19, PSMA, CD123, CD20, CD33. Median binding affinities for human CD3 and tumor antigens were 14.4 nM (0.057 nM – 1,520 nM) and 1.5 nM (0.0009 nM – 12,718 nM), respectively. Some (14%) protocols used modeling and simulation to support FIH dose, with quantitative systems pharmacology (QSP) models being the most common approach (7%). Most (78%) protocols contemplated using step-up dosing (SUD) to mitigate cytokine release syndrome; only 46% incorporated SUD into their initial design. Some (29%) of protocols contemplated subcutaneous administration, with a smaller proportion (12%) incorporating it from the outset. RP2Ds had been identified for 12% of TCEs. On average, two step-up doses were used prior to administering target doses. This survey highlights the current landscape and trends in TCE clinical trials, identifies potential areas for improvement, and recognizes gaps where innovative methods can be introduced, such as QSP models.

- **Purpose**
    - Bispecific T-cell engagers (TCEs) are an expanding class of antibody-based therapies that are primarily employed in cancer treatment. Although great progress has been made, the development of TCE continues be challenging due to their unique PK/PD characteristics, acute toxicities during treatment initiation, and narrow therapeutic windows. This has resulted in novel and non-standardized approaches for the development of these products with regards to first-in-human (FIH) study design, including methods for determining initial doses for human use, dose escalation, and dose optimization for initial step-up and treatment doses. By compiling and presenting this survey, we aim to stimulate fresh perspectives and inspire the development of novel approaches that could advance the field of TCEs.
- **Methods**
    - We reviewed FIH studies for 111 novel TCE INDs submitted to the FDA between January 1, 2015, and December 31, 2022, to identify emerging trends in FIH clinical study design.

- **Results**
    - Based on the current stage of analysis, 56% of protocols were designed for hematological malignancies, while 44% were designed for solid tumor related indications. The most targeted tumor antigens included BCMA, CD19, PSMA, CD123, CD20, CD33. Median binding affinities for human CD3 and tumor antigens were 14.4 nM (0.057 nM – 1,520 nM) and 1.5 nM (0.0009 nM – 12,718 nM), respectively. Seven percent (7%) of novel TCEs were trispecific. MABEL approach was the predominant (84%) method for justifying first-in-human dose. However, 14% of protocols used modeling and simulation to support FIH dose, with quantitative systems pharmacology (QSP) models being the most common approach (7%). In FIH studies, 79% of FIH protocols used accelerated titration. While a majority (78%) of protocols contemplated using step-up dosing (SUD) to mitigate cytokine release syndrome (CRS), fewer than half (46%) incorporated SUD into their initial design. Roughly one third (29%) of protocols contemplated subcutaneous administration, with a smaller proportion (12%) incorporating it from the outset. RP2Ds had been identified for 12% of TCEs, 77% and 23% of which were for hematological and solid tumors, respectively. On average, at least two step-up doses were used prior to administering target doses.
- **Implications**
    - This survey highlights the current landscape and trends in TCE clinical trials, identifies potential areas for improvement, and recognizes gaps where innovative methods can be introduced, such as QSP models. Our objective with this survey was not only to summarize existing practices, but also to encourage thoughtful consideration in the design of future clinical trials for TCEs.

34) **Abstract Title**: *Pediatric Clinical Trial Designs for Testosterone Replacement Therapy (TRT)*
**Authors**: Hyeonglim Seo, FDA/CDER (Student); Chongwoo Yu, FDA/CDER (Mentor); Kim Shimy, FDA/CDER (Researcher); Yanhui Lu, FDA/CDER (Researcher); Gilbert Burckart, FDA/CDER (Researcher); Jingyu Yu, FDA/CDER (Researcher); Yun Wang, FDA/CDER (Researcher); Suresh Kaul, FDA/CDER (Researcher)
**FDA Regulatory Strategic Priority**: Increasing Choice and Competition through Innovation
**Abstract**:

- **Synopsis**
    - Testosterone replacement therapy (TRT) in adult males is indicated for conditions associated with a deficiency or absence of endogenous testosterone (T). Under the Pediatric Research Equity Act (PREA), pediatric studies are required when a product presents with any of the following: (1) new indication; (2) new dosage from; (3) new dosing regimen; (4) new route

of administration; and (5) new active ingredient. The Agency has deferred the submission of the pediatric trial for males ages 12 years to less than 18 years of age when some new TRT products were ready for approval in adults and the pediatric trial has not been completed. Recent approvals of some new TRT products are triggering PREA and the agency is starting to receive submissions with pediatric clinical trial protocols to fulfill post marketing requirements (PMRs). However, there has not been any well-designed pediatric trial with TRT conducted to date supporting regulatory decisions. Various aspects of things to be considered in designing of future pediatric TRT trials were investigated and summarized in this presentation.

- **Purpose**
  - The primary purpose of this investigation is to identify and address the challenges in designing adequate pediatric clinical trials with TRT and provide recommendations in designing future trials involving this treatment.
- **Methods**
  - The study includes regulatory reviews available at Drugs@FDA (https://www.accessdata.fda.gov/scripts/cder/daf/) that covers adult clinical trial protocols with TRT and published literature relevant to hypogonadism, TRT, and clinical assessment following testosterone exposure.
- **Results**
  - Challenges in designing pediatric trials with TRT were identified: i) Lack of formal clinical practice guidelines regarding the use of TRT in pediatric patients; ii) Variable clinical parameters for puberty; iii) Published standards for growth/pubertal development derived from healthy children; iv) Assessment of pubertal stage in hypogonadal boys is not being straightforward; v) Wide and overlapping range for T concentration during puberty are; vi) Lack of standardization on target normal T concentrations for each Tanner stage. To address the challenges, information and data on important clinical and clinical pharmacology aspects that need to be considered in designing adequate pediatric clinical trials were collected. For example, establishing an adequate dose titration scheme for each product together with the optimal exposure parameter (e.g., Cavg or Cmid) considering the dosage regimen and route of administration to determine the need for dose adjustment is an important clinical pharmacology consideration that needs to be made. Very little is known about the exposure-response (E-R) relationship in the pediatric population for TRTs. Therefore, measurable clinical parameters including specific component of body composition (e.g., lean body mass), bone density of certain skeletal sites (e.g., posterior-anterior spine) and stretched penile length were

identified as an potential response parameter(s) to be used in the E-R analysis that may be utilized in determination of the optimal pediatric dose for TRT.

- **Implications**
    - This investigation provides valuable insights into the clinical and clinical pharmacology parameters that should be considered when designing pediatric clinical trials for TRT. The identified challenges, such as variable parameters as clinical endpoints for TRT in pediatric patients, highlight the need for further research and guidance in this area. The collected information and data can serve as cornerstone of recommendations for designing future pediatric clinical studies with TRT.

35) **Abstract title:** *Comparing Multiple Test products with the Same Reference for Bioequivalence with Normal Data*
**Authors:** Zhang, Xuze, FDA/CDER (Student); Shen, Meiyu, FDA/CDER (Mentor); Tsong, Yi, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - For the assessment of bioequivalence between test and reference products with normal response data, the ratio of means will be tested against the pre-specified margin. It is conventionally performed using two one-sided tests, each at the significance level of 5%. When the sponsor conducts a study with multiple candidate test products with one reference product, the significance level for each test product should be adjusted to control the familywise type I error rate under 5%. This project is to determine the critical values for the bioequivalence tests with multiple test products against the same reference product since the adjusted significance level for each equivalence test depends on the joint distribution of the test statistics of one-sided tests.
- **Purpose**
    - For the assessment of bioequivalence between a single test product and a reference product with normal response data, the ratio of means will be tested against the pre-specified margins. It is conventionally performed using two one-sided tests, each at the significance level of 5%. This will guarantee that the joint test (union of the two one-sided tests) is at the significance level of 5%. When there are multiple test products tested against a reference product, multiplicity adjustment is required for each test product. In this case, the joint test is considered as the intersection of the union of the two one-sided tests for each test product. Thus, the

significance level of the two one-sided tests should be adjusted in order to guarantee that the significance level of the joint test remains 5%. This project is to determine the critical values and significance levels of the one-sided tests while controlling the significance level of the joint test to be 5%.

- **Methods**
    - The two one-sided tests, lower margin test and upper margin test, correspond to the tests of two pre-specified margins. The joint test, intersection of the union of the two one-sided tests for each test product, is equivalent to the union of the intersection of one of the two one-sided tests for each test product. For K test products, the joint test is a union of 2^K intersections. In each intersection, either lower margin test or upper margin test is included for each test treatment. The intersection of lower margin tests is controlled at the significance level of 5%. Since all test products are treated equally, then the critical values are the same for all lower margin tests. This critical value can be determined by the joint distribution of the test statistics of all lower margin tests. Similarly, the critical value for all upper margin tests is determined such that the significance levels for the rest 2^K-1intersections are less than 5%. This procedure guarantees that the joint test at the significance level of 5%.
- **Results**
    - The table of critical values and adjusted significance levels for lower and upper margin tests is created for different sample sizes.
- **Implications**
    - When multiple test products are tested against a reference for bioequivalence, the joint test appears to have a complicated form. Thus, the common procedure for multiple comparison such as Bonferroni correction cannot guarantee the significance level to be 5% for the joint test. Moreover, it is discovered that the critical values for lower and upper margin tests are not symmetric about 0 if one wishes to maintain the joint test at the significance level of 5%.

36) **Abstract title***: Dissecting the mechanistic pathway of Anti-HER2 Antibody Drug Conjugates through the development of cell-based potency assays*
**Authors:** Zhao, Madison, FDA/CDER (Student); Garg, Ria, FDA/CDER (Student); Patterson, Kamai, FDA/CDER (Student); Bacot, Sylvia, FDA/CDER (Mentor); Gorospe, Jordon, FDA/CDER (Student); Simhadri, Venkateswara, FDA/CDER (Mentor), Wang, Tao, FDA/CDER (Mentor); Feldman, Gerald, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
    - Antibody Drug Conjugates (ADCs) have shown noteworthy clinical efficacy in a variety of cancer patients and are a rapidly growing area of clinical

research because of their targeted approach to chemotherapy. Specifically, ADCs such as Trastuzumab emtansine (T-DM1), have shown dramatic effects in HER2-positive breast cancer patients. However, there are growing concerns regarding its lack of efficacy in a sub-cohort of patients, and its apparent role in inducing severe toxicities. While there are already two federally approved ADCs for anti HER2 cancers (Trastuzumab emtansine and trastuzumab deruxtecan), there are gaps in our knowledge regarding the specific mechanism(s) utilized by these ADCs to kill HER2-positive tumor cells. In this study, we demonstrate the development of an assay that is capable of determining not just the biological activity of an ADC but the mechanism by which it exerts its killing effect. Using a combination of flow cytometry and spectrum cello-meter analysis, we have developed a mechanistically driven potency assay to test for different types of cell death such as apoptosis and ferroptosis. The results of these analyses are supported by western blot assays to more fully explore the mechanism by which cell death occurs. Using T-DM1 as a reference product, we have developed this assay to determine how the major cellular death pathways (i.e., necroptosis, apoptosis, autophagy, or ferroptosis) contribute to the efficacy of T-DM1 induced cell death. Our preliminary data suggests the development of this assay paves the way for a more comprehensive approach to the understanding of the mechanisms underlying ADC-induced cell death.

- **Purpose**
    - The overexpression of human epidermal growth factor receptor-2 (HER2) is found in about 20%–30% of breast cancer patients. Although these patients suffered clinically unfavorable outcomes previously, the development of antibody-drug conjugates (ADCs)— therapeutic entities consisting of monoclonal antibodies chemically linked to cytotoxic payloads drastically changed this. The development of ADCs is a rapidly growing area of clinical research because of their targeted approach to chemotherapy. Specifically, ADCs such as Trastuzumab emtansine (T-DM1), have shown dramatic effects in HER2-positive breast cancer patients. However, there are growing concerns regarding its lack of efficacy in a sub-cohort of patients, and its apparent role in inducing severe toxicities. While there are already two federally approved ADCs for anti HER2 cancers (T-DM1 and trastuzumab deruxtecan, or T-DXd), the specific mechanism(s) utilized by these ADCs to kill HER2-positive tumor cells remains unclear.
- **Methods**
    - In this study, we demonstrate the development of an assay that is capable of determining not just the biological activity of an ADC but the mechanism

by which it exerts its killing effect. For assessing the anti-tumor effects of T-DM1 on HER2-positive cancer cells we cultivated two cell lines with varying levels of HER2 expression (SK-BR-3, and BT-474) as well as a HER2 negative cell line (MCF7) to act as a control. We then exposed them to T-DM1 treatments for at least 24 hours. Following treatment, we conducted a flow cytometry analysis to compare viability across the three cell lines, and Western blot analyses were conducted to more fully expand on the mechanism by which cell death occurs.

- **Results**
  - Our preliminary data show an obvious difference in the percentage of viable cells between treated and untreated samples, indicating that T-DM1 did induce cell death in the HER2-positive cell lines, with cell death correlating with the level of expression of HER2. Furthermore, western blot data demonstrates that T-DM1 triggers both autophagy and apoptosis in HER2-positive SK-BR-3 and BT-474 cell lines.
- **Implications**
  - Overall, using T-DM1 as a reference product, we have developed a cell-based potency assay to determine how the major cellular death pathways (i.e., necroptosis, apoptosis, autophagy, or ferroptosis) contribute to the efficacy of T-DM1 induced cell death. The development of this assay paves the way for a more comprehensive approach to the understanding of the mechanisms underlying ADC-induced cell death. Despite the availability and efficacy of current HER2 targeting agents, a large number of patients eventually acquire therapeutic resistance to anti-HER2 monoclonal antibodies, and an understanding of their mechanism of action would be the first step in any approach to reducing the development of drug resistance.

37) **Abstract title:** *Adaptive Comparative Clinical Endpoint Study Design for Biosimilars with Comparative Sample Size Re-estimation and Optional Effect Size Re-estimation*
**Authors:** Zhou, Grace, FDA/CDER (Student); Hinds, David, FDA/CDER (Student); Sun, Wanjie, FDA/CDER (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
  - In comparative clinical endpoint trials, biosimilar products need to demonstrate that there are no clinically meaningful differences between the test biosimilar (T) and the reference biologic (R). While a fixed design has traditionally been used and is more straightforward, it relies on initial assumptions made for unknown study design parameters, potentially

resulting in over- or under-powered trials when those assumptions do not hold. Previously, Fuglsang (2014) proposed a two-stage adaptive design for parallel two-arm bioequivalence studies where the alpha adjustment was based on simulation results. In this paper, we seek to adapt the two-stage adaptive approach for a three-arm (test, reference and placebo) clinical endpoint bioequivalence study proposed by Hinds and Sun (2023) to a parallel two-arm study design comparing the equivalence of T vs. R which utilizes standard/maximum combination method and optional re-estimation of the effect size in unblinded sample size re-estimation. The proposed adaptive design safeguards against over- and under-powered trials of a fixed design by re-estimating the sample size based on the observed data from Stage 1 to ensure adequate power is maintained. The proposed method also guarantees control of the Type 1 error rate analytically in all scenarios as compared to Fuglsang's method which may not guarantee the Type 1 error rate under certain scenarios. This design can also be adapted for parallel two-arm bioequivalence trials. The proposed methods will help to cut cost for applicants and promote the availability of affordable biosimilar and generic drugs to the public.

- **Purpose**
    - In comparative clinical endpoint trials, biosimilar products need to demonstrate that there are no clinically meaningful differences between the test biosimilar (T) and the reference biologic (R). While a fixed design has traditionally been used and is more straightforward, it relies on initial assumptions made for unknown study design parameters, potentially resulting in over- or under-powered trials when those assumptions do not hold. Previously, Fuglsang (2014) proposed a two-stage adaptive design for parallel two-arm bioequivalence studies where the alpha adjustment was based on simulation results.
- **Methods**
    - In this paper, we seek to adapt the two-stage adaptive approach for a three-arm (test, reference and placebo) clinical endpoint bioequivalence study proposed by Hinds and Sun (2023) to a parallel two-arm study design comparing the equivalence of T vs. R which utilizes standard/maximum combination method and optional re-estimation of the effect size in unblinded sample size re-estimation.
- **Results**
    - The proposed adaptive design safeguards against over- and under-powered trials of a fixed design by re-estimating the sample size based on the observed data from Stage 1 to ensure adequate power is maintained. The proposed method also guarantees control of the Type 1 error rate

analytically in all scenarios as compared to Fuglsang's method which may not guarantee the Type 1 error rate under certain scenarios.

- **Implications**
    - This design can also be adapted for parallel two-arm bioequivalence trials. The proposed methods will help to cut cost for applicants and promote the availability of affordable biosimilar and generic drugs to the public.

38) **Abstract title**: *Bioproduction strategy for a cetuximab biobetter without highly immunogenic alpha Galactosylated glycans*

**Authors**: Zidan, Yousof, FDA/CDER (Student); Ju, Tongzhong, FDA/CDER (Mentor); Biel, Thomas, FDA/CDER (Mentor); Falkowski, Vincent, FDA/CDER (researcher); Ortega-Rodriguez, Uriel, FDA/CDER (researcher); Houchens, Tylee, FDA/CDER (researcher)

**FDA Strategic Initiative**: Increasing Choice and Competition through Innovation

**Abstract**:

- **Synopsis**
    - N-glycosylation is a common critical quality attribute for human IgG1 monoclonal antibody (mAb) drugs because N-glycans in the Fc region can impact antibody effector functions and pharmacokinetic properties. Cetuximab is an FDA approved N-glycosylated therapeutic humanized IgG1 monoclonal antibody (mAb) that inhibits epidermal growth factor receptor (EGFR) and induces antibody-dependent cytotoxicity (ADCC) for colorectal carcinoma and metastatic colorectal carcinoma indications. Cetuximab, which is manufactured using a murine myeloma cell line (NS0) as a host cell substrate, is a glycoprotein that contains four N-glycan sites: two within the constant region (Fc) and two within the variable region (Fab). For the outcomes, in the reference material, we found N-glycan species bearing the highly immunogenic α-Galactosylated lactosamine moieties, while sialylated N-glycan species were untraceable, as well as no detectable levels of immunogenic N-glycolylneuraminic acid glycan. The cetuximab manufactured using CHO cells contained N-glycans species that were sialylated N-glycan without any α-Galactosylated lactosamine moieties. Moreover, CHO-manufactured cetuximab did not contain immunogenic N-glycolylneuraminic acid. These preliminary data support that the cetuximab biobetter manufactured using CHO cell lines as host cell substrate may produce a potentially less immunogenic drug product as compared to a cetuximab drug product manufactured using a NS0 cell substrates. Further studies are warranted to assess the immunogenic potential of the CHO-manufactured cetuximab.
- **Purpose**
    - N-glycosylation is a common critical quality attribute for human IgG1 monoclonal antibody (mAb) drugs because N-glycans in the Fc region can impact

antibody effector functions and pharmacokinetic properties. It is known that the glycoproteins produced from Chinese hamster ovary (CHO) cells have human-like N-glycans, while the glycoproteins from murine cells can contain non-human glyco-epitopes including α-Galactose (αGal) and N-glycolyl-neuraminic acid (Neu5Gc) on their glycans which are highly of immunogenicity due to naturally occurring anti- αGal and anti- Neu5Gc antibodies in humans. Cetuximab is an FDA approved N-glycosylated therapeutic humanized IgG1 monoclonal antibody (mAb) that inhibits epidermal growth factor receptor (EGFR) and induces antibody-dependent cytotoxicity (ADCC) for colorectal carcinoma and metastatic colorectal carcinoma indications. Cetuximab, which is manufactured using a murine myeloma cell line (NS0) as a host cell substrate, is a glycoprotein that contains four N-glycan sites: two within the constant region (Fc) and two within the variable region (Fab). The impact of N-glycosylation on Cetuximab's Fab region on product safety, efficacy, and quality remains elusive. Here we present the comparative N-glycosylation characterization of cetuximab manufactured using CHO cells to that manufactured using murine myeloma NS0 cells.

- **Methods**
    - Freestyle CHO S cells expressing cetuximab were expanded and inoculated for a 6-day bioproduction campaign using 1L shake flasks in an environmentally controlled incubator and FreeStyle CHO medium. Harvest cell media was pooled, clarified by centrifugation, and filtered prior to a sequential three-step purification process (Chromatography: Protein A, Anion exchange, and Cation exchange). The final eluant was buffer exchanged into a cetuximab drug product formulation. N-glycans from cetuximab manufactured using CHO cells (CHO cetuximab) and cetuximab manufactured using murine myeloma NS0 (reference material) were released using a filter aided N-glycan separation (FANGS) using filtered aided sample prep (FASP). Cetuximab containing samples were reduced using DTT, subsequently alkylated with iodoacetamide (IAA) and then buffer-exchanged into ammonium bicarbonate. N-glycans were released by treatment of PNGase F, recovered, dried, and purified. N-glycans were reduced with an ammonia borane complex then dried under vacuum and a series of methanolic evaporations to remove excess reactants. Reduced N-glycans were subsequently permethylated using solid-phase permethylation method and the permethylated N-glycans were purified by C18 micro spin columns. The final eluate was dried and analyzed on MALDI-TOF (Matrix-assisted Laser Desorption/Ionization time-of-flight Mass Spectrometry). N-glycan profiling of cetuximab drug product of CHO Cetuximab and reference material was performed in parallel and compared.

- **Results**
    - Mass spectrometry analysis by MALDI-TOF-MS revealed major differences in N-glycosylation between the reference material and the CHO cetuximab. In the reference material, we observed evidence of N-glycan species bearing α-Galactosylated lactosamine moieties (αGal) which are typical in murine-based glycoproteins. These α-Gal glycotopes are highly immunogenic to humans because human glycoproteins do not contain α-Galactosylated glycans. We did not identify any sialylated N-glycan species, including immunogenic N-glycolylneuraminic acid (Neu5Gc) glycans in the reference material. In the CHO-based cetuximab, we observed sialylated N-glycan species-namely N-acetylneuraminic acids (Neu5Ac) but not immunogenic N-glycolylneuraminic acids (Neu5Gc) on these N-glycans. Importantly, we did not observe any α-Galactosylated glycans in the CHO cetuximab that were present in the reference material and reduced levels of high mannose N-glycans in CHO-based cetuximab when compared to the reference material.
- **Implications**
    - It is well known that murine myeloma NS0 cells synthesize non-human glycans, such as αGal, that are highly immunogenic. CHO cells are known to produce untraceable quantities of αGal, and the use of CHO cells as an alternative cell substrate may lead the biomanufacturing of cetuximab biosimilars with less immunogenic potential, e.g., cetuximab biobetter. Here, we present a CHO cell-based biomanufacturing tool that produces cetuximab with 1) more mature complex N-glycans with a greater number of sialylated species as compared to the reference material, and 2) N-glycans that do not harbor the highly immunogenic aGal moiety. These preliminary data support that the cetuximab biobetter manufactured using CHO cell lines as host cell substrate may produce a potentially less immunogenic drug product as compared to a cetuximab drug product manufactured using a NS0 cell substrates. We plan to continue biomanufacturing N-glycovariants of cetuximab to determine the role N-glycans can have on drug product safety, efficacy, and quality.

## Center for Devices and Radiological Health (CDRH)

1) **Abstract title:** *Tunability of Microstructure Parameters in an In-silico Trabecular Bone Model*
**Authors:** Wang, Andrew, FDA/CDRH (Student); Cao, Qian, FDA/CDRH (Mentor); Marupudi, Sriharsha, FDA/CDRH (Mentor); Samala, Ravi, FDA/CDRH (Mentor); Petrick, Nicholas, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**
    - **Synopsis**
        - Bone microstructure is an important indicator of bone health. However, there is currently a lack of high-quality image data of bone microstructure

for developing robust imaging features for assessing bone health. In this work, we evaluated an in silico trabecular bone model by characterizing the range and tunability of microstructure parameters with respect to its input parameters. We found that the model can generate a wide range of trabecular bone with varying trabecular thickness, bone volume, and anisotropy that resembles trabecular bone found in human trabeculae.

- **Purpose**
  - Bone health is dependent on both bone mineral density (BMD) and microstructure. Currently, the standard of care for bone health evaluation is based on BMD. High quality image data of trabecular bone microstructure is needed to develop robust texture features for evaluation of bone microstructure. To address the lack of high quality trabecular bone data, we have developed a tool for in silico generative modeling of trabecular bone microstructure. The generative model uses Voronoi tessellation of randomly distributed seed points in 3D to generate simplices, from which rods and plates (edges and faces of the Voronoi simplices) are sampled to form the final trabecular bone structure. The parameters of the model can be adjusted to generate bone structures with different microstructure. In this work, we investigate our model's ability to generate synthetic trabecular bone structures with prescribed microstructure metrics. This tunability is an important feature for our in silico model because it will allow the user to control the distribution image data to be generated.
- **Methods**
  - The input parameters of the model, consisting of seed point density (1 to 10 points/mm), the ratio of retained rods and plates (0 to 1), mean thickness (0.01 to 1 mm), and standard deviation of thickness (0.001 to 0.1 mm), were randomly sampled (n=191) from a uniform distribution. The model generated 5 bone volumes for each input parameter, for a total of 955 volumes. Then, microstructure metrics for each volume were computed, these include mean trabecular thickness, degree of anisotropy (DA), and volume fraction (BV/TV). For each set of input parameters, the mean microstructure metrics of the 5 instances were calculated. The mean values were interpolated into the input parameter space. The 2-dimensional slices of the metrics in the input parameter space were plotted to observe relationships between different combinations of input parameters and their corresponding microstructure metric results. The microstructure metrics were then compared with those of a dataset of real human lumbar vertebrae. The similarity between these two distributions of microstructure metrics was evaluated using Nearest Neighbor and Mahalanobis Distance.
- **Results**
  - The interpolated grid data reveals that the model can generate a range of values for each microstructure metric that encompass the corresponding true range of values in trabecular bone. The range of BV/TV values that was generated was 0.015 to 1.00, the range of mean thickness was 79.6 to 4000 microns, and the range of DA was 0.004 to 0.665. For comparison, the target metric ranges for trabecular bone are 0.03 to 0.20 for BV/TV, 90 to

400 microns for mean thickness, and 0.10 to 0.40 for DA. This demonstrates that the model has the capacity to generate a comprehensive set of trabecular bone volumes. Additionally, when the model metrics were compared to the dataset of real bone metrics, the joint distribution of DA and BV/TV was close to that of real trabecular bone, as shown by a mean Mahalanobis Distance of 0.18. However, when analyzed together with mean thickness, the difference in distribution between the model and actual data was much more substantial, due to the discrepancy in the mean thickness distribution between the model and real bone, resulting in a mean Mahalanobis distance of 3.48.

- **Implications**
    - This model shows promise for producing trabecular bone volumes similar to human vertebrae, as each microstructure metric's range individually captures the range of metrics in real trabecular bone. Currently, the joint distribution of DA and BV/TV overlaps with the joint distribution in real bones, but less so with the incorporation of other metrics, such as mean thickness. Next steps include further analysis of the variance of these metrics with respect to the input parameters as well as further revisions to the model to improve the realism of synthetic trabecular bone structures.

