Poster Abstract Booklet

Posters will be presented in-person at FDA White Oak Great Room
on Thursday, September 12, 2024, unless otherwise noted.

Posters

01. Evaluation of RNA extraction and Illumina NGS library preparation methods to detect viral RNA from different sample matrices

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Objective: Current pathogen and adventitious agent detection assays face limitations, such as specificity to certain pathogens and reduced sensitivity, which challenge the safe use of HCT/Ps. This project aims to evaluate various RNA extraction and NGS library preparation methods to achieve rapid, broad, sensitive, and specific detection of pathogens and adventitious agents.

Method: We used three study designs to assess RNA extraction methods: spiking Zika virus (ZIKV) into naïve cells, using a cell line persistently infected with ZIKV, and spiking persistently infected ZIKV cells into buffy coat samples. RNA was extracted using five methods and ZIKV quantification was performed using qRT-PCR. To evaluate RNA library preparation methods, we spiked virus panels HEV RR.1 and BEI- NR-59622 (containing PCV1, Reo, FeLV, RSV, EBV) into U937 cells, extracted total RNA, and constructed libraries with various commercial kits and combinations. The libraries were sequenced on the Illumina NovaSeq 6000 platform. NGS data is being analyzed to determine virus genome mapping, genome coverage, and percentage of mapped reads.

Results and conclusion: Overall, TRIzol extraction with silica column purification proved to be the best method for extracting viral RNA. As anticipated, we observed significant variability between different NGS library preparation methods. rRNA depletion effectively reduced human rRNA in the NGS libraries. Further data analysis is underway to study the impact of various library preparation methods on the total number of NGS reads generated from reference viral RNAs.

02. Evaluation of plasma proteome and miRNA changes related to COVID-19 patient severity response

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The severity outcome of COVID-19 disease resulting from SARS-CoV-2 infection has shown that many cases are related to underlying health conditions like diabetes and cancer. There remains an urgent need to gain mechanistic insights at the molecular level to understand the differences in severity of the infection and discover early biomarkers that enable prediction of severity among COVID-19 patients. These insights could help ease the burden of care and aid in evaluation and development of treatments. In this study, COVID-19-positive patients were categorized into 3 symptom response categories using a 12-point patient response scoring system: mild (score 0-1), moderate (score 2-4), and severe (score 5-12). Blood samples from 93 COVID-19 patients collected at the time of initial diagnoses were processed to plasma and deidentified for proteomic and miRNA analyses. A total of 2,921 proteins and 2,083 miRNAs were analyzed in the plasma samples. Ingenuity Pathway Analysis (IPA) of significant proteins revealed that the top pathways were neutrophil degranulation, cytokine storm, role of osteoblasts in rheumatoid arthritis signaling, interleukin-10 signaling, and wound healing signaling pathways in severe patients. Interleukin-6 (IL6) was involved in nine of the top ten IPA pathways. MicroRNA associations with protein targets were extracted from IPA using experimentally observed and highly predicted criteria. Proteomics and miRNA analyses of plasma from recently diagnosed COVID-19 patients revealed 1,353 protein and 1,167 miRNA biomarkers of severity response to COVID which are associated with preexisting health status and acute response to the COVID infection. Additional experiments are needed to validate the biomarkers.

03. Investigating the impact of replicates for different food matrices on non-targeted analysis data and results

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Most analytical methods employed for food safety target specific compounds or compound classes. Non-targeted analysis (NTA) using liquid chromatography coupled with high-resolution mass spectrometry (LC/HR-MS) facilitates a more global analysis, allowing potential detection and identification of a wider range of compounds. This study aims to investigate how replicate number and type may impact results obtained for food matrices with different macronutrient content. Composite samples were prepared in quintuplicate for three matrices from the FDA Total Diet Study and injected up to thirty times using a non-targeted LC/HR-MS method. Data processing was performed using Compound Discoverer 3.3, with different groupings of sample preparation and injection replicates submitted for each matrix. Each data analysis group was evaluated for the total number of features detected as well as number of spiked quality control (QC) compounds extracted from the data. A comparable number of features, with %RSD routinely less than 6%, was detected for both triplicate injections of the same preparation and single injections of triplicate preparations in most matrices. However, more molecular features and spiked QC compounds were identified as more files were processed together. Replicates also facilitated additional metrics for feature prioritization, such as %CV for peak area, that would otherwise not be possible. These preliminary results indicate replicates are important for helping ensure data quality; however, their type and number may be situational and of particular benefit when analyzing novel matrices. This work will give practical guidance for how replicates can be utilized when designing experiments using NTA for food samples.

