FDA Projects in Scientific Priority Areas

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FDA’s Overarching Scientific Priority Areas

**Rapid Detection:** Development and implementation of rapid, sensitive, high throughput methodologies to detect and identify microbial or other contamination in human derived materials, animals and regulated products and in manufacturing and production sites.

**Adverse Event Detection and Analysis:** Development, implementation and qualification of improved methods for detection and analysis of adverse events associated with use of marketed products.

**Biomarkers:** Development and implementation of new or improved biomarkers, models and methods to predict safety and efficacy of regulated products including drugs, biologics, devices and foods.

**Clinical Trial Design and Analysis:** Implementation of clinical trial design and analysis methodologies to more rapidly and efficiently evaluate safety and efficacy of FDA regulated products.

**Microbial Ecology and Contamination Mitigation Strategies:** Development and implementation of programs to reduce or eliminate the contamination of products by microbial pathogens based on a characterization of routes of contamination and transmission and an understanding of microbial ecology.

**Manufacturing Science:** Development and implementation of innovative, novel technologies in manufacturing science to enhance manufacturing efficiency and product safety, quality and traceability.

**Personalized Medicine and Nutrition:** Development of individualized approaches to therapeutics and nutrition, such as toxicogenomics, pharmacoselection, and complex prognostic and predictive devices, and the use of these techniques to accelerate product development and provide enhanced product and food safety.
Center for Biologics Evaluation and Research (CBER)

Priority Areas:

Rapid Pathogen Detection
Adverse Event Detection and Analysis
Biomarkers
CBER Priority Area:
Rapid Pathogen Detection

Overview

Background:
CBER is responsible for maintaining the safety of the blood supply as well as tissues used for transplantation—essential for the health care system and for our nation’s preparedness. These products, given to millions annually, are, based on their source, always at risk for contamination by known or new infectious diseases. Further, due to their labile nature, they cannot safely be subjected to currently available sterilizing methods to ensure the absence of bacteria, fungi, parasites, viruses or prions. Therefore, in addition to safety measures such as assessment of donor history and travel to defer higher risk donors, blood and tissue donors are specifically tested for relevant transmissible pathogens including hepatitis B and C; human immunodeficiency virus 1 and 2; human T-lymphotropic virus I and II, West Nile virus (WNV), T. cruzi (Chagas disease), syphilis, and, during product manufacturing, in process testing for bacteria and fungi. However, there are additional agents constantly emerging to threaten product safety and availability, whether due to spread of disease vectors (e.g. dengue, babesiosis), travel and immigration (e.g. malaria, HIV O, Leishmania), terrorism (e.g. anthrax), previously rare or unknown pathogens (e.g. vCJD, SARS, Chikungunya). CBER successfully faced a recent major challenge, from WNV, through leadership in facilitating collaborative, rapid development and deployment of nucleic acid screening tests that prevented thousands of infections. This effort showed the potential in enabling collaborative public/private action to foster product development to meet emerging public health needs, particularly where markets are uncertain or will only kick in too late to meet the need. While this effort was successful, there are many more potential threat agents and risks for which screening tests are not available, or not optimal and for which market incentives for development are inadequate to drive preparedness prior to the development of a full blown public health problem. In addition, even the state of the art, current testing technologies will not detect unexpected or unknown pathogens and, they consume product and add significant costs whenever new pathogens are added.

Therefore, the scientific focus in Priority Area 1 is to build on the success of the WNV model to support a systematic, proactive, comprehensive approach toward pathogen detection and response for new and emerging safety threats. We propose three integrated projects—each builds upon the other requiring investment as a package. These projects will support a systematic, coordinated approach to:

1) Periodically identify, assess, monitor and prioritize emerging threats, and initiate staged responses, through improved partnership and risk assessment;
2) For priority threat agents, develop a core scientific team to facilitate development and availability of reagents, samples and methods to enable, through public-private collaboration, the development, validation and deployment of screening tests for new pathogens; and
3) Assess and encourage development of new technologies to enable more rapid, cost-effective screening for blood, tissues (and donors), and other biologics for known, emerging and unknown pathogens (including monitoring and testing during manufacturing).
Rapid Pathogen Detection

**Project Title:**
Proactive Identification, assessment, monitoring of and response to Top Priority Pathogen Threats to Blood and Tissue Supply

**Description:**
This project will support and engage a systematic, ongoing, coordinated process that includes FDA, CDC, NIH, HRSA, WHO, sister regulatory laboratories, academia, and the blood, tissue and diagnostics industries, and consumers to identify, monitor and assess emerging threats and to prioritize and drive the scientific guidance, preparedness and product development response needed.

**Hypothesis:**
Active periodic evaluation of surveillance and other scientific data will enhance preparedness and facilitate product development efforts to allow earlier, more rapid detection and response to maintain the safety of blood and tissues.

**Methods:**
CBER will develop and support a new collaborative partnership that includes relevant government agencies, WHO and other global partners, academia, blood and blood product suppliers, diagnostic developers and consumers to periodically meet to consider emerging scientific and surveillance data to 1) identify and rank the top threats to blood and tissue safety; 2) develop plans to prepare for these threats, including providing needed communications, guidance, project planning and management and helping to direct the efforts of the collaborative partnership in development of new diagnostic methods, reference materials, etc (this component is described in more detail in the next section of this proposal). In order to facilitate this work, CBER must initially build in-house capacity and develop risk assessment models for evaluating and ranking potential impacts of different pathogens (and mitigations) on the blood and tissue supply, while also incorporating surveillance data into the model for informed decision-making. The identification, monitoring and ranking of high priority threat pathogens that will then be accomplished through the partnership will be used directly to drive the work of project 2: providing tools to proactively promote development and deployment of tests needed to screen for the high priority threat agents.

Examples of the types of work which the collaborative partnership could make possible include enhancing collection of blood safety relevant surveillance information such as to support a nationally and globally coordinated approach to surveillance of novel HIV strains that are not detected by current screening assays. A recent site visit at CBER made a specific recommendation for this type of effort. HIV is but one example of many potential pathogens for which improved coordination and data monitoring focused on threats to blood safety would be beneficial. Information from such efforts, such as coordinated evaluation of trends in other emerging pathogens such as dengue and Chikungunya viruses, would be used to model and prioritize risks for both enhanced domestic blood safety surveillance and (Part 2) for reagent and product development (Part 2)

**Deliverables:**
Identification, risk assessment, and prioritization of emerging safety threats in a proactive, systematic, coordinated, data-driven, and more transparent manner to provide needed data for risk-based and proactive “ahead-of-time” preparedness allowing more rapid responses to emerging threats.
Provision of guidance for test development, implementation and other measures, where needed, to protect against high priority threats prior to test availability.

Project leadership and planning to identify and prioritize scientific needs to facilitate assay development and deployment for the threats identified (then implemented collaboratively through the new, dedicated Emerging Pathogen Methods Laboratory described in the next proposal).
Rapid Pathogen Detection

Project Title:
Development of standards, reagents and assays to facilitate rapid response to emerging pathogens that threaten the blood and tissue supply

Description:
For priority threats, we propose to both strengthen internal capacity and to support work collaboratively with other public health partners and the blood and diagnostics industry to proactively develop, test and deploy reagents, reference panels, samples and methods to facilitate rapid response when risks to safety merit advanced preparedness for potential implementation of new screening strategies and tests.

Hypothesis:
Availability of test methods, panels, samples and reagents will facilitate and incentivize rapid, early and effective responses to high priority threats to blood and tissue safety.

Methods:
We will develop and set up a dedicated, new, Emerging Pathogen Methods Laboratory (EPML), composed of both existing and new scientific staff. The EPML’s responsibility will be to acquire or develop needed reagents and panels of samples, and perform pilot testing for assay methods that would be applied to screening blood and tissues for high priority threat pathogens identified by the Collaborative Partnership (see Project 1). Examples of likely current agents of interest include dengue hemorrhagic fever, Chikungunya, malaria, Babesia, and Leishmania. The EPML will work collaboratively with laboratories both inside and outside government, and internationally, to achieve the following tasks to enhance preparedness: 1) develop protocols and plans to generate a global repository of specimens that can be used to evaluate test performance; 2) provide well-characterized specimens for validating different assay platforms as they are introduced into blood banks and other settings; 3) validate standards, panels and reagents for assay performance; 4) coordinate with and transfer materials to CBER’s Division of Product Quality (our centralized program for quality controlled and accredited product testing, lot release and standards) to provide capacity for vial filling, labeling, and making needed reagents and samples available to interested, qualified outside parties. In order to provide rapid response, the methods supported will, where feasible, be based on current state-of-the-art available rapid high throughput screening platforms, such as nucleic acid testing and immunologic-based automated technologies. In the longer term, the EPML will also be tasked with providing needed samples and standards to facilitate the development and evaluation of novel technologies to facilitate novel, currently non-commercial, high throughput, multi-pathogen approaches (see Project 3). Those activities to promote development and evaluation of new technologies will be greatly enabled by the development and availability of the standards and samples developed in the Methods Laboratory in Project 2 and are described under Project 3.