2) **Abstract title:** *Differences in IMU Signals between Parkinson's Patients Relative to Staggering Events During Gait Exercises*
**Authors:** Ngoh, Brian, FDA/CDRH (Student); Watkinson, Sophia, FDA/CDRH (Student); Caiola, Michael, FDA/CDRH (Mentor); Nyman, Edward, FDA/CDRH (Mentor); Eguren, David, FDA/CDRH (Mentor); Velazquez, L. Moro, FDA/CDRH (Mentor); Dehak, Najim, FDA/CDRH (Mentor); Moukheiber, Emile, FDA/CDRH (Mentor); Motley, Chelsie, FDA/CDRH (Mentor); Mills, Kelly, FDA/CDRH (Mentor); Butala, Ankur A., FDA/CDRH (Mentor), Kontson, Kimberly, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - Falling is a concern for PD patients and patients who stagger are prone to falls. A patient who staggers loses balance and requires an additional step to regain their balance. This study will evaluate the angular velocities of PD patients who do and do not stagger during the tandem gait task. The IMU data of angular velocities will be compared between two groups of participants (those who exhibit multiple staggering events and those who do not) to explore the differences in the variation of data. The results show that PD patients with a stagger had more variability in their angular velocities over time compared to those without a stagger which could contribute to a future fall.
- **Purpose**
    - Gait disturbances such as staggering can lead to falls which can cause serious injury, particularly in PD patients. A stagger occurs when a participant loses balance while walking and needs additional steps to regain balance. Inertial Measurement Unit (IMU) data may be useful to identify patterns and find differences for various walking tasks for those with and

without a stagger. This research can help to detect stagger events for clinicians to implement physical therapy or other clinical interventions to prevent future falls for PD patients.

- **Methods**
  - A sample size of 6 participants performing a tandem walking task (i.e., heel-toe walking) was included in the analysis. Three participants had multiple stagger events while the remaining three did not have any stagger events. The angular velocities from IMU data from the three participants with a stagger were compared to those without a stagger. IMU data collected from the head, lower back, ankles, and chest were used since these are informative body areas to assess the balance of PD patients. The variation of IMU angular velocities (e.g., standard deviation and range) were quantified and compared across the two groups.
- **Results**
  - The average SD of angular velocity about an axis approximating the anterior-posterior direction was generally greater in the stagger group compared to the no stagger group at the right ankle IMU sensor location (stagger: SDx = 1.10 rad/s, NO stagger: SDx = 0.89 rad/s). The average range of angular velocity values about that same axis during the tandem gait task was also greater in the stagger group at the right ankle IMU sensor location (stagger: Rx = 17.98 rad/s, NO stagger: Rx = 12.24 rad/s). The head IMU sensor location measured the second largest difference in angular velocity ranges about an axis approximating the superior-inferior direction for the stagger group compared to the no stagger group (stagger: Ry = 6.75 rad/s, NO stagger: Ry = 4.20 rad/s).
- **Implications**
  - The loss of balance can lead to staggering which can cause falls. Wearable devices using IMUs to measure the angular velocities could determine when a stagger event occurred. When there is variable and inconsistent data from IMUs, this could be an indicator of the possible balance issues PD patients experience. This analysis may also contribute to the development of a stagger detection algorithm that predicts angular velocities using the variable data from the analysis and notifies the PD patient and caregiver before it happens.

3) **Abstract title:** *Development of a Regulatory Science Tool for the Growth, Detection, and Extraction of Biofilm from Surfaces of Medical Device Materials*
**Authors:** Canagarajah, Christa, FDA/CDRH (Student); Pandey, Ruchi, FDA/CDRH (Mentor); Weeks, Jon, FDA/CDRH (Mentor); Vishwakarma, Apoorva, FDA/CDRH (ORISE Fellow)
**FDA Strategic Initiative:** Empowering Patients and Consumers
**Abstract:**

- **Synopsis**
  - There is currently a need for a reproducible method for biofilm testing pertaining to medical devices. Biofilm may grow on devices, such as heater

coolers, despite device cleaning instructions. Creating a standard method for biofilm testing can assist in validating cleaning methods or instructions to reduce biofilm from medical device surfaces. Quantifying residual biofilm will help demonstrate effective reduction.

This research will involve growing biofilm using a drip flow reactor, quantifying biofilm material through microbiological techniques and biochemical analysis. Quantification methods such as bacterial plating and counting, protein quantification, and crystal violet staining will be employed. The data obtained through these experiments aims to help development of a regulatory science tool and eventually may aid future standard development.

- **Purpose**
  - Biofilms represent a dynamic community of bacteria that grow on surfaces encased in an exopolymeric matrix. Biofilms can be a significant problem especially in healthcare settings. In some cases, they can potentially grow on medical devices despite instructions to clean the device surface. Healthcare-associated infections (HAIs) have been linked to biofilm formation on medical devices. New regulatory science tools could help mitigate these risks by supporting validation of cleaning methods for reduction of biofilm. This research aims to develop a regulatory science tool regarding growth and quantification of biofilms on medical device surfaces.
- **Methods**
  - To develop the regulatory science tools biofilm, relevant gram-positive and gram-negative bacteria will be used. Biofilm will be grown under low fluid shear, close to the air–liquid interface, using the drip flow reactor (DFR) based on ASTM E2647-20. Gram-negative bacteria will be the primary focus of these experiments.

    A relationship will be correlated between optical density and CFU of each bacterial species prior to the cultivation of the biofilm. Using this relationship, concentration of bacteria will be known, allowing us to find the difference in colonies before and after culturing in the DFR. These studies will include both gram-positive and gram-negative bacteria to understand the correlation in optical density and colony forming units. Data obtained on gram-positive bacteria will inform future experiments.
- **Results**
  - Preliminary results show a linear correlation between optical density and colony forming units for the gram-positive and gram-negative bacteria used in the study. This suggests that once the relationship is established for each test microorganism, optical density can be an appropriate indicator of bacterial concentration when seeding bacteria into the drip flow reactor.

- **Implications**
    - This research is anticipated to support method development for creating and quantifying biofilm on medical device material surfaces. This will help future development of acceptance criteria for biofilm extraction and will inform residual limits for biofilm material remaining after cleaning of medical devices. Once established, these methods may be utilized to validate instructions to effectively reduce biofilm as part of the cleaning methods.

4) **Abstract title:** *Designing a Microfluidic Recirculating Flow Loop*
**Authors:** Emily Cheung, Matthew Hirschhorn, Gavin D'Souza, Jean Rinaldi, Richard Malinauskas, Suvajyoti Guha, Luke Herbertson
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
    - Flow characterization is a critical step in determining if a blood-contacting device is safe and effective. Benchtop flow systems are important, multi-disciplinary tools that can be used to assess the pressure-flow performance characteristics and flow-related issues associated with medical devices. In this study, we designed a microfluidic flow loop capable of evaluating the hemodynamics of microscale components and microfluidic chips. This type of miniaturized recirculating flow loop has the potential to be used for testing long-term durability, leakage, blood damage, coating integrity, or mixing of microfluidic devices on the bench. The benefits of such a system are its small volume, capability for running multiple loops simultaneously, the potential for high sensitivity measurements, optical access to the device under investigation, high attainable pressures, its simplicity for modeling, and reproducibility. Here, characterizing different pumping mechanisms and flow profiles is an important first step in determining the capabilities and specifications of the flow loop. This proof-of-concept study can be built upon to study different microfluidic applications over a range of operating conditions in a reproducible manner. Overall, we hope to improve device performance testing by developing well-defined, reproducible, bench test methods using a recirculating flow loop to simulate different microfluidic applications.
- **Purpose**
    - Benchtop flow systems are important, multi-disciplinary tools that can be used to assess the pressure-flow performance characteristics and flow-related issues associated with medical devices. Flow characterization is a

critical step in determining if a blood-contacting device is safe and effective. The purpose of this study is to develop a microfluidic flow loop capable of evaluating the hemodynamics of microscale components and microfluidic chips by applying principles learned from larger cardiovascular mock circulatory loops used to assess mechanical circulatory support devices. To develop a microfluidic recirculating flow loop for a variety of biomedical applications, it is critical to select an appropriate pumping mechanism that can achieve a wide range of pressures and flows. A standard miniaturized recirculating flow loop rather than traditional single-pass, syringe-driven flow could enable long-term in vitro testing of microfluidic devices to test durability, leakage, damage to blood elements, coating integrity, and mixing.

- **Methods**
    - We initially characterized the dynamic pump-driven flow in existing cardiovascular mock circulatory test loops to understand how pump control, compliance, and resistance impact the pressure and flow waveforms. Using a piston pump in a simple flow loop with valves, compliance chambers, and resistance clamps, we simulated the pumping action of the heart and systemic circulation. We recorded the generated pressure and flow waveforms throughout the cardiac cycle. 3D-printed adapters were designed and fabricated to integrate uni-directional valves into the circuit. Pressure and flow at multiple locations in the loop were recorded and evaluated to characterize the functionality and limitations of each flow loop component under worst-case operating conditions. The fluid mechanics knowledge gained in these studies was then applied to design a novel microfluidic recirculating flow loop.Using a peristaltic microfluidic pump, we designed a proof-of-concept recirculating flow loop that could generate forward flow during high-pressure applications. Several tubing sizes and materials, with varying stiffnesses, were tested to optimize pump-tubing compatibility and flow stability. The simple test loop was composed of primed tubing, a fluid reservoir, in-line pressure transducers, resistance sources, and a microfluidic test article. Computational fluid dynamics modeling was performed using ANSYS CFX software to simulate the maximum pressure generated, and the simulations were compared to empirical test results.
- **Results**
    - A standard test protocol identifying the necessary flow loop components, test fluid properties, instrument calibration procedures, data acquisition hardware and software, test methodology, measured quantities of interest (i.e., device pressures and flows), and data processing and analysis

techniques is being established using the microfluidic recirculating flow loop. Key metrics for quantifying the flow performance of devices with microfluidics technologies (i.e., loop pressures, tubing specifications, pump occlusion settings, interconnections) were identified. Initial results from the microfluidic flow loop indicate that the circuit produces a negligible resistance (i.e., pressure drop of 1-2 mmHg with 1.5 mm ID tubing) when no additional sources of resistance are applied to the system. When the tubing is completely clamped downstream of the pump, the differential pressure gradually rises to a maximum of 150 mmHg over a two-hour period. If the tubing is pre-conditioned in the peristaltic pump prior to testing, the pressure plateaus within minutes of starting the test. Further investigation of tubing size and composition, as well as the occlusion settings of the pump, will allow us to determine the maximum pressure of the flow loop and the sensitivity of the measurements. The pressure and flow data appear to be reproducible based on three replicate tests (n=3) and they match the computational model predictions, suggesting that the simple flow loop may be able to reliably generate a range of microfluidic operating conditions up to 45 mL/min at 45 rpm.

- **Implications**
    - In this study, we designed a microfluidic flow loop capable of evaluating the hemodynamics of microscale components and microfluidic chips. This type of miniaturized recirculating flow loop has many possible applications for evaluating medical devices. For instance, a standardized, small flow loop has the potential to be used for long-term durability, leakage, blood damage, coating integrity, or mixing testing of microfluidic devices on the bench. The benefits of such a system are its small volume, capability for running multiple loops simultaneously, the potential for high sensitivity measurements, optical access to the device under investigation, high attainable pressures, its simplicity for modeling, and reproducibility. Here, characterizing different pumping mechanisms and flow profiles is an important first step in determining the capabilities and specifications of the flow loop. This proof-of-concept study can be built upon to study different microfluidic applications over a range of operating conditions in a reproducible manner. Overall, the outcomes of this research can be used to improve device performance testing by developing well-defined, reproducible, bench test methods using a recirculating flow loop to simulate different microfluidic applications or physiologic conditions.

5) **Abstract title:** *Accelerated Aging Test Method for Assessing Susceptibility to Oxidative Degradation of Non-resorbable Polymers*

**Authors:** Danesi, Hunter, FDA/CDRH (Student); Jain, Tanmay, FDA/CDRH (Mentor); Vorvolakos, Katherine, FDA/CDRH (Sponsor)

**FDA Strategic Initiative:** Empowering Patients and Consumers

**Abstract:**

- **Synopsis**
  - The goal of this project is to develop an accelerated aging test method for implantable polymer-based medical devices. Specifically, it is to determine a non-resorbable polymer's susceptibility to undergo oxidative degradation. The accelerated aging test method involves a system that uses electrochemistry to maintain a constant $H_2O_2$ concentration in a reaction flask at elevated temperatures and for predetermined periods. A Raspberry Pi-coded feedback loop between the electrochemical activity of $H_2O_2$ and a peristaltic pump that delivers H2O2 ensures consistent oxidative degradation conditions that will be verified using UV-Vis. The oxidation of UHMWPE can be monitored by using FTIR to measure the Oxidative Index as recommended in ASTM F2102. The deliverables for the summer project are to contribute to the development of the method, verify consistent H2O2 concentration and to finalize the SOP for the method.
- **Purpose**
  - To develop an accelerated aging test method for implantable polymer-based medical devices, to determine a novel non-resorbable polymer's susceptibility to oxidative degradation. Accelerated testing is needed because real time aging could take several years. UHMWPE has been shown to oxidize over time and will serve as a model polymer. The test method involves immersion of UHMWPE into a solution of PBS and hydrogen peroxide ($H_2O_2$) held at constant concentration. In the future, the accelerated aging test method developed in this project may assist in correlating in vitro testing and in vivo performance of polymer-based devices.
- **Method**
  - The developed accelerated aging test method is a system that uses electrochemistry to maintain $H_2O_2$ concentration in a reaction flask at elevated temperatures and for long periods. The reaction vessel consists of a five-neck European-style 125mL with a magnetic stirrer and was filled with 150ml of 200 mM hydrogen peroxide in 1x phosphate buffered solution (PBS). The flask was heated by immersing it in a mineral oil bath kept on top of a controlled heating hot plate with a stirrer and an external temperature probe. A platinum working microelectrode and a graphite rod counter electrode were placed in two of the ports of the flask which were connected to a potentiostat. Two ports of the flask were used for perfluoroalkoxy (PFA) semi-flexible tubes, one for delivery of 400 mM $H_2O_2$ stock solution and the other for removal of excess solution to maintain a constant volume and $H_2O_2$ concentration using a 4-channel peristaltic pump. The system uses a loop to maintain the $H_2O_2$ concentration and it relies on the electrochemical activity of $H_2O_2$. Voltage is applied between the electrodes for a current to be recorded. The system allows for testing at different conditions such as various $H_2O_2$ concentrations and temperatures.

- **Results**
    - Oxidation of the UHMWPE will be assessed using ATR-FTIR per the Oxidative Index method described ASTM F2102. Depending on the results, the concentration of the oxidative solution and/or the duration of exposure may be increased to better mimic real-time reports.
- **Implications**
    - The long-term goal of this work is to help correlate in vitro testing with in vivo performance, which in turn will help eliminate some unnecessary animal testing. For this method, however; it will assist the industry and other stakeholders to assess susceptibility of novel materials to undergo oxidative degradation, rank resistance to oxidation between materials or target devices, and estimate long-term in vivo oxidative stability of polymer-based devices. This may further assist in streamlining the Center for Disease and Radiological Health (CDRH) review process and reduce the time to market of medical devices. The results from this work will improve public's access to safe, effective, and innovative medical devices, and provide the industry with predictable, consistent, and efficient regulatory science tools to expedite device development and introduction of innovative medical devices to the market.

6) **Abstract title:** *Extending the Computer-Aided Triage and Notification (CADt) evaluation framework to multi-disease, multi-CADt scenarios*
**Authors:** Deshpande, Rucha, FDA/CDRH (Student); Thompson, Yee Lam Elim, FDA/CDRH (Mentor); Samuelson, Frank, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Empowering Patients and Consumers
**Abstract:**

- **Synopsis**
    - Although the deployment of computer-aided triage and notification (CADt) devices in clinical workflows has increased in recent years, a quantitative evaluation of such workflows in terms of patient wait-time-savings and time delay under realistic scenarios remains a challenge. Our group had previously proposed a Monte Carlo simulation framework and a corresponding theoretical model based on queuing theory to predict wait-time savings for a single-disease and single-CADt scenario. In the present work, we extend this framework for multi-disease and multi-CADt settings. Via the new framework, the effect of two parameters: (i) the number of CADt devices and (ii) the specificity of CADt devices, on the wait-times of a diseased patient-group not targeted by CADt were studied. Initial results suggest that the non-targeted patient-groups may suffer increased wait-times when more CADt devices or when CADt devices with low specificity are included in the clinical workflow. These results highlight the need for a comprehensive quantitative evaluation of the wait-time benefits and risks for all diseased patient-groups, including those not targeted by any CADt

device, in a clinical workflow before the deployment of multiple CADt devices.

- **Purpose**
    - Computer-aided triage and notification (CADt) devices have seen increased deployment in clinical workflows in recent years. The purpose of a CADt device is to prioritize radiologists' reviews for patients that may suffer from time-sensitive conditions, as identified by the device from the patient's radiological images. Although CADt devices are expected to reduce waiting times for diseased patients, especially those suffering from time-critical diseases, a quantitative evaluation of such devices in a realistic clinical workflow remains a challenge. Previously, our group had proposed a queuing theory-based method for evaluating CADt devices when deployed in the single-disease and single-CADt scenario. This method involved Monte-Carlo simulations as well as theoretical predictions of per-patient wait-times while accounting for various components in a clinical workflow such as traffic intensity in a clinic, radiologist reading rates, disease prevalence and device performance. In the present work, this method is extended to a multi-disease, multi-CADt scenario, via a hierarchical queue and with the assumption of uncorrelated disease occurrence. Thus, the present work is aimed as a step in the direction of evaluating CADt wait-time savings in a more realistic clinical workflow by quantifying the relative wait-time benefits in various diseased patient-groups with different time-sensitivities.
- **Methods**
    - The existing simulation framework for the evaluation of wait-time savings due to a CADt device allows a Monte Carlo simulation of queues with two patient classes: CADt-positive, and all other patients. Our initial work focused on two kinds of queues: first-in-first-out (FIFO) without CADt, and pre-emptive resume priority queuing (PRQ) with CADt. In the latter, all CADt positive patients are allocated equal priority. With the goal of including the hierarchy of time-sensitivities across multiple diseases, a multi-disease, multi-CADt scenario is modelled by extending the existing framework to include a third queue type: hierarchical pre-emptive resume priority queuing (HRQ), wherein a user-defined hierarchy of diseases is employed to determine prioritization within all CADt-positive patients. Furthermore, the existing theoretical framework based on Markov-matrix representations and recursive dimensionality reduction, was also extended. Specifically, the total wait-time for a certain diseased patient-group is now obtained as the difference between the total wait times of (i) all diseased patient-groups up to and including the group under consideration and (ii) all diseased patient-groups with greater priority than the group under consideration. To obtain

the wait-times in each of the two cases, all diseases and CADt devices are represented via their equivalent prevalence and diagnostic performance respectively.

- **Results**
    - First, the extended simulation framework was validated against theoretical predictions via multiple trials of 5000 patients each, for a three-disease, three-CADt scenario. Next, two preliminary studies were conducted to assess the impact of (i) the number of CADt devices and (ii) the specificity of CADt devices on the wait-times of the diseased patient group not seen by a CADt. In both studies a three-disease scenario was simulated. In the first study, increasing the number of CADt devices (1 to 2) in a clinical workflow led to over a 100% increase in the mean per-patient wait-time for a diseased patient-group that is never assessed by CADt. This indicates that increase in wait-times for certain diseased patient-groups may offset benefits derived from wait-time savings for CADt-targeted diseased patient-groups. In the second study, it was observed that as specificity was lowered (from 0.9 to 0.7) for both devices in the workflow at a fixed sensitivity (0.9), the wait-times for diseased patients in the group not targeted by CADt increased by about 50%, as expected due to the unnecessary prioritization of false positive patients within each diseased patient-group.
- **Implications**
    - The new framework enables the investigation of various scenarios involving multiple diseases and multiple AIs within a clinical workflow. Time-saving benefits and time delay risks for each diseased patient-group, irrespective of whether it is targeted by a CADt, can potentially be quantified via both simulations and theoretical predictions in terms of wait-time savings. Initial results from the two studies above indicate that increasing the number of CADt devices in a clinical workflow might not always be beneficial to all classes of diseased patients and thus, warrants more investigation. In addition, factors such as low specificity of the AIs in the workflow that may increase the number of false positive patients in the prioritized queue might also have a negative impact on the wait-times of diseased patient-groups not targeted by any CADt. The effects of other parameters that may potentially impact wait-time savings such as disease prevalence, time-sensitivity rankings of diseases in queue, and the combined performance of CADt devices remain to be studied.

7) **Abstract title:** *Development of in Vitro Test Method for Assessing the Mechanical Performance of AVBT*
**Authors:** Sonia Ezenwajiaku, FDA/CDRH (ORISE Student); Vivek Palepu, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Empowering Patients and Consumers

**Abstract:**

- **Synopsis**
  - Adolescent Idiopathic Scoliosis (AIS) is a spinal deformity that accounts for 80-90% of scoliosis cases, this condition is most prevalent in children ages 10-18 and occurs equally among sexes, however, females are eight times more susceptible to requiring advance treatment. Traditional treatment includes bracing for mild cases, and surgical intervention for severe cases. The traditional surgical method, spinal fusion (SF), has several disadvantages (e.g., reduced spinal mobility). Anterior vertebral body tethering (AVBT) is a novel surgical technique that serves as an alternative option for AIS patients that mitigates many challenges of SF. However, tether-related adverse events (e.g., tether breakage) occur in approximately 50% of patients. No current non-clinical method assesses the initial mechanical integrity of the device. Therefore, this research focuses on the development of in vitro mechanical test methods to examine factors of AVBT device failures. To complete the objective, three aims were developed: 1) characterize static and dynamic properties of the tether, 2) develop a benchtop testing construct capable of replicating clinically observed failure of tether material, and 3) evaluate how screw angulations and set screw repositioning influence tether fatigue life.
- **Purpose**
  - Scoliosis is a complex 3D spinal deformity that effects approximately 6 to 9 million people in the United States. Adolescent Idiopathic Scoliosis (AIS) accounts for 80-90% of cases, this condition is most prevalent in children ages 10-18 and occurs equally among sexes, however, females are eight times more susceptible to requiring advance treatment. Traditional treatment includes bracing, (curves 20-40°), in more severe cases (curves > 45°) surgical intervention is necessary. Spinal fusion is a traditional surgical technique, however, it is linked to reduced spinal mobility, straining of mobile segments, and reduced function compared to normal populous. Anterior vertebral body tethering (AVBT) is a novel surgical technique that serves as an alternative option for AIS patients. This technique uses vertebral body screws connected to a tethering device; the tethering device applies tension across the convex side of the spinal deformity. This provides spinal correction upon implantation and continues to apply a tension to modulate the spinal deformity. However, tether-related adverse events (e.g., tether breakage) occur in approximately 50% of patients, resulting in revision surgery. Currently no established non-clinical method assesses the mechanical integrity of the device. Therefore, the objective of this research

is to develop in vitro mechanical test methods to examine factors contributing to AVBT device failures.

- **Methods**
  - To complete the objective, we investigated the: 1) characterization of static and dynamic properties of the tether and 2) development of a bench-top test method capable of replicating clinically observed failure of tether material. Aim 1 methods include performing static tension, creep, stress relaxation, and fatigue testing on mock tether cord material with mechanical strength, dimensions, and material similar to commercially available tether devices. These cords will be tested with two-gauge lengths, one based on ASTM F1717 standards (76mm), to maintain uniformity with current spinal implant standards, the second length (25 mm) based on the single unit VBT construct dimensions, to assess results at implantation lengths. Aim 2 includes modification of ASTM F1717 test blocks, and the construction of a single-unit VBT construct. Each tether test sample will be placed between a pair of vertebral body screws and locked into position according to the surgical technique. Samples will undergo the same testing as aim 1. All testing will be conducted in a saline bath to mimic in vivo environment.
- **Results**
  - For the tether only static tensile characterization, the preliminary results indicate there is no significant correlation between the gauge length and strain rate to the max breakage force of the material. However, it can be observed that faster strain rates slightly increase the displacement at breakage of the tethers. Additionally, compared to the tether only, the tether construct exhibited significantly lower displacement and force at initial tear, this demonstrates the significance of set screw force on the tether. Overall, the current test design demonstrates similar failure mechanisms observed clinically. This indicates potential for this method to be used as a procedure for further investigating characteristics and factors causing failure of these devices.
- **Implications**
  - Further refinement of test methods developed during this project will provide industry and regulators with more predictive tools to assess the mechanical integrity of an AVBT device.

8) **Abstract title:** *Establishment of a Method to Chronically Stimulate 3D Human Engineered Cardiac Tissues with Cardiac Contractility Modulation Device Signals*

**Authors**: Avila, Anna, FDA/CDRH (Student); Feaster, Tromondae K., FDA/CDRH (Mentor); Ewoldt, Jourdan K., FDA/CDRH; Casciola, Maura, FDA/CDRH; Narkar, Akshay, FDA/CDRH; Chen, Christopher S.; Blinova, Ksenia, FDA/CDRH
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**


- **Synopsis**
  - Human 3D microphysiological systems (i.e., 3D human engineered cardiac tissues, 3D ECTs) respond to discontinuous chronic cardiac contractility modulation (CCM) stimulation. This work provides an in vitro tool to evaluate structural, molecular, and functional effects of long-term (i.e., chronic) cardiac electrophysiology medical device signals, to support safety and performance studies.
- **Purpose**
  - Cardiac contractility modulation (CCM) is a medical device-based therapy delivering non-excitatory electrical simulations to the heart during the absolute refractory period to enhance cardiac function. We previously evaluated the acute (i.e., seconds) effects of CCM in 2D human induced pluripotent stem cell derived cardiomyocyte (hiPSC-CM) monolayers, on flexible substrate, and found enhanced calcium and contractility. In the present study, we sought to develop a chronic CCM assay to evaluate the long-term (i.e., hours to days) effects of CCM in vitro using 3D human engineered cardiac tissues (ECTs) as a model. While the clinical CCM device is approved for 5 hours (i.e., intermittent) per day, in the US, the continuous and discontinuous effects of CCM on human cardiac tissue are largely unknown.
- **Methods**
  - Custom carbon electrode stimulation dishes were fabricated to electrically stimulate 3D ECTs, in parallel, with control (Pacing only, 20 V/cm) and CCM (Pacing 20 V/cm + CCM 28 V/cm, phase amplitude). HiPSC-CMs and cardiac fibroblasts in a fibrin-based gel were combined to form 3D ECTs. ECTs were cultured for 5 days then exposed to stimulation. 3D ECTs were removed from stimulation and morphology and contractile properties were evaluated to elucidate permanent changes induced by stimulation.
- **Results**
  - We found discontinuous CCM stimulation (i.e., 5 hours per day increments in 24 hours, on/off) comparable to the schedule used in patients resulted in enhance contractile, molecular, and structural changes relative to control.
- **Implications**
  - This study provides a comprehensive characterization of long-term CCM stimulation in vitro on intact human cardiac tissues. Future studies will

investigate prolonged time points. These data provide an in vitro model to assess physiologically relevant mechanisms and evaluate safety and performance of future cardiac electrophysiology medical devices.