04. Metabolite profiles distinguish exposure to Zika and Dengue flaviviruses in human induced pluripotent stem cells (hiPSCs)

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Background: Previous studies suggest that flaviviruses can be transmitted through cells and tissues threatening safe use of human cells, tissues and cellular and tissue-based products (HCT/Ps). Therefore, developing sensitive and specific tools to detect flavivirus contamination is critical to ensure safety of HCT/Ps. Here, we studied metabolite profiles following flavivirus infection of hiPSCs with goals to develop metabolite markers based-detection assay.

Methods: hiPSCs were infected with Zika-MR766 or Dengue-3 viruses, and cell viability, cytopathic effect (CPE), and viral load were measured using immunofluorescence assay (IFA) and qRT-PCR respectively. We performed mass spectrometry (FIA-MS and LC-MS) of hiPSCs at 0, 8, 24, 48 and 96 hours post infection to identify virus-specific shift in metabolome. LC-MS raw files were converted to mzML using msConvert and peak extraction was performed using MZmine. Features with a signal-to-noise ratio below three in pooled quality control (QC) samples compared to blank, missing in more than 20% of study samples, or with a coefficient of variance greater that 20% in pooled QC samples were removed.

Results: We did not observe significant differences in cell viability and CPE in hiPSCs following infection with Zika or Dengue compared to mock infection. However, IFA and qRT-PCR results indicated higher infectivity of ZIKV compared to DENV. FIA-MS results demonstrated that 0.2 million cells were sufficient to detect a maximum number of metabolites. The positive ionization mode in LC-MS yielded 10,077 features. After filtration, 1,052 features remained. Currently data from LC-MS is further evaluated via various machine learning techniques and statistical methods to identify a panel of biomarker specific for Zika and Dengue virus.

Conclusions: Preliminary data demonstrate that metabolomic profile can distinguish between Dengue or Zika virus infected and uninfected hiPSCs. This suggest that metabolite-based biomarkers have potential to detect flavivirus infection of hiPSCs.

05. Whole genome sequencing (WGS) analyses: Room for the benchtop?

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Third generation sequencing technologies are actively contributing to food safety monitoring. However, small and large plasmids may present a number of challenges to both sequencing platforms in different ways. In order to understand the extent of that challenge in *Salmonella*, we evaluated how PacBio Sequel II (PBS) and Oxford Nanopore Technologies (ONT) whole genome sequencing (WGS) compares to traditional Illumina sequencing pipelines and the impact those pipelines have on plasmid recovery. Nine strains of Salmonella were sequenced across the three major platforms: ONT, PBS, and Illumina. The plasmids from the strains were predicted to range from 6 KB to over 400,000 kb. Putatively identified plasmids were harvested from the sequences and a BLAST analysis was performed to identify the plasmid and PCR was used to confirm plasmid replicon type. The plasmids were bioinformatically predicted via two independent plasmid programs, MOB-SUITE and PlasmidFinder. The resulting data varied where an isolate had six plasmid replicons identified via the Illumina platform; whereas, PBS only identified four plasmids (PBS: IncC, IncFIA, Inc FIB, IncX4 vs. Illumina: IncA/C, IncFIA, IncFIB, IncX4, ColpVC and IncFII (pCoo) using the same annotation pipeline (PlasmidFinder). When using MOB-SUITE (Typer), plasmid annotations shifted significantly in PBS sequences, with plasmid replicons identified from the same strain via PBS as: IncFIB, IncFII, IncFIA, ColRNAI_rep_cluster_1857, MOBP, IncA/C2, and IncX4. Two of the plasmids identified by their replicons appear to be re-arranged and merged (ColRNAI_rep_cluster_1857 and MOBP). The overall predicted plasmid sizes for that particular isolate ranged from 3.176 to 126.356 kb with PBS and 2 to 120 kb when sequenced via Illumina MiSeq for a single isolate. Additionally, mobility predictions and antibiotic resistance annotations on each plasmid (Abricate) varied significantly between the sequencing and bioinformatics platforms, as did virulence genes. On the bench top and via sequencing, each of the strains contained multiple resistance plasmids, were resistant to at least eight antimicrobials, had at least one plasmid that transferred resistance to the recipient. Data indicates that while long read sequencing can be advantageous, benchtop validations still hold a place in the analytical toolbox.