Deliverables:
Increase preparedness for highest priority pathogen threats by reducing lead time required to rapidly and reliably develop, validate and deploy the new tests needed to protect the safety and availability of the blood and tissue supply.
Rapid Pathogen Detection

Project Title:
Harness new cutting edge science for pathogen detection to enhance prevention and rapid response to emerging and unknown threats and to improve product quality through in-process testing and process analytic technologies

Description:
In order to develop long-term solutions to screening blood and tissues/donors and biologics themselves for emerging pathogens, we need to develop different testing paradigms that move qualitatively beyond one assay/one pathogen (and even beyond current multiplexed approaches we have recently approved), in order to overcome development and implementation time and cost problems, to reduce sample volumes consumed in testing, and, perhaps most important, to allow detection of hundreds to thousands of unsuspected or unknown threats. Therefore, we propose to evaluate emerging novel high throughput multi-pathogen detection methods for applicability to screening biologics products including blood, tissues, vaccines, cellular and gene therapies, as well as product manufacturing intermediates (such as cell banks and vaccine bulks). In order for a new technology to be applicable to screening of biologics products, product intermediates, blood and tissues, the method must have a high degree of sensitivity, able to detect single organisms, rather than hundreds-thousands as typical for other applications, such as clinical diagnostics or even detection of food-borne pathogens. The lack of needed sensitivity in addition to their lack of assessment for the spectrum of blood born/transmissible pathogens has been a major problem with many such cutting-edge highly multiplexed technologies (such as microarrays or PCR linked to mass spec). Very low levels of emerging pathogens are typically infectious through parenteral routes vs. typically higher infection and detection thresholds for foods, clinical diagnosis etc. Therefore carefully assessing, and where feasible, enhancing sensitivity of new assays is a critical task in enabling their application to blood and tissue safety.

Hypothesis:
Safety of blood, tissues, and other biologics will be enhanced by evaluation and deployment of validated novel high throughput and high sensitivity ultra-multi-pathogen screening methods. We will ultimately be able to detect all known and many unknown pathogens, and rapidly adopt novel “plug-in” platforms (e.g. best nucleic acid, antibody, and host response assays) to include new and emerging infectious disease agents.

Methods:
We will work collaboratively to assess the feasibility (including sensitivity and specificity for relevant agents), improve, and where possible validate and implement pilot application of new high throughput multi-pathogen methods to screening of donors and biologics. While we will seek collaborators based on an open solicitation, and choose to evaluate the most promising leads, the following are likely examples of what we propose to evaluate. 1) The ViroChip developed at University of California, San Francisco, which utilizes microarray, and may prove to be a sensitive and specific platform for detection of viruses. The ViroChip has been designed to detect both known and unknown viruses due to inclusion of both virus-specific and conserved virus family sequences (over 20,000 sequences currently). While proven useful for detection of known and novel viruses in clinical samples, the limits of sensitivity, a major issue in the application of its use in biologics testing, have not been determined or, likely, optimized. 2) Nanoparticle-based methods: Preliminary assessments in CBER indicate promising sensitivity for blood-borne pathogens using nanoparticles to enhance the sensitivity of traditional PCR or
antibody-based assays. For example Biobarcode nanoparticle detection assays increased the sensitivity of detection of HIV-1 in blood donors over 150-fold compared to conventional ELISA, allowing detection approximately 3 days earlier after infection. We will assess the utility and limits of detection of nanoparticles to enhance pathogen detection by nucleic acid or antibody-based methods using microarray and other formats. 3) The IBIS Biosensor System, which utilizes PCR coupled to analysis by mass spectrometry. While IBIS has been useful to identify and speciate bacterial genomes, the applicability for detecting viruses, and in particular, whether the sensitivity will be sufficient, or can be enhanced for donor, blood, tissue and biologics screening has not been evaluated. Studies of this device may be able to be performed with limited resources and collaboratively with other Centers using the technology for different purposes. 4) Exploratory studies of non-traditional approaches: In addition to the studies described above, CBER proposes to explore non-specific methods that could be deployed to screen potential blood donors for signs of infections. For example, microarray studies of host response genes in donor peripheral blood leukocytes would be used to the feasibility of using a panel of immune response mediators to ask whether a specific host response signature correlates with acute or chronic infection.

For pathogen detection methods found to have similar or better sensitivity, specificity, reliability and reproducibility, and in some cases, reduced time to obtain results, compared to conventional screening methods, CBER will facilitate further collaborative efforts to create next generation donor, in-process and product testing assays.

**Deliverables:**
Enhanced and more rapid pathogen testing for biologics, earlier and more economical detection of known, emerging and new pathogens.
CBER Priority Area:
Adverse Event Detection and Analysis

Project Title:
Enhanced analytic capability: Develop tools to more quickly and reliably identify adverse events caused by administration of biologics

Description:
The goal of this project is to develop and implement methods to simultaneously monitor and analyze a broad range of potential vaccine related adverse events. Together, this work will provide tools to help understand health care data and metadata, and provide improved methods for evaluating vaccine effectiveness using large observational databases. Once developed, these methods could also be applied to, adapted and validated for monitoring other medical products.

Hypothesis:
Analysis of large datasets will increase the rapidity and sensitivity of detection and increase understanding of the significance of adverse events caused by biologics, and may also be useful, when needed (e.g. in a pandemic, or for seasonal influenza) in assessing effectiveness.

Methods:
We will develop and assess methods that determine signal thresholds for further analysis and that integrate different signal streams, while developing potential approaches to, and thresholds for, confirmatory studies, application of modeling and simulation approaches. The principal approach used to date requires selection of pre-specified events of interest and adapts sequential statistical procedures developed for clinical trials. We will develop, test and deploy approaches such as data mining to identify unexpected adverse effects in near real time in large medical encounter and claims databases. In addition, we will evaluate existing approaches to account for the potential "healthy vaccinee effect" and other confounding factors when estimating vaccine safety and effectiveness using observational data in large medical encounter and claims databases and adapt them for near real time application, including for pandemic influenza vaccine.

Deliverables:
1. Improved methods for monitoring safety and effectiveness of vaccines, biologics, and other medical products in near real time, including rapid signal detection of potential adverse events and improved analytic ability, including to exclude false positive signals and to determine cause and effect.

2. Increased preparedness for pandemic influenza vaccine and other emergency product deployments. 3) Increased public confidence in vaccine safety and increased ability to more rapidly assess whether passively reported adverse events and clusters are or are not of concern.
CBER Priority Area:

Biomarkers

Project Title:
Build and apply genomics and personalized medicine to biologics safety

Description:
We propose to build on CBER’s core genomics capabilities, heretofore focused on improving product characterization and quality to provide dedicated review, laboratory and population science/bioinformatics capacity to plan and implement collaborative projects utilizing bioinformatics/genomics to identify molecular markers that predict adverse events and/or allow enhanced targeting or dosing of biologics. In addition, the new project team will enhance collaborative work to advance needed underlying analytic methods and standards. The fundamental objective is to systematically and fully build in and realize the capabilities of genomics throughout the product life-cycle, from planning of studies that lead to licensure, to investigation of potential adverse events.

Hypothesis:
Application of the science of genomics will lead to an era of “Personalized medicine” when a patient’s genetic information can be used to optimize treatment leading to improved outcomes in terms of clinical benefit and reduced risk of adverse events. Given that we anticipate the widespread availability of individualized genomic information within a few years, it is essential that we prepare to realize its potential benefits by fostering the development of genomic knowledge and capacity in areas, such as vaccine and blood safety and effectiveness, that may not be as market driven as and are lagging behind pharmaceuticals.