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9) **Abstract title:** *Manufacturing and Evaluation of 3D-Printed Phantoms for Adaptive Optics Ophthalmic Imaging Devices*
**Authors:** Fitzgerald, Declan, FDA/CDRH (Student); Rosenthal, Ian (Contributing Author); Zhoulin, Liu, FDA/CDRH (Mentor); Hammer, Daniel, FDA/CDRH (Mentor), Agrawal, Anant, FDA/CDRH (Mentor), Sochol, Ryan (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **<u>Synopsis</u>**
    - Adaptive optics (AO) is an emerging ophthalmic technology that provides in-vivo resolution of individual photoreceptor cells, among other cells and microscopic targets in the human eye. Leveraging the 3D printing techniques of direct laser writing (DLW) and two-photon polymerization (TPP), we fabricated microarray phantoms designed to resemble small patches of the human photoreceptor mosaic. Our analysis ensures that our phantoms possess the high level of consistency required for a regulatory science tool (RST). We developed an evaluation method with ImageJ software that compared the location of specific structural elements of the printed array in high-resolution scanning electron microscopy (SEM) images. We compared the resulting locations for several phantoms to the same location on the intended design. This analysis procedure provides qualitative insight into the potential strengths and flaws of the manufacturing process as well as quantification of differences between the design and the actual print. Additionally, we have developed a new low-cost method to efficiently replicate accurate copies of our phantoms, using only commonly-available inexpensive laboratory equipment (hot plate). The replicas were created using a PDMS mold of both the phantom and its substrate. PMMA dissolved in anisole was poured into the mold and cured, taking the form of the PDMS mold. Throughout the course of this research, we have both continued to develop and improve evaluation methods to validate our new phantom as a regulatory science tool for AO ophthalmic

imaging devices. We have also explored a way to efficiently scale production to ensure expanded access to the wider community of device developers.

- **Purpose**
  - Adaptive optics (AO) is an emerging technology with significant potential in the field of ophthalmology. AO-enabled devices can resolve individual photoreceptors, among other cells and microscopic structures within the retina of the human eye, which has important implications for ophthalmic medicine. We produced microarray phantoms to emulate photoreceptor cells in the eye as a regulatory science tool (RST) to ensure consistency between devices. These phantoms are produced via direct laser writing (DLW), a 3D printing technique. To ensure the validity of these phantoms as a stable, repeatable standard for AO image device calibration, factors that affect production consistency must be determined. The phantoms must demonstrate high uniformity and robustness to environmental variables to be used as a calibration standard and RST. Additionally, to scale production while accounting for variability between prints, a fabrication method to produce optically identical replicas of a single DLW-formed phantom is desired. A manufacturing technique that leverages the benefits of a variety of well-known lithographic approaches is demonstrated to effectively duplicate the phantom with nanometer-level accuracy.
- **Methods**
  - A 3D printing technique called two-photon polymerization (TPP) was used to fabricate the phantoms (Nanoscribe Photonic Professional GT2). Printing was performed on a glass disc with preselected surface texture. The phantoms were evaluated with ImageJ by overlaying reference points across high-resolution scanning electron microscopy (SEM) images. The arrangement of these reference points was then compared to the intended design to calculate deviations with a dimensional accuracy of tens of nanometers. Phantom replicas were manufactured using a combination of microtransfer molding (μTM) and solvent-assisted microcontact molding (SAMIM). The replication procedure involves creating a polydimethylsiloxane (PDMS, a.k.a. “silicone”) mold of the printed phantom, then pouring polymethyl methacrylate (PMMA) dissolved in anisole into the mold, filling both the contours made by the phantom and the overall shape of the phantom substrate. This mixture then cures, hardening as the anisole dissolves. A solid replica of the 3D-printed phantom is then removed from the mold.
- **Results**
  - The 3D-printing fabrication process was successfully implemented to yield three phantoms, and we produced three PMMA replicas that were similar in

dimension and structural fine features to the original print. The quantitative structural deviation analysis with SEM is currently underway. Qualitative analysis of deviations between design and SEM for multiple phantoms revealed manufacturing process issues, including shrinkage and sparse defects. Overall, the PMMA replicas faithfully reproduced the features from the 3D-printed phantoms, but also with occasional defects.

- **Implications**
    - The overarching goals of this research are to facilitate the clinical translation and regulatory evaluation of AO-enabled devices. Successful translation will increase the availability of this important technology for clinical studies into new therapies for the benefit of patients with blinding diseases. Evaluation of the repeatability of TPP-DLW manufactured phantoms will provide further validation of this manufacturing approach for consistent RST production. Furthermore, RSTs for AO imaging can play a role in ensuring consistent and standardized performance of devices used at individual sites in multisite clinical investigations. In addition, accurate phantom replication provides a means to achieve low-cost, efficient, and scalable phantom production in moderate quantities, potentially helping to solve a long-standing problem of wider dissemination of FDA-developed phantoms. These benefits will all contribute to a more effective regulatory evaluation of AO device performance, improving patient access to this significant technology.

10) **Abstract title:** *Evaluating the performance of AI rule-out devices in mammography screening with relative utility*
**Authors:** Fan, Kwok Lung FDA/CDRH (Student); Thompson, Yee lam Elim, FDA/CDRH (Mentor); Samuelson, Frank, FDA/CDRH (Mentor); Chen, Weijie, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data,Empowering Patients and Consumers
**Abstract:**

- **Synopsis**
    - We apply the theory of relative utility to evaluate the effectiveness of recently published deep learning model as an AI-based rule-out device for screening mammography, as described in previous literature. Relative utility measures the trade-off between true positives and false positives and helps explain how radiologists make decisions regarding follow-up tests for screening patients. Therefore, we can assess the AI rule-out device by determining whether the new workflow with radiologists and AI provides a better trade-off compared to radiologists alone. Based on the AI performance in the previous study, we cannot conclusively state that the devices offer a significantly better utility ($p=0.2$) at a significance level of 0.05. However, this method can be employed to evaluate the effectiveness of AI rule-out devices in other diagnostic tests as well.

- **Purpose**
  - With the advancement of deep learning and artificial intelligence (AI), the performance of AI in computer vision has improved drastically, generating interests in its applications to medical imaging. Currently, all FDA-approved/cleared AI-based Computer-Aided Detection and/or Diagnosis devices are intended to assist radiologists in making diagnoses rather than to make independent decisions without reviewal by radiologists. In contrast, an AI-based rule-out device aims to exclude patients with a low probability of disease from the radiologist's reading list. Although such devices could significantly reduce the workload of radiologists in cancer screening and potentially achieve better overall performance than radiologists working alone, an appropriate evaluation metric to assess the overall diagnostic performance with the rule-out device remains unclear. Therefore, this study investigates the use of relative utility in evaluating the performance of AI-based rule-out devices specifically in mammography screening.
- **Methods**
  - We apply the theory of relative utility in our study. Relative utility measures the trade-off between a radiologist's true positive and false positive rates when making diagnoses and can be expressed as the utility of true positives divided by the utility of false positives. Previous studies have shown that relative utility can be estimated using the radiologist's true positive rate, false positive rate, and receiver operating characteristic (ROC) curve. In the context of US mammography screening without the device, these studies have provided an estimate of relative utility at 150, indicating that radiologists are willing to perform additional follow-ups on 150 non-diseased patients to detect one patient with the disease. Therefore, a rule-out device is considered effective if the with-device workflow provides an improved trade-off compared to the standard-of-care relative utility of 150. As a demonstration on how relative utility can be used to assess the performance of these rule-out devices, we apply this technique to investigate the effectiveness of a recently published deep learning model that retrospectively rule out AI-negative screening mammograms below certain thresholds.
- **Results**
  - Based on the information from a recently published deep learning model for mammograms in US, we estimated the relative utility of the radiologists involved in the study with a binormal model. The result is consistent with the standard-of-care estimation of 150. With the use of their deep learning model, we found that the relative utility of the with-device workflow suggests an improved trade-off compared to the radiologist-alone performance. However, the 95% confidence interval does not cover the estimated relative utility. There is only 80% chance (p=0.2) that a radiologist with the device is truly superior to the radiologist alone in terms of utility. Therefore, based on the theory of relative utility, we cannot definitively conclude that the use of their deep learning model as a rule-out device is superior to the standard-of-care workflow of the radiologist alone.

- **Implications**
    - We apply the theory of relative utility to assess the effectiveness of a recently published deep learning model as an AI-based rule-out device. This method provides an innovative way to evaluate whether the alternative workflow with an AI-based rule-out device can achieve a better trade-off between true positives and false positives without conducting a new reader study. One advantage of this method is its suitability for large-scale clinical datasets, as it does not require radiologist ratings and can be applied in a realistic setting. Furthermore, this approach can be extended to other large scale diagnostic tests with AI rule-out devices, such as lung cancer screening.

11) **Abstract title:** *Preliminary Assessment of Concurrent Validity of Moticon Pressure Insole System for Gait Analysis in Parkinson's Disease Patients*
**Authors:** Watkinson, Sophia, FDA/CDRH (Student); Kontson, Kimberly, FDA/CDRH (Mentor); Caiola, Michael, FDA/CDRH; Nyman, Edward, FDA/CDRH; Eguren, David, FDA/CDRH; Ngoh, Brian, FDA/CDRH (Student); Moro-Velazquez, Laureano; Dehak, Najim; Moukheiber, Emile; Motley, Chelsie; Mills, Kelly; Butala, Ankur A.
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - Gait impairments are one of the most well-known and observable symptoms of Parkinson's disease. Assessment of gait via wearables has the potential to improve early diagnosis through identification of wearable-derived biomarkers, to monitor disease progression, and to assess the efficacy of therapeutic interventions. Pressure insole sensors are one example of a wearable sensor that could be useful in quantifying gait in patients with PD outside of the clinical environment. However, a limited number of studies explore gait measurement properties of pressure insole sensors in clinical populations, and those that do present on a condensed list of measures and differ in usage of a reference system, making it difficult to characterize the system for all gait measures. In this study, pressure insoles sensors were placed directly into participant's shoes and data was collected simultaneously as they performed self-selected and hurried pace walking tasks along the electronic walkway. Three participants with a diagnosis of Parkinson's disease and a score of 2.5 of the Modified Hoehn & Yahr score were included in this analysis and several gait parameters (stride length, step time, swing time, and double support time) were evaluated to explore concurrent validity between systems via coefficient of determination and Bland-Altman analysis. Preliminary findings of this research show concurrent validity of the Moticon pressure insole system to compute spatiotemporal gait parameters. If limitations of the insole system are well understood and tendencies of the system to under- or over- report certain parameters are characterized, the system can be a valuable tool to supplement current methods of diagnosis and disease monitoring.

- **Purpose**
    - In recent years, there has been a push to evaluate gait impairment in Parkinson's disease (PD) patients quantitatively using wearable sensors, such as inertial measurement units (IMUs) and insole pressure sensors, that enable continuous monitoring outside of clinical settings. This has the potential to improve early diagnosis through identification of wearable-derived biomarkers, to monitor disease progression, and to assess the efficacy of therapeutic interventions. However, a limited number of studies explore gait measurement properties of pressure insole sensors in clinical populations, and those that do present on a condensed list of measures and differ in usage of a reference system, making it difficult to characterize the system for all gait measures. Therefore, the purpose of this research was to conduct a preliminary assessment of a commercially available insole pressure sensor system for gait analysis in comparison to a gold-standard pressure-sensitive electronic walkway and characterize any potential differences between systems in calculating gait parameters in the Parkinson's disease population.
- **Methods**
    - The data for this research comes from an ongoing FDA effort to curate an open-access database of gait data from patients with Parkinson's disease. Three participants (1F/2M; mean age 71.5 ± 5.6 years) with a diagnosis of Parkinson's disease and a score of 2.5 of the Modified Hoehn & Yahr score were included in this analysis. Data were collected simultaneously using the Moticon pressure insole sensor system and the 16-foot Protokinetics Zeno electronic walkway. The insole sensors were placed directly into participant's shoes as they performed self-selected and hurried pace walking tasks along the electronic walkway. Several gait parameters were calculated using the software associated with each system including a spatial parameter (mean stride length) and temporal parameters (step time, swing time and double support time). Mean and standard deviation were computed for each parameter from each system. Concurrent validity of the systems was analyzed via coefficient of determination and Bland-Altman analysis. The R-squared values were interpreted as weak ($R2 \leq 0.13$), moderate ($0.13 < R2 \leq 0.26$), and substantial ($R2 > 0.26$). The systematic mean bias from the Bland-Altman analysis was compared to acceptable error limits for the specific parameters.
- **Results**
    - Results showed substantial R-squared values for stride length ($R^2 = 0.81$), step time ($R^2 = 0.29$), and double support time ($R^2 = 0.47$) and a weak R-squared value for swing time ($R^2 = 0.07$). The insoles tended to overestimate the spatial parameter of stride length by a mean of 7 cm with 95% limits of agreement (LoA) between -7.4 cm and 21 cm. Swing time was also slightly overestimated by the insoles with a mean bias of 0.08 seconds and LoA between -0.11 and 0.26 seconds. The other temporal parameters of step time and double support time were underestimated by the insoles by

0.05 and 0.15 seconds, respectively. For all measures except swing time, all points fell within the 95% limits of agreement.

- **Implications**
    - The preliminary findings of this research show concurrent validity of the Moticon pressure insole system to compute spatiotemporal gait parameters. The mean bias of the spatial parameter, stride length, showed the most substantial difference of the calculated measures. However, literature reports that the stride length of PD and healthy control populations have a mean difference of 22 cm. Thus, the error in calculating stride length using the insole pressure sensing system may be within acceptable limits such that the differences in calculated stride length can be attributed to PD gait impairments and not measurement bias. As long as limitations of the insole system are well understood and tendencies of the system to under- or over- report specific gait parameters are characterized, the system can be a valuable tool to supplement current methods of diagnosis and disease monitoring. Future work should focus on evaluating these pressure insoles outside of clinical settings.

12) **Abstract title:** *Son of Grid Engine to Slurm Migration*

**Authors:** Liou, Martin, FDA/CDRH (Student); Mikailov, Mike, FDA/CDRH (Mentor); Cha, Kenny, FDA/CDRH (Mentor); Luo, Fu-Jyh, FDA/CDRH (Mentor)

**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response

**Abstract:**

- **Synopsis**
    - Slurm migration is the process of migrating applications from the Son of Grid Engine (SGE) to Slurm Workload Manager. SGE is no longer being actively developed, and Slurm is a newer job scheduler that is more scalable, efficient, and better supported. The migration process typically involves converting application run scripts from using SGE commands and parameters to using those of Slurm. This can be a relatively straightforward process, as most of the changes are only to the scripts and not to the application code itself. However, there are a few things to keep in mind when migrating to Slurm. First, Slurm has more features and specifications for applications using GPUs. This means that if your application uses GPUs, you will need to learn more about Slurm parameters in order to get the best performance. Second, Slurm is actively developed, which means that new features and fixes are being added all the time. This can be a good thing, as it means that Slurm is constantly being improved. However, it also means that you need to keep up with the latest changes in order to make sure that your applications are running on the latest version of Slurm. Overall, Slurm migration is a worthwhile investment. It can help you to improve the performance of your applications and to scale your cluster as needed.

However, it is important to be aware of the challenges involved in the migration process and to plan accordingly.

- **Purpose**
  - The purpose of this research is to investigate the feasibility of migrating applications from Son of Grid Engine (SGE) to Slurm Workload Manager. Slurm and SGE are both job schedulers used to manage and schedule jobs on high-performance computing clusters. There are several reasons why migrating to Slurm is a good idea. First, Slurm is more scalable than SGE. This means that it can handle a larger number of jobs and resources, which is important for large-scale HPC clusters. Second, Slurm is more efficient than SGE. This means that it can better optimize the use of resources, which can lead to improved performance. Third, Slurm has better support for GPUs than SGE. This is important for HPC applications that use GPUs. Another reason to migrate to Slurm is that it is a newer job scheduler and is actively developed and maintained. This means that new features and fixes are being added to Slurm on a regular basis. SGE, on the other hand, is no longer actively developed, so it is not receiving the same level of support.
- **Methods**
  - If not adequately planned and executed, migrating from SGE to Slurm can be a daunting task. The following steps could be followed for an efficient migration from SGE to Slurm: a) Assessing the current SGE environment, identifying needed resources and applications; b) Choosing migration strategy by assigning priorities to the applications based on their urgency and feasibility; c) Mapping SGE directives to Slurm directives and resolving cases in which direct mapping does not exist; d) Converting SGE scripts to Slurm manually and using available automation tools, such as Bard, ChatGPT, uge2slurm; e) Testing converted scripts and verifying the results; f) Monitoring the resource usage, accounting records, the job queue and the error logs.
- **Results**
  - More than 100 applications have already been migrated to become Slurm compatible. Application batch run scripts have been converted from SGE to Slurm; submitted and run on Slurm to ensure that they are working as expected. Interactive applications (without batch run scripts) have been run in a terminal by using ‘srun --pty bash’ to start an interactive session on a node and then directly execute them on the node. The overall migration process has been relatively smooth with exception of a few challenges. The main changes have been made in the run commands and script parameters, so the application codes themselves did not require any modifications. The main complications with migration process has involved specifying GPU

resources. Although Slurm has more support for GPU applications, the process of allocating GPU resources is more complicated than in SGE. These challenges can be overcome by carefully planning the migration process and by following the appropriate documentation.

- **Implications**
    - The implications of migrating to Slurm include the potential for the application scripts to be optimized even further. Slurm is actively developed, so new features can arise and make running jobs on Slurm even more efficient. For example, Slurm recently added support for preempting jobs that are running longer than their allotted time. This can help to ensure that resources are used efficiently and that jobs that are waiting to run can be started sooner. Not only that but because Slurm is very scalable and is suited for larger HPC clusters, as HPC clusters are expanded, there is little concern that Slurm will not be able to keep up. The most important part of migrating to Slurm is learning to maximize its usage. Slurm has many more features than SGE and is different from it in more than one aspect. Therefore, it is important to learn about Slurm's features and how to use them before being able to take advantage of its many benefits.

13) **Abstract title:** *Alternative anticoagulant strategy to improve the test sensitivity of ASTM F2888-19 Standard for Platelet and Leukocyte Count Assay*
**Authors:** Arjun Gupta, Mehulkumar Patel, Anna Parrish, Carlos Serna III, Megan Jamiolkowski, Qijin Lu
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
    - The ASTM F2888-19 standard for platelet and leukocyte count testing is one of the only two standards available for the in vitro thrombogenicity evaluation of medical devices. This standard has been increasingly used in FDA regulatory submissions and is applicable to various blood-contacting devices. However, two major limitations exist for the standard: (1) it uses a high surface area to blood volume ratio (12 cm2/mL), which often requires cutting the test samples into many small pieces to fit in the test tube and the excessive exposure of the cut surfaces/edges to the blood may confound the test results. (2) It lacks test sensitivity for leukocyte count to effectively differentiate between thrombogenic and thromboresistant materials. To address these issues, this study evaluates an alternative anticoagulant strategy, using ACDA anticoagulated blood instead of 3.2% sodium citrate blood to increase the sensitivity of the test method. Preliminary results showed that the use of recalcified and heparinized ACDA

blood increased test sensitivity and enabled the test to differentiate materials with different thrombogenic potentials at a lower surface area to blood volume ratio (6 cm2/mL) than the ratio recommended in the ASTM F2888-19 standard (12 cm2/mL). The use of ACDA blood also enabled the test to differentiate materials with different thrombogenic potentials based on leukocyte count. Further experiments are ongoing to verify these preliminarily results. The outcomes from this study can be used to update the F2888-19 standard.

- **Purpose**
    - Appropriate pre-clinical thrombogenicity evaluation of blood-contacting devices is important to minimize thrombosis-related risks to patients. Because platelets and leukocytes play a critical role in thrombus formation, platelet and leukocyte count testing (ASTM F2888-19 test method) is one of the recommended tests for material mediated thrombogenicity assessment of medical devices/materials in the ISO 10993-4 hemocompatibility standard and the FDA Biocompatibility Guidance document. Although the test sensitivity of the current ASTM F2888-19 standard has improved from its original 2013 version, two major limitations exist: (1) The use of a high surface area to blood volume ratio (12 cm2/mL) requires substantial cutting of test samples to tightly pack them into test tubes. This often exposes materials that are not typically blood-contacting to the blood, which can confound the test results. (2) It lacks test sensitivity for leukocyte count to effectively differentiate between thrombogenic and thromboresistant materials. To overcome these limitations, this study aims to evaluate alternative anticoagulant strategies to further improve test sensitivity, allow the test to be performed at a lower surface area to volume ratio, and evaluate whether leukocyte count is a sensitive marker under the new test conditions.
- **Methods**
    - Fresh donor human blood from healthy adults was obtained from the National Institutes of Health Blood Bank. For each test day, blood from the same donor was drawn into polypropylene tubes containing 3.2% sodium citrate (recommended in ASTM F2888-19) or the alternative anticoagulant citrate dextrose solution A (ACDA). Seven materials were investigated, including thrombogenic controls (latex, buna, and glass beads) and commonly used biomaterials (silicone, stainless steel, polytetrafluoroethylene [PTFE], and high-density polyethylene [HDPE]). Platelet and leukocyte testing was performed in accordance with ASTM F2888–19, except for the alternative anticoagulation strategy and surface area to blood ratio. Briefly, the blood was first recalcified, heparinized, and

then incubated with the test materials at a 6 or 12 cm2/mL surface area to blood volume ratio. The samples were incubated for 1 hour at 37 ± 2°C in a shaking water bath at a rotational speed of 60 rpm. After the incubation, 500 mM K3-EDTA (Ethylenediaminetetraacetic Acid Tripotassium) solution was added to the blood for a final K3-EDTA concentration of 5 mM to inhibit further reactions. The blood was transferred to a new tube and kept on ice until a complete blood count of the samples was measured using a hematology analyzer.

- **Results**
    - Preliminary results suggested that the use of the alternative ACDA anticoagulation strategy can improve the test sensitivity when compared to the sodium citrate anticoagulant specified in the current ASTM F2888-19 standard. Test sensitivity is dependent on the heparin concentration level used and it may be improved with the use of donor-specific heparin concentrations (based on the heparin concentration that yielded activated clotting time (ACT) between 200-230 sec) instead of a fixed concentration for all donors. When using the ACDA blood that was re-calcified and heparinized to a donor-specific level (ACT 200-230 sec), the test was able to effectively differentiate the materials with varying thrombogenic potentials at a 6 cm2/mL surface area to blood volume ratio based on the resulting platelet count values. This ratio is half the amount of surface area recommended in the current ASTM F2888-19 standard. Additionally, compared to citrated blood, the use of ACDA blood also significantly improved test sensitivity for leukocyte count, enabling the differentiation of the test materials base on the resulting leukocyte count values.
- **Implications**
    - Currently, there are only two standardized in vitro test methods (ASTM F2888-19 for Platelet and Leukocyte Count testing and ASTM F2382-18 for Partial Thromboplastin Time assay) available for the material-mediated thrombogenicity evaluation of medical devices. As such, the ASTM F2888-19 standard has been increasingly used in FDA regulatory submissions and is applicable to various blood-contacting devices. However, the high sample surface area to blood volume ratio (12 cm2/ml) and the insensitivity of the leukocyte count are two major issues for the standard. This study directly addresses these two issues. The results of the current study are meaningful because our proposed alternative anticoagulant approach was shown to significantly improved the sensitivity. This enables this test method to differentiate materials of different thrombogenicity potentials using half of the recommended surface area to blood volume ratio (6 cm2/ml vs 12 cm2/ml). Additionally, the sensitivity for leukocyte count was improved.

Further experiments are ongoing to verify these preliminarily results. The outcomes of this study can be used to update the F2888-19 standard.

14) **Abstract title:** *Evaluating Transfer Function Methods and Excitor Probes Used for Evaluation of MR Safety for Active Implantable Medical Devices*
**Authors:** Marchini, Charles, FDA/CDRH (Student); Jeong, Hongbae, FDA/CDRH (mentor); Kumar, Ananda, FDA/CDRH (mentor);
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
  - Active implantable medical devices can be a safety hazard when an MRI scan is required due to the heating that comes from an incident electric field. A transfer function (TF) approach has previously been developed that can drastically decrease simulation times for predicting heating caused by a lead. In this study, different TF methods were compared and showed agreement. However, higher errors in the TFs were found in the non-insulated tips of the lead.
- **Purpose**
  - Active implantable medical devices contain leads that need to be evaluated for their safety during an MRI scan. Leads interact with the radiofrequency (RF) electric fields, which causes excessive heating at the electrode (tip of the lead). If the temperature exceeds safety limits, it causes tissue damage. Simulating how a lead responds to given RF pulses can take a long time (multiple days). A new approach uses a TF, which is a function of how far away an applied electric field is from the electrode tip and gives the value for the resulting scattered electric field (Park et al. JMRI 2007). One method to find the TF is by simulating a lead being excited piecewise along its length using plane waves while measuring the scattered electric field at the electrode. This approach typically requires an excitor probe loop coil, which was analyzed in this study for how it compares to an ideal plane wave excitation. Another way to solve for the TF is called the reciprocity method and is done by applying current to the electrode and measuring the resulting current along the lead (Feng et al. IEEE 2015). These two methods were compared using experiments (non-simulations) and simulations.
- **Methods**
  - The piXE128HPV1 from Zurich Med Tech was used for piecewise excitation TF measurements, simulated using the finite differences time domain (FDTD) method, and compared to an ideal plane wave excitation. Resonance tuning and impedance matching was done to ensure a transmission of RF power that results in an electric field with 128MHz (for 3T MRI) frequency tangent to the lead under study. The simulation and experimental setup for the plane wave excitation consisted of a phantom, tissue simulating liquid, and a 20cm lead with insulation except for 1cm of non-insulated wire at both ends. Simulating the TFs using the plane wave excitation was done

using FDTD in Sim4life. For piecewise experiments, three TFs were taken and averaged. The simulated TF using the measured current was performed in FEKO using method of moments (MoM). A voltage source was placed near the tip of the electrode and oscillated at 128MHz to stimulate the current in the electrode to obtain the TF. The lab experiment for this method utilized a wide band current monitor to measure the TF.