06. Identifying the *Plasmodium falciparum* sexual RNA-protein interactome using novel in vivo capture methods

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As sexual development is necessary for onward transmission of *Plasmodium falciparum*, identifying and characterizing molecular factors that regulate parasite sexual maturation and transmission is essential in our efforts to design novel intervention strategies. While it is well known that post-transcriptional regulation is essential for parasite transmission, the identification and function of factors involved in transition remains poorly characterized due to a lack of effective *in vivo* methodologies. In this study, we comprehensively identify RBPs throughout sexual development using the Photoactivatable Ribonucleoside-enhancing Crosslinking (PAR-CL) technique combined with isolation of crosslinked RNA of all biotypes. We utilized a *P. falciparum* strain that conditionally expresses PfAP2-G and can biosynthetically label nascent RNAs via pyrimidine salvage (NF54$^{AP2\text{-}Gdd\text{-}3xHA}$::yFCU). Following the addition of Shld-1 for stable expression of PfAP2-G-ddFKBP to induce gametocytogenesis, parasites were incubated with a 4-thiouracil for different durations and crosslinked to capture zero-distant RBP-RNA interactions at Stages I, III, and V of gametocyte development. Through Data Independent Acquisition mass spectrometry (DIA-MS) of the crosslinked proteins, we have identified RNA interactions with known RBPs, but also with non-canonical type proteins yet to be annotated as RNA-binding. Interestingly, the RBPs captured vary depending on the length of 4-thiouracil labeling. From this, we can begin to attribute RBPs that are likely associated with post-transcriptional stabilization (DOZI/CITH complex) versus processing (CCR4-NOT complex). Our results expand our knowledge of RNA-protein crosstalk and provide the first comprehensive network of RNA-protein interactions in sexual gametocyte stages of development with continued efforts toward finding RBPs that are critical for host-to-vector transmission.

07. FDA and NIST collaboration: Evaluation of assays and control materials for characterizing animal biotechnology products generated by genome editing

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Genome editing technology has revolutionized the ability to make targeted changes to an animal's genome (intentional genomic alterations [IGAs]), offering promise for the development of animal biotechnology products that address animal and public health needs. Characterization of these IGAs is an important part of the regulatory process to ensure that the edit to the animal is as intended and to identify any unintended changes. However, there are currently no validated measurements and standards for characterizing unintended edits in animals. FDA-CVM has established a collaboration with NIST to generate resources for characterizing both intended and unintended alterations in animal biotechnology products resulting from genome editing. These resources will provide developers and FDA regulators with example characterization approaches that they could use as part of the development and regulatory process for IGAs in animals as well as for validating methods, materials, and data.

NIST qualified porcine and bovine cell lines as potential control materials by characterizing their DNA sequences at on-target and potential off-target loci before and after genome editing with multiple guides. The off-target analyses performed include comparisons between off-target loci identified by in silico methods and biochemical assays (CHANGE-seq and SITE-Seq), and assessment of editing at nominated loci in edited animal cells. Resulting protocols and datasets will be published and made accessible to developers and the general public. Future work involves generating prototype DNA spike-in control materials used for qualifying DNA sequencing methods that may be used for characterizing animal biotechnology products.

08. Multiomics analysis of optimal cryopreservation conditions for the storage of various cell-based therapies

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Cellular therapies present immense therapeutic opportunities and challenges. Identifying critical quality attributes to assure product safety and effectiveness and preserving these during storage and transport are vital considerations from a regulatory standpoint. Previously, we used induced pluripotent stem cells (iPSCs) to compare various cell fixation methods with conventional cryopreservation (with DMSO). At several time points we found that when compared with fresh cells, conventional cryopreservation compromised cells less than the other methods we tested. We then compared the effect of cryopreservation on the transcriptome and epigenome of retinal pigmented epithelial (RPE) cells versus fresh cells analyzed at two timepoints: frozen-thawed and frozen-thawed cells allowed to recover at 4°C for 48 hours. scRNA-seq and scATAC-seq revealed significant differences in the transcriptomes of cryopreserved cells: the expression levels of RPE-related genes were decreased in the former. Interestingly, the chromatin accessibility profiles of fresh and cryopreserved cells were strikingly different, indicating that chromatin accessibility data provides better discriminatory power. Data mining to identify candidate attributes will be done to identify improved preservation conditions for cell therapy products. Confirmatory molecular analyses will be done to validate candidate proteins or transcripts using several single cell methods, followed by in vivo functional studies. We will also include additional timepoints and protocols for iPSCs and RPE cells and other cell therapy products to explore cellular responses to preservation and reconstitution. Such a comprehensive analysis will help create a 'best practices' set of standards to evaluate and optimize storage and transportation of cell therapy products.