Methods:
We propose to build in-house review, analytic and laboratory capacity focused on genomics and safety while also leveraging with ongoing initiatives, such as the NIH program for Genome-wide association studies (GWAS, http://grants.nih.gov/grants/gwas/) and the CDC’s genomics initiative (CISA, http://www.cdc.gov/vaccinesafety/cisa/genomics.htm). The GWAS initiative is based on analysis of single nucleotide polymorphisms to identify genetic associations with various observable traits involved in health and disease, while CDC’s genomics initiative is focused on identification of genetic factors predisposing to known adverse events associated with vaccines. We will partner with these initiatives, including by providing funding to conduct 1-2 studies/year focused on identification of genetic markers that correlate with severe adverse events associated with selected vaccines, potential examples might include severe local reactions to anthrax vaccine, thrombocytopenia after MMR, Guillain-Barre syndrome following influenza or meningitis vaccine, viscerotrophic infection with live yellow fever vaccine. In addition, we will collaborate with NIH, CDC and industry to develop standard protocols for sample and data acquisition and storage.

We will also build a core group of review scientists with the required expertise to routinely identify needs and opportunities for obtaining genomic information, partnering with both public sector (e.g. NIH, CDC) and with industry in both pre and post-approval studies, and to analyze bioinformatic data to determine the significance of genomic associations with specific adverse events and the role of interventions such as vaccination (vs. natural genetic susceptibility). In some cases, the core group of review scientists may also identify areas where increased
guidance to industry is necessary and would then be provided to facilitate the types of genomics studies envisioned both pre- and post-approval.

**Deliverables:**

1. Routine inclusion of host genomics samples and studies, where appropriate, in pre and post-marketing assessments by CBER of vaccines and other biologics.

2. Identification of genetic markers that predispose to serious AEs or other clinical outcomes of interest will allow development of strategies to reduce such AEs.

3. Understanding the genetic basis of these AEs, and differences in host response, will help lead to improvements in product design and quality.

4. Guidance to Industry on study design and sample collection to facilitate both pre- and post-approval genomic studies.
Biomarkers

Project Title: Development and use of improved preclinical models to identify and assess biomarkers for the safety and efficacy of cellular therapies, including stem cells and engineered tissues

Background: Advances in materials science, developmental, cell and molecular biology have converged into a new field, regenerative medicine. Regenerative medicine products being actively developed (several hundred IND's at CBER) hold great potential for definitive treatment of a wide array of human medical conditions, ranging from genetic disorders, neurological conditions, diabetes, arthritis and cancer to repair of damaged tissue and ultimately to organ transplantation. One particularly active area of investigation is the use of human stem cells to derive cells of desired function and structure to treat disease or replace failed organs, tissues or functional capacity. While these developments are exciting, the technologies are so novel that there is a lack of information to fully assess and understand even the most basic and serious questions about potentially associated risks and about how best to assess efficacy. Scientific and regulatory concerns include where stem cells migrate after transplantation, whether they will cause tumors, and whether the desired differentiated function remains stable. These challenges are further complicated by the lack of relevant, useful in vitro and animal models, a serious challenge when seeking to assess the survival, safety and function of candidate human stem cell-derived products in animal models with very different physiology, anatomy, and immune systems. Some major questions that arise as CBER is working with NIH, academia, and industry innovators to facilitate safe development and evaluation of these products include: 1) What are the key characteristics of stem cell-derived products to assess product quality, safety, and potency? 2) What is the influence of the local microenvironment on cell survival, differentiation, phenotype expression following implantation? 3) What is the stability of the phenotype of the derived cells before and after implantation? 5) Where will cells migrate after implantation and what adverse effects such as ectopic location and tumorigenicity are likely to occur? 6) What are the mechanisms of action – direct effect of the cell product or indirect effect on recipient cell function to induce endogenous repair, or both, or others? 7) What are the best models to utilize before human studies that could help assure safety and better predict likely efficacy? 5) What cell characteristics should be measured to characterize the product?

The development of improved, more practical and predictive preclinical models for evaluation of safety and efficacy of cellular therapies and tissue engineered products represents a critical step in moving the entire field forward. The project proposed under this priority provides an integrated approach towards identification of critical biomarkers of product safety, quality, and potency while developing models that could be critical for evaluating stem cell-derived cellular therapies and with potential applicability towards evaluation of tissue engineered products. Embracing such approaches could significantly facilitate development of new clinical interventions to treat disease. Once relevant models are established, key cellular characteristics measured in vitro can be correlated both with specific biological outcomes in relevant animal models and ultimately bridging to human clinical trials. State-of-the-art imaging techniques will be used to provide as much in-life, in vivo observation of cell fate, cell migration, and cell function to the extent that the technology and animal models permit.

Description: We propose helping to support the collaborative step-wise development and analysis of three preclinical models while concurrently developing tools to identify and measure the key characteristics that can be measured in vitro that correlate with biological outcomes in vivo.
Characterization of stem cell-derived products: Human embryonic stem cells will be induced to differentiate using published methods proven to cause certain cell phenotypes. The differentiated cells that will be used for animal studies will be analyzed by gene expression microarrays, mass spectroscopy to identify proteins, and cell surface phenotype by flow cytometry.

Improved murine cell therapy models: Currently available mouse strains that carry reporter genes under the influence of cellular promoter/enhancers that are known to influence cell fate, differentiation, and function (i.e., cell signaling pathways) will be used in disease or tissue injury models to assess host responses and the differentiation and function of cell therapies. Effects of changes in manufacturing and cell culture conditions will also be assessed. Initial studies will be performed in available transgenic mouse models using murine counterparts to human cellular therapies. The murine cells will be assessed to provide as many functional and phenotypic characteristics for comparison to the human counterparts as possible (this will provide critical bridging data for later studies described in this proposal).

Immunodeficient mouse models to allow human cell transplants: Due to immune rejection of human cells in immuno-competent mice, the models proposed above would not allow direct evaluation of human cell-based therapies in vivo. In order to allow for preclinical assessment of human cell therapies, we will derive analogous immunodeficient mouse strains to yield animal models that will allow engraftment and survival of human cells, thus facilitating correlation of observations made in the mouse cell-mouse transplant model. Results with the chimeric mouse-human models will be compared with the morphological and functional data collected in the mouse-mouse model to allow for identification of the most critical pathways and biomarkers that provide information about cell fate, differentiation, and function. Thus, the mouse-human chimera will be used to identify and evaluate potential safety and efficacy biomarkers for human stem cell-derived therapies intended for use in humans.

Large animal model for human cell transplantation and tissue engineering: Data generated in murine models do not always translate to clinical outcome due to limitations of size, anatomy, and physiology. Therefore, we will seek collaborations with relevant academic and industry scientists who are able to develop large animal model(s) that allows engraftment and survival of human cells and tissues, allowing safety and efficacy assessment in a relevant and reproducible large animal model.

Hypothesis:
Scientifically relevant rodent and nonrodent new animal models, including models that do not reject transplanted human cells, can be developed to better evaluate the safety and effectiveness of human stem-cell based products.

Methods:
1. Initial studies will be performed in available transgenic mouse strains that carry fluorescent reporter genes under the transcriptional control of enhancer/promoter elements from genes involved in signal transduction pathways or other genes known to be important during cell development, differentiation, wound repair, and inflammation. Cell-cell interactions that trigger expression of these reporter genes would therefore provide an in vivo marker for the effect of the stem cell-derived cells on signaling in host environment and vice versa, by designing the studies using the transgenic mice as donors or recipients of the genetically modified cells. The use of a fluorescent marker that would only be expressed during certain conditions of cell differentiation or specific activation states allows one to determine the cell differentiation status and function while
simultaneously tracking the localization of the cell therapy. Initial models to be utilized include heart, brain, and peripheral limbs damaged by induced ischemia, followed by use of stem cells to assess their ability to repair ischemic damage.

2. As data emerge from the mouse-mouse model, the transgenic mouse strains that are most informative with regard to determining cell localization, differentiation, and activation state will be back-crossed onto the NOD/SCID mouse strain. Development of an immunodeficient mouse strain that carries the same fluorescent marker genes would allow expansion of the exploratory mouse-mouse studies to studies using human cellular therapies that can be directly purchased or obtained by MTA or CRADA from relevant academic and private industry sources. Similar analyses that were described above would be applied to the chimeric mouse-human models.