- **Results**
  - The excitor was shown to have good impedance matching for both simulation and experiment at 128MHz, so the emitted electric field matched the Lamour frequency of hydrogen at 3T. The full width at half maximum of the electric field tangent to the lead was 13.64mm in the direction tangent to the lead, which is comparable to the ideal 5mm. The TFs for the simulated loop coil excitation, simulated plane wave excitation, reciprocity simulation, loop coil experiment, and reciprocity experiment were in good agreement. For the insulated regions, the average percent errors comparing the excitor lab experiment to the plane wave simulation, excitor simulation, and reciprocity simulation was 9.0%, 8.2%, and 3.2% for the magnitude and 36.0%, 11.0%, and 27.4% for the phase, respectively. For the insulated regions, the percent errors for comparing the reciprocity lab experiment to the plane wave simulation, excitor simulation, and reciprocity simulation was 10.0%, 8.3%, and 8.2% for the magnitudes and 33.2%, 14.0%, and 26.0% for the phase, respectively. When lead tip measurements were included, percent error did not exceed 141%. Comparisons with the reciprocity method lab experiment yielded higher average percent error due to low current at the edge of the lead.
- **Implications**
  - The simulations of the loop coil excitor show how the resonance tuning and impedance matching can be done using simulations to get an acceptable power transfer of electric field similar to a plane wave. The loop coil was not close to being perfectly impedance matched because it is made entirely of reactive components, with the resistance of the load coming from the surrounding medium. The TFs agreed with each other. The percent errors across simulation and experiments were highest for values near the tips of the lead. The highest percent error was ~4000% between the excitor simulation and reciprocity method magnitudes at the edge of the lead, which was likely caused by a simulation error and the current being measured as near zero for the tip of the lead. A factor between the MoM method and the FDTD method is the amount of time a simulation takes. Using the FDTD method, a TF with 41 measurements (τ from 0 to 200mm in 5mm increments) took over 24 hours using a GPU, where as a reciprocity TF simulation took less than a minute to get 100 measurements across the lead without a GPU.

15) **Abstract title:** *Exploring Stir Bar Sorptive Extraction (SBSE) as an Alternative Method for Volatiles Analysis from Medical Device Extracts*

**Authors**: Naimi, Isabella, FDA/CDRH (Student); Wickramasekara, Samanthi, FDA/CDRH (Mentor); Patabandige, Milani, FDA/CDRH (Mentor); Hill, Jacob, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**


- **Synopsis**
    - Volatile analysis of aqueous extracts using gas chromatography mass spectrometric (GC-MS) techniques is a challenge due to the lack of solvent compatibility with the system. To overcome this challenge, headspace analysis has emerged as the preferred alternative, but accumulation of water at the injector interface when using the aqueous samples in headspace vials caused many analytical challenges. Stir Bar Sorptive Extraction (SBSE) has been successfully used in the environmental and food industry for extracting volatiles from aqueous samples and therefore exploration of SBSE may reveal an appropriate alternative volatile analysis method for medical device extracts. Preliminary experiments were conducted with PDMS (polydimethylsiloxane) coated Twister stir bars immersed in 5 mL of volatile standard mix (50 ng/mL) in saline. Samples were stirred for specific time intervals followed by thermal desorption before injecting into the GC-MS system for analysis. We tested two different incubation times (30 minutes and 60 minutes) and calculated the extraction efficiency of the SBSE method, as well as signal variability in triplicate extractions. Preliminary results showed that longer incubation time provided more efficient extraction and the sample-to-sample variation ranges between 1-25%. Extraction efficiency was higher for the non-polar and semi-polar volatile organic compounds and lower efficiency for more polar volatiles such as alcohols. Extraction efficiency of the SBSE method depends on the coating material, incubation time, stirring speed, sample volume, pH of the solution and sample matrix etc. Optimization of these parameters will be conducted before applying this method to medical device extract analysis. These optimized volatile analysis methods will be useful in evaluating biocompatibility of the devices and improve our readiness for future public health emergency preparedness and response.
- **Purpose**
    - Extractables can be released during clinical use of medical devices. Among those chemicals, volatile compounds are known to be toxic and pose a serious threat to patient health. Identifying an efficient method of extracting these volatiles is crucial. Methods currently being used for this purpose include Dynamic Headspace (DHS), Static Headspace (SHS), Solid-Phase Microextraction (SPME), and Stir Bar Sorptive Extraction (SBSE). SBSE is a less widely used technique that relies on the use of a magnetic stir bar

coated with adsorbent polydimethylsiloxane (PDMS). One primary advantage of SBSE compared with other methods is improved sensitivity; this is not only because of direct desorption of the stir bars within the thermal desorption unit (TDU), but also due to larger sorptive phase volume (specifically compared to SPME). The purpose of this project is to determine whether SBSE is more sensitive in detecting volatiles compared to other headspace methods, and a more efficient method for chemical characterization.

- **Methods**
    - Residual solvents class 3-mix A with a concentration of 50 ng/mL was prepared in 0.9% saline. Each Twister stir bar was conditioned before and after use in the TDU. All parameters were controlled using the GERSTEL MAESTRO software, and each method was completed using the GERSTEL MPS connected to the Agilent GC-MS system. The Twisters were separated into two sets: one to be run for an incubation time of 30 minutes and another for 60 minutes. For each set, Twisters were immersed in an extraction vial containing either saline (blank) or the standard solution. After extraction, each stir bar was removed from the vial, rinsed with water, patted dry, and returned to its desorption tube. Both sets of Twisters, along with blanks, were desorbed in the TDU followed by injection into the GC-MS system sequentially. Several more vials of the original standard and saline were also run using an optimized dynamic headspace (DHS)-GC-MS method to obtain the pre- and post-extraction concentrations of standards and saline blanks. The Unknown Analysis software was first used to identify components in the standard and saline solutions, followed by MS Quantitative software to determine the peak areas of each of these compounds.
- **Results**
    - A total of 18 out of 24 compounds (in the residual solvents class 3-mix A standard) were identified using the SBSE method. The general trend is that semi-polar organic compounds, such as ethyl acetate and anisole, show a higher percent recovery than polar compounds such as 1-propanol. However, preliminary data illustrates that, for both categories of compounds, the extractions with a 60-minute incubation time have improved percent recovery than those with a 30-minute incubation time. For example, the percent recovery of isopropyl alcohol (a polar compound with low recovery) increases by about 30 % when raising the incubation time by 30 minutes. The overall extraction efficiency for this method varies from 5% to 30%, which was explored with two DHS methods including two separate drying options to reduce the residual moistures. While the recovery of each compound varies by property, sample to sample variation is low within each drying method. Chromatographic profiles showed significant siloxane elution from the Twister stir bars, specifically from their

PDMS coating and therefore, conditioning steps need to be optimized to minimize the interferences.

- **Implications**
    - Currently, SBSE proves useful in avoiding issues with water accumulation at the system's injector interface. However, to improve extraction efficiency through higher percent recovery, minimize compound loss during extraction, and to reduce siloxane elution, continued optimization of the Stir Bar Sorptive Extraction Method is necessary. This includes adjusting the incubation time, as well as current conditioning and desorption parameters. Going forward, the optimized method will be used to analyze more volatile standards and medical device material extracts. Once optimized, SBSE may be a better alternative for volatile extraction compared to other headspace methods.

16) **Abstract title:** *Mock circulatory loop generated database for dynamic characterization of pressure-based cardiac output monitoring systems*
**Authors:** Nair, Keerthi, FDA/CDRH (Student); Masoud Farahmand, FDA/CDRH (Mentor); Christopher Scully, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Pulse contour cardiac output (CO) monitoring systems allow real-time and continuous estimation of hemodynamic variables such as CO and stroke volume variation (SVV) by analysis of arterial blood pressure waveforms. Evaluating the performance of CO monitoring systems to measure small variations in CO and SVV is a challenge due to limitations in clinical reference methods for tracking patient hemodynamics. We developed a non-clinical database of pressure and flow waveforms with known perturbations as a potential tool for assessing the dynamic attributes of pressure-based CO monitoring systems, including CO response time, and CO and SVV resolutions.
- **Purpose**
    - Minimally and noninvasive cardiac output (CO) estimation methods based on pulse contour analysis of arterial blood pressure (BP) waveforms enables tracking rapid changes in CO and/or stroke volume variation (SVV) to understand patient responses to treatment. Testing of CO monitoring systems is generally limited to comparing the device measurements against traditional, well-established methods such as triplicate thermodilution readings of CO using central catheters. However, the "gold standard" thermodilution method has practical limitations when evaluating the performance of CO devices in tracking rapid changes of parameters such as SVV and CO. Here, we created a database of physiologically-relevant pressure and flow waveforms under known conditions for characterizing the dynamic attributes of arterial blood pressure based cardiac output

monitoring devices including CO resolution, SVV resolution and CO time response.

- **Methods**
  - A mock circulatory loop (MCL) was developed that can simulate rapid changes in different parameters, such as CO and SVV. The MCL was configured to simulate three different hemodynamic states (i.e., normovolemic, cardiogenic shock, and hyperdynamic) representing a range of flow and pressure conditions. Controlled stepwise changes in flow rate were simulated in the MCL, and nine datasets were collected for characterizing dynamic attributes of pressure-based CO systems.
- **Results**
  - Three types of datasets were collected for each hemodynamic state: 1) CO resolution, 2) SVV resolution, and 3) CO response time. Overall, the nine datasets contain peripheral pressure, central flow, and central pressure waveforms for each hemodynamic state.
- **Implications**
  - We presented nine MCL-generated datasets for evaluating key dynamic attributes of pressure-based CO monitoring systems. This benchtop testing approach enables the characterization of a CO monitoring system and accounts for the effects of different equipment (e.g., sensors and monitors with different settings, bandwidths, resolutions, accuracies, fluid-filled tubing with different damping properties, and different interface connections) on the dynamic attributes of the system. This database is intended to be a useful tool for characterizing dynamic attributes of pressure-based CO monitoring systems and algorithms (i.e., CO response time, CO resolution, and SVV resolution), and provides insight into the performance of these attributes.

17) **Abstract title:** *Semantic Distribution Shift in Evolving Artificial Intelligence Segmentation Algorithms*

**Authors:** Najarian, Daniel, FDA/CDRH (Student); Burgon, Alexis, FDA/CDRH (Student), Petrick, Nicholas, FDA/CDRH (Mentor), Sahiner, Berkman, FDA/CDRH (Mentor), Cha, Kenny H, FDA/CDRH (Mentor), Samala, Ravi K FDA/CDRH (Mentor)

**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**

- **Synopsis**
  - The recently released draft guidance for Predetermined Change Control Plan (PCCP) for evolving artificial intelligence (AI) algorithms is a step in the direction of harnessing the power of data. Our work addresses a novel problem in evolving AI segmentation algorithms using a case study in neuro-oncology. Medical image segmentations provide useful information for clinical decision making and treatment planning. Semantic segmentation labels pixels in an image individually, causing it to be more sensitive to pixel-level changes. Any change in the semantics of the background pixels results

in semantic distribution shift, which may decrease algorithm stability. For evolving AI algorithms, data from different sources might be used for AI development, which would consequently increase annotator variability and induce semantic distribution shift. In this study, we systematically modified the distribution of the annotations in the training data to simulate annotator variability. The performance of the algorithm trained on the modified data was compared to a baseline to observe the effects of semantic distribution shift. The results show that semantic distribution shift has a significant negative impact on the algorithm's performance and that annotator variability decreases algorithm stability. This suggests that semantic distribution shift should be taken into consideration for the development of future evolving AI segmentation algorithms to maintain a high standard for patient care.

- **Purpose**
    - Artificial intelligence (AI) algorithms used for medical image segmentation delineate regions of interest to identify structural boundaries and to support the extraction of characteristics useful for clinical decision making and treatment planning. Semantic segmentation is a type of dense prediction where pixels in the images are labeled individually. This pixel-level prediction results in performance that is less stable due to changes in the reference standard than with classification or detection algorithms, which predict at the image- or patient-level. Semantic distribution shift, or any changes in background pixel semantics, can be triggered by inter- or intra-annotator variability which produces an inconsistent reference standard. Evolving AI algorithms currently in clinical use are further trained on newly available data with the goal of improving algorithm performance and patient outcomes. The incremental acquisition of data increases the likelihood that evolving AI algorithms will be affected by distribution shift. The goal of this work is to demonstrate the impact of semantic distribution shift on evolving AI segmentation algorithm stability.
- **Methods**
    - Multi-institutional pre-operative multi-parametric MRI from 1,251 patients are used to develop and evaluate an AI algorithm for segmentation of brain tumors. Images of each patient include T1, T1-weighted, T2-weighted, T2-FLAIR, and an annotated segmentation reference standard. The labeled regions are background, edematous tissue (ED), necrotic tumor core (NCR), and gadolinium-enhancing tumor (ET). ET and NCR combined form the tumor core (TC), which is the target in resections, while TC and ED combined form the whole tumor (WT). Two U-Net AI algorithms (A and B) are trained in an evolving algorithm framework consisting of two sequential training

steps with different data sets. A and B are trained on the same step 1 training data. The same training data is used in step 2 for A and B, however for A the training data is modified systematically while B uses the unmodified data. The data is modified by dilating the TC region using a ball filter with a radius of 3 millimeters (mm). After training, both step 2 algorithms are evaluated on a test set with a similar distribution as the step 1 training data, and a significant difference in performance between A and B indicates that the distribution shift affects algorithm stability. The evaluation metrics used are the Dice coefficient and 95% Hausdorff distance.

- **Results**
    - The experiment was repeated using 12 algorithm initializations with the same data sets. Across all repetitions, the 95% confidence interval for the difference in Dice coefficient between A and B are -0.266±0.017, -0.182±0.012, and -0.042±0.003 for the ET, TC, and WT regions, respectively. For the 95% Hausdorff distance, the respective 95% confidence intervals are 3.647±0.326mm, 2.556±0.331mm, and 0.394±0.158mm for the ET, TC, and WT regions. The decrease in the Dice coefficient and the increase in the 95% Hausdorff distance across all three regions was found to be statistically significant between A and B. The Dice coefficient is an overlap-based metric indicating the overlap between the algorithm's segmentation and the reference standard. The agreement is measured between 0 (no overlap) and 1 (complete overlap). The 95% Hausdorff distance is a spatial distance-based metric that captures the agreement between the edges of the algorithm's segmentation and the reference standard while accounting for outliers. A value of 0 indicates complete agreement excluding outliers.
- **Implications**
    - Many AI algorithms are currently deployed to assist clinicians and with evolving AI these algorithms can continue to learn and adapt. The FDA's draft guidance for Predetermined Change Control Plan (PCCP) helps support iterations upon machine learning-enabled device software functions. In the PCCP, planned modifications and testing plans are outlined in advance, allowing the algorithms to be updated over time and streamlining the process of improving public health. Adding more data and compiling them from different sources can increase performance of data-driven AI algorithms. However, this type of iterative device modifications comes with additional considerations. In evolving AI segmentation algorithms, the likelihood of using data from different sources increases, consequently increasing annotator variability and inducing semantic distribution shift. Our results show that the effects of semantic distribution shift, specifically in

evolving AI segmentation algorithms, can be detrimental to the stability of machine learning-enabled devices.

18) **Abstract title:** *Determining Clinically Meaningful Performance Goals Diagnostic Test Accuracy Studies*
**Authors:** Ngoc Ty Nguyen, FDA/CDRH(Student); Genne Pennello/CDRH (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
**Abstract:**

- **Synopsis**
  - In the assessment of diagnostic tests, it is common to establish targets for accuracy measures like specificity, sensitivity, and positive and negative diagnostic likelihood ratio (PLR, NLR). These performance goals are particularly appealing for tests that identify uncommon conditions, as they can be assessed through case-control studies that focus on the specific condition. In this work, we introduce our regulatory science tool to illustrate how to determine classification accuracy goals based on desired risk stratification. Using the Shinny app, we demonstrate goals for rule-out tests, rule-in tests, and those that do both. The input for the Shiny app contains performance types including standalone and comparative, goals consisting of rule-in, rule-out or both, prevalence and threshold, and collected data from users. The output includes performance goals, likelihood ratio graph and estimated points from the input data. Confidence interval for estimations are also plot and used as evidences to support for the conclusion that whether the data meet the performance goals.
- **Purpose**
  - Commonly used measures of diagnostic test accuracy include sensitivity, specificity, and positive and negative diagnostic likelihood ratio (PLR, NLR), and receiver operating characteristic curve (ROC). These are measures of classification accuracy in that they summarize the ability of a test to correctly classify the status of the target condition (absent, present). They are popular because they do not depend on the prevalence (pre-test probability) of the target condition and thus for low prevalence conditions can be estimated without bias in a moderately-sized, well-conducted study enriched with subjects having the condition.
    Unfortunately, performance goals for classification accuracy are often chosen with only a vague understanding of whether they confer that the test would be clinically useful in practice. Clinicians think the performance goals should be set by the statisticians. Statisticians think the performance goals should be set by the clinicians! A framework is needed for how to set clinically meaningful goals for classification accuracy. Such a framework

would improve conversations among stakeholders (Patients / FDA / Industry / Payers) on appropriate acceptance criteria for validating diagnostic tests for their intended use.

- **Methods**
  - We propose a framework in which clinically meaningful performance goals for sensitivity, specificity, PLR, and NLR are determined based on desired risk stratification, defined by the triple (p0,p,p1) = (1 – NPV, p, PPV), where p is the assumed prevalence of the target condition, PPV > p is the desired positive predictive value of the test, and NPV > 1 - p is the desired negative predictive value. Selection of risk stratification thresholds p0 and p1 may be guided by medical guidelines specifying risk thresholds at which treatment for the target condition should be withheld or received.
    Classification accuracy goals for determining if a test B is non-inferior or superior to a comparator test A are based on non-inferiority margins gam0 for the difference d0 =p – p0 and gam1 for the difference d1 = p1 – p, where gam0 and gam1 are between 0 and 1 and gam0 = gam1 = 1 is an evaluation of whether Test B is superior to Test A.
- **Results**
  - For standalone test accuracy, the PLR goal corresponds to points above the PLR line sensitivity = PLR * (1 – specificity). The NLR goal corresponds to points above the NLR line sensitivity = 1 – NLR + NLR * (1 – specificity). The plot of the PLR and NLR lines is known as the likelihood ratio graph (Biggerstaff, 2000). Independent goals for sensitivity and specificity are defined by the intersection of the PLR and NLR lines and are a subset of the pairs of sensitivity and specificity that meet the NLR and PLR goals. That is, NLR and PLR goals are least burdensome and thus should be preferred.
    For comparative accuracy. Test B is superior to Test A in d0 = p – p0 if it is superior in NLR. Test B is superior to Test A in d1 = p1 – p if it is superior in PLR. Test B in non-inferior to Test A in d0 if the ratio of (1 – NLR)s is > gam0. Test B in non-inferior to Test A in d1 if the ratio of (PLR – 1)s is > gam1. Wald confidence intervals for these ratios were developed to evaluate if non-inferiority was met with statistical significance. We developed software to determine the performance goals, perform data analysis, and provide the likelihood ratio graph and other graphs to facilitate understanding of the goals and their analysis.
- **Implications**
  - Our framework for setting clinically meaningful performance goals for sensitivity, specificity, and the diagnostic likelihood ratios PLR and NLR should improve conversations among stakeholders (Patients / FDA / Industry / Payers) on appropriate acceptance criteria for validating

diagnostic tests for their intended use. The goals should improve the design of diagnostic test accuracy studies, especially retrospective studies enriched for low prevalence target conditions, We developed software as a regulatory science tool to determine the goals and perform data analysis on them and thus can be utilized to implement the framework.

19) **Abstract title:** *Design reference standards mixtures suitable for different material of construction based on a priori knowledge (Gap6&9)*

**Authors**: Leon Oblaender (Student), Ying Jin, Omar Rivera Betancourt, Kerry Bolton, Byeong Hwa Yun (Mentor)

**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**


- **Synopsis**
  - ISO 10993, Part 18:2020 provides methodologies for chemical characterization (CC)of extractables and leachable (E&Ls) in medical devices, enhancing safety and improving manufacturing practices. The chemical characterization can be leveraged for several biocompatible tests that can address the toxicological risk of medical devices. Therefore, quantitation and identification of E&Ls profile in medical devices is critical to assess human health risk. As the profile of E&Ls is often unpredictable, non-targeted analysis is commonly used to quantitate the target analytes. This study aims to develop a set of reference standards for GC/MS technique that can enhance reproducibility and reliability of data analysis. A list of reference standards for different chemical mixtures was developed, considering availability, relevance, signal response, toxicological assessments, reproducibility, and suitability for GC/MS. Calibration curves, LOD, LOQ, and uncertainty factors (UF), were used to create qualitative reference standards. GC/MS techniques analyzed 25 high signal chemicals used in polymer device analysis. The result of this study will streamline chemical analysis of E&Ls in medical devices and their timeline of medical device development in our advancing technological age.
- **Purpose**
  - By providing validated set of reference materials, we aim to provide reliable tools for proper quantitation of E&Ls profile in medical device. The result of this study can facilitate improved practices in medical device manufacturing. Moreover, it plays a pivotal role in the toxicological risk assessment and biocompatibility tests for medical devices, which is a prerequisite for their successful submission to the FDA review for market approval. Due to the wide range of construction materials of medical device, the analyses of E&Ls in medical devices are often performed using non-targeted analysis. The

quantitation of E&Ls using non-targeted analysis is relatively determined using a set of reference standards. However, the current ISO 10993, Part 18 (2020) does not provide a clear guideline for the selection of reference standard for non-targeted analysis resulting in inconsistent CC results from different laboratories. The purpose of this study is to develop a regulatory science tool that can harmonize CC of polymer materials analyzed by mass spectrometry and reduce the burden for internal and external stakeholders during medical device review processes.

- **Methods**
    - As polymers are the primary source of extractable constituents in medical devices, we selected the polymer additives to develop a GCMS method. The polymer additives were prioritized according to availability, relevance to potential analytes, sufficient signal response, toxicological risk, and suitability of analytical methods. Then, we categorized the polymer additives into three tiers to develop an analytical method using mass spectrometry. Twenty-five chemicals with high responses were selected for method development and were prepared in LCMS grade methanol at 5 and 20 ug/mL concentrations. All samples were analyzed in triplicates. To validate the sensitivity of GCMS technique, we measured a 5-point calibration curve and determined limits of detection and limits of quantitation. Statistical analysis including linear regression was performed using Excel and GraphPad PRISM 9.
- **Results**
    - We analyzed the signal intensity of each 25 chemicals at two concentration levels (5 and 20 ug/mL) using the peak areas of extracted ion chromatograms. We also monitored the system contamination and sample carryover between each sample run to confirm the purity of sample signals. In 20 ug/mL concentration, we observed various ranges of signal intensities of the reference standards in our GCMS. The signal responses of some reference standards were matching with that of reported from external test laboratories. The signal intensities of the 25 chemicals at 5 ug/mL showed different distribution than that of 20 ug/mL concentration. Following these two concentration tests, we analyzed 6 of the 25 chemicals at low concentration (2.5-10 µg/mL) and high concentration ranges (12.5-50 µg/mL). The measurement of signal intensities for the remaining 25 chemicals in GCMS is currently in progress.
- **Implications**
    - A non-biased and appropriate list of reference standard can provide consistent results in chemical characterization reports between test laboratories, which help accurate toxicological assessments of E&L profiles

in medical devices. This can streamline the review process for FDA reviewers and reduce the burden for external stakeholders The implications of a cohesive list of chemical standards can also provide a significant tool for an analytical system evaluation during FDA testing. This guideline for appropriate selection of chemicals standards for non-targeted analysis can also be extended by including various extractables such as byproducts of polymer additives and degradation products of polymer materials in future studies. Efficient and rigorous medical technology testing is crucial for the release of new medical devices and products to the public, ensuring their safety and efficacy in serving the healthcare needs of nations and the global population.

20) **Abstract title:** *Interdependence of Cross-Sectional Area and Exposed Perimeter on Spinal Cage Subsidence*

**Authors:** Pennington, Rebecca, FDA/CDRH (Student); Dooris, Andrew, FDA/CDRH; Shetye, Snehal, FDA/CDRH (Mentor)

**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation

**Abstract:**


- **<u>Synopsis</u>**
  - Patients with degenerative disc disease often suffer from substantial pain due to a loss of disc height. Surgical implantation of an interbody fusion device intended to facilitate fusion between the upper and lower vertebral bodies can also restore the lost disc height. However, a common undesirable effect of the implantation is subsidence of the cage into the vertebral body. It is widely understood that cages with larger cross-sectional area have a lower risk of subsidence, but it is important to consider how other factors, like the perimeter of the device, could contribute to this risk. Mock lateral (LLIF) and anterior (ALIF) lumbar intervertebral body fusion cages were designed in Solidworks. Three different designs were created for each cage type with the same cross-sectional area and varying perimeters. Per ASTM F2267, cages were placed between two PCF 15 foam bocks and compressively loaded the system to measure the maximum force and stiffness of the system. The results of the tests demonstrated that with a constant cross-sectional area, cages with a greater exposed perimeter reached a higher maximum force, indicating a lower risk of subsidence.
- **<u>Purpose</u>**
  - Patients afflicted with degenerative disc disease can experience substantial pain due to nerve root impingement within the intervertebral foramen, which is a direct result from loss of disc height and concomitant closing of the foramen. Restoration of disc height is commonly achieved by surgical

implantation of a spinal cage, which is designed to facilitate fusion between the superior and inferior vertebral bodies. However, the inherent mismatch in strength of a spinal cage device and vertebral endplates can result in post-surgical subsidence. Under a given load, it is usually assumed that devices with a lower cross-sectional area will result in greater subsidence, and these devices can be considered as worst-case. However, when comparing cages with similar cross-sectional area, the total exposed perimeter might be an important factor to consider. This study aims to investigate the correlation, if any, between cage subsidence and the total perimeter of two mock intervertebral body fusion device design. It was hypothesized that with a constant cross-sectional area, the cage with a higher exposed perimeter will experience greater subsidence.

- **Methods**
    - Mock lateral (LLIF) and anterior (ALIF) lumbar intervertebral body fusion cages were designed in Solidworks. Three different designs were created for each cage type. Each of the three designs were prescribed a constant cross-sectional area, while the total perimeter value was varied by changing external dimensions and adding internal features. External dimensions were restricted to realistic cage sizes as reported by Peck et al. Mock cage designs were additively manufactured from nylon using an EOS powder bed fusion printer. ASTM F2267, which outlines a standard for measuring load-induced subsidence of intervertebral body fusion devices under static axial compression, was utilized to measure subsidence of the mock LLIF and ALIF cage designs. Each cage design (n = 4) was placed between PCF 15 foam blocks and 5mm of compression was applied at a rate of 0.1mm/s. Force (N) and displacement (mm) data were collected at 100Hz. Force-displacement data were used to obtain the system stiffness (N/mm) and maximum force during the test. All cages were also tested under axial compression per ASTM F2077 to obtain stiffness of the intervertebral body fusion device. Groups were compared with a one-way ANOVA followed by Tukey post-hoc tests. Significance was set at $p < 0.05$.
- **Results**
    1) LLIF cages had a statistically significant higher maximum force at 5mm of subsidence with an increase in exposed perimeter. The solid LLIF cage had maximum force of 3908.6 ± 170.8N, the two-hole design had a maximum force of 4491.0 ± 52.5N, and the six-hole design had a maximum force of 4800.7 ± 84.9N. Similarly, the solid ALIF cage had maximum force of 3556.0 ± 22.9N, the one-hole design had a maximum force of 3893.3 ± 69.1N, and the four-hole design had a maximum force of 4058.5 ± 102.5N. For both LLIF and ALIF cages, the solid design had a significantly lower stiffness when

compared with the one/two-hole designs and the four/six-hole designs. No significant differences were observed in stiffness between the one/two-hole signs when compared with the four/six-hole designs, respectively.