09. Large alterations in the genomes of pigs edited using CRISPR-Cas9

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CRISPR-Cas9 genome editing is being used to produce pigs harboring intentional genomic alterations (IGAs) to serve as source animals for human-compatible cells, tissues, and organs for xenotransplantation. Unintended large genomic alterations are known to occur during genome editing. Their putative effect on gene function may present hazards to the health of the animal and the safety and efficacy of the human product. Compared to humans, there is relatively little data on the genome-wide effects of CRISPR-Cas9 editing in pigs. This study sought to characterize large alterations in pig genomes following in vitro CRISPR-Cas9 editing of three porcine genes (*B4GALNT2*, *CMAH*, and *GGTA1*) either singly or in combination (concurrent or sequential editing). Edited and unedited cells were subjected to 30X Illumina whole genome sequencing to identify large alterations. CasOFFinder was used to identify predicted cut sites based on gRNA homology. We found that 90% of the edited cells had at least one unintended large alteration that was not present in the unedited genome. The most common types of alterations were deletions (~50%) and tandem duplications (~25%). Large alterations were identified in cells regardless of approach (single edit, combination concurrently, and combination sequentially). The majority of the alterations did not have a breakpoint within 10kb of a predicted Cas9 cut site, suggesting a blind spot in prediction algorithm and/or somatic events including naturally occurring mobile element insertion events. Ongoing work includes editing pig zygotes to directly compare in vitro and in vivo genome editing.

10. Evaluation of a targeted amplicon sequencing method for detection of contaminating microorganisms in a probiotic product

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In recent years there has been an increase in consumption of live microbial dietary supplements. One of the challenges in maintaining the safety of probiotic products is the detection of low levels of contaminating microbes and/or pathogens in the presence of large number of beneficial microbes. Whole metagenomic sequencing (WMS) and target amplicon sequencing (TAS) has proven to be robust methods for detection of pathogens in food matrices. Here we evaluate and compare how accurate and sensitive these two sequencing methods are in detecting low levels of microbial contaminants in a probiotic matrix. Probiotic products which contained varying bacterial species combinations were chosen to spike with the foodborne pathogen, *L. monocytogenes* (Lm) ranging from 20 to $2x10^9$ CFUs. Target amplification was done using a custom primer panel that targets 135 pathogens which includes ten Lm genes and eight Lm virulence genes. Respective library preparation methods for TAS and WMS were used, and the libraries were sequenced on the Illumina MiSeq platform. GalaxyTrakr and BLAST matching of the amplicons were used for data analysis. Here we show that compared with WMS, TAS is a more sensitive method that can detect spiked Lm in the probiotic products tested to as low as $2x10^6$ CFU, 2 logs more sensitive than WMS. We are working on conditions that will allow TAS to detect Lm at CFUs lower than $2x10^6$. We show that TAS is a sensitive method for detection of low-level contaminants that may be present in probiotic products thus enhancing public health safety.

11. Metagenomic analysis of the microbial community of an experimental hydroponic system growing leafy greens

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Hydroponics is gaining popularity as a sustainable farming method. By using less water and land, and providing a controlled environment, hydroponics can increase food production and reduce contamination risks. However, recent outbreaks of foodborne pathogens (e.g., *Salmonella* in hydroponically grown lettuce) highlight knowledge gaps regarding the microbiomes of hydroponic systems and their potential role in harboring pathogens. To address these gaps, romaine and butterhead lettuce were sprouted in rockwool and then grown in a benchtop deep water culture hydroponic system. Metagenomic sequencing was performed on samples from the root zone, leaves, nutrient solution, and the interior surfaces of the cultivation tank, to characterize the microbiome and to identify potential food safety hazards. The rockwool and water were sampled twice, 5 weeks apart, to provide a temporal dimension to the analysis. The communities were dominated by environmental Proteobacteria, previously observed in hydroponic systems and the lettuce microbiome. The microbial communities varied by sampling location, timepoint, and lettuce type. However, the ten most abundant genera were often similar across samples, although they varied by their ranked abundance. No foodborne pathogens were detected in the metagenomes; however, in some samples *Legionella*, a human pathogen often found in water, was amongst the top ten most abundant genera. Functional profiling of the metagenomes revealed a high abundance of metal resistance genes for copper, mercury, and nickel. These results will inform upcoming projects exploring the role of hydroponic microbiomes in supporting or suppressing foodborne bacterial pathogens.