3. Presently, large animal models are limited to short-term studies due to immune rejection of administered cells. Studies providing information on the long-term function and safety of stem cell-derived products are needed to evaluate stability of the cell phenotype, tumorigenic potential, and long-term efficacy. The ability to apply transgenic and nuclear transfer methods to generate genetically modified pig strains have been reported (Exp.Biol. Med. (2004)229:1120-1126 Science (2002) 295: 1089 – 1092). We would seek collaborations with laboratories that have successfully made genetically modified pigs in order to generate immunodeficient pigs using knock-out or transgenic technologies, as necessary, to allow for in vivo assessment of the safety and effectiveness of human stem cell based therapies. It is likely to be practical to produce immunodeficient pigs since pigs can be bred and raised in germ-free environments (eg: Gnotobiotic pigs J Virol (1998) 72,:330-338). The biomarkers identified as possible predictors of safety and efficacy in the mouse models will be re-evaluated using disease/injury models in genetically modified immunodeficient pigs. If the technical and animal husbandry challenges of creating and maintaining an immunodeficient pig strain are met, a future extension of this project could include addition of reporter transgenes to gather information about cell-cell interactions in this model. Another expansion of this project would be to utilize the system of Pratt et al (Tissue Engineering A, (2008)DOI: 10.1089=ten.tea.2008.0117) to generate transgenic pigs with a "humanized" immune system that potentially would allow assessment of the impact of human immune responses to allogeneic cell transplants in a large animal model.

In all phases of the proposed studies state-of-the-art imaging techniques that are available and relevant to the proposed model will be employed to perform in-life analyses including: 1) ferromagnetic or radiolabelled cells will be detected by MRI or PET, respectively, or fluorescently tagged cells detected by whole body fluorescence imaging will be used to determine biodistribution; 2) micro-CT will measure structural changes related to tissue regeneration and repair; 3) ultrasound can be used to measure cardiac function; 4) intravital imaging using endoscopic analysis will measure expression of the fluorescent transgenes to determine cell survival and fate. Currently, CBER investigators use imaging facilities available at NIH, but we propose that similar capabilities will be required as we move to our new laboratories at the White Oak campus in FY12 in order to support in-life imaging as proposed for intramural projects.

**Deliverables:**
Improved characterization of stem cell-derived products and relevant preclinical models that allow for direct assessment of candidate human cell therapies for safety and effectiveness. The mouse and large animal models proposed could also be used for studies to determine
biomarkers for host cellular responses to a variety of tissue engineered products such as cells seeded onto structural matrices for bone, bladder, or skin repair.
Improved guidance to sponsors about how to perform preclinical assessment of human cell and tissue engineered therapies.
Center for Drug Evaluation and Research (CDER)

Priority Areas:

Adverse Event Detection and Analysis / Biomarkers
Clinical Trial Design and Analysis
Manufacturing Science / Rapid Detection
CDER Priority Area:
Adverse Event Detection and Analysis / Biomarkers

Project Title:
Analysis of Medical Product Adverse Events Utilizing a Distributed Network: Efforts toward building a Sentinel System

Description:
In May 2008, the Secretary of Health and Human Services and the FDA Commissioner announced the Sentinel Initiative. The Sentinel Initiative is a long-term effort by the FDA to create a national electronic system for monitoring medical product safety. The System is intended to augment FDA’s existing post-market (primarily passive) safety surveillance systems and to allow the Agency to actively gather information about the post-market safety and performance of its regulated medical products.

The Sentinel Initiative will include the development of a new electronic system, called the Sentinel System, that will enable FDA to conduct active medical product safety surveillance using large electronic healthcare databases (e.g., electronic health records, patient registries, insurance claims). During at least the initial phases of Sentinel, the data would remain with their owners, to whom FDA would send queries. In accordance with existing privacy and security safeguards, dataholders would evaluate their data and send results to FDA for review.

The Sentinel Initiative is a response to various calls for this type of effort from the Agency. In September 2005, the HHS Secretary asked FDA to expand its current system for monitoring medical product performance and to explore the possibility of working with multiple healthcare data systems to augment the Agency’s current capabilities of identifying and evaluating product safety information. The Secretary recommended that FDA explore creating a public-private collaboration as a framework for such an effort, leveraging large, electronic healthcare databases.

In 2006, the IOM issued a report, entitled The Future of Drug Safety—Promoting and Protecting the Health of the Public. Among other suggestions, this IOM report recommended FDA identify ways to access other health-related databases and create a public-private partnership to support safety and efficacy studies.

In 2007, Congress enacted the Food and Drug Administration Amendments Act of 2007 (FDAAA). Section 905 of this statute calls for the HHS Secretary to develop methods to obtain access to disparate data sources and to establish an active post-market risk identification and analysis system that links and analyzes healthcare data from multiple sources. The law sets a goal of access to data from 25 million patients by July 1, 2010, and 100 million patients by July 1, 2012. The law also requires FDA to work closely with partners from public, academic, and private entities. FDA views its Sentinel Initiative as a mechanism through which some of the requirements mandated in this legislation can be carried out.

2 Food and Drug Administration Amendments Act of 2007, Public Law 110-85, was signed into law in September 2007. See Title IX, Section 905.
The initial stage of the Sentinel Initiative has allowed the Agency to further refine the requirements and develop the scope. FDA has funded 8 contracts to support this initial stage. As a next step, FDA would like to conduct a proof-of-concept pilot that would integrate the learnings from not only its current contracts, but other activities ongoing in both the public and private sectors to further understand the feasibility and utility of these data and methodologies for active medical product surveillance.

The current pilot would involve developing working agreements with databases from different healthcare data sources and the infrastructure to perform statistical queries of these data. This pilot would also evaluate the ability of these queries to detect defined medical product adverse events that may represent increased risks over baseline. The pilot will inform the development of an active medical product surveillance system as envisioned by the Sentinel Initiative.

Hypothesis:
1. It is possible to devise an organizational structure for targeted queries about medical product-adverse event pairs to enable parallel, real-time interrogation of different types of healthcare databases.
2. Such queries can add information to traditional sources of early safety signals (spontaneous reporting and clinical trials).
3. Statistical techniques (which may include datamining) can be used to interrogate such databases for signal identification and strengthening.
4. Barriers to such a system (legal, functional, structural or methodological) can be addressed and overcome.

Methods:
Develop a distributed data model
1. Solicit at least three health data environments with varied attributes to conduct analyses.
2. Convene a Planning Board, representing the contracted data environments and the FDA, to develop governing documents that specify the governance structure of the consortium that will enable the conduct and methods of the proposed evaluations.
3. Under the planning board, establish a Safety Science committee charged with the day-to-day execution of analysis and evaluation.
4. Develop a means (infrastructure) to allow for communication and queries with contracted data repositories.
5. Identify and prioritize potential medical product-event pairs for evaluation.

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6. Engage appropriate legal counsel to identify and address data privacy and security issues related to the conduct of this pilot.

**Evaluate emerging methods in safety science**

7. Identify and apply appropriate statistical methodologies employed for evaluation of product-event pairs (hypothesis strengthening); these statistical methodologies should be appropriate for data environments queried, the research questions posed and selected in discussion with the researchers representing the data environments.

8. Identify and apply appropriate statistical techniques for adverse event identification (hypothesis generation) for evaluation in contracted data sources.

**Synthesize lessons learned**

9. Identify existing obstacles to the development of a working Sentinel System. The following areas should be considered:
   - Technical capacity;
   - Infrastructure needed;
   - Incentives;
   - Legal impediments;
   - Conflicting state and federal laws;
   - Funding.

10. Prioritize identified obstacles in order of importance, and identify means and methods to overcome each obstacle.

11. Identify the time and resources required to remove each obstacle, and demonstrate potential resources to address these obstacles.