- **Implications**
    1) This study investigated the interdependence between cage subsidence and exposed perimeter of the mock intervertebral body devices. The results demonstrated a significant correlation between cage subsidence and the total perimeter of two mock intervertebral body fusion device types. Cages with a greater exposed perimeter are less prone to subsidence than cages with less exposed perimeter when cross-sectional area is held constant. When comparing two spinal cages with similar cross-sectional area, the total perimeter may be considered to determine potential for subsidence. Moving forward, medical devices manufacturers may be able to use this observed correlation during development and review of novel intervertebral body fusion devices.

21) **Abstract title:** *Impact Analysis of Network Failure Modes on MXR Medical Devices in 5G Networks*

**Authors:** Parisa Rafiee (Student), Yongkang Liu (Mentor), Weichao Gao, Mohamad Omar Al-Kalaa

**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation

**Abstract:**

- **Synopsis**
    - This research project focuses on identifying network failure modes in 5G networks and their effects on Medical eXtended Reality (MXR) devices in healthcare applications. Through a comprehensive analysis of various network entities and interfaces, common failure scenarios are identified. A 5G testbed is utilized to emulate selected failure scenarios where network behaviors and device performance are analyzed to provide insights into the impact of connectivity failures on MXR devices. Candidate mitigation strategies are proposed and validated. This research contributes to OSEL regulatory science tool (RST) efforts for accelerating the safety and effectiveness evaluation of MXR devices in 5G networks.
- **Purpose**
    - This research aims to identify and analyze network failure modes in 5G networks and assess their impact on Medical eXtended Reality (MXR) applications. By surveying the variety of system components and interfaces within the 5G network, the study seeks to pinpoint potential failures in the network operations and measure such hazards to MXR device functionality. Through this analysis, we investigate the connectivity reliability and

resilience as enablers of MXR devices operating in the dynamic 5G network environment. Findings provide technical insights for developing 5G-enabled medical device regulatory science tools (RST), such as practical testing methodologies. These testing methods are developed and verified in FDA's 5G medical device testbed. The outcome of this research is shared with FDA stakeholders and the general public to facilitate the development and evaluation of MXR applications enabled by 5G connectivity.

- **Methods**
  - This research employs a hybrid approach involving system analysis and experimental development to emulate and analyze identified network failure modes and their impact on the application performance. Firstly, a comprehensive review of existing literature, industry standards, and technical specifications is conducted to identify the key network entities and interfaces that serve as essential system components in typical 5G network deployments. Potential failure modes in 5G networks and their corresponding impact on MXR medical device users are analyzed through the 3rd Generation Partnership Project (3GPP) protocol stack, the de facto 5G network standard, in individual interfaces per representative network operation scenarios. Next, selected failure scenarios are replicated in the FDA 5G testbed, where application data and network status are collected for post-analysis. The test data is analyzed to quantitatively verify the 5G network performance and its impact on medical device functionality.
- **Results**
  - This research project aims to achieve three main goals:
    (1) The study identifies and analyzes 5G network failure scenarios such as link failures and network congestions, illustrating the intricacies of these failure modes within 5G networks that enable medical device services.
    (2) The study assesses the impact of network failure modes on the performance and functionality of MXR medical devices, enabling stakeholders to proactively address these failure modes in their design of risk mitigation approaches, e.g., identifying principal factors and prioritizing associated risks. Through experimentation in the testbed, context-rich data is gathered to assess the effects of failure scenarios on device responsiveness, network behaviors, and data transmission reliability.
    (3) The research showcases examples of mitigation methods that effectively reduce the impact of network failures on device functionality and user experience. These mitigation strategies offer practical approaches to device manufacturers, enabling them to implement measures that enhance device resilience, minimize downtime, and ensure uninterrupted performance of MXR medical devices in 5G networks.

- **Implications**
    - The identification of network failure modes and their impact on MXR medical devices in 5G networks contributes to the development of regulatory science tools and standards for promoting patient safety and reliable device performance. The research outcomes enable manufacturers to evaluate and enhance the compatibility and reliability of their devices in 5G network environments. By utilizing the provided testing framework, manufacturers can assess device behavior during different failure scenarios, improving overall device performance and user experience. Furthermore, the research findings can inform healthcare regulators in formulating policies and guidelines related to the deployment and usage of 5G networks in the healthcare industry. This includes considerations for network resilience, fault tolerance, and failure mitigations for MXR medical devices operating within 5G networks.

22) **Abstract title:** *Analyzing Medical Texts: Exploratory Research on NLP Algorithms and Large Language Models*
**Authors:** Sharma, Gaurav, FDA/CDRH/DIDSR (Student); Thompson, Yee Lam Elim, FDA/CDRH/DIDSR (Mentor); Garcia, Victor, FDA/CDRH/DIDSR (Mentor)
**FDA Strategic Initiative:** Unleashing the power of Data
**Abstract:**
    - **Synopsis**
        - Our main objective is to build a robust Natural Language Processing (NLP)-based pipeline that uses cutting-edge architectures to carry out a variety of text analyses related to medical scholarly articles. Advanced NLP and Large Language Models (LLMs) are used to perform tasks including summarization, keyword extraction, and general question answering. Our goal is to create a highly functional and adaptable system that can handle various text processing requirements. Through this process, we gain regulatory insights on the potential capabilities and limitations of NLP algorithms.
    - **Purpose**
        - In this research, we investigate the use of natural language processing (NLP) as it pertains to medical devices. Exploring tasks like keyword recognition, summarization, and question answering, we want to examine how NLP algorithms and large language models (LLMs) perform on medical datasets. We build models using publicly accessible datasets (medalpaca/medical_meadow_cord19), assess their performance using

established measures, and provide visualization strategies. Through this exploratory work, we gain initial insights into the regulatory aspects of NLP algorithms and LLMs in the context of medical scholarly articles.

- **Methods**
  - Using open-source datasets, we investigate methods for question-answering text generalization, keyword recognition, and summarization. Our techniques include token extraction using frequency-based algorithms (TF-IDF, RAKE, YAKE, KeyBERT), graph-based algorithms (TextRank, TopicRank, SingleRank, and MultipartiteRank algorithms), transfer learning using pre-trained Hugging Face transformer models for summarization (BERT, BART, T5), LLMs for question-answering, and other token extraction techniques. We evaluate model performance using parameters including ROUGE, F1, Recall, Precision, and Discounted Cumulative Gain. In order to visualize model outputs and performance, we plan to use PowerBI to exhibit the extracted tokens as clusters, highlighting their linkages in an embedding space. Though we are still in the initial phase of exploration, our goal is to learn more about how these techniques, which make use of transfer learning and pre-trained models, can effectively find labels that can be used for clustering.
- **Results**
  - Based on our current findings, we can deduce that the summarization technique may not be the most effective approach to label text in a few words, especially when we are dealing with complex input texts.
    The performance of keyword extraction algorithms varied, with KeyBERT and Multipartite Rank algorithms yielding better results compared to others. KeyBERT demonstrated the ability to extract twice as many relevant keywords compared to other frequency-based methods, while Multipartite Rank exhibited a higher frequency of extracting important keywords, surpassing other graph-based methods by 1.5 to 2 times.
    The most favorable outcomes were achieved by LLMs in conjunction with keyword extraction algorithms, particularly the combination of a question-answering model with the Multipartite Rank algorithm. By providing a simple prompt as input and extracting relevant keywords from the text, this approach demonstrated satisfactory performance in handling over 75% of complex texts. However, we also found that this pipeline is highly sensitive to the prompt provided by the user, as even slight modifications can lead to different outcomes.
- **Implications**
  - Our research offers insight for regulatory scientists into the capabilities and limitations of cutting-edge NLP techniques in text analysis. We illustrate the

models' capacities to extract relevant information from challenging input texts using publicly accessible datasets of COVID-related scholarly articles. We work to define appropriate evaluation metrics while improving the precision and quality of our summarization and token extraction approaches through iterative experimentation and improvement. Our final objective is to create a reliable pipeline that can carry out multiple operations on complex textual data related to medical devices. This project fills the gap between state-of-the-art NLP/LLM-based methods and regulatory science, making it possible to evaluate the safety and effectiveness of LLM-based software as medical devices.

23) **Abstract title:** *Simethicone Impact on Microbicidal Efficacy of Disinfectants*
**Authors:** Ullman, Jeremy, FDA/CDRH (Student); Anderson, Greg, FDA/CDRH (Mentor); Linden, Sara, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
  - This project seeks to evaluate if the presence of simethicone has an impact on the microbicidal efficacy of selected high-level disinfectants on the Gram-negative bacterium Pseudomonas aeruginosa. To accomplish this, a method was developed to test the effects of disinfectant on bacteria inoculated on coupons. Initial experimentation verified the proof of concept, as inoculated and uninoculated coupons treated with disinfectant only produced plates with no colonies grown while inoculated coupons not treated with disinfectant produced plates with colonies. Later research will rely on this proof of concept when incorporating simethicone into the procedure.
- **Purpose**
  - The chemical properties of simethicone can support reduced surface tension of mucus, permit air bubbles to fuse and disperse, and promote expulsion of intestinal air. As a result, simethicone is used in many applications, including as a lubricant and anti-foaming agent during endoscopic procedures. Based on the literature, bubbles in the gastrointestinal lumen can adversely affect these procedures, potentially lengthening their duration by impairing mucosal assessment and lesion detection. Simethicone is sometimes used during these procedures to inhibit bubble formation in the gastrointestinal lumen. However, some published studies have found simethicone residue on endoscopes even after cleaning and disinfecting according to instructions. Some literature suggests that this residue may mask bacterial contaminants on used devices,

potentially leading to contamination and risk of infection. This project seeks to evaluate the impact of simethicone on the microbicidal efficacy of selected disinfectants.

- **Methods**
  - Pseudomonas aeruginosa was grown overnight and 10^6 colony forming units (CFU) were inoculated onto sterile stainless-steel coupons, as a surrogate for a device surface. Coupons were then treated with disinfectant for approximately 45 minutes. Remaining bacteria were extracted by vortexing and sonication, and they were quantified by plating and enumerating CFU. Colony forming units were then compared between treated and untreated coupons. In future experiments, replicate coupons will be similarly treated but with the addition of simethicone over the bacterial spot to investigate the impact of simethicone on the microbicidal efficacy of a high-level disinfectant.
- **Results**
  - Initial experimentation helped to verify proof of concept. No colonies were evident from inoculated and uninoculated coupons treated with disinfectant, while inoculated coupons not treated with disinfectant contained an average of $6.8 \cdot 10^4$ CFU. This equates to a loss of roughly 1.5 log of CFU when considering that around $10^6$ CFU was added to each coupon, and this may be attributed to the washing that occurred during the washing steps.
- **Implications**
  - Considering that lubricant may be used during medical procedures, and that simethicone is sometimes used as a lubricant, this research seeks to understand the impact, if any, that simethicone has on microbicidal action of disinfectants commonly used during reprocessing. Future research on different bacterial strains and other disinfectants while using simethicone could help to broaden understanding of the impact that simethicone may have on reprocessing.

24) **Abstract title:** *Assessing Damage to Red Blood Cells by Medical Device Materials: Improving the ASTM F756 Hemolysis Testing Standard*
**Authors:** Williams, Justin, FDA/CDRH (Student); Malinauskas, Richard, FDA/CDRH (Mentor); Skoog, Shelby, FDA/CDRH (Mentor); Lu, Qijin, FDA/CDRH (Mentor)
**FDA Strategic Initiative:** Increasing Choice and Competition Through Innovation
**Abstract:**

- **Synopsis**
  - To ensure the safety of materials used in blood-contacting devices, the FDA collaborates with industry to develop various testing standards. This project aims to improve the ASTM F756 standard which tests the potential of materials to damage red blood cells (i.e., cause hemolysis), as released

hemoglobin can adversely affect patients. To identify reliable positive control materials (those that cause %hemolysis > 5%), one brand of nitrile glove, surfactant-containing PVC pellets, and varying concentrations of DMSO (dimethyl sulfoxide) were tested according to the procedures described in the standard. Results showed that the nitrile glove was a reliable positive control (>79% hemolysis with %CV of 6%) for all samples. The PVC pellets were a suitable positive control when tested at 0.2 g/mL and had an average %hemolysis of 14.5% (%CV up to 15%). DMSO concentrations at 15% and above consistently yielded hemolysis values greater than 5% (e.g., 17.5% DMSO caused 24 ± 6 % hemolysis). Non-medical grade high-density polyethylene (HDPE) sheet was shown to be a negative control (hemolysis < 2%) after proper cleaning with alcohol. The PVC pellets, developed as a positive hemolysis control material, did not have hemolysis levels as high as previously reported by the supplier (56%) using similar test conditions. Interference testing revealed that particles of certain sizes may spin out of solution at the centrifugation condition listed in the standard, but colored dyes and small particles (< 1 um) remain and could interfere with sample absorbance readings when measuring released hemoglobin. Methods of correcting for this interference must be considered when performing the test. The results of this study will be used to update the ASTM F756 hemolysis testing standard of device materials.

- **Purpose**
  - Blood-contacting medical devices such as stents and catheters are crucial to treating patients, but they have the potential to damage blood elements, especially when new materials or coatings are utilized. Hemolysis occurs when red blood cell membranes become compromised and free, unbound hemoglobin is released into circulation that may adversely affect vascular and renal tissues. The FDA collaborates with industry to develop and improve testing standards to ensure the safety of materials used in blood-contacting devices. This project evaluates the procedures in the ASTM F756 testing standard for determining the hemolytic potential of materials. Factors studied include the identification of more relevant positive control test materials and a method to correct for material-released absorbance interference when determining the amount of free hemoglobin. The standard defines hemolytic potential (% hemolysis) as the ratio of free hemoglobin concentration to the total blood hemoglobin exposed to the test material. Materials with a hemolytic potential over 5% (relative to a negative control material) are considered hemolytic, while those below 2% are considered non-hemolytic.
- **Methods**
  - To identify reliable positive and negative control materials, high-density polyethylene (HDPE) sheet, one brand of nitrile glove, varying concentrations of dimethyl sulfoxide (DMSO), and surfactant-containing polyvinyl chloride (PVC) pellets were evaluated using the ASTM F756 standard for direct contact materials. Test materials were cut or weighed to maintain the standard-specified material-to-phosphate buffered saline (PBS)

ratio (3-6 cm2/mL or 0.2 g/mL for pellets). Rabbit blood from 3 donors was pooled and diluted with PBS to a hemoglobin concentration of 10 mg/mL. Materials were tested in triplicate using a 7:1 ratio of PBS to dilute blood in a total volume of approximately 2 mL. Samples were incubated in a water bath at 37°C for 3 hours and gently inverted three times every 30 minutes to re-suspend settled red blood cells. After incubation, fluid samples were centrifuged at 800g for 15 minutes. The supernatants were mixed 1:1 with cyanmethemoglobin reagent to convert free hemoglobin into a stable form and measured using a spectrophotometer at 540 nm. To study the effect of optical interference on the measurement of free hemoglobin, PBS spiked with dyes or microparticles (0.1 - 5 um), which may dissociate from device materials, was also investigated.

- **Results**
    - Non-medical grade HDPE sheet required washing with alcohol to reliably produce less than 2% hemolysis and meet the acceptance criterion for a negative control material. For the potential positive control materials, the sample amount was based on surface area (nitrile glove), weight (PVC pellet), or concentration (DMSO). The nitrile glove produced 80-98% hemolysis during all tests, which is consistent with previous data that identified it as a positive control (% hemolysis > 5%). Surfactant-impregnated PVC pellets tested at 0.2 g/mL caused about 15% hemolysis with low variability (%CV = 10% for n=6 samples tested using one blood pool), while lesser amounts of pellets (0.014-0.1 g/mL) resulted in less than 5% hemolysis. DMSO concentrations at 15% and above consistently yielded hemolysis values greater than 5% (e.g., 17.5% DMSO caused 24 ± 6 % hemolysis). Optical interference testing showed that particles are removed as a function of their size (i.e., small particles remain suspended while large particles spin out) at the centrifugation conditions used in the standard, while color dyes remained in the supernatant. As such, background interference correction of the absorbance readings for free hemoglobin needs to be considered when performing the test.
- **Implications**
    - We identified test conditions for potential positive control materials (nitrile glove at 6 cm2/mL, PVC pellet at 0.2 g/mL, DMSO at 17.5% concentration) that reproducibly caused hemolysis greater than 5% above that of the HDPE negative control material with low variability (%CV: 6% for nitrile gloves, 15% for PVC pellets, and 24% for 17.5% DMSO), which make them acceptable positive controls according to the testing standard. The hemolysis levels of the PVC pellets, developed as a positive hemolysis control and listed for that purpose in another standard (ISO 10993-12;2021), were not as high as previously reported by the commercial supplier (i.e., 56% by the supplier versus 15% in our tests). Non-medical grade HDPE reproducibly caused less than 2% hemolysis as a suitable negative control material only after proper cleaning. Further research needs to be performed for interference testing to determine the methodology to follow when different types of interferents affect the absorbance readings

of samples by altering the supernatant color and clarity. The results of this study will be used to update the ASTM F756 hemolysis testing standard for evaluating device materials.

25) **Abstract title:** *Evaluating Vaporized Hydrogen Peroxide Diffusion Through Medical Device Materials For Sterilization Applications*
**Authors:** Liu, Shan, FDA/CDRH (Student); Liu, Yunzhi, FDA/CDRH (Mentor, staff fellow); Winogradoff, David, FDA/CDRH (Staff fellow); Arsano, Iskinder, FDA/CDRH (ORISE fellow)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Approximately 50% of sterile medical devices in the US are sterilized by ethylene oxide (EtO). In recent years, there has been increased awareness around reducing reliance on EtO sterilization, driven in part by EPA interest in decreasing EtO emissions. To mitigate potential impacts on availability of sterile medical devices, alternative gaseous sterilants are a research priority. Vaporized hydrogen peroxide (VHP) has been identified as a candidate for the EtO alternative, as it degrades into water and oxygen and is used as a sterilant in hospitals. The published literature describing VHP penetratation through materials is limited. Penetration of gaseous sterilant is important for the sterilization process. Herein, experimental measurements are set up utilizing free evaporation to create a dataset of the diffusion coefficients of VHP through medical device polymers and primary packaging materials of varying geometries based on Fick's law. This dataset can be utilized not only as a reference value for sterilization process development but also as a basepoint for computational model validation. So far, measurements are completed on polyether urethane (TPU) 55D, and polyether block amide (Pebax) 25D, two polymers widely used in medical devices, showing diffusion coefficients in the order of 10-8 and 10-7 cm2/s, respectively. In summary, the combination of experimental data and the computational model will be used to develop a regulatory science tool to help identify the conditions necessary for sufficient sterilant concentration at the interior device surface. This tool aims to shorten the time needed to develop the VHP sterilization process for medical devices with various materials and geometries.
- **Purpose**
    - Approximately 50% of sterile medical devices in the US are sterilized by ethylene oxide (EtO). In recent years, there has been increased awareness around reducing reliance on EtO sterilization, driven in part by EPA interest in decreasing EtO emissions. To mitigate potential impacts on availability of sterile medical devices, alternative gaseous sterilants are a research priority. Vaporized hydrogen peroxide (VHP) has been identified as a candidate for

the EtO alternative, as it degrades into water and oxygen and is used as a sterilant in hospitals. The published literature describing VHP penetration through materials is limited. The penetration of gaseous sterilant is important for the sterilization process. For instance, the sterilant needs to reach the interior surface of sterile medical devices,and the sterilant needs to penetrate through the package to reach the device surface for terminal sterilization. To contribute to available literature, this research aims to create a dataset of the diffusion coefficients of VHP through medical device polymers and primary packaging materials of varying geometries.

- **Methods**
  - The test material was sealed in a diffusion cell with a downstream VHP probe placed behind it to measure any diffusion through the material. The upstream side of the material is exposed to known concentration VHP as measured by a separate VHP probe. The vapor is formed by free evaporation from hydrogen peroxide aqueous solution. Once the evaporation reaches saturation, the material is exposed to the vapor. The test apparatus is isolated from the surrounding environment, and is dehumidified prior to VHP evaporation. By monitoring the VHP concentration in both probes, diffusion curves are obtained with VHP concentration change measured over time. Based on Fick's law, the diffusion coefficient of VHP through representative medical device materials can be calculated.
- **Results**
  - Measurements were completed on polyether urethane (TPU) 55D, and polyether block amide (Pebax) 25D, two polymers widely used in medical devices. Samples of varying thickness were measured for each material. The diffusion curves suggest that within the same material in this thickness range, the diffusion behavior is similar. After the data processing steps, the diffusion coefficient (D) of VHP through various representative medical device materials can be calculated. So far, Polyether Urethane (TPU) 55D and Pebax 25D are showing diffusion coefficients in the order of $10^{-8}$ and $10^{-7}$ $cm^2/s$, respectively.
- **Implications**
  - This research aims to provide a publicly available dataset of diffusion coefficients of VHP into materials, indicating that VHP penetrates through the material rather than only sterilize the surface. In addition, this dataset may provide reference values for sterilization process development and as a basepoint for computational model validation. In summary, the combination of experimental data and the computational model will support development of a regulatory science tool to help identify the

conditions necessary for sufficient sterilant concentration at the interior surface. The proposed tool aims to streamline incorporating the VHP sterilization process for medical devices with various materials and geometries.

## Center for Food Safety and Applied Nutrition (CFSAN)

1) **Abstract title:** *High-Throughput Automated DNA Extraction: Is It Possible to Obtain High-Quality Shiga Toxin-Producing Escherichia coli DNA from Different Environmental Matrices?*
**Authors:** Akshaya Balaji1, Ai Kataoka2, Roberto Guzman2, Andrew Battin2, Jennifer Wolny2, Natalie A. Brassill3, Channah M. Rock4 and Julie A. Kase2
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**
    - **Synopsis**
        - Leafy greens-associated Shiga toxin-producing Escherichia coli (STEC) outbreak responses have involved the collection and evaluation of water, sediment, soil, and air samples in addition to produce. Such large-scale sampling efforts necessitate the assessment of automated high-throughput processes that yield DNA compatible with PCR. The purpose of this study is to investigate the different methods of extracting E.coli DNA from different environmental samples. Using methods from both manual (wash spin boil) and automated (Maxwell) procedures, the rate of positive samples was compared across water, passive air, soil and sediment samples. In air, water, and soil samples with low-level STEC contamination as judged by PCR Ct values, the automated PFP and FM methods yielded detectable DNA targets when compared to manual procedures. However, the processing of sediment samples illustrated that performance of methods differed in their ability to remove PCR inhibitors. While the Maxwell kits are automated, they are not necessarily more efficient as they still require manual manipulation.
    - **Purpose**
        - We evaluated the recovery and quality of STEC DNA from different environmental matrices using the Maxwell® RCS 48 Instrument with two different protocols as compared to manual DNA preparation.
    - **Methods**
        - From 77 enriched samples, DNA was extracted from aliquots using the PureFood Pathogen (PFP) and Fecal Microbiome DNA (FM) kits and manually processed by either a wash-spin-boil or Qiagen DNEasy step. DNA products were analyzed for STEC genes using PCR according to FDA BAM Chapter 4A.
    - **Results**
        - No statistical difference between the manual and automated DNA preparation methods was observed for air, water, and soil samples when PCR Ct values were < 30.0 ($p < 0.05$). Notably, of the 39 soil samples manually extracted without STEC targets detected, DNA from the PFP and FM kits yielded positive results for 39 (Ct values > 33.4) and 13 samples (Ct

values > 37.4), respectively. Similarly, 10 air samples without positive STEC detection from DNA manually prepared produced values when either PFP (n = 10, Ct > 33.7) or FM (n = 4, Ct > 38.3) extracted. Although only eight sediment samples were tested, all but one yielded PCR inhibition of the amplification control in DNA extracted using the PFP method but none in samples extracted using FM or manual methods.

- **Implications**
    - In air, water, and soil samples with low-level STEC contamination as judged by PCR Ct values, the automated PFP and FM methods yielded detectable DNA when compared to manual procedures. However, the processing of sediment samples illustrated that performance of methods differed in their ability to remove PCR inhibitors.

2) **Abstract title:** *Detection and persistence of Cyclospora cayetanensis in soil and fresh herbs grown under two different water content conditions in controlled growth chambers*
**Authors:** Arida, Joseph, FDA/CFSAN (Student); Grocholl, John, FDA/CFSAN (Mentor); Njoroge, Joyce, FDA/CFSAN (Mentor); Almeria, Sonia, FDA/CFSAN (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Cyclospora cayetanensis causes a diarrheal illness called cyclosporiasis. Outbreaks of cyclosporiasis linked to fresh produce have affected thousands of persons in the U.S. in a multi-state fashion. Cyclospora oocysts are highly resistant in the environment. Contact with soil has been found to be a risk factor for C. cayetanensis infection and may play an important role in the contamination of foods. The main objective of the present study was to determine the effects that water content in combination with type of soil and temperature could have in C. cayetanensis detection in soil and herbs artificially contaminated with the parasite. Detection of the parasite in soil artificially contaminated with oocysts as well as in leaves of herbs grown was analyzed using the GEN1000 CONVIRON growth chambers, under arid/low water content conditions and under wet/high water content conditions. After the plants were considered mature, the soil was inoculated with 400 C. cayetanensis oocysts at different spots and individual leaves of the herbs (cilantro, parsley, and basil) were inoculated with 100 oocysts/each. Samples of soil and leaves were collected weekly up to 52 days post-inoculation (DPI) in dry conditions and 49 dpi in wet conditions. The presence of the parasite in soil was confirmed by concentration via flotation in high density sucrose solutions, DNA extraction and molecular detection by real-time PCR specific for the parasite. The detection of the presence of the parasite in leaves of the herbs followed BAM chapter 19b method. Our results indicate the possibility of long-term persistence of

oocysts in soil and in herbs when grown in wet/high water content conditions, while in arid/dry conditions the parasite showed short-lived persistence in soil but persisted in the leaves over the experimental period. This data will allow FDA to better understand ways of C. cayetanensis transmission and to establish control measures to disrupt the cycle of transmission.