12. Whole genome sequencing (WGS) as an analytical tool in the *Pseudomonas aeruginosa* in artificial tears outbreak investigation

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In December 2022, the FDA began collaborating with the CDC to investigate a multistate outbreak of antibiotic-resistant Pseudomonas aeruginosa infections associated with artificial tears. As part of this investigation, the FDA collected samples from lots of opened and unopened product. The samples were sent to FDA medical products laboratories for testing where *Pseudomonas aeruginosa* was recovered from both open and unopened products. The genomic DNA of fifteen isolates was extracted and sequenced using Illumina MiSeq systems. Bioinformatic analyses were performed using tools available in GalaxyTrakr. The sequence data was subsequently submitted to the NCBI database. The CFSAN SNP Pipeline was used to compare the FDA and outbreak isolates. Thirteen isolates clustered with the outbreak isolates with a mean of 5.27 SNPs. The isolates had a sequence type (ST) 1203 and exhibited one of two distinctive antimicrobial resistance (AMR) profiles. Seven isolates encoded the $bla_{\text{VIM-80}}$ and $bla_{\text{GES-9}}$ genes which was the profile associated with the outbreak and six isolates encoded the $bla_{\text{VIM-2}}$ and $bla_{\text{GES-9}}$ genes. Additionally, two isolates matched each other but did not cluster with the outbreak isolates. These isolates had an ST 357 and did not encode any of the bla_{VIM} and bla_{GES} genes. Antibiotic-resistant Pseudomonas aeruginosa is a major healthcare-associated pathogen worldwide. The variants associated with this outbreak had not been previously detected in the US. This investigation led to a nationwide recall of all lots of this product and underscored the value of WGS as an analytical tool for the FDA medical products labs.

13. Understanding the host regulatory circuits behind host defense against flavivirus infection

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Background: Rising global temperature is boosting vector (ticks, mosquitoes) populations, leading to more vector-borne flavivirus cases worldwide. Also, it is well documented that human bodily fluids, tissues, and cells can transmit flaviviruses. Flaviviruses such as Zika (ZIKV) and Dengue (DENV) virus can remain in infected tissues for many months even when the individuals are no longer viremic. Herein, we investigate the molecular basis of high tissue tropism exhibited by flaviviruses.

Methods: Vero (monkey) and SK-N-SH (human) cells exhibit cytopathy in response to ZIKV, but not DENV, infection. Cells were infected with ZIKV-MR766 and DENV3 strains at an MOI of 1. Post-infection, cells were collected at various time points to investigate transcriptomic and protein level changes. RNA-seq was performed on the NovaSeq 6000, and differential gene expression was analyzed using DESeq2. Normalized RNA-seq data was visualized with IGV.

Results: Pathway analysis revealed differential expression of RNA processing factors following flavivirus infection. Transcript heatmaps indicated that genes involved in nonsense-mediated decay (NMD), RNA degradation, and the nuclear pore complex (NPC) pathways are upregulated in a time-dependent manner. Notably, our protein study found that ZIKV degrades specific NMD factors in host cells, a mechanism not observed with DENV. These findings demonstrate that flaviviruses employ a multifaceted strategy to hijack host defenses, impacting both transcript and protein levels.

Conclusion: We believe ZIKV, but not DENV, drives host cell cytopathy through targeted protein degradation. We've identified potential biomarkers of flavivirus infection and plan to test them via qRT-PCR on donor PBMCs infected with flaviviruses.

14. Single-cell transcriptomics reveals the immune landscape of the mouse colon during chronic Trypanosoma cruzi infection

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Chagas disease (CD), caused by the blood-borne protozoan *Trypanosoma cruzi* (*T.cruzi*), affects nearly 6 million people worldwide. An estimated 300,000 infected individuals live in the United States, where transmission occurs mainly by local triatomine bugs and from mother-to-baby. Chronic CD can lead to life-threatening cardiac and gastrointestinal complications. Using bioluminescent parasites and ex-vivo imaging, we have shown that the colon is a major site of *T.cruzi* persistence in a chronically infected mice disease model. However, little is known about gastrointestinal CD and how the parasite evades the local host immune response to establish a lifelong infection. This study aims to evaluate the immune cell landscape of the chronically infected mouse colon. Lamina propia single-cell suspensions extracted from infected and naïve mice, were processed using the BD Rhapsody single-cell analysis system. scRNA-Seq analysis identified 17 cell clusters that were assigned to specific cell types based on their marker genes profiles. We found that the percentage of immune cells, such as T/NK cells and B cells, significantly increased (4 and 2.5 folds, respectively, compared to controls) in the colon of chronically infected mice, suggesting their role in gut immunity to *T.cruzi*. Ingenuity Pathway Analysis of differentially expressed genes predicted the activation of canonical pathways associated with the mouse immune response to *T. cruzi* such as S100 protein family, pathogen induced cytokine storm and phagosome formation in macrophages, T-cytotoxic and T-helper cells. Future functional studies will help elucidate how parasites modulate the mouse defense mechanisms to persist long-term in the colon.