**Deliverables (Fiscal Year):**

**2009:**
- Report on potential medical product-event pairs for evaluation
- Report on appropriate potential methodologies for evaluation
- Report on potential data sources for inclusion in pilot
- Award contract to support project (if funding notification before March OAGS deadline)

**2010:**
- Develop infrastructure for pilot
- Convene planning board and develop appropriate governance
- Report on initial analyses and findings
- Report on potential statistical techniques for evaluation
- Report on obstacles and plans to overcome in order to create and implement Sentinel Initiative

**2011:**
- Report on query analyses and findings, to include utility
- Report on subsequent analyses
- Report on recommendations for Sentinel Initiative
Project Title:
Risk assessment of drug-induced phospholipidosis in the CNS

Description:
Over 180 marketed and investigational drugs can induce phospholipidosis (the excessive intracellular accumulation of phospholipids) in laboratory animals in preclinical toxicology studies. The clinical significance of drug-induced phospholipidosis (DIPL) is difficult to assess in the absence of a qualified biomarker or other noninvasive test to monitor its incidence. DIPL, especially in the CNS, presents a concern to regulators because phospholipid accumulation is associated with inherited lysosomal storage disorders such as Niemann-Pick disease. The development of DIPL during preclinical testing in animals may result in delay or discontinuation of new drug development. In order to resolve the concerns about DIPL, there is a need to develop a fundamental understanding of the process (including identification of valid biomarkers) such that regulatory policy can be developed on DIPL.

The tissue distribution of drug-induced phospholipidosis is usually generalized, occurring in multiple tissues of the body. Although brain is a less commonly reported site of occurrence, chloroquine, quinacrine, 4,4'-diethylaminoethoxyhexestrol, chlorphentermine, iprindole, 1-chloro-amitriptyline, clomipramine and azithromycin have all been reported to cause lipidosis in the choroid plexus (Acta Neuropathol. 1979 May 15;46(3):203-8; product monograph, Pfizer Canada 1994). Additionally, classes of centrally acting drugs like the selective serotonin reuptake inhibitors and tricyclic antidepressants are associated with generalized phospholipidosis.

There is evidence that bis(monoglycerol) phosphate (BMP) levels in the urine, assayed using liquid chromatography coupled to mass spectrometry, may have utility as a noninvasive biomarker of generalized phospholipidosis that could be translatable to the clinic (Baronas et al., 2006). However, there is currently no data available on the sensitivity and specificity of levels of BMP or other phospholipids in cerebral spinal fluid as a potential diagnostic assay of DIPL incidence in the CNS. This assay could provide a valuable tool for monitoring drug safety in the clinic.

With the aim to address the gaps in our knowledge about DIPL, a database of DIPL findings in various species (e.g., rats, dogs, mice, monkeys, humans) is currently under construction by members of FDA/CDER’s Phospholipidosis Working Group. The database is being constructed with information from regulatory submissions including clinical as well as non-clinical data. This database will help CDER develop regulatory guidance on how to address DIPL findings during drug development. However, the database will also serve to help identify scientific gaps by allowing the design and conduct of appropriate research projects. This is why the DIPL database will form the foundation of this proposal.

Animal models of drug induced phospholipidosis in the CNS will be used to assess whether BMP or other phospholipid components provide good diagnostic correlation. The CDER phospholipidosis database will be expanded to include recorded incidences of DIPL in the CNS based on NDA and literature reports. The structures of the DIPL inducing drugs and their metabolites from the chemistry database and physicochemical properties such as log P/log D and pka will be used to build developmental models to predict which investigational drugs have potential to induce DIPL in the brain. Subsequently, a predictive model of permeability across the blood-brain barrier (BBB) based on chemical structure and physico-chemical properties will
be used to predict which drugs with known phospholipidotic activity could potentially cause DIPL in the CNS. The identified drugs will be used in animal studies to generate an animal model of DIPL in the CNS. Histopathology and lipid analysis in blood, urine, brain and the CSF will be conducted to acquire a dataset to evaluate the correlation of DIPL in the brain with BMP in the CSF and blood and urine. AY-9944 will also be used as a model compound for assessing the correlation of PL in the brain with BMP in the CSF. The cholesterol synthesis inhibitor AY-9944 can be used to induce an animal model of Neimann-Pick disease that exhibits lamellated cytoplasmic inclusions and reduced sphingomyelinase activity in oligodendroglial cells and other tissues (Sakuragawa et al., Science 196: 317, 1977).

Hypothesis:
Phospholipidosis in preclinical animal models is the result of organ toxicities and can be predictive of adverse effects in clinical settings. Molecular “structural alerts” have previously been used to predict the likelihood of inducing DIPL, and it is hypothesized that, given adequate training data, a quantitative structure-activity relationship (QSAR) model can be constructed to predict organ-specific DIPL in animal models, which can be used to extrapolate to organ-specific DIPL risk in humans. The use of predictive computational toxicology tools in combination with appropriate biomarkers that contribute to the accurate prediction of phospholipidosis in the brain, can prevent the appearance of serious and irreversible damage to the CNS.

Methods:
This project seeks to investigate the underlying relationship between DIPL findings in animals and clinical populations and explore ways to predict not only the incidence of DIPL, but also the toxicity that may be a direct result of DIPL. The project involves a multi-faceted approach that is centered around the enhancement of a comprehensive database of DIPL findings. The database currently contains chemical structures for all records to facilitate the investigation of molecular structural and physico-chemical attributes that are associated with DIPL activity, allowing the data to be used for modeling QSAR. Furthermore, the database contains some organ-specific data that can be used to select marketed drugs that are known to cause DIPL, and that can serve as candidates for further animal studies for biomarker identification. The various parts of the project are as follows:

1. Expand the phospholipidosis database to contain organ-specific data that can be the foundation for organ-specific QSAR modeling, thereby expanding the scope and usefulness of the current models. Specific attention will be placed on recording incidences of DIPL in the CNS and retina.

2. Enhance the existing QSAR models previously developed by CDER/FDA in order to address organ-specific predictions of DIPL. Once the database has been populated by adequate organ-specific DIPL data, software programs such as MC4PC, MDL-QSAR, Leadscope Predictive Data Miner, and BioEpisteme can be used to develop QSAR models.

3. Acquire and validate a predictive model of permeability across the blood-brain barrier based on chemical structure and physico-chemical properties. The enhanced database and QSAR model will be used to screen a list of drugs in the database for which there is evidence of generalized phospholipidosis. These drugs will be selected for use in animal and clinical studies to evaluate a potential biomarker of DIPL.
4. Develop animal models of phospholipidosis in the CNS using model compounds with known or predicted phospholipidotic activity to use for assessing the sensitivity of lipomic biomarkers. The presence of the lipomic biomarkers in blood, urine and CSF will be correlated with the use of advanced fluorescence imaging technologies for detection of DIPL.

5. Conduct a clinical study in patients currently taking drugs shown to preclinically result in DIPL, in order to evaluate the usefulness and sensitivity of the lipomic biomarkers being considered. Concomitantly, a study of post marketing human adverse events reported for all known drugs causing DIPL in animals will be undertaken in collaboration with OSE, in order to establish a link between the rat tissue genomic profile, blood, urine and CSF lipomic biomarker and the reported adverse events from post marketing data.

**Deliverables:**

2009:
- Enhance existing QSAR models (using MC4PC and MDL-QSAR) with recent data findings (~100 new molecules).
- Perform study to determine whether other modeling software (Leadscope Predictive Data Miner and BioEpisteme) offer feasibility.
- Add data fields to PL database to report each drug’s ability to cross the BBB.
- Develop a comprehensive database on DIPL which will provide information on various aspects of DIPL. The database will actually be composed of several databases which are the following: toxicology, chemistry, ADME, clinical, structure and summary database.

2010:
- Develop BMP as a biomarker of DIPL in an animal model.
- Initiate a study to evaluate the pattern of post marketing adverse events reports in patients treated with DIPL-causing drugs.
- Continue development of the DIPL database with enhanced organ-specific DIPL and BBB data.
- Develop and validate QSAR models to predict DIPL in target organ systems.
- Begin building models based on physicochemical properties of drugs, in order to predict DIPL.