- **Purpose**
    - The main objective of this study was to evaluate the effect that two different water content conditions have on C. cayetanensis detection and persistence in soil and herbs artificially contaminated with the parasite. Using the controlled environmental conditions of growth chambers, we aim to analyze the role of rainfall and humidity in the persistence and detectability of C. cayetanensis oocysts in both soil and produce. This data will allow FDA to better understand ways of C. cayetanensis transmission and to establish control measures to disrupt the cycle of transmission. This will provide the U.S. FDA with information needed to determine the survival and persistence of C. cayetanensis oocysts in the soil, and the potential role of soil and water as a source of C. cayetanensis food outbreaks.
- **Methods**
    - The effect of arid conditions in the detection of C. cayetanensis compared to more humid conditions was analyzed in soil and herbs grown at temperatures ranging from 180C-250 (moderate/high temperature) in a daily cycle in a CONVIRONTM growth chamber, in farm soil rich in sand (21.9% clay, 22.8% silt, and 55.3 sand) using two experiments. Plants in low rainfall/arid conditions received 500 mL of water once a week while plants in high rainfall/humid conditions received 500 mL of water every other day. The soil was autoclaved before seeding of herbs to avoid insect interference. Once the plants were considered mature, the soil was inoculated with 400 C. cayetanensis oocysts at different spots and individual leaves of the herbs (cilantro, parsley, and basil) were inoculated with 100 oocysts each. Samples of soil and leaves were collected at weekly intervals post inoculation. The presence of the parasite in soil was confirmed by flotation in high density sucrose solutions, DNA extraction and molecular detection by real-time PCR specific for the parasite. The detection of parasite presence in leaves followed the BAM chapter 19b method, with three main steps including washing and concentration of the parasite, DNA extraction, and molecular detection by real-time PCR.
- **Results**
    - In low rainfall/arid conditions, with a daily humidity ranging from 55-65%, and plants being only watered once a week, herbs started as seeds, grew

very slowly and were relatively small. In dry/arid conditions, the parasite was detected at soil samples collected at 7 dpi, but not at any other collection date while the parasite could be detected in leaves of the herbs at all the sample collection points from 7 dpi to 45 dpi. On wet/high water content conditions, plants grew faster, looked healthier and reached larger sizes. The parasite was detected in all soil samples collected up to 49 dpi as well as in the leaves of the herbs up to 49 dpi. Additional samples will be collected in the next month until the end of the experimental period of the second experiment. Our results indicate the possibility of long-term persistence of oocysts in soil and in herbs when herbs are grown in wet/high water content conditions, while in arid/dry conditions the parasite showed short-lived persistence in soil but persisted in the leaves over the experimental period.

- **Implications**
    - There are still significant gaps in our knowledge of the epidemiology of C. cayetanensis. Outbreaks of cyclosporiasis, linked to fresh produce such as fresh herbs, leafy greens, and berries, have affected thousands of persons in the U.S in recent years. Contact with soil has been included as a risk factor for C. cayetanensis infection in several studies. For FDA to fulfill its mission to protect consumers and improve food safety, the agency must ascertain detection and persistence of the parasite in soil. This study used a fast and sensitive method for the detection of C. cayetanensis in soil recently developed by our group. The comparison of different environmental conditions in the detection of C. cayetanensis detection in soil and herbs artificially contaminated with the parasite will improve the ability of the FDA to understand the role of environmental factors on the detection/persistence of C. cayetanensis in the environment and in fresh herbs, frequently linked to outbreaks, and to close critical gaps in our knowledge of the epidemiology of C. cayetanensis needed for the control of this important parasitic infection.

3) **Abstract Title:** *S. Agona survival on extreme low moisture food*
**Authors:** Meeks, Ellie, FDA/CFSAN (Student); Hoffamnn, Maria, FDA/CFSAN (Mentor); Solaiman, Sultana, FDA/CFSAN (Mentor); Zheng, Jie, FDA/CFSAN (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Low-moisture foods are at risk of bacterial growth if not properly processed and stored. Outbreaks from specific strains of Salmonella have led to research being conducted to understand how and with what tools bacteria

uses to survive in these typically unfavorable environments. The goal of this study is to understand the functionality of these bacteria more in the hopes to prevent future outbreaks from food-borne illness.

- **Purpose**
  - There have been recurrent outbreaks of Salmonella agona in the same processing facility in both 1998 and 2008. From SNP analysis of the two strains, it has been determined that these bacteria are related and have lasted in low moisture environments for around a decade. To understand the growth patterns, morphology, and genetic factors that S. Agona utilizes to survive in desiccation stress, further analysis is required. To determine the survivability and changes in morphology of S. Agona strain in low moisture environment and in LMF, establish different growth patterns in low moisture versus standard moisture conditions, and determine the different genetic factors responsible for these changes.
- **Methods**
  - S. Agona strain (CFSAN 000477) from the latter outbreak has been selected for these further analyses. Preliminarily, a growth curve and desiccation stress survival growth were run with 3 trials each at separate times. A single colony was suspended in 50ml TSB and incubated 35.0℃ at 135rpm for the growth curve, samples taken every 30 minutes for the initial 8 hours and again starting between 23-24 hours for 6-8 additional datapoints. 10g of off-brand rice cereal was inoculated with 100µl of ~10 log cfu/g S. Agona, then placed in chambers with a desiccation stress (aw≤0.25) with uninoculated controls at room temperature (25$^{o}$C) for 30 days. 15 timepoints were taken from the samples; the experiment was replicated twice more for consistent data. Morphology was determined by developing cells in both standard and desiccated states and reviewing under electron microscopy. Future work will include knocking out genes randomly with transposon and inserting segments of plasmid that allows for growth on kanamycin limited plates, then determining which gene has desiccation stress functionality by exposing mutants to desiccation and monitoring growth.
- **Results**
  - The desiccation stress-exposed S. Agona was analyzed and compared across the triplicate experiments. Directly after inoculation, there was ~9 log cfu/g Salmonella cells recovered, but 8 hours into desiccation, the population decreased by a factor of 2 log cfu/g ($P>0.05$). Throughout the remainder of the 30 days of desiccation exposure, however, colony count held consistent at ~7-8 log cfu/g. This survival within the desiccated sample indicates a potential resistant subpopulation that can successfully survive in low moisture environments. Population levels of Salmonella were tracked across

26.5 hours with an initial ~6 log cfu/g and grew to a peak average of ~9 log cfu/g after 8 hours and ~10 log cfu/g at the endpoint. Cell morphologies had different appearances between the control and desiccated stress samples.

- **Implications**
    - This data suggests that there are different growth patterns between liquid and desiccated stress S. Agona, however maintained growth across both states indicates that low humidity environments provide an adequate climate for these bacteria. This stresses the importance of proper sanitization for low moisture food processing environment.

4) **Abstract title:** *Determining Vitamin B12 Concentration in Breakfast Cereals using HPLC-ICP-MS*
**Authors:** Escavage, Jordan, FDA/CFSAN (Student); Wolle, Mesay, FDA/CFSAN (Mentor)
**FDA Strategic Initiative:** Empowering Patients and Consumers
**Abstract:**

- **Synopsis**
    - Vitamin B12 is used in vital biological processes for humans such as red blood cell and DNA formation. Fortified food products such as breakfast cereals supply humans with the necessary vitamin B12 to fuel these processes. Reliable analytical methods are needed to ensure the fortified foods meet the necessary vitamin B12 requirements. This study quantifies vitamin B12 in breakfast cereals by first converting the cobalamins to CNCbl using NaCN. Then, the starch is broken down by α-amylase, and the CNCbl is preconcentrated using solid phase extraction. Using extraction, HPLC, and ICP-MS, the cyanocobalamin is separated from the sample and quantified. This work contributes to an FDA validated method for quantification of vitamin B12 in breakfast cereals.
- **Purpose**
    - Vitamin B12 represents a group of vitamins called cobalamins, which are important in red blood cell and DNA formation. Cyanocobalamin (CNCbl) is the most stable form of cobalamin and is a commonly used form of B12 added to foods including breakfast cereals. In the work described here, breakfast cereals with various major ingredients (such as rice, wheat, corn, and oat) were analyzed for vitamin B12 after sample extraction using sodium acetate (NaAc) buffer and analyte preconcentration on a solid phase extraction (SPE) sorbent. High-performance liquid chromatography (HPLC) temporally isolates the cobalamin for analysis and quantification by inductively coupled plasma-mass spectrometry (ICP-MS). Each cobalamin molecule contains cobalt which allows vitamin B12 to be quantified using ICP-MS. The method was recently developed and validated at the FDA for

infant formula analysis and the present study aims to examine the possibility of its extension to include breakfast cereals. [1]

1. Mesay M. Wolle. Jordan Escavage and Patrick Gray, Method development for the determination of vitamin B12 in infant formulas and toddler beverages, Poster presented at European Winter Conference on Plasma Spectrochemistry, Ljubljana, Slovenia, Jan. 2023.

- **Methods**
  - A measured portion of homogenized cereal (1–2 g) was mixed with 10 mL of 0.25 M NaAc (pH 4.5). The mixture was treated with 0.25 mL of 1% (w/v) sodium cyanide (NaCN) and heated in a hot block to 95°C for 45 min to convert the cobalamins in the sample to CNCbl. The extraction mixture was cooled to room temperature, mixed with α-amylase (300 µL) and incubated in a water bath at 40°C for 30 min to breakdown the starch in the matrix. The mixture was centrifuged, and the supernatant was filtered through a 0.45 µm pore size filter. Extracted vitamin B12 was preconcentrated on an Oasis HLB C18 SPE sorbent, eluted with methanol, and reconstituted with 0.5 mL of 0.1% (v/v) formic acid after evaporating the organic solvent. Finally, the reconstituted extract was analyzed by HPLC-ICPMS with isocratic separation using a Agilent Zorbax Eclipse C18 reversed-phase column with a mobile phase consisting of 0.1% (v/v) formic acid in a 30:70 methanol-water mixture.
- **Results**
  - The concentration of vitamin B12 was determined in breakfast cereal samples containing various ingredients.
- **Implications**
  - The results of this study verified the extension of the above-described method to analyze breakfast cereals for vitamin B12. The method will be single-lab validated to be used as a standard method at the FDA for monitoring vitamin B12 in such products.

5) **Abstract title:** *Evaluation of limits of quantification (LOQs) of per- and poly- fluoroalkyl substances (PFAS) in foods using FDA's Method regarding the European Union Reference Laboratory Guidelines*
**Authors:** Maria Moreno Santiago, FDA/CFSAN (Student); Christine Fisher, FDA/CFSAN; Brian Ng, FDA/CFSAN; Susan Genualdi, FDA/CFSAN (Mentor); Lowri deJager, FDA/CFSAN
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**

- There are limited data on per- and poly- fluoroalkyl (PFAS) compounds in regularly consumed foods. More data is needed to better assess dietary exposure to these chemicals in foods. The European Union Reference Laboratory (EURL) for Halogenated Persistent Organic Pollutants in Feed and Food (POPs) has published a guidance document with different recommendations that state PFAS of interest, analytical method parameters, and limits of quantification (LOQs), among other aspects. Also, the EURL has published Commission Regulation 2022/2388, where the maximum levels of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) that are considered acceptable in foods have been established. Hence, this project focused on evaluating the FDA method for PFAS in foods using these guidelines for its performance to meet EURL's required LOQs. The quantification and comparison of LOQs for four PFAS components in five food matrices (lettuce, eggs, milk, clams, and salmon) using three different liquid chromatography and mass spectrometry instruments (Sciex 6500 plus, Sciex 7500, and Sciex 7600) was carried out. Samples from these various matrices were extracted using QuEChERS and Solid Phase Extraction (SPE). This presentation will include details about the outcomes, challenges, and interferences associated with this project.

- **Purpose**
  - To evaluate the FDA method for its performance to meet European Union Reference Laboratory (EURL) for Halogenated Persistent Organic Pollutants in Feed and Food (POPs) required LO To evaluate the FDA method for its performance to meet European Union Reference Laboratory (EURL) for Halogenated Persistent Organic Pollutants in Feed and Food (POPs) required LOQs
- **Methods**
  - Samples were extracted using QuEChERS and Solid Phase Extraction (SPE). Three different liquid chromatography and mass spectrometry instruments were used for analysis. (Sciex 6500 plus, Sciex 7500, and Sciex 7600)
- **Results**
  - Limits of quantification for PFAS were determined for 5 different food matrices on three different instruments and compared.
- **Implications**
  - This determination of LOQs for PFAS in different foods on three different instruments provides insight and information on which type of instrument can meet method requirements set by the European Union Reference

Laboratory (EURL) for Halogenated Persistent Organic Pollutants in Feed and Food (POPs).

6) **Abstract title:** *Evaluation of a Polymerase Chain Reaction (PCR) to detect toxic Digitalis species*
**Authors:** Fo, Sydnee, FDA/CFSAN (Student); Hunter, Elizabeth Sage, FDA/CFSAN (Mentor); Literman, Robert, FDA/CFSAN (Collaborator); Wolny, Jennifer, FDA/CFSAN (Collaborator); and Handy, Sara, FDA/CFSAN (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
  - Digitalis, a botanical genus known for its colorfulness, toxicity, and medicinal properties, possesses bioactive compounds, which even in minute quantities can cause adverse effects. Recent research conducted by Hunter, Literman, and Handy (Foods, 2021), leveraged advanced data analysis and single nucleotide polymorphisms (SNPs) to identify Digitalis species, the present study aims to capitalize on the SNP library established in their work to develop a rapid PCR assay to detect Digitalis species. By doing so, the study endeavors to enhance the range of DNA-based identification tools available to FDA-CFSAN, thereby contributing to the agency's efforts in promoting consumer safety.
- **Purpose**
  - Safeguarding consumer well-being and maintaining public health necessitates ensuring that Food and Drug Administration (FDA) regulated products do not contain toxic ingredients or contaminants. This can become particularly challenging when dealing with complex mixtures of botanical ingredients, where there is a need to both identify and confirm the presence of known botanical ingredients and determine if any undeclared botanical ingredients are present. Therefore, developing methods which can address both issues in a single, rapid test is beneficial to effectively and efficiently monitoring FDA regulated products.
- **Methods**
  - Based on previous work from our group, we have developed 10 pairs of Digitalis-specific primers and are testing a streamlined assay to effectively detect these plant species in a simplified way. The primer sets will be tested on ~60 Digitalis species, Digitalis plant parts and other closely related relatives to determine specificity. DNA extractions were done with the Qiagen DNeasy Plant kit and PCRs with the Invitrogen Platinum Taq DNA Polymerase.

- **Results**
    - For this work, ~60 samples have been extracted from leaf tissue of vouchered specimen. Ten pairs of PCR primers are being tested against these samples which produce PCR amplicons that range in size from 211 to 802 bases. General plant primers are also being used to determine if extracted DNA is usable and free of inhibitors.
- **Implications**
    - Digitalis, a botanical genus known for its colorfulness, toxicity, and medicinal properties, possesses bioactive compounds, which even in minute quantities can cause adverse effects. Recent research conducted by Hunter, Literman, and Handy (Foods, 2021), leveraged advanced data analysis and single nucleotide polymorphisms (SNPs) to identify Digitalis species, the present study aims to capitalize on the SNP library established in their work to develop a rapid PCR assay to detect Digitalis species. By doing so, the study endeavors to enhance the range of DNA-based identification tools available to FDA-CFSAN, thereby contributing to the agency's efforts in promoting consumer safety.

7) **Abstract title:** *DNA barcoding of Cannabis-derived products*
**Authors:** Turner Jr.**,** Michael A., FDA/CFSAN (Student); Dubrow, Geoffrey A., FDA/CFSAN (Collaborator); Handy, Sara M., FDA/CFSAN (Mentor); Hunter, Elizabeth Sage FDA/CFSAN (Mentor)
**FDA Strategic Initiative:** Empowering patients and consumers
**Abstract:**

- **Synopsis**
    - DNA barcoding in processed consumer products can be challenging due to degradation of genetic material during processing and storage, so it is important to determine which types of consumer products retain adequate amounts of DNA for amplification. In this study, we attempt to define the most efficient method for recovering DNA from raw materials, crude extracts, refined extracts, and laboratory-formulated model products for amplification. This work will serve as a proof-of-concept and aid in the development of an effective identification system for plant-derived material in consumer products.
- **Purpose**
    - There is a growing market within the United States for Cannabis-derived products (CDPs), including those containing hemp-derived cannabidiol (CBD). The CDP market is highly diverse and includes products ranging from raw plant materials to edible products formulated with crude hemp extracts or purified isolates. Methods to facilitate the identification of plant-derived

material in consumer products will help better understand this diverse and growing market. DNA barcoding is a technique that examines specific regions of DNA to identify inter- and intra-species genetic differences; it has been used successfully in many systems, including raw Cannabis plant material, and could be an effective detection method for identification of botanical ingredients in consumer products. DNA barcoding in processed consumer products can be challenging due to degradation of genetic material during processing and storage, so it is important to determine which types of consumer products retain adequate amounts of DNA for amplification.

- **Methods**
    - Crude extracts were generated via extraction of dried hemp. Crude extracts were then refined by a series of steps including vacuum concentration followed by thermal decarboxylation of cannabinoids, and subsequent formulation of extracts into model products. At each stage, DNA extraction was attempted using Qiagen extraction kits (DNeasy Plant Mini Kit and DNeasy mericon Food Kit). Protocol modifications were tested to increase the yield of DNA recovered.
- **Results**
    - The quantity and quality of DNA recovered from extractions were visualized and quantified using a Qubit fluorometer and NanoDrop spectrophotometer. A Cannabis specific mini barcode assay was tested for specificity and then evaluated for use in amplifying highly degraded DNA from different CDP materials, using unprocessed Cannabis tissue as a control.
- **Implications**
    - Future work could exploit the polymorphisms in the tetrahydrocannabinol acid (THCA) synthase gene, which are linked directly to plant phenotype, and may be able to differentiate between THC-dominant and CBD-dominant strains of Cannabis in processed products. This work will serve as a proof-of-concept and aid in the development of an effective identification system for plant-derived material in consumer products.

8) **Abstract title:** *Hazard Identification of Printing Ink Substances Used on the Exterior of Food Packaging*
**Authors:** Verma, Risa, FDA/CFSAN (Student); Markley, Laura, FDA/CFSAN (Mentor); Bandele, Omari, FDA/CFSAN (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**

- Photoinitiators are molecules that create reactive species when exposed to radiation and are widely used in UV-cured printing inks applied to the exterior of food contact materials (e.g., coatings and varnishes). New data indicates that, for some intended uses, photoinitiators applied to the exterior of food packaging may migrate to food. This project performed a hazard characterization and prioritization analysis of photoinitiators to determine those that may need further investigation and potential regulation.

- **Purpose**
  - The purpose of this project is to perform hazard characterization of ~100 photoinitiators used in printing ink substances applied to the exterior of food packaging.
- **Methods**
  - A new approach methodology (NAM) was applied using the ChemTunes-ToxGPS database and in silico workflows to determine the Cramer classification and genotoxicity predictions for each of the identified photoinitiators. The Cramer Decision Tree (CDT) uses chemical structure and predicted chemical reactivity of a substance to categorize substances into three classifications with Class III substances representing the highest predicted toxicological hazard.
- **Results**
  - Based on the Cramer classifications and predicted genotoxicity, the photoinitiators were characterized and prioritized for further analysis to identify existing toxicity data in FDA's Chemical Evaluation and Risk Estimation System (CERES) and available literature.
- **Implications**
  - This analysis helped determine those photoinitiators that may need further investigation for their intended use, potential migration into food, and possible consideration for further regulation. This qualitative hazard identification approach supports the Agency's mission of incorporating the 3 R's (i.e., Replacement, Reduction, and Refinement for promoting ethical research, testing, and education using animals) into safety assessments. Future work will be to use this new approach methodology (NAM) to characterize and prioritize an estimated >5,000 ink substances and ink components potentially used in printing inks applied on the exterior of food packaging in U.S. markets.

9) **Abstract title:** *Investigating State Food Freedom Laws: Implications for the Future of Retail Food Safety*

**Authors:** Wright, Madison, FDA/CFSAN (Student); Liggans, Girvin, FDA/CFSAN (Mentor); Williams, Laurie, FDA/CFSAN (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**


- **Synopsis**
    - This study examined the influence that food freedom laws and the autonomy of food freedom operations have on the dynamics of retail food safety. The overall objective was to thoroughly investigate how the presence or absence of these laws may impact food safety practices in the aspects of production, sale, and distribution. The research methodology that was utilized involved reviewing argumentative essays and literature on food freedom laws. To assess the current relationship between food freedom and retail food safety, a comprehensive review was conducted. The findings intended to present a well-constructed argument that would influence the readers perspective on the subject. As a result, the study offered valuable insights to the U.S. Food and Drug Administration regarding the potential impacts of expanding food freedom laws. Ultimately, the research enhanced the understanding and clarification on the broader implications of cottage food operations' ability to operate outside of traditional protocols. It was able to highlight the perceived inequity in food freedom laws permitting cottage farms, home-based restaurants, etc., that produce, cook, and sell foods from private residences with minimal regulatory oversight, although public retail food establishments must still abide by specific state laws to operate and are subject to inspections by local jurisdictions, which may align with the FDA's Food Code guidelines.
- **Purpose**
    - This study investigated the impact of food freedom laws and the autonomy of food freedom operations such as cottage farms, home-based restaurants, etc. on the dynamics of retail food safety. It has aimed to examine how regulatory oversight, or lack thereof, influences the nature of food safety practices in the aspects of production, sale, and distribution.
- **Methods**
    - The research methodology that was utilized for this study entailed learning about argumentative essays and literature reviews. To investigate the status of the relationship between food freedom laws and retail food safety, a preliminary review of resources was conducted. A full-scale review was then performed by carefully gathering, analyzing, and synthesizing information to address the research objective and ultimately curate and present a well-

constructed argument influencing the readers perspective on the subject matter.

- **Results**
  - The results of this study provided the U.S. Food and Drug Administration with insight on whether the expansion of food freedom laws will hinder regulatory agencies' ability to prevent all incidence of food borne illnesses. Overall, the research yielded more knowledge and clarity on the overall implications of states allowing for food freedom operations to operate beyond traditional protocol.
- **Implications**
  - For the retail food industry, it may be seen as inequitable for food freedom laws to allow food freedom operations to produce, cook, and sell food from a private residence with little to no regulatory oversight. While public retail food establishments are required to abide by certain state laws to keep the establishment up and running. These retail food establishments are also subject to inspection by local jurisdictions. Beyond that, these local jurisdictions may follow the guidelines set forth by the FDA via the Food Code.

10) **Abstract title:** *The Impact of Live Microbials and Emulsifiers on Gut Epithelial Barrier Integrity and Function*
**Authors:** Xi, Cynthia, FDA (Student); Khuda, Sefat, FDA (Mentor)
**FDA Strategic Initiative:** Empowering Patients and Consumers
**Abstract:**

- **Synopsis**
  - The gut epithelium barrier serves the unique function of selectively allowing passage of nutrients into the body, while limiting entry of harmful molecules and foreign antigens. Dietary components have the potential to alter gut barrier permeability and functions. Establishment and utilization of intestinal epithelial cell co-culture models will assist in providing scientifically important information for understanding the complex effects of various dietary components on intestinal barrier function.
- **Purpose**
  - The gut epithelium barrier serves the unique function of selectively allowing passage of nutrients into the body, while limiting entry of harmful molecules and foreign antigens. Dietary components such as live microbials, polyphenols, lipids, proteins, or a combination thereof have the potential to alter gut barrier permeability and may affect the expression of tight junction genes, mucin production, or inflammatory signals. Compromised tight junctions may contribute to the pathology of obesity, inflammatory bowel

disease, autoimmune disease, allergic disease, and colon cancer, among other diseases. The interaction of common food ingredients (emulsifiers and live microbials) with the intestinal epithelial barrier has not been thoroughly evaluated; thus, the purpose of this study is to establish co-culture models that simulate the intestinal epithelium in order to investigate the effect of mixed food components on gut barrier function and integrity.

- **Methods**
  - Human intestinal epithelial cells (Caco-2: goblet HT-29-MTX) will be mixed to represent small (90:10) and large (75:25) intestinal barriers. Monolayers of Caco-2, HT-29-MTX, and co-cultures will be treated with emulsifier polysorbate (P)-80, and live microbials: Lactobacillus acidophilus (LA), Streptococcus thermophilus (ST), and Bacillus subtilis (BS). The trans-epithelial electrical resistance (TEER), reactive oxygen species (ROS), cellular viability, and expression of biomarkers (tight junction, mucin, and cytokines genes) will be examined.
- **Results**
  - In our previous study, about 20% reduction of monolayer integrity was observed with the use of 0.2% low cytotoxic concentration P-80 in TEER measurements (Khuda et al., 2022). Other published research indicated that live microbial-containing foods such as yogurt have the potential to improve gut barrier function and to regulate tight junction-associated proteins (Putt et al., 2017). Results of our ongoing project have yet to be determined. Based on the above-mentioned studies, we hypothesize that in the presence of emulsifiers (P-80) and live microbials (LA, ST, BS), monolayer integrity can be altered, and the potencies may vary among the selected microbials. Biomarkers (tight junction-associated proteins, cytokines, mucin production) and cell metabolic activity of this model will be analyzed to examine effects seen in other experiments conducted on live microbial research. Reference Citations: • Khuda, S. E., Nguyen, A. V., Sharma, G. M., Alam, M. S., Balan, K. V., Williams, K. M., Effects of Emulsifiers on an In vitro Model of Intestinal Epithelial Tight Junctions and the Transport of Food Allergens. Mol. Nutr. Food Res. 2022, 66, 2100576. https://doi.org/10.1002/mnfr.202100576 • Putt KK, Pei R, White HM, Bolling BW. Yogurt inhibits intestinal barrier dysfunction in Caco-2 cells by increasing tight junctions. Food Funct. 2017 Jan 25;8(1):406-414. doi: 10.1039/c6fo01592a. PMID: 28091645.
- **Implications**
  - Establishing intestinal epithelial cell co-culture models will assist in providing scientifically important information for understanding the complex effects of various dietary components on intestinal barrier

function. Additionally, these co-culture models more accurately mimic the gut epithelial barrier in comparison to monoculture models, allowing the obtained data on gut mucosal responses to be more reliable.

11) **Abstract title:** *Determination of patulin in apple juice and apple-based food products using an automated sample preparation system and LC-MS*
**Authors:** Zhang, Lauren, FDA/CFSAN (Student), Zhang, Kai, FDA/CFSAN (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
  - An atmospheric pressure chemical ionization (APCI) with liquid chromatography tandem mass spectrometry (LC-MS/MS) method will be developed to determine patulin in apple juice and apple-based food. Different ionization parameters, extraction solvents, and LC conditions will be evaluated. Apple juice and apple-based food samples will be fortified with 13C uniformly labelled patulin and extracted using dichloromethane, followed by LC-APCI-MS/MS analysis. The method will be validated following the FDA Guidelines for the Validation of Chemical Methods for the FDA Foods Program (3rd Ed.).
- **Purpose**
  - Patulin is a mycotoxin produced by Penicillium, Aspergillus, and Byssochylamys molds that grow on fruit, grains, and cheese. The best-known example is patulin in juice or cider made from apples. The FDA has set an action for patulin in apple juice and apple juice products at 50 ppb. To monitor this mycotoxin in apple-derived food products, there is a need for adequate analytical methods that can reliably determine patulin. In this study, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method will be developed and validated for patulin in apple juice and other apple-derived food products. The method will assist the Agency in gathering data on the occurrence of patulin in these commonly consumed food products.
- **Methods**
  - Following the FDA Guidelines for the Validation of Chemical Methods for the FDA Foods Program (3rd Ed.), we will single laboratory validate (Level 2) an LC-MS/MS method for the quantification of patulin by stable isotope dilution in apple juice and apple-derived food products. Method performance metrics including accuracy, precision, and method limit of detection will be evaluated, and the influence of sample matrix interferences will also be assessed. The study will also compare manual sample preparation to an automated workflow using robotic tools to streamline sample preparation for routine analysis.