15. Untargeted metabolomics and lipidomics in COVID-19 patient plasma reveals severity biomarkers

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has widely varying clinical severity responses. Currently, biomarkers used to assess severity of COVID-19 patients include interleukin-6 (IL-6) and C-reactive protein (CRP), which are inflammatory markers and not specific to COVID-19 disease. The goal of this study is to gain mechanistic insights at the molecular level using LC/MS-based metabolomics/lipidomics to discover predictive biomarkers of severity of infection and outcomes among COVID-19 patients. This cohort study (n=76) included patients aged 16–78 years who tested positive for SARS-CoV-2 and were enrolled from inpatient hospitals and outpatient testing centers in Memphis, TN, between August 2020 and July 2022. The protocol was approved by UTHSC and the FDA Research Involving Human Subjects Committee. Severity of COVID-19 patients was classified as Mild (n=39) or Severe (n=37) based on cumulative points scores of ≤3 and ≥4, respectively. The metabolomics data showed that the tryptophan pathway was altered in severe COVID-19 patients—specifically, increases in kynurenine. Activation of the kynurenine pathway might cause an increased susceptibility to infection. Based on correlation analysis to total severity score, IL-6, and creatine (a kidney function biomarker), a biomarker panel was discovered that contains glucose (pro-inflammation), ceramide and S1P (inflammation related), 4-hydroxybutyric acid (oxidative stress related), testosterone sulfate (immune related), LPC(20:0) (infection related), and creatine. This panel provides better severity prediction with an area under the curve (AUC) of 0.946 versus 0.875 from IL-6.

16. *E. coli* exploits co-infection with *Enterococcus faecalis* to enhance biofilm formation and virulence on antimicrobial urinary catheters

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Infections associated with urinary catheters are often caused by biofilms, which are composed of various bacterial species, that form on the surface of the catheters. This study investigates the intricate interplay between *Escherichia coli* and *Enterococcus faecalis* during biofilm formation on urinary catheter segments using a dual-species culture model. We analyzed biofilm formation and global proteomic profiles to understand how these bacteria interact and adapt within a shared environment. Our findings demonstrated dynamic population shifts within the biofilms, with *E. coli* initially thriving in the presence of *E. faecalis*, followed by a decline in its populations during biofilm development, while *E. faecalis* exhibited a rapid decrease in cell numbers by 48 h in both mono- and dual-species biofilms. Interestingly, the composition of the dual-species biofilms was remarkably diverse, with some biofilms predominantly composed of *E. coli*, others primarily of *E. faecalis*, and still others showing a balanced ratio of both species. Notably, elongated *E. faecalis* cells were observed in dual-species biofilms, a novel finding in mixed-species biofilm cultures. Proteomic analysis revealed distinct adaptive strategies employed by *E. coli* and *E. faecalis* within biofilms. *E. coli* exhibited a more proactive response, emphasizing motility, transcription, and protein synthesis for biofilm establishment, whereas *E. faecalis* displayed a more reserved strategy, potentially downregulating metabolic activity, transcription, and translation in response to cohabitation with *E. coli*. Both *E. coli* and *E. faecalis* displayed significant downregulation of virulence-associated proteins when coexisting in dual-species biofilms. By delving deeper into these dynamics, we can gain a more comprehensive understanding of biofilm-associated infections, paving the way for the development of novel strategies to combat these challenging infections.

17. Visualizing the evolution of RNA viruses: A web-based platform for tracking genetic and antigenic changes

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RNA viruses, such as noroviruses and influenza viruses, are notorious for their ability to rapidly mutate. This facilitates adaptation to new environments and evasion of immune responses, posing a significant challenge to vaccine development. While online tools exist to track the evolution of influenza viruses, there is a lack of similar resources for human noroviruses. To address this gap, we have developed a web-based application that provides a global, spatiotemporal view of norovirus genotype distribution over the past three decades, as well as a tool to track the temporal diversification of residues involved in the antibody-mediated neutralization of GII.4 noroviruses, the most common norovirus genotype. This innovative tool allows users to visualize the changes occurring in viruses circulating each year, including the combination of multiple residues. By combining this tool with antibody binding information, we have demonstrated that current GII.4 norovirus strains have reconstituted conformational epitopes from ancestral variants. The web application, built using the Shiny R platform, is highly flexible and can be easily expanded to track the evolution of other medically relevant viruses, such as influenza H5 and H7 viruses. We anticipate that this tool will facilitate the surveillance and antigenic characterization of emerging noroviruses and influenza viruses with pandemic potential.