2011:
- Design and conduct a clinical study in human subjects treated with DIPL causing drugs, in order to evaluate BMP as a non-invasive biomarker.
- Correlate results of biomarker studies in animal and clinical study and compare with adverse events reports from post marketing data.
- Develop a framework for a DIPL guidance document.
- Complete and release phospholipidosis database to include data on physico-chemical properties, organ-specificity of DIPL and likelihood that drugs can cross BBB.
- Complete and release final version of QSAR models to predict organ-specificity of DIPL-causing drugs.
**Project Title:**
Effect of proton pump inhibitors (PPIs) on the pharmacokinetics and pharmacodynamics of clopidogrel: Impact of CYP2C19 genotypes and PPI class effects

**Description:**
Clopidogrel, a prodrug, is an inhibitor of platelet aggregation. Biotransformation to the active metabolite involves two sequential steps which are mediated via several cytochrome P450 isozymes such as CYP2C19, CYP1A2, CYP2B6, CYP3A4/5 and CYP2C9. Several published articles have pointed to the important role of CYP2C19 in the active metabolite formation. One recent article reported that carriers of a reduced-function CYP2C19 allele had significantly lower levels of the active metabolite of clopidogrel, diminished platelet inhibition, and a higher rate of major adverse cardiovascular events.\(^1\)

Patients taking clopidogrel are often receiving a proton pump inhibitor (PPI) to prevent GI bleeding. There have been reports that patients on concomitant PPIs as a whole had higher cardiovascular events than those without concomitant PPIs.\(^2\) In vitro studies\(^3\) showed that PPIs inhibit CYP2C19 (among other CYP isozymes) to various degrees with lansoprazole having the highest interaction potential and pantoprazole the lowest. Therefore, concomitant PPI use may reduce the formation of the active metabolite and consequently reduce the efficacy of clopidogrel, resulting in more cardiovascular events.

Several PPIs, including omeprazole, lansoprazole, pantoprazole and esomeprazole have been evaluated by various investigators for their interactions with clopidogrel. So far, only omeprazole was reported to have significant interactions. However, the results of some studies appeared contradictory.\(^4,5\) It is noted that these studies differed in study design and assay methodology for evaluating platelet aggregation and lacked individual genotyping information. Some articles did not report the doses/dosing regimens for PPIs. As such, the interactions between various PPIs and clopidogrel remain unclear and confusing.

The purpose of this project is to determine how genetic polymorphism in CYP2C19 impacts the interactions between PPIs and clopidogrel and whether there is a class effect for the interactions.

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3 Xue-Qing Li, Tommy B. Andersson, Marie Ahlstrom, and Lars Weidolf, “Comparison of Inhibitory Effects of the Proton Pump-Inhibiting Drugs Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole, and Rebeprazole on Human Cytochrome P450 Activities” Drug Metabolism and Disposition, 32(8),821-7, 2004
Hypothesis:
1. Genetic polymorphism in CYP2C19 impacts the pharmacokinetics of both clopidogrel (particularly its active metabolite) and PPIs. Therefore, the interaction between clopidogrel and PPIs depends on the CYP2C19 genotype.

2. Interaction between clopidogrel and PPIs is not a class effect and the degree of interactions differs among PPIs depending on their CYP2C19 inhibitory potency.

Methods:
A study in human subjects will be conducted.

Study design: This will be a randomized, open-label, crossover study with sufficient washout between study periods to allow regeneration of the platelet P2Y12 ADP receptors.

Subjects: The study will be conducted in healthy volunteers to minimize confounding factors.

Genotyping: CYP2C19 genotyping will be conducted to enroll sufficient number of extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM). Genotyping for additional genes, such as CYP2C9, P2Y12, may be considered for covariate analysis.

Treatments:
Three PPIs, including omeprazole, lansoprazole, and pantoprazole as well as a placebo will be coadministered with clopidogrel. Lansoprazole and pantoprazole are chosen as they represent the highest and lowest CYP2C19 inhibition, respectively, based on in vitro studies. Omeprazole is also included because of its significant interactions as reported in the literature.

The highest PPI dose for the indication will be used (once daily) in the study. In each study period, volunteers will be pretreated with a PPI (or placebo) for 3 days to reach a “steady state” for PPI since PPI concentrations increase following multiple dosing. PPIs will be continued for another 6 days. On Day 4, volunteers will also receive a 300 mg dose of clopidogrel followed by clopidogrel 75 mg once daily for 5 days.

PK and PD analyses:
Samples will be collected for both PK and PD analyses following the first and last clopidogrel doses.
PK: Samples will be assayed for the clopidogrel active metabolite and PPI concentrations following the clopidogrel loading dose and on day 5 of clopidogrel.
PD: The antiplatelet activity of clopidogrel will be assessed by VASP assay, since this is specific for P2Y12 receptor inhibition, following the clopidogrel loading dose and on day 5 of clopidogrel.

Deliverables:

2009-2010:
Complete the study conduct, study report and manuscript regarding the impact of CYP2C19 genotyping on drug interactions between PPI and clopidogrel. Clear recommendations regarding concomitant PPI use will be made based on the study results.
CDER Priority Area:
Clinical Trial Design and Analysis

Project Title:
New Outcome Measures and Data Collection Methods for Improving CDER Bioresearch Monitoring Compliance Programs

Description:
Performance evaluations of CDER Bioresearch Monitoring (BIMO) Compliance programs currently do not include measures of the outcomes regarding public health and regulated populations. The sole use of measures of output, such as the number of inspections and enforcement actions, provides only a partial performance assessment that limits the optimal strategic allocation of resources and activities. This project proposes the following hypothesis: that CDER BIMO Compliance programs can be much more effective in meeting program goals if better metrics and methods are developed for program evaluation.

The mission of the BIMO programs is to ensure human subject protection and data integrity in bioresearch studies. Challenges to the current BIMO Compliance programs include globalization of clinical trials, increasing numbers of generic drug applications, and increasing reliance on electronic records. Meeting these challenges will require changes to BIMO Compliance priorities and policies that should be guided by informed program evaluations in order to support the public health mission of the organization.

Performance metrics are critical to the continuous reassessment of program effectiveness and progress. As a subset of performance metrics, outcome measures are particularly helpful because they can assess the mission impact of a regulatory program. Using appropriate performance measures, allocating limited resources can be substantially more informed and strategic. This proposal describes a New Outcome Measures Program for the development and use of outcome measures and data collections methods for BIMO Compliance.

Outcome measures are intended to assess the impact of BIMO Compliance programs on public health and the behavior of regulated populations. The EPA Office of Enforcement and Compliance (OECA) has organized outcomes into the following categories: changes in understanding, changes in behavior, and public health improvements. The development of useful outcome measures will require simultaneous development of new data collection methods. The data collection methods that can be used to generate data for outcome measures can be quantitative or qualitative. As noted by OECA, some of the available data collection methods “include surveys (mail, fax, email, Internet, and phone) and observed data (on-site revisits, pre/post tests, and reviews of self-reported data).”

An example of a “change in behavior” outcome measure is the rate at which Institutional Review Boards (IRB) complete follow-up reviews for clinical study protocols. An online survey could be created in which IRB board members self-assess their follow-up review rate. This survey could be run every two years to assess the performance of compliance assistance programs aimed at increasing the follow-up review rate. Many other potential outcome measures could be

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1 Guide for Measuring Compliance Assistance Outcomes, Revised October 2007, Office of Enforcement and Compliance Assurance (OECA), Environmental Protection Agency, EPA 300-B-07-002
developed for CDER BIMO Compliance programs in the following areas: Good Clinical Practice, Bioequivalence, and Good Laboratory Practice.

The effective development and use of outcome measures is greatly facilitated by having a systematic process for evaluating the effectiveness of public health programs. One system that could be used as a model for BIMO Compliance is the Framework for Program Evaluation in Public Health published by the CDC. The framework describes six steps in program evaluation: engage stakeholders, describe the program, focus the evaluation design, gather credible evidence, justify conclusions, and ensure use and share lessons learned. This system and others will be evaluated as part of the development of the Outcome Measures Program.

After a ramping up period, the New Outcome Measures Program would be able to continuously conduct data collection and analysis for defined outcome measures while also developing new outcome measures as needed for BIMO programs in the Division of Scientific Investigations (DSI), Office of Compliance. In order for the New Outcome Measures program to succeed, several skill sets would be needed including data analysis, survey development, BIMO program knowledge, and project management. The program would require development, pilot, and production phases with significant input and guidance from DSI in the Office of Compliance.

A contractor should be used for the development and pilot phases and the program should be run entirely by CDER personnel once sufficient expertise is available. The contractor should have expertise in data collection methodology for evaluating the effectiveness of public health programs.

**Hypothesis:**
CDER BIMO Compliance programs can be much more effective in meeting program goals if better metrics and methods are developed for program evaluation.