- **Results**
    - A liquid chromatography–tandem mass spectrometry (LC-MS/MS) method will be developed for the detection and quantification of patulin. Briefly, juice samples will be processed using stable isotope dilution procedures, followed by LC-MS determination. Multiple reaction monitoring (MRM) conditions will be optimized in negative ionization mode. Preliminary recovery studies will be performed to evaluate feasibility of the method in an apple juice, apple sauce, and apple cider matrix fortified at 10, 50, 200, and 1,000 ng/g patulin.
- **Implications**
    - Presently, FDA field laboratories use AOAC 995.10 for the quantification of patulin in apple juice by liquid chromatography with optical detection, and confirmation by mass spectrometry. The development of an LC-MS/MS based method provides a modernized approach to enable the quantification and confirmation of patulin in a single method, further expanding testing capabilities for monitoring patulin in apple juice and apple-derived food products.

12) **Abstract title:** *Cost-Benefit Analysis LC-MS vs. TLC/LC-UV/FLD for Mycotoxin Determination in Foods*

**Authors:** Zhang, Lauren, FDA/CFSAN (Student), Zhang, Kai, FDA/CFSAN (Mentor)

**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**

- **Synopsis**
    - Cost-effectiveness is an important but underexplored factor to consider alongside specificity and sensitivity when comparing different analytical methods for mycotoxins. A major benefit of liquid chromatography-tandem mass spectrometry (LC-MS/MS) is its ability to perform multi-mycotoxin analysis. However, its high initial cost seems to be a drawback, preventing mycotoxin laboratories from assuming the method for routine analysis. To compare the cost-effectiveness of different methods, a cost benefit analysis model was developed taking into consideration various contributing factors which demonstrated the savings possible with LC-MS.
- **Purpose**
    - An important factor in deciding between different mycotoxin methods with different fixed (instrumentation, training, etc.) and variable (materials and labor) costs is comparative cost effectiveness. In the current regulatory sample analysis model used by the FDA mycotoxin program, single or single class mycotoxin methods are frequently used to screen for various regulated mycotoxins separately. On the other hand, LC/MS can analyze multiple mycotoxins simultaneously and has been demonstrated to be superior to TLC and LC-UV/FLD in terms of identification (specificity) and quantitation (sensitivity). However, the initial investment for LC/MS instrumentation impedes the adoption of this technology by mycotoxin laboratories for routine sample analysis. In this presentation, we compare

cost effectiveness of screening models by single mycotoxin methods (TLC and LC-UV/FLD) and LC/MS over predefined time horizons and sample throughput capacities using total present value of costs and benefits, and cost per analysis/sample.

- **Methods**
  - The costs of TLC, LC-UV/FLD, and LC-MS were estimated using labor costs (time per analysis and hourly wage), initial costs, material costs required per sample, discount rates, and the number of samples and mycotoxins needed to be analyzed over a ten-year time period. Then the benefits in the form of cost savings by using LC-MS for multi-mycotoxin analysis were estimated.
- **Results**
  - Our findings show that with the baseline of 1,000 samples, LC-MS could save around $2.12 million compared to TLC and $2.21 million compared to LC-UV/FLD. With the 3% discount rate applied, LC-MS still saves $1.8 million compared to TLC and $1.89 million compared to LC-UV/FLD, and with a 7% discount rate, $1.47 million compared to TLC and $1.56 million compared to LC-UV/FLD. However, for a laboratory with a low sample throughput, LC-MS would be a costly and unrecommended method. For 100 samples/year, LC-MS costs about $0.2 million more than to TLC and $0.14 million more than LC-UV/FLD. The cumulative costs associated with the three yearly sample capacities (100, 1,000 and 10,000 sample per year) clearly illustrate the benefit of LC-MS for the analysis of large number of samples (e.g., 1,000 or 10,000 per year).
- **Implications**
  - With this mathematical representation of the costs associated with each method, it becomes clear that LC-MS is an efficient and beneficial alternative to legacy methods of sample analysis. Despite higher initial costs, LC-MS can save organizations millions of dollars in the long run, depending on their throughput. The main factor regarding cost that sets LC-MS apart is its ability to perform multi-mycotoxin analysis, which saves both effort and money. Technological advancements such as LC-MS could drastically change the paradigm of mycotoxin analysis and replace conventional single mycotoxin analysis, but only laboratories that are willing to invest in expensive LC-MS equipment could take advantage of the benefits of this technology. The best way to take advantage of LC-MS technology in the laboratory is to estimate and to reach the economies of scale for sample analysis capacity.

## Center for Veterinary Medicine (CVM)

1) **Abstract title**: *A Review of the Information Available of the Risks and Benefits of Copper Sulfate and Potassium Permanganate for Therapeutic Use in Aquaculture*
   **Authors:** Baldonado, Kenny, FDA/CVM (Student), Cornwell, Emily FDA/CVM (Mentor)
   **FDA Strategic Initiatives:** Unleashing the Power of Data
   **Abstract:**

- **Synopsis**
    - Aquaculture is a growing industry that involves the harvesting, breeding, and rearing of many organisms in aquatic environments. With the growth of this industry there is an increased need for therapeutics to prevent and treat diseases in aquaculture. Bacterial, viral, fungal, and parasitic outbreaks can cause significant losses in breeding efforts and even the death of fish, resulting in economic consequences for farmers. Two particular compounds, copper sulfate and potassium permanganate, have been extensively studied over time for possible therapeutic use in aquaculture but are not approved by the U.S. Food and Drug Administrations (FDA). Currently these two compounds are listed in a special deferred enforcement category (pending further study) in the Policy and Procedures Manual 1240.4200 "Enforcement Priorities for Animal Drugs Used in Aquaculture" (2002). This deferred enforcement has created a lack of clarity on their use for the aquaculture community. Since 2002, additional information and data are available regarding these compounds that may allow FDA CVM to determine a permanent category for these two compounds.
- **Purpose**
    - The purpose of this study is to review currently available information on human, environmental, and animal safety related to the drugs copper sulfate and potassium permanganate for use as therapeutics in fish.
- **Methods**
    - This study will be conducted by gathering and analyzing published literature from online databases and from CVM files. A thorough review of the peer-reviewed literature will be conducted by searching multiple online databases. In addition, CVM will review internal records, including public master files.
- **Results**
    - More than twenty-five peer-reviewed studies and CVM files were found. These resources will be reviewed and assessed to evaluate the risks and benefits of copper sulfate and potassium permanganate. This evaluation can serve as an internal resource for CVM in determining a permanent category for these two compounds.
- **Implications**
    - The results of this review may assist CVM in reconsidering any changes needed to the Policy and Procedures Manual to provide more clarity to the aquaculture community regarding the use of these two compounds to treat and prevent disease in fish.

2) **Abstract title:** *Exploring Biological Effects of Excipients: Development of a User-Friendly Database*

**Authors:** Thierry Laguerre, FDA/CVM (Student), Marilyn N. Martinez (Mentor)

**FDA Strategic Initiatives:** Empowering Patients and Consumers

**Abstract:**

- **Purpose**

- Dosage forms contain excipients which can influence drug pharmacokinetics. The influence of many excipients can be identified through in vitro dissolution testing. However, there are others that exert an in vivo effect not identifiable by traditional in vitro methods. The FDA's Center for Veterinary Medicine (CVM) has been developing a spreadsheet of published in vivo excipient effects, but that spreadsheet has been difficult to navigate. Therefore, the purpose of this project was to build a reliable, consistent, and easily accessible database that can be used by the CVM review staff to improve the efficiency of their search for research materials and in the managing of data. An additional purpose of this database is also to ensure autonomy of updates. Creating one database tool that can be accessed by Center of Veterinary Medicine professionals with consistent and updated information is a prime goal.

- **Methods**
  - Formulas were developed that simplified the data such that the spreadsheet could be easily searched. The file was divided into several sheets containing varying levels of information and corresponding citations, thereby allowing the reviewer to interrogate the files at levels of complexity consistent with their objective. The primary objective was to create an accurate database, therefore ensuring that every section was properly labeled and reviewed was of utmost importance. Next, with the anticipation of added information along the road, the design had to be scalable. This led to an increase in the use of application formulas that would be referenced and dereferenced throughout the datasheets. Furthermore, efficiency was key in making a consistent database that could be interpreted by professionals without third-party mediation. This took the shape of reducing redundant data and allowing new features such as isolated comparison charts. We have also emphasized constant testing of the database to ensure that the database is fully utilized in real-world scenarios. It was also important that formulas and referencing was used as much as possible as well.

- **Results**
  - The initial version of the spreadsheet was shared with the review staff within CVM's Office of New Drug Evaluation (ONADE), providing them with an overview of the attributes and requesting input. The Excipient Database was placed on an internal SharePoint site so that it could be accessed by anyone within CVM/ONADE. The project provided an opportunity to not only explore the current contents but also to expand the entries as more information becomes available. Prior to completion of the summer internship program, an instruction sheet will be written that informs all users how to search and modify entries, thereby making this a living

document. The searchable tool has drastically decreased the time it takes to find information. The search tool is not case sensitive, and it is able to find partial matches and display all information needed. Furthermore, within the database professionals can selectively filter and isolate numerous sections and compare them hand in hand. Next, we have been able to successfully improve the readability of data with text abbreviations, aliases, and correction of misleading words/symbols.

- **Implications**
    - As the ONADE review staff receive submissions containing changes in formulation, for example, the type of excipient used or the amount of excipient added), this tool will be a valuable resource to support their regulatory research decisions. Ultimately, the goal is to prevent unnecessary in vivo study requirements when in vitro dissolution would suffice for addressing product comparability and will flag those changes where in vitro dissolution may not adequately reflect in vivo product performance (and therefore, an in vivo bioequivalence study should be required). Additionally, the database will positively influence data sharing and security. Professionals will be able to share data simultaneously and respond effectively. It also helps that data will be integrated, in the case that information is updated in the database, providing an updated version of the database. This improved format will help reduce the number of mistakes and errors in the research process

## National Center for Toxicological Research (NCTR)

1) **Abstract title:** *Challenges and solutions in measuring commonly used hepatotoxicity biomarkers in a liver-on-a-chip platform*
   **Authors:** Dai, Emily, FDA/NCTR (Student); Ren, Lijun, FDA/NCTR (Mentor); Schnakenberg, Laura, FDA/NCTR (Mentor); Shi, Qiang, FDA/NCTR (Mentor)
   **FDA Strategic Initiative:** Increasing Choice and Competition through Innovation
   **Abstract:**
    - **Synopsis**
        - Liver-on-a-chip (liver-chip) presents a novel way to culture hepatic cells in vitro and holds the promise to recapitulate in vivo liver responses. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are proposed translational biomarkers for liver-chips. When Emulate liver-chips were used to examine acetaminophen-induced hepatotoxicity, it was found that ALT activity was unchanged in the presence of significant cell death. Follow up studies showed that ALT activity decreased by 50% every 24 h in the culture medium, suggesting ALT activity was not well preserved under cell culture conditions. In

contrast, AST activity was stable in the liver-chips, but its activity level was too low to be used to determine the percentage of cell death. To address these issues, an enzyme-linked immunosorbent assay (ELISA) was used to measure protein levels of ALT and AST. It was found that ALT and AST protein levels correlated very well with the gold standard cell death assay, the release of lactic dehydrogenase (LDH). These data suggest that ALT and AST activities are not ideal translational biomarkers in Emulate liver-chips, but their proteins levels might be an alternative to observe in vivo and in vitro correlations regarding liver responses.

- **Purpose**
  - Liver-on-a-chip (liver-chip) is a microphysiological system (MPS) designed to better maintain hepatic functions and viability of liver cells cultured under in vitro conditions. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are clinically used biomarkers for drug-induced liver injury (DILI) and are therefore the proposed hepatotoxicity biomarkers in liver MPS. In our study of acetaminophen hepatotoxicity, we used Emulate® liver-chips, which coculture primary hepatocytes (PH) with non-parenchymal cells (NPCs) consisting of sinusoidal endothelium, Kupffer, and stellate cells. We unexpectedly found that commonly used assays for ALT and AST activities did not work in liver-chips, and efforts were undertaken to address this issue.
- **Methods**
  - Commercial human PH and NPCs were thawed and cultured in the top and bottom channels, respectively, of the Emulate® liver-chips. The cells were perfused using culture medium. The spent medium, also called effluent, was collected to determine ALT and AST. The cells were lysed using 1% Triton X-100, and then the lysate was stored in the cell culture incubator for 0, 24, 48, and 72 h. ALT and AST activities were measured using the method recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC method). In parallel, an enzyme-linked immunosorbent assay (ELISA) was used to determine protein levels of ALT and AST.
- **Results**
  - The activities of ALT and AST of the effluent from intact cells were undetectable. In the lysed cells, ALT and AST activity were 20 and 70 U/L, respectively. However, the activity of ALT, but not AST, spontaneously decreased by about 50% every 24 h when the effluents were stored in cell culture incubator, suggesting that ALT activity is not suitable for measuring cell death in the liver-chips. In contrast, the protein levels of ALT and AST measured by ELISA were stable over time and readily detectable in both

intact and lysed cells, and their changes showed good correlation with the release of lactic dehydrogenase (LDH), the gold standard cell death assay.

- **Implications**
    - Our results suggest that assays of clinically used biomarkers need to be optimized for liver MPS for the development of translational biomarkers bridging the gaps between in vitro and in vivo findings.

2) **Abstract title:** *Lipidomics Evaluation of the Impact of Fentanyl Treatments on Neural Cells*
**Authors:** Donakonda, Rohini, FDA/NCTR (Student); Sun, Jinchun, FDA/NCTR (Mentor); Wang, Cheng, FDA/NCTR (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Untargeted lipidomics analysis was conducted to assess the vulnerability of neural stem cells (NSC) exposed to fentanyl, a potent opioid analgesic used for medical pain management. To examine the impacts of different dosages of fentanyl on neural cells, particularly focusing on lipidome changes. Lipidomics data show that fentanyl exposure might cause ceramide pathway disturbance, potentially due to fentanyl-induced neurotoxicity. The study's preliminary data provided insights into the underlying mechanisms of fentanyl-induced neurotoxicity on NSCs.
- **Purpose**
    - Fentanyl is a potent opioid analgesic used for pain management in medical settings. Because it is impossible to obtain critical dose-response and time-course data on anesthetic/analgesic-induced neural damage in human infants or children, the utilization of NSC models, especially cells derived from humans, might be a good tool to evaluate the vulnerability of the nervous system. This study aims to investigate the underlying mechanisms of fentanyl-induced neurotoxicity on NSCs using ultra-high-performance liquid chromatography (UHPLC) and high-resolution mass spectrometry (HRMS)-based lipidomics approaches.
- **Methods**
    - The neural stem cells (NSCs) were treated with a vehicle (control) or fentanyl at concentrations of 1 µM, 10 µM, or 100 µM. Lipids were extracted from the NSCs using a modified Bligh and Dyer extraction protocol. After extraction and drying, the samples were reconstituted in ethanol and centrifuged before LC/MS analysis. UHPLC/MS was performed using a Thermo Accucore C30 column coupled to a Thermo Orbitrap Exploris 240 mass spectrometer. The chromatography conditions included a 30-minute gradient with a flow rate of 0.4 mL/min and a temperature of 40 °C. The mobile phase consisted of solvent A (60% acetonitrile with 0.1% formic acid) and solvent B (10% acetonitrile, 90% isopropanol with 0.1% formic acid). Mass spectrometric data were collected using the Thermo Orbitrap Exploris 240 mass spectrometer in full-scan mode. Data analysis was performed using LipidSearch software, and statistical analysis included

supervised partial least squares discriminant analysis (PLS-DA) and hierarchical clustering. The significance level was set at $p < 0.05$. The untargeted analysis involved normalization and log transformation of ion features before statistical analysis.

- **Results**
  - In total, 919 lipid species from 20 lipid classes were detected and identified. Among the detected 20 lipid classes, the total abundance of cholesterol ester and sphingosine classes significantly decreased while ceramide and di-hexosylceramide classes significantly increased (>2 folds increases) in the high-dose group vs. the control. The unchanged Acyl carnitine (16:0) was consistent with the MTT data (a marker of mitochondrial health) that mitochondrial was not damaged by fentanyl treatments, Ceramide pathways (play important roles in neural cell inflammation) disturbance was consistent with substantial increases in PSA-NCAM immune-reactivity that fentanyl treatments might induce neurotoxicity.
- **Implications**
  - This preliminary data indicated that the ceramide pathway was disturbed by fentanyl treatments, which might provide the underlying mechanisms of fentanyl-induced neurotoxicity. The findings of this study might contribute to a broader understanding of the effects of fentanyl on neural cells and may help develop targeted interventions to reduce adverse effects.

3) **Abstract Title***: Longitudinal Study to Assess Change in the mRNA Expression of Genes related to Intestinal Permeability During Long term consumption of High Fat High Sucrose Diet*
**Authors**: Gannon, Adelaide, NCTR (Student), Kumari Karn, NCTR (Mentor), Kuppan Gokulan, NCTR (Mentor), Sangeeta Khare, NCTR (Mentor)
**FDA Strategic Initiative:** Empowering Patients and Consumers
**Abstract:**

- **Synopsis**
  - Disturbance in the gastrointestinal homeostasis, specifically the disruption of the intestinal barrier plays an important role in the development and progression of extraintestinal diseases. The current study is focused to delineate if there are differences in the intestinal permeability in two strains of CC mice (CC042 and CC011) that showed highly distinguished responses to the diet. Moreover, in this study, a longitudinal assessment of long-term (20, 40 and 60 weeks) consumption of High Fat/High Sucrose diet was conducted to see the impact in the progression of changes at the gastrointestinal tract. Ileum tissue from male and female mice fed either control or HF/HS diets for 20, 40, and 60 weeks were used in this study. RNA from the ileum was extracted and converted to cDNA to determine mRNA expression of the genes involved in gut permeability using Real Time PCR. The fold change in the gene expression in the HF/HS diet as compared to control, for each of the time point groups were then computed to determine changes in gene expression. The data has suggested that a high fat, high sucrose diet affects the expression of genes responsible for gut permeability. Detailed comparison of diet effect (20, 40 and 60 weeks) in

these two CC strains is ongoing and will be presented. This research addresses the knowledge-gap how the genetic/microbial diversity respond to a selection pressure (diet).

- **Purpose**
  - The purpose of the experiment was to determine if mRNA expression of intestinal permeability related genes were altered when mice with different genetic background were fed a high fat, high sucrose (HF/HS) diet. Disturbance in the gastrointestinal homeostasis, specifically the disruption of the intestinal barrier plays an important role in the development and progression of extraintestinal diseases. Towards this, the intestinal barrier effects and its role in the gut-liver axis is an emerging area of research in the Non-Alcoholic Fatty Liver Disease (NAFLD). The NAFLD is also associated with the life-style factors such as a high fat, high sugar diets. In an earlier study 25 strains of Cross Collaborative mice (a population of mice designed to approximate the genetic variability of the human population; hereon called CC mice), showed strain and sex-specific differences in the development of liver pathologies when fed high fat, high sucrose diet (HF/HS) for 16 weeks. The current study is focused to delineate if there are differences in the intestinal permeability in two strains of CC mice (CC042 and CC011) that showed highly distinguished responses to the diet.
- **Methods**
  - Two strains of cross collaborative mice (CC011, which is known to be susceptible to NAFLD, and CC042, which is known to have natural resistance to NAFLD) were divided into two groups: i) control, which has a diet of 0% sucrose and 18% calories from fat, and ii) high fat, high sucrose diet, which had a diet consisting of 45.6% sucrose and 35% calories from fat. Banked samples (ileum tissue) from male and female mice fed either control or HF/HS diets for 20, 40, and 60 weeks, mice were used in this study. RNA from the ileal mucosa was extracted, converted to cDNA to determine mRNA expression of the genes involved in gut permeability using Real Time PCR. The fold change in the gene expression in the HF/HS diet as compared to control, for each of the time point groups were then computed using the gene globe analysis tool to determine fold changes in gene expression.
- **Results**
  - The results have suggested that there was limited change in the gene expression in CC042 at 20 weeks. However, there was a differential regulation of several genes responsible for intestinal permeability in the CC042, 40 weeks cohort. At 40 weeks, in HF/HS female mice, there is an upregulation of two genes responsible for focal adhesion, and one gene responsible for tight junctions compared to control females. In males, there is a downregulation of a gene responsible for focal adhesions. At 60 weeks, in HF/HS female mice, there is an upregulation of a gene responsible for tight junctions, and compared to control males, HF/HS mice at 60 weeks showed a downregulation of a gene responsible for gap junctions and an upregulation of a gene responsible for focal adhesions. Compared to control females, control males exhibited an upregulation of a variety of genes

responsible for focal adhesions, desmosomes, and tight junctions. HF/HS Male also experience an upregulation of two genes responsible for desmosome formation and gap junctions in comparison to HF/HS females.

- **Implications**
    - The implications of this experiment show progression in the change in intestinal permeability of HF/HS diets. Earlier studies with the same strain measured gene expression at 20 weeks and found little to no change in permeability, which contrasts with this study. Further examination of the 60 weeks cohort will be able to determine how intestinal gene expression changes as mice feed on a high fat, high sucrose diet. Furthermore, the CC042 strain is naturally resistant to NAFLD. Additional studies have been conducted on susceptible strain CC011, which shows large fluctuations of gene expression even in the 20-week cohort. Lastly, ongoing experiments investigating the fluctuation and dysbiosis of gut microflora in HF/HS diets are being performed. Gut microflora can also affect intestinal permeability when in imbalance, and this can further exacerbate the effects of a HF/HS diet. Therefore, this data could provide additional information about the advancement of NAFLD in at risk patients, while also being a target for therapeutic intervention.

4) **Abstract title:** *Evaluation of sampling volume for the detection of Burkholderia cepacia complex in water sources*
**Authors:** Yang, Hannah, FDA/NCTR (Student); Daddy Goah, Soumana, FDA/NCTR (Mentor); Ahn, Youngbeom FDA/NCTR (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Non-sterile aqueous medical products implicated in health care–associated outbreaks due to *Burkholderia cepacia* complex (BCC) contamination include ultrasound gel, nasal sprays, mouthwashes, preoperative skin solutions, and hand sanitizers. The purpose of our experiment was to evaluate different sampling volumes with direct enrichment, and membrane filtration methods to detect BCC based on feasibility. *Burkholderia cenocepacia* AU1054 cells were seeded to autoclaved distilled water, tap water, ground water, chlorhexidine gluconate (CHX) and benzalkonium chloride (BZK) solutions at known concentrations (1 colony-forming unit (CFU)/L, 10 CFU/L, 100 CFU/L and 1000 CFU/L) to evaluate different sampling volumes (10 mL, 20 mL, 100 mL and 200 mL). To confirm the presence of BCC on direct enrichment, quantitative polymerase chain reaction (qPCR) was performed. All tested sampling volumes can successfully lead to positive detections as low as 100 – 1000 CFU/L. We demonstrated that the 200 mL sampling volume, with a range of positive detections observed as low as 1 CFU/L, is a more sensitive alternative to 100 mL (>10 CFU/L) to detect BCC in autoclaved distilled water as well as antiseptic samples. These results highlight the importance of collecting

adequate volumes of samples using the traditional culture method for more sensitive detection of BCC.

- **Purpose**
  - *Burkholderia cepacia* complex, also known as BCC, are a group of bacteria species that are commonly found in aqueous environments. These species are known to proliferate in environments with limited resources. Predictably, these species have caused numerous contamination outbreaks in pharmaceutical products as well as water sources, and have caused serious respiratory infections in auto compromised individuals, and in patients diagnosed with Cystic fibrosis. It is imperative that water, one of the main raw ingredients for pharmaceutical products, is up to standard and that detection of BCC in water sources is reliable. In this study, we are evaluating different sampling volumes to recover and enrich BCC from distilled water, tap water, ground water, and antiseptics solutions (10 µg/mL chlorhexidine dihydrochloride (CHX), and 50 µg/mL benzalkonium chloride (BZK)). Our purpose was to evaluate data to understand whether the sampling volumes tested in standard examination techniques are sensitive enough to detect low enough concentrations of BCC. We also aimed at testing a higher sample volume (200 mL membrane filtration method) to evaluate detection sensitivity and determine if a higher sample volume is necessary to detect lower concentrations of BCC.
- **Methods**
  - *B. cenocepacia* AU1054 cells were grown on 1/10× Trypticase Soy Agar (TSA) at 30 °C for 24 hrs before being inoculated into autoclaved nuclease-free water (Qiagen) with a final optical density of 0.1 (approximate cell density = $1.5 \times 10^8$ colony-forming units (CFU/mL)) using the Synergy MX spectrophotometer. The cells were then serially diluted and spiked with 10 mL, 20 mL, 100 mL volume of distilled water, tap water, and antiseptic solutions (10 µg/mL CHX, and 50 µg/mL BZK) into specific concentrations of BCC (1, 10, and 100 colony forming units (CFU)/L). The samples were enriched with 1/10 TSB solution and incubated for 48 hrs at 30 °C. The detection of BCC was tested by measuring growth using optical density, and was confirmed with quantitative polymerase chain reaction (qPCR). Subsequently, to assess whether a higher volume can provide better sensitivity, we evaluated 200 mL sampling volume using filtration. We then finally assessed the presence of live BCC using the 200mL sampling volume, followed by 10 µM Propidium Monoazide (PMAxx)-qPCR assay.
- **Results**
  - All sample volumes containing 100 CFU/L BCC could be detected at different sampling volumes (*i.e.*, 10 mL, 20 mL, 100 mL and 200 mL). In general, the target organism can be recovered at a rate of at least 90% using 10 mL inoculation volume from 100 CFU/L, whereas less than a 30% recovery rate was observed in 10 CFU/L samples. In contrast, when 100 mL inoculation volume was used, more than 50% recovery rate can be observed in 10 CFU/L samples. Moreover, a 200 mL sample volume can invariably lead to the detection of as low as 1 CFU/L, regardless of the nature of the water

source. The 200 mL sample volume was tested for its ability to assess the presence of live BCC in surface waters using 10 µM PMAxx-qPCR. Only 2 out of 8 surface water samples examined were BCC positive, in contrast to 4 out of 4 tap water samples examined using RibB67 primers. Although the resulting amplicons were not sequenced yet, the 200 mL sample volume makes it useful as a presumptive test to detect BCC.

- **Implications**
    - These results highlight the importance of collecting adequate volumes of samples using the traditional culture method for a more sensitive detection of BCC. Although standard methods can detect higher concentrations of BCC, these methods are not sensitive enough for the detection of lower concentrations. The use of a sample volume of at least 200 mL was shown to be effective at detecting BCC, even at low concentrations of 1 CFU/L.