18. Baseline proteomic biomarkers for predicting chemotherapy-induced cardiotoxicity in breast cancer patients

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Treatment with the anthracycline, doxorubicin (DOX), is associated with cumulative dose-dependent cardiotoxicity in a subset of cancer patients – limiting its use in those patients. However, there are no qualified clinical biomarkers to predict cardiotoxicity. In this study, 83 breast cancer patients were enrolled and treated with a combination of DOX (60 mg/m^2) and cyclophosphamide (600 mg/m^2). Thirty-nine patients were randomly selected for biomarker discovery, in which nine patients experienced treatment-related cardiotoxicity after completion of chemotherapy. The remaining 44 patients were assigned to the biomarker-validation cohort, in which ten patients experienced cardiotoxicity. SOMAscan analysis of plasma samples before treatment for the biomarker-discovery cohort identified 48 proteins with differential baseline levels between the patients with and without cardiotoxicity. Olink proteomic analysis of the validation cohort confirmed 6 proteins, of which baseline levels of biglycan, carbonic anhydrase 6, cadherin-5, CD109, and thrombospondin-4 were higher, and level of cystatin-F was lower in the patients with cardiotoxicity. A logistic regression analysis indicated that these 6 proteins and higher baseline levels of left ventricular ejection fraction (LVEF) were associated with an increased risk of cardiotoxicity. A model using partial least squares discriminant analysis of these proteins in combination with baseline LVEF, predicted cardiotoxicity with a sensitivity of 80%, specificity of 88%, and overall accuracy of prediction of 86%. These biomarkers and the predictive model could provide new tools for identifying cancer patients at high risk of DOX-induced cardiotoxicity, and multi-center qualification of these biomarkers is underway.

20. Supporting innovation through Sci-Assist bioinformatics method development on precisionFDA

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FDA's Center for Veterinary Medicine (CVM) leverages precisionFDA to support developers through development and approval of intentional genomic alterations (IGAs) in animals. These products have many applications that include improving the health of humans (e.g., xenotransplantation) and animals (e.g., disease resistance).

PrecisionFDA Review Spaces provide developers an option for rapid, secure electronic submission of large next generation sequencing (NGS) datasets to CVM. The precisionFDA platform additionally enables CVM's hands-on bioinformatics method development assistance ("Sci-Assist") to developers of products enrolled in the Veterinary Innovation Program (VIP). The goal of VIP is to facilitate advancements in development of innovative animal products by providing greater certainty in the regulatory process, encouraging development and research, and supporting an efficient and predictable pathway to market for IGAs in animals and animal cell- and tissue-based products (ACTPs). One particular benefit of VIP, Sci-Assist, provides developers help in navigating scientifically challenging questions, such as those related to analytical methods for product characterization. In these cases, CVM may offer technical advice on the design, execution, and analysis of product characterization data. One such example is employing NGS as the identification method for detecting intentional and unintentional genomic alteration(s) in the animal with the IGA.

CVM bioinformatics reviewers have developed over 70 apps on precisionFDA to assist developers in their NGS bioinformatics method development. While developed with VIP in mind, these apps on precisionFDA are not exclusive to developers in VIP and are available to all precisionFDA users.

21. Dashboard to monitor foodborne pathogen sequences available via NCBI's Pathogen Detection Portal

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Background: FDA's GenomeTrakr (GT) program is a network of laboratories sequencing bacterial pathogens isolated from food and environmental samples. Data Management of WGS and associated metadata primarily occurs at the National Center for Biotechnology Information (NCBI) for all the network laboratories. The NCBI Pathogen Detection (PD), which is essential for surveillance and outbreak investigation, analyzes data and builds SNP trees to identify relatedness among DNA sequences from bacterial. We created an exploratory dashboard utilizing metadata from NCBI PD, enabling the visualization of large amounts of metadata for rapid comparison of foodborne pathogens across years, source types, genomic relatedness, and geographical distribution.

Methods: We use Tableau to build a user-centric dashboard interface, applying data visualization best practices. Each visualization highlights one of five components: pathogen diversity, pathogen diversity data object model (DOM) compliant, epi type, genomic relatedness, geographical distribution. Data was obtained from NCBI Sequencing Run Archive (SRA) and NCBI PD through access to Amazon Web Services (AWS) and ftp site, respectively.

Results: We built a dashboard called Characterization of Foodborne Pathogen Sequences Available via NCBI's Pathogen Detection. This resource allows users to review pathogen diversity across years, epi type, genomic relatedness, geographical distribution for the sequenced bacterial pathogens, and the extent to which environmentally sourced bacterial pathogens are related to human isolates. In the process of creating the tools, we identified nearly 100,000 records submitted to NCBI PD without raw sequencing data. Within organism groups, sequences with unassigned epi types ranged from 7 to 63% of all sequences due to submissions using incorrect BioSample metadata packages. More than 50 SNPs above 30% data for organisms other than *Salmonella*. We expect these dashboard statistics will prompt NCBI submitters to address gaps in previously submitted metadata to allow for better performance of NCBI PD algorithms and more powerful analyses based on cleaner, more accurate data.