**Methods:**
Initially, DSI staff will work with the contractor to identify program outcomes and public health impacts relevant to high priority compliance areas. Some possible areas for outcome measures include levels of compliance in regulated populations (e.g. CROs conducting BEQ studies) and responses of violative entities (e.g. rates of improvement in clinical investigators found to have GCP violations).

Outcome measures are intended to assess the impact of BIMO compliance programs on public health and the behavior of regulated populations; these measures can be organized into the following general categories: changes in understanding, changes in behavior, and public health improvement.

The data collection methods that can be used to generate data for outcome measures can be quantitative or qualitative. As noted by OECA, some of the available data collection methods “include surveys (mail, fax, email, Internet, and phone) and observed data (on-site revisits, pre/post tests, and reviews of self-reported data).”

Data analysis methods will include standard statistical methods of description and association as appropriate for each data collection project.

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Deliverables:

2009:
- Complete the Project Definition Phase; determine and verify the assumptions related to metric attributes for quality and quantity data collection.
- Collect bids from contractors
- Select contractor
- Develop specific outcome measures for 1-2 program areas

2010:
- Create data collection methods for top 2 outcome measures
- Conduct data collection for top 2 outcome measures
- Analyze data and generate reports
- Evaluate and make change to programs based on reports
- CDER has in-house expertise to create outcome measures and data collection methods
- Develop data collection methods for other outcome measures

2011:
- CDER is self-sufficient to conduct routine data collection and analysis
- CDER is self-sufficient to develop new outcome measures
Clinical Trial Design and Analysis; Adverse Event Detection and Analysis

Project Title:
Development of bioinformatic tools to improve drug safety and to consistently predict and assess complex drug interactions including genetic components

Description:
The FDA recommends that during drug development, the effect of individual ethnic factors on its safe and effective use be evaluated. Intrinsic factors (e.g., age, gender, race, organ dysfunction, and genetic polymorphism of drug metabolizing enzymes, transporters, and receptors) and extrinsic factors such as food, juice, dietary supplements, and concomitant drugs that may affect individual’s drug exposure and response are often separately evaluated and the results listed in the drug label with recommendations for dosing adjustments for any specific factor when appropriate. However, evaluation of their combined effect (such as the presence of two or more concomitant drugs that are enzyme inhibitors; the presence of drugs that are both enzyme inhibitors and inducers; presence of a drug that is an enzyme inhibitor in renal impairment patients or patients with variant genotype for an enzyme or transporter; or the presence of a drug that is an enzyme inhibitor in pediatrics) has only been variably conducted and dose recommendation in the labeling is not always clear. The increasing prevalence of “poly-pharmacy” (concurrent use of > 5 medications) may render patients greater vulnerability to adverse drug reactions caused by drug interactions. A current Medco study regarding the potential effect of proton pump inhibitors on the efficacy of clopidogrel is one example of critical drug interactions to evaluate and prevent. Results from recent publications on differential efficacy of clopidogrel in patients with various CYP2C19 genotype and the effect of CYP3A inhibitors on the decreased exposure of clopidogrel active metabolite have added to the complex situation. How should one label the safe and effective use of clopidogrel in view of various patient factors? The most recent revision of the FDA Guidance on Drug Interactions (http://www.fda.gov/cder/drug/drugInteractions/default.htm) has added recommendations that complex drug interactions such as the concomitant use of multiple inhibitors with a new molecular entity (NME) be considered during drug development. However, the scientific knowledge base and tools required to rationally address this issue are insufficient to help us fully analyze and label these interactions.

Since not all combined effect can be adequately assessed and not all combinations can be feasibly evaluated via clinical studies, development of database and bioinformatic tools to simulate the effect of multiple factors on drug exposure (and response) should improve our understanding of the multiple impairment to help clinical trial design and facilitate labeling recommendations in the safe and effective use of a drug for an individual patient.

Currently the Office of Clinical Pharmacology (OCP) is evaluating the utility of the University of Washington Drug interaction Database (UWDIDB) and the SimCYP® modeling and simulation software (Sheffield, UK). The UWDIDB allows reviewers and industry scientists to search and retrieve published in vitro and in vivo human drug interaction data. The SimCYP® simulator incorporates database capturing inter-individual variability associated with drug absorption, metabolism, distribution and excretion processes (including polymorphism of CYPs) in a variety of study populations and allows researcher to evaluate a drug molecule using a physiological-based pharmacokinetic (PBPK) approach. Our recent application has resulted in a recommendation of optimal study designs when ketoconazole is used as an inhibitor for evaluating CYP3A-based interactions (Zhao, Ragueneau, Zhang, Strong, Reynolds, Levy, Thummel, Huang et al, JCP, in press; Huang and Lesko, JCP, in press).
Hypothesis:
Development of state-of-the-art bioinformatic tools to simultaneously evaluate the effect of multiple factors on drug exposure and response will facilitate our decision-making in clinical trial design, consistent evaluation and prediction of complex drug interactions (including genetic components) and useful labeling recommendations for safe and effective use of a drug in patients.

Methods:
An internal database will be constructed to house *in vitro* and *in vivo* metabolism- and transporter-based drug interaction data, which include the confidential data from IND/NDA submissions and public data from the UWDIDB database. The newly constructed database will provide reviewers rapid access to organized and up-to-date drug interaction data and enhance our ability to cross-labeling for critical drug interactions. The data from the database will also be used in the establishment of a compound profile in order to construct individual pharmacokinetic and pharmacodynamic model for any new molecular entity. Initially, the simulation of the effect of multiple inhibitors or impairment on the drug exposure will be performed on several index drugs, such as repaglinide, loperamide, warfarin, and darifenacin, where clinical data under certain multiple inhibition conditions are available. The PBPK model will be constructed in SimCYP using its platform for compound profiles and built-in population database to perform simulations. Note the extraction and validation of the necessary *in vitro* and *in vivo* metabolism data from UWDIDB and SimCYP will be an iterating process not limited by using these two software products. In addition, collaboration within FDA or contractors will be identified to generate needed *in vitro* and *in vivo* human data (as described in the deliverables).

Once the internal database and initial models of index drugs are established, internal training workshops will be held. Reviewers will be able to apply these tools to evaluate multiple impairment for NMEs or marketed drugs (e.g., the evaluation of how chronic use of proton pump inhibitors that are CYP2C19 inhibitors, combined with a CYP3A inhibitor may affect the safe and effective use of clopidogrel in patients with varying CYP2C19 genotypes). The model can be applied to other multiple impairment scenarios and in pediatric populations where clinical data are rare.

Deliverables:
2009:
- Establishment of a drug interaction database on the CDER/FDA server
- Development of a physiological pharmacokinetic and pharmacodynamic model (initiating collaboration to construct compound profiles of index compounds for the simulation of multiple impairment on drug exposure) and to identify appropriate contractors to produce *in vitro* and *in vivo* clinical pharmacology data (e.g., PK/PD of clopidogrel in humans with multiple impairment conditions)
- Organization of an internal training workshop for FDA reviewers for both the drug interaction database and the modeling and simulation software
- Organization of a public workshop to share scientific advancement in the multiple impairment area (e.g., evaluation of complex drug interactions-*in vitro, in vivo* and *in silico*)
- Collaborating with pediatric hospitals to obtain key complex drug interaction data in pediatrics

2010:
- Initiating data entry of confidential IND/NDA data into the FDA drug interaction database and merging the UW database with the FDA database (accessible by the FDA reviewers only)
- Maintenance of the drug interaction database on the CDER/FDA server
• Continuing the development of a physiological pharmacokinetic and pharmacodynamic model with additional index drugs and marketed drugs

2011:
• Model validation by meta-analysis
• Continuing data entry and maintenance of the drug interaction database on the FDA server
• Continuing development of a physiological pharmacokinetic and pharmacodynamic model with additional index drugs and marketed drugs
• Organization of a workshop to share scientific advancement in the multiple impairment area focusing in specific populations (e.g., pediatrics)
Clinical Trial Design and Analysis

Project Title:
Development of an FDA-EMEA Collaboration Program for Good Clinical Practice Inspection of Clinical Trials Supporting Drug Development Worldwide

Description:
The clinical development of drugs for human use is a global undertaking with areas of shared interest between international regulatory agencies. In most cases, the same pivotal clinical trials are used to support marketing applications submitted to the FDA and the European Medicines Agency (EMEA). Subjects participating in these pivotal clinical trials have been recruited in the European Union (EU), the US and the remaining countries of the world. FDA and EMEA share the mission for ensuring human subject protection and data integrity for these pivotal clinical trials. The increasing number of clinical trials and the evolving globalization, coupled with limited available GCP inspection resources, means that only a sample of these clinical sites can be inspected for GCP compliance. Currently there is a huge challenge in selecting sites for GCP inspections due to the large number submitted in any given application. While there are ongoing studies to produce risk models to select sites which are believed to be the most informative as to how the study was conducted and to the integrity of the data, collaborating with other regulators can add significantly to the small amount of sites being inspected now and can greatly enhance that risk model.