5) **Abstract title:** *Impact of training data size on developing quantitative structure-activity relationship (QSAR) models – A case study of Ames test*
**Authors:** Kumar, Annika, FDA/NCTR (Student); Tong, Weida, FDA/NCTR; Li, Ting, FDA/NCTR (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - The Ames assay is required by regulatory agencies worldwide to assess the mutagenic potential risk of consumer products. The abundance of Ames assay data available in the public literature provides an opportunity to enhance the performance of QSAR models for predicting Ames mutagenicity. In this study, we compared the impact of four progressively larger training sets, starting from Phase 1 with 3,783 compounds to Phase 4 with 18,008 compounds, using five conventional machine learning (ML) methods: Logistic Regression (LR), KNN, SVM, Random Forest, and XGBoost. From Phase 1 to Phase 3, these five ML methods were evaluated on the Ames Challenge test set of 1,508 compounds and the external validation set of 6,337 compounds. We observed that XGBoost consistently benefited from the increasing training size, with the Matthews correlation coefficients (MCC) improving from 0.247 to 0.368 for the test set and from 0.109 to 0.272 for the external validation set. Meanwhile, LR yielded comparable results to XGBoost. Furthermore, when the external validation set was combined with the Phase 3 training set to create the Phase 4 training set, XGBoost achieved the highest MCC of 0.368, followed by LR, KNN, RF, and SVM with MCC values of 0.347, 0.211, 0.175, and 0.077 respectively. This study demonstrated that increasing the training data size could improve model performance, but the impact varied across different ML methods. Additionally, the generally higher performance on the test set compared to

the external validation set indicates that the importance of interpreting the predictions within the context of the same Ames test guidelines.

- **Purpose**
  - The Ames Test is a bacterial reverse mutation assay designed to detect a wide range of chemical substances capable of inducing gene mutations. Its purpose is to assess the mutagenic potential of new chemicals and drugs. By adhering to the International Council for Harmonization (ICH) guidelines, quantitative structure-activity relationship (QSAR) models can be employed to predict Ames mutagenicity test results. With the abundance of Ames assay data available in the public literature, there is an opportunity to enhance the performance of QSAR models in predicting Ames mutagenicity. This study aims to evaluate how increasing the sample size of the training dataset influences the performance of these models.
- **Methods**
  - We utilized two datasets, the Ames Challenge dataset (13,179 compounds) and the benchmark Ames dataset (6,337 compounds), to assess the impact of training size on the performance of QSAR models. The Ames Challenge dataset was divided into three training sets (3783 compounds, 3644 compounds, and 4244 compounds) and one test set (1508 compounds). These training sets were grouped into three phases: phase 1 with 3783 compounds, phase 2 with 7427 compounds, and phase 3 with 11671 compounds. Five different conventional machine learning (ML) algorithms (Logistic Regression (LR), KNN, SVM, Random Forest, and XGBoost) were trained on these three phases of training sets and evaluated on the Ames Challenge test set, as well as the benchmark Ames dataset serving as an external validation set. Additionally, the benchmark Ames dataset was combined with the phase 3 training set to form the phase 4 training set, on which all methods were trained and evaluated using the Matthews correlation coefficients (MCC) on the test set.
- **Results**
  - In this study, we compared the impact of four progressively larger training sets, starting from Phase 1 with 3,783 compounds to Phase 4 with 18,008 compounds, using five conventional ML methods. From Phase 1 to Phase 3, these five ML methods were evaluated on the Ames Challenge test set of 1,508 compounds and the external validation set of 6,337 compounds. We observed that XGBoost consistently benefited from the increasing training size, with the Matthews correlation coefficients (MCC) improving from 0.247 to 0.368 for the test set and from 0.109 to 0.272 for the external validation set. Meanwhile, LR yielded comparable results to XGBoost. Furthermore, when the external validation set was combined with the

Phase 3 training set to create the Phase 4 training set, XGBoost achieved the highest MCC of 0.368, followed by LR, KNN, RF, and SVM with MCC values of 0.347, 0.211, 0.175, and 0.077 respectively.

- **Implications**
    - Building accurate QSAR models to predict Ames mutagenicity is crucial as it can save costs and enhance efficiency in assessing the mutagenicity of chemicals. Among the various ML methods evaluated in this study, XGBoost demonstrated the best performance for the Ames Challenge test dataset, utilizing comprehensive Ames datasets. This study demonstrated that increasing the training data size could improve model performance, but the impact varied across different ML methods. Additionally, the generally higher performance on the test set compared to the external validation set indicates that the importance of interpreting the predictions within the context of the same Ames test guidelines.

6) **Abstract title:** *Random Forest Model for Predicting μ Opioid Receptor Binding Activity for Assisting Development of Opioid Drugs*
**Authors:** Li, Jerry, FDA/NCTR (Student); Liu, Jie, FDA/NCTR (Mentor); Hong, Huixiao, FDA/NCTR (Mentor)
**FDA Strategic Initiative:** Unleashing the Power of Data
**Abstract:**

- **Synopsis**
    - The opioid epidemic is one of the most prominent and severe public health crises in U.S. The devastating consequences of the opioid crisis are not only the increased number of deaths caused by opioids but also the increased economic burden of combating this crisis. The highly addictive nature of the opioids is closely related to the overdose fatalities caused by prescription opioids, heroin, and illicit fentanyl. However, the therapeutic benefits of prescription opioids acting as the most potent analgesic make prohibition of the drugs impossible. Opioids exert their analgesic effect by binding to the μ opioid receptor (MOR), which then activates its downstream signaling pathway, eventually leading to the inhibition of spinal cord pain transmission. Since the discovery of MOR in the 1970s, many efforts have been endeavored to understand the structure activity relationship between the receptor and its ligands, hoping to shed some lights on the development of non- or less-addictive opioid analgesics. Yet, many questions remain unanswered, and the development of non- or less-addictive opioid analgesics has had limited success. Here, we present a machine learning model that can be used to predict the binding activity of small molecule compounds to the MOR based on chemical structures. The random forest model was evaluated using 5-fold cross validations and external validation, resulting in 89.5% and 78.0% MOR binding activity prediction accuracy,

respectively. Our results suggest that this model could be useful to identify MOR binders, which may aid the development of non- or less-addictive drugs targeting on MOR.

- **Purpose**
  - The opioid epidemic is one of the most prominent and severe public health crises in U.S. The devastating consequences of the opioid crisis are not only the increased number of deaths caused by opioids but also the increased economic burden of combating this crisis. The highly addictive nature of the opioids is closely related to the overdose fatalities caused by prescription opioids, heroin, and illicit fentanyl. However, the therapeutic benefits of prescription opioids acting as the most potent analgesic make prohibition of the drugs impossible. Opioids exert their analgesic effect by binding to the μ opioid receptor (MOR), which then activates its downstream signaling pathway, eventually leading to the inhibition of spinal cord pain transmission. Since the discovery of MOR in the 1970s, many efforts have been endeavored to understand the structure activity relationship between the receptor and its ligands, hoping to shed some lights on the development of non- or less-addictive opioid analgesics. This study aims at developing machine learning models for predicting MOR binding activity of chemicals to assist the development of non- or less-addictive opioid analgesics.
- **Methods**
  - Chemicals with MOR binding activity data were first curated from public databases and literatures. Next, molecular descriptors of all the curated chemicals were calculated using the software Mold2. Then, the chemicals and MOR binding data were split into training and testing sets, which contain 11,876 and 608 chemicals, respectively. The random forest model was developed on the training set and evaluated using 5-fold cross validations and the testing set.
- **Results**
  - MOR binding activity data of 12,484 chemicals were curated. The developed random forest model performed well in the 100 iterations of 5-fold cross validations with average accuracy, sensitivity, specificity, and Matthews Correlation Coefficient (MCC) of 89.5%, 96.5%, 53.4%, and 0.574, respectively. The predictions on the testing set resulted in accuracy, sensitivity, specificity, and MCC of 78.0%, 79.4%, 70.7%, and 0.408, respectively.
- **Implications**
  - Our results suggest that this developed random forest model could be useful to identify MOR binders, which may aid the development of non- or less-addictive drugs targeting on MOR.

7) **Abstract title:** *Bioinformatics approaches to pig genome annotation*
**Authors:** Perez-Cuevas, Michelle, FDA/CVM (Student); Norris, Alexis, FDA/CVM (Mentor)

**FDA Strategic Initiative:** Unleashing the Power of Data

**Abstract:**

- **Synopsis**
  - The Center for Veterinary Medicine (CVM) reviews intentional genomic alterations (IGAs) in animals. IGAs in animals have many different intended uses, including the production of human therapeutics. Genome annotation information is used to identify potential effects of alterations to an animal's health. The richness of available genome annotation data varies by species and is found in multiple database sources. Here, we automated genome annotate alterations in the pig genome using R programming. We compared three approaches and found that AnnotationHub R package generally outperforms the other two R packages (biomaRt and rtracklayer). While our focus was on pig genome annotation data, the R code we developed can be used broadly for any species with public genome annotation in Ensembl or UCSC Table Browser databases.
- **Purpose**
  - Intentional genomic alterations (IGAs) in animals have many different intended uses, including production of human therapeutics; improvements to animal health, well-being, and husbandry practices; and enhanced production and food quality. IGAs are introduced into the animal's genome using recombinant DNA, genome editing, or other technologies. The molecular characterization step of review of IGAs in animals assesses the intended change to the animal's genome and seeks to identify any potential unintended alterations that may have arisen during the introduction of the IGA. Genome annotation is used to guide our hazard determination for each unintended alteration, and thus identify potential risk to animal or human health. This project sought to improve genome annotation methods for IGAs in pigs (Sus scrofa), as they are one of the common species used by developers of IGAs in animals.
- **Methods**
  - First, we sought to identify databases of available annotation data for the most recent version of the pig reference genome (11.1), through searches of the Bioconductor R package repository and the literature. Then, we developed approaches to annotate genome regions of interest using the R programming language. This included the R packages used, the data wrangling, and adding overlapping annotation to the alterations. For each approach, we compared the: computational time, simplicity and user friendliness of the code, potential errors, and amount of available data.

- **Results**
    - From our survey, we identified three R package databases with ample pig genome annotation data: biomaRt, rtracklayer, and AnnotationHub. We developed R code to retrieve desired annotation from the package and add overlapping annotation to our regions of interest (unintended alterations). Our comparison of the three approaches found that AnnotationHub was overall the best choice for our specific needs: the code is more simplistic than the other two methods; it is faster computationally than rtracklayer; it does not truncate data like rtracklayer; and it includes more datasets than biomaRt.
- **Implications**
    - Our direct comparison of genome annotation approaches helps inform reviewers when they are deciding on a bioinformatics pipeline for their genome annotation. While this project focused on the pig genome, this can be broadly applied to other genomes, as the tools (AnnotationHub, biomaRt, and tracklayer) are not limited to the pig genome. The code will be available to all FDA scientists using FDA GitLab: https://git.fda.gov/alexis.norris/mpc_genome_annotation.

8) **Abstract title:** *Microbiological Survey of Commercial Tattoo and Permanent Makeup Inks Available in the United States*
**Authors:** Khare, Prakshi, FDA/NCTR (Student); Kondakala, Sandeep, FDA/NCTR (Mentor); Kim, Seong-Jae, FDA/NCTR (Mentor)
**FDA Strategic Initiative:** Empowering Patients and Consumers
**Abstract:**

- **Synopsis**
    - In three previous surveys of tattoo inks on the market done by the FDA, 39% (62 out of 159 inks) of tattoo and permanent makeup inks (PMU) were found to have bacterial contamination with some pathogenic species having the potential to cause infections if injected into the skin. The present study aims to expand upon these findings by increasing the sample size and including inks from various manufacturers. We surveyed 10 tattoo and PMU inks (five tattoo inks and five PMU inks) from different manufacturers. The inks were tested for bacterial and fungal contamination using the aerobic plating and enrichment methodologies outlined in FDA's Bacteriological Analytical Manual (BAM) Chapter 23 "Methods for Cosmetics." Briefly, inks were first diluted in Modified Letheen Broth (MLB) and then plated on Modified Letheen Agar (MLA) and Potato Dextrose Agar (PDA) plates to test for bacterial and fungal contamination, respectively. In parallel, diluted ink samples were first enriched in MLB for seven days and then plated on MLA to ensure any microbial contamination within the ink sample would be identified. Plates were incubated at 30°C for 2-7 days and examined for

microbial contamination. Isolated bacteria were lysed, and the cell lysate was used for PCR amplification of the 16S rRNA gene. The species of the bacteria was identified by the 16S rRNA gene sequence. Four samples have been found to be contaminated with seven different bacterial isolates, three were identified to the genera level and three were identified to the species level, one was unable to be identified. The genera *Bacillus, Lysinibacillus*, and *Paenibacillus* were identified and the species *Pseudomonas aeruginosa* was identified.

- **Purpose**
  - The prevalence of tattoos and the amount of the population with at least one tattoo have been increasing significantly and with it the rate of people getting infections from tattoos. With tattooing being so prevalent in modern day society, the safety of those who choose to get them is of utmost importance. There are three main aspects from where one can get infected while getting a tattoo: the ink used in the tattoo, the tattooing process itself, and aftercare from getting a tattoo. Previous studies done by the FDA on tattoo and permanent makeup (PMU) inks focused on the inks themselves, testing them for contamination. The three previous surveys found that 39% (62 of 159 inks) of the unopened inks they tested were found to have microbial contamination, with some of the species found being known to cause infections. This study aims to further expand the sample size of the previous studies by including inks from a variety of manufacturers.
- **Methods**
  - In this study, 10 tattoo and PMU inks from different manufacturers were surveyed. Randomly, five tattoo inks and five PMU inks were selected to test for microbial contamination. Inks were tested for microbial contamination using the aerobic plating and enrichment methodologies that are outlined in the FDA's Bacteriological Analytical Manual (BAM) Chapter 23 "Methods for Cosmetics." Briefly, inks were first diluted up to 10-3 in Modified Letheen Broth (MLB) before being plated on Modified Letheen Agar (MLA) and Potato Dextrose Agar (PDA) plates to test for bacterial and fungal contamination respectively. Then plates were incubated at 30°C for 2-7 days. Diluted inks samples were also directly incubated for seven days before being plated on MLA plates to isolate the bacteria. A single colony of each bacterium was lysed, and its cell lysate was used for PCR amplification of the 16S rRNA gene. The 16S rRNA gene sequence was used to identify the species of bacteria.
- **Results**
  - It was observed that forty percent (4 out of 10 inks) were found to have microbial contamination. Two out of five (40%) tattoo inks and two out of five (40%) PMU inks were found to be contaminated. A total of seven bacterial isolates, five from tattoo inks and two from PMU inks, were isolated. Out of the seven isolates, three were identified to the genus level, three were identified to the species level, and the sequence for one sample was inconclusive. The genera identified were Bacillus, Lysinibacillus, and

Paenibacillus. Pseudomonas aeruginosa was the species identified; it was identified three times.

- **Implications**
    - The results found in this study were consistent with those found in previous studies. Our findings containing bacteria that can be pathogenic highlight the importance of monitoring these products for microbial contaminants, including potentially pathogenic microorganisms. Further surveys on the contamination rates of tattoo inks should be conducted to monitor these products along with research looking into the other aspects of tattooing that can cause infections such as the tattooing process itself and improper aftercare of a healing tattoo.

9) **Abstract title:** *Validating qPCR Assay to Quantify HCoV-NL-63*
**Authors:** Raghav, Sreelakshmi, FDA/NCTR (Student); Feye, Kristina, FDA/NCTR (Mentor); Johnson, Shemedia, FDA/NCTR (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
    - Being able to detect and quantify a virus during an experiment simply and quickly is important. Currently, the alphacoronavirus NL-63 is proposed to be a safe surrogate for the SARS-CoV-2 virus, which is the etiological agent of COVID-19. A surrogate virus has the same molecular mechanism as a more deadly virus, but produces self-limiting infections. While the pandemic is over, finding suitable surrogates to study viruses safely is essential to help head off future outbreaks. Also, finding ways to increase research in BSL-2 environments using surrogates serves to enhance research speed and efficiency in the future. However, little is known about ways to quickly quantify NL-63. The current gold-standard is immunofluorescence; however, this takes time and requires expensive reagents and equipment to read results. We propose a qPCR assay that is specific and sensitive for the nucleocapsid of NL-63. First, we designed the qPCR primers to the conserved ORF1 region of the NL-63 virus and validated them for specificity. The amplification efficiency of the qPCR assay was determined and consistently performed at 90-110%, with melt curves indicating a lack of non-specific amplifications and dimer formation. Additionally, using cDNA from an experimentally infected animal with NL-63 that was previously quantified, we ran a full doubling dilution curve and ran the amplicons on a 2% agarose gel. Data indicates that using native NL-63, semi-quantitatively, we have excellent amplification through the full range of dilution. The repeatability of the stability of the curve and lack of untargeted amplification indicates that the primers are likely suitable for downstream analyses. Additionally, the qPCR protocol only required four hours compared to two days using immunofluorescence. Further analyses will include evaluating the primers for sensitivity and specificity using quantified RNA standards and evaluating how closely the NL-63 qPCR assay compares to immunofluorescent technique.

- **Purpose**
    - Being able to detect and quantify a virus during an experiment simply and quickly is important. Currently, the alphacoronavirus NL-63 is proposed to be a safe to use surrogate for the SARS-CoV-2 virus, which is the etiological agent of COVID-19. A surrogate virus has the same molecular mechanism as the more deadly virus, but produces self-limiting infections.
      However, not much is known regarding the way to quantitate the NL-63 virus quickly. The current gold-standard is immunofluorescence; however, that takes days to get data, requires expensive reagents, and special equipment to read the results. We propose a qPCR assay that is specific and sensitive for the nucleocapsid of NL-63.
- **Methods**
    - First, the qPCR primers were designed to the conserved ORF1 region of the NL-63 virus and validated for specificity. qPCR with melt curves of varying concentrations of the primers was performed to determine the optimal concentration for the primers. Additionally, using cDNA from an experimentally infected animal with NL-63 that has previously been quantified, a full tens dilution curve with a melt curve was run to create a standard curve and determine efficiency. NL63 probes were then added to the assay and qPCR was run with varying concentrations of the probes were used to determine the optimal concentration. Similarly, a full tens dilution was run to create a standard curve and determine efficiency and the amplicons were run on a 2% agarose gel electrophoresis.
- **Results**
    - Data indicates that using native NL-63, semi-quantitatively, there was excellent amplification through the full range of dilution for all assays, with melt curves and agarose gel indicating a lack of non-specific amplifications and dimer formation. The concentration of 5 μM was optimal for both the primers and the probe. The repeatability of the stability of the curve and lack of untargeted amplification indicates that the primers are likely suitable for downstream analyses. Additionally, the qPCR protocol only required four hours compared to two days using immunofluorescence.
- **Implications**
    - While the pandemic is over, finding suitable surrogate species to study pandemic virus safely is
      essential to help us head off future pandemics. Additionally, finding ways to increase research using surrogates for more dangerous viruses for scientists who only have access to a BSL-2 environment only serves to enhance research speed and efficiency in future outbreaks. Further analyses will include evaluating the primers and probe for sensitivity and specificity using quantified RNA standards and evaluating how closely the NL-63 qPCR assay compares to immunofluorescent technique.

10) **Abstract title:** *Assessment of Alternative Human Skin Barrier Models to Predict Dermal Absorption*

**Authors:** Swat, Sandra, FDA/NCTR (Student); Salminen, Alec, FDA/NCTR (Mentor); Camacho,

Luísa, FDA/NCTR (Mentor)

**FDA Strategic Initiative:** Increasing Choice and Competition through Innovation

**Abstract:**


- **Synopsis**
  - Skin permeation assessments are an important component of the safety evaluations of cosmetic ingredients and topical drugs, as they inform on the potential for local and systemic exposures. Although excised human skin (EHS) is the 'gold standard' for in vitro permeation testing (IVPT), the inherent cost and limited supply of EHS hinders its use in dermal absorption studies. In addition, current IVPT methods have limited throughput, which can be restrictive when performing early dermatological product safety screenings. Here, we developed a 96-well format reconstructed human epidermis (RhE) model, consisting of human neonatal epidermal keratinocytes cultured at the air-liquid interface on permeable support membranes. The in-house RhE model, EHS, and a commercially available RhE model (MelanoDerm, MatTek, Ashland, MA) were tested for their potential to predict percutaneous absorption in vitro using [$^{14}$C]caffeine. Caffeine was selected as the test compound because it is a reference hydrophilic compound for IVPT as per OECD guidelines. The barrier function of the three skin barrier models was also evaluated by histology and by measuring their transepidermal water loss (TEWL) and/or trans-epithelial electrical resistance (TEER). Overall, the side-by-side evaluation of several skin models with various methodologies contributes to a better understanding of strengths and limitations of each model to predict the absorption of topical chemicals of interest to the FDA.
- **Purpose**
  - Skin permeation assessments are an important component of the safety evaluations of cosmetic ingredients and topical drugs, as they inform on the potential for local and systemic exposures. Although excised human skin (EHS) is the 'gold standard' for in vitro permeation testing (IVPT) studies, the inherent cost and limited supply of EHS hinders its use in dermal absorption studies. In addition, current IVPT methods have limited throughput, which can be restrictive when performing early dermatological product safety screenings. The goal of our research is to develop and evaluate alternative models of the human skin barrier to help identify effective and useful tools for examining the skin permeation of chemicals and formulations of interest to the FDA.
- **Methods**
  - A commercially available reconstructed human epidermis (RhE) model (MelanoDerm, MatTek, Ashland, MA) was tested in parallel with EHS to

assess its potential to predict percutaneous absorption in vitro using [$^{14}$C]caffeine as the test chemical. The commercial RhE model was received from the manufacturer and matured at the air-liquid interface for an additional 14 days. The EHS was collected from female donors undergoing abdominoplasties and dermatomed to a thickness of 500 µm. Skin barrier models were mounted on flow-through diffusion cells (0.196 cm$^2$ diffusion area) and assembled on an automated fraction collection system (PermeGear, Hellertown, PA), allowing for continuous collections of basal receptor fluid (1X phosphate buffered saline) over 24 h. Upon completion of the permeation test, the apical skin barrier surface was washed and the tissue collected. Additionally, a 96-well format RhE model was developed, consisting of human neonatal epidermal keratinocytes cultured at the air-liquid interface on permeable support membranes, and the permeation of [$^{14}$C]caffeine was assessed in situ. All samples were analyzed via scintillation counting. The barrier function of the three skin barrier models was also evaluated by histology and by measuring transepidermal water loss (TEWL) and/or trans-epithelial electrical resistance (TEER).

- **Results**
    - EHS had a lower mean TEWL and 24 h [$^{14}$C]caffeine permeation when compared to that of the commercial RhE model. MelanoDerm tested at Day 0 of maturation was found to be a poorer barrier to [$^{14}$C]caffeine permeation and had a lower mean TEER and higher mean TEWL when compared to MelanoDerm at Day 14. [$^{14}$C]Caffeine permeation (24 h post-dose) was higher in the in-house RhE model compared to EHS and matured MelanoDerm; nonetheless, the 96-well format of the in-house RhE model facilitated higher throughput experimentation when compared to the two other skin barrier models. Both RhE models (in-house and commercial) exhibited human epidermis-like stratification when examined by histology.
- **Implications**
    - Side-by-side evaluation of human skin barrier models with various methodologies contributes to a better understanding of the strengths and limitations of each model to predict the absorption of topical chemicals in humans. Although there has been tremendous growth and improvement toward alternative human skin barrier models, continued development and evaluation of these models will be required to understand their potential to be adopted for IVPT in regulatory science. Future directions include the assessment of in-house models of cultured keratinocytes to evaluate the feasibility of manufacturing improved skin barrier models in the laboratory. Increasing the experimental throughput of such models has the potential to

support the use of these models in applications such as product development and quality control.

## Office of Regulatory Affairs (ORA)

1) **Abstract Title:** *DART-MS Databases For Rapid ID of Additives/Contaminants in Retail Food Contact Articles*
**Authors:** Harris, Sydney, FDA/ORS (Student); Lindahl-Ackerman, Luke, FDA/ORS (Mentor)
**FDA Strategic Initiative:** Public Health Emergency Preparedness and Response
**Abstract:**

- **Synopsis**
  - When already approved food contact materials are reviewed or reassessed, exposures or incidences need to be assessed from broad retail packaging surveys. Current methods to identify polymers and additives/contaminants in retail food contact articles are too slow, too limited, and/or do not identify both polymers and additives/contaminants. We are developing direct mass spectrometric databases of these food contact additives and contaminants to permit rapid, high-volume, survey-based screening to support incidence/exposure estimates. Using a literature review to target the 46 most common additives/contaminants, prior laboratory work, and careful database curation, we are constructing DART-MS databases for over 230 of the most common additive/contaminants to supplement the DART-MS polymer ID method development. Together the polymer ID and contaminant/additive ID should allow the identification of the large majority of polymers and additives/contaminants present in retail food contact articles in less than 1 minute, supporting survey-based food packaging incidence/exposure/compliance estimates.
- **Purpose**
  - Concerns have been raised over the years about some materials used in contact with food. Because the FDA conducts pre-market safety assessments of all food contact substances, anytime the agency wants to review safety of existing food contact articles (FCA), new retail-based incidence or exposure estimates are often requested. The current methods to screen and identify materials and/or substances in retail FCA's are very long and complex. The best current method (FTIR) identifies many but not all materials, and very few additives or contaminants. Direct Analysis in Real Time (DART) is a rapid way to ionize molecules directly off the surface of samples so they can be identified by mass spectrometry (MS) without any sample preparation. DART-MS has been shown to be excellent at identifying a very wide array of additives and contaminants on FCAs, and to identify several polymers. A DART-MS method is being developed to identify most of the remaining food contact polymers in the same analysis as additives/contaminants.

- **Methods**
  - We are developing comprehensive DART-MS databases and target lists to robustly screen for additives/contaminants in the same DART-MS analysis used to identify polymers. The DART-MS database for small molecule additives/contaminants is being developed from a target list of the 46 most commonly and frequently detected FCA additives/contaminants and ~200 additives and contaminants analyzed to-date. Small molecule additives/contaminants were tabulated in excel from literature, industry, and publicly available sources. Chemical identifiers were triangulated by CAS#, INCHI-key, PubChem CID#, and IUPAC name. Additive/contaminants' existing DART-MS data were located, listed, cross-referenced against existing standards in stock, and new standards were acquired and analyzed by (+/-)DART-MS for analytes with incomplete data. Existing additive/contaminant DART-MS spectra were tabulated, cleaned, listed by m/z, major ion forms identified, ion-formula tabulated, and ion ID's, ion formula's, theoretical m/z's, and observed abundances reported to the DART-MS database.
- **Results**
  - The compounds in the literature review target list were observed in at least 3 of 25 multi-analyte or non-targeted FCS studies and detected at the highest frequency. While the target list compounds cover 15 additive functions, the combined database covers 19 functions/sources of over 230 compounds with over 800 DART ions, 550 molecular formula, 320 structural identifications, and 400 relative intensities tabulated to-date. Prior (+)DART-MS spectra of additives/contaminants is 75% complete but about 44% of all DART-MS spectra are not yet collected. Database tabulation of already acquired (-)DART-spectra (35% collected) is underway, and standards for remaining 32 target additives/contaminants are being sought. The separate development of the polymer identifier ions is ongoing.
- **Implications**
  - This work seeks to flush out the positive and negative ion DART-MS additive/contaminant database to permit suspect screening of the majority of polymer additives and contaminants. Based on recent work DART-MS has proven to be reliable in ensuring that polymers and FCA additives/contaminants are accurately identified in <1 minute in post-market FCAs. Validation of this single material & additive ID method can dramatically accelerate incidence/exposure estimation and compliance assessments for a much broader array of food contact articles and additives/contaminants.