Conclusions: This GT dashboard provides rapid access to large metadata sets and alleviates users' reliance on a time-consuming process of manually downloading metadata from multiple databases at NCB. Real time availability of data in a dashboard format allows GT data exploration, tracking data releases, identification of trends and needs for data curation.

22. PrecisionFDA: A sandbox for innovative collaborative omics advancement

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precisionFDA is a collaborative, high-performance computing environment that brings together data scientists, regulators, and the global scientific community to analyze multi-omics and real-world datasets and advance regulatory science to improve public health. precisionFDA provides access to data analytic and artificial intelligence (AI) research capabilities, multi-omics applications, shared workspaces for secure data-sharing, virtual workstations supporting relational databases, RStudio, SAS Studio, Jupyter notebooks, and Public Data Challenges.

It advances omics regulatory science, best practices, and research through public Challenges and evolving informatics and AI capabilities.

Since 2017, precisionFDA hosted 41 Challenges and received over 850 submissions, thereby engaging the public and scientific community to optimize innovative statistical, bioinformatics, and AI solutions to advance regulatory science. Omics-focused Challenges include assessing the accuracy/consistency of identifying genomic variations (Olson et al., 2022), identifying/correcting accidental mislabeling of biospecimen samples (Boja et al., 2018, Yoo et al., 2021), identifying pathogens in sequencing data (Sichtig et al., 2019), and benchmarking indel calling pipelines for oncopanel sequencing (Gong et al., 2024).

precisionFDA also supports FDA AI exploration, including running an AI Challenge series. precisionFDA provides capabilities to advance FDA omics and AI research, including a sandbox to fine-tune generative AI large language models, and integration with the FDA Intelligent Decision Lab and Ecosystem (FIDLE) GovCloud platform to empower data transfer into secure FDA environments.

precisionFDA Challenges advance omics regulatory science and help establish best practices/standards. Additionally, precisionFDA supports future omics research by providing a generative AI sandbox environment that can be used for integrative analysis and interpreting multi-omics data.

23. Independent FDA analyses of Nirmatrelvir/Ritonavir resistance in the phase 2/3 trials EPIC-HR and EPIC-SR

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Introduction: PAXLOVID consists of nirmatrelvir, an inhibitor of SARS-CoV-2 main protease (Mpro), copackaged with ritonavir, a pharmacokinetic enhancer. Nirmatrelvir/ritonavir received emergency use authorization in the United States in 2021 and was approved in 2023. However, there is limited published information on SARS-CoV-2 clinical resistance to nirmatrelvir/ritonavir.

Methods: To investigate SARS-CoV-2 resistance development to nirmatrelvir/ritonavir in treated patients, we analyzed baseline and matching post-baseline SARS-CoV-2 next-generation sequencing data from 1,862 participants (912 nirmatrelvir/ritonavir, 950 placebo) in EPIC-HR and EPIC-SR, which were Phase 2/3, randomized, double-blind, placebo-controlled trials in participants with mild-to-moderate COVID-19. Potential resistance-associated substitutions (RAS) were defined as those that were enriched in nirmatrelvir/ritonavir-treated participants or occurred at Mpro positions of interest, defined using nonclinical data. SARS-CoV-2 sequence databases were analyzed to characterize temporal frequencies of nirmatrelvir/ritonavir RAS in circulating viruses.

Results: In EPIC-HR, nirmatrelvir/ritonavir RAS included Mpro T21I (n=1), E166V (n=3), A173T (n=1), and T304I (n=1), with E166V being the clearest RAS observed. In EPIC-SR, no RAS were detected. Nirmatrelvir/ritonavir RAS were not associated with hospitalization or death. Analyses of SARS-CoV-2 sequence databases did not reveal concerning increases in the frequencies of nirmatrelvir/ritonavir RAS over time.

Conclusions: In clinical trials, emergence of SARS-CoV-2 resistance to nirmatrelvir/ritonavir was infrequent (<0.3%-1.1%). Surveillance data currently indicate a low frequency of circulating SARS-CoV-2 variants with nirmatrelvir/ritonavir RAS. Collectively, these results provide the most comprehensive analysis of SARS-CoV-2 resistance to nirmatrelvir/ritonavir in the clinical setting to date. Viral sequences should continue to be closely monitored to identify the potential emergence of nirmatrelvir/ritonavir-resistant variants.