This project proposes developing programs for FDA and EMEA to work in a collaborative and synergistic manner in carrying out GCP inspections and exchanging timely actionable information, in order that optimal and strategic use of GCP resources worldwide may be achieved. This project will inform the potential future expansion of these proposed programs to include other international regulatory partners.

FDA and EMEA each have systems and programs in place to verify compliance with provisions of GCP; including inspection of clinical trials, in particular those included in Marketing Authorization Applications (MAAs) submitted to EMEA and NDAs/BLAs submitted to FDA. However, each system’s available resources are stressed. In addition, each regulatory authority’s programs do not include bilateral, systematic coordination and conduct of GCP-inspections on marketing applications of common interest, nor do they include a systematic and timely mechanism for sharing relevant GCP-related information. Communication and cooperation between FDA and EMEA on GCP harmonization and inspection has long been a strategic initiative of FDA’s Good Clinical Practice Program (GCPP), Office of International Programs (OIP), and Centers. As such, long-standing informal communication channels have been enhanced through formal information-sharing confidentiality arrangements; however, effective and timely use of these arrangements has largely been limited.

This proposal supports the development of enhanced and systematic GCP-related information exchanges between EMEA and FDA combined with collaboration in the conduct of GCP inspections of clinical trials included in MAAs and NDAs/BLAs submitted to EMEA and FDA, respectively.

Hypothesis:
If FDA and EMEA can work in a collaborative and synergistic manner in carrying out GCP inspections and exchanging timely actionable information, then optimal and strategic use of GCP resources worldwide may be achieved.
**Methods:**
The Division of Scientific Investigations (DSI) verifies the integrity of efficacy and safety data submitted to the Center for Drug Evaluation and Research (CDER) in support of new drug applications, and assures that the rights and welfare of human research subjects are protected through coordination, co-conduct and interpretation of good clinical practices (GCP) inspection findings.

In order to optimize GCP inspection efficiency and effectiveness DSI/CDER and EMEA will; (i) develop a joint GCP program to forecast, coordinate, schedule and conduct joint and/or collaborative GCP inspections, and (ii) establish and implement procedures to share information with EMEA related to applications for approval of new drugs.

The timely and transparent sharing of GCP-related information between FDA and EMEA should; (i) optimize use of limited GCP inspection resources within the FDA and the European Union/EMEA, (ii) enhance consistency in inspectional findings between FDA and EMEA, and (iii) may decrease burden to clinical investigators targeted for inspection and (iv) may decrease burden on New Drug Applicants. This project will include significant infrastructure for information management and exchange to match sites of common interest, coordinate inspections, share information, and to incorporate this into ongoing risk modeling efforts.

**Deliverables:**

2009:
- Develop CDER expertise in EMEA GCP Inspection Programs
- Develop Pilot GCP Joint Program Procedures with EMEA (18 month pilot)
- Initiate Pilot GCP Inspections
- Establish Methods for Periodic Information Exchange: GCP Global
- Initiate Pilot Periodic Information Exchange
- Initiate Cross Training GCP Experts from EMEA and Member States on FDA/CDER GCP Programs
- Develop database to archive international activities and findings

2010:
- Host and Train EMEA GCP Inspection Sector Visiting Experts
- Present Findings/Participate in the EMEA Annual GCP Inspectors' Training Course
- Feasibility Assessment of a CDER GCP Clinical Monitoring Program in Conjunction with International State Health/Regulatory Authorities
- Conduct Pilot Joint GCP Inspections
- Conduct Pilot Periodic Information Exchange

2011:
- Conduct analysis comparing inspectional findings from Pilot Joint GCP Program
- Deliver Report on Pilot Program (18 month)
- Joint FDA-EMEA Joint Workshop GCP Inspection Best Practices and Path Forward
- International GCP Regulators Symposium
- Propose Best Practices Approach to GCP Inspections for Global Consideration
- Establish and implement long-term program
CDER Priority Area:
Manufacturing Science / Rapid Detection

Project Title:
Rapid screening of pharmaceutical products and ingredients

Description:
Globalization of the pharmaceutical ingredient and finished drug supply chains appears to increase the risk of consumer exposure to counterfeit, contaminated and mislabeled drugs. In addition, little if any surveillance is done on excipients. Urgency of these emerging public health risks prompted an executive order in July 2007 to form an Interagency Import Safety Task Force led by HHS and the Food and Drug Import Safety Act of 2007 was proposed in Congress to increase FDA testing of imports. The early reports from the president's task force concur with recent findings of an FDA-CDER task force on pharmaceutical ingredient safety. Both groups find an import supply chain with traceability weaknesses which needs improved surveillance. In recognition of this concern, critical path funds were awarded to CDER in 2008 to begin a proof of concept study with portable spectrometers for rapid screening of products and raw materials. With these funds, four types of portable spectrometers and chemometrics software to evaluate data were purchased and an ORISE fellow was funded for development of methods. With these funds, proof of concept of the use of modern analytical tools to rapidly and effectively screen ingredients for aberrations in quality is being carried out including method development, instrument calibration and method transfer. Continued support of this program will ensure a smooth transition of the developed technologies to the field and provide support for continued evaluation of new rapid screening techniques. This new paradigm for the surveillance of pharmaceutical products and raw materials will allow for rapid screening of a large number of samples to identify those materials that need in depth analysis. More time and resource consuming laboratory testing can be focused on testing materials that fail rapid screening resulting in an overall dramatic increase in surveillance of pharmaceutical ingredients and finished doses.

Hypothesis:
FDA needs to deploy analysts with portable spectrometers for rapid screening of products and raw materials at points of entry, distribution centers, pharmaceutical manufacturers and pharmacies. Proof of concept is currently being performed in the CDER laboratory. Focused resources for field deployment of the instrumentation and methods will ensure successful establishment of these types of rapid detection systems. Funds requested in this proposal include ORA resources to allow for deployment of rapid screening methods including chemists to accompany inspectors, and CDER Office of Compliance resources to prepare and establish GMP, import, and quality guidances and regulations to implement these programs and to incorporate these methods into our supply chain strategy. Additional equipment money is also requested to continually increase and improve the number of screening instruments available.

Methods:
The proposed project has identified suitable analytical tools that can be used by FDA field personnel for compliance, counterfeit and counterterrorism applications in finished dosage products, compounded products and raw materials. Drugs and drug components may be screened for identity, potency, content uniformity and/or impurities, depending on the nature of
the risk. Methods using Raman and Near-Infrared spectroscopies coupled with chemometric techniques have been successfully developed on the bench top to screen pharmaceutical products and ingredients. Examples include diethylene glycol contamination in glycerin and over sulfated chiontrotin sulfate in heparin sodium. These methods are now being transferred to portable instruments. Portable X-Ray fluorescence and ion mobility spectrometers have also been purchased and are being explored to expand the ability to screen for impurities such as heavy metals in pharmaceuticals and erectile dysfunction drugs in herbal supplements. Collaborative studies for method validation will begin between CDER and four ORA laboratories before the end of fiscal 2009. Once the methods have been validated, the portable instruments will be ready to deploy in the field to screen materials. Deployment will involve chemists accompanying inspectors to screen materials on site and determine which materials need to be sampled for further analysis. Additional FTEs and instruments will be needed to support this deployment to allow for simultaneous development, validation and deployment of methods.

**Deliverables:**

**2009:**
The following 2009 deliverables are complete:

- Identification of a total of 10 raw materials, drug products and compounded products for rapid screening method development with Office of Compliance (OC) and ORA.
- Purchase of portable near infrared, Raman, ion mobility and X-ray spectroscopic instrumentation.

Remaining deliverables for 2009 include:

- Installation of purchased instrumentation.
- Development and validation of methods including chemometric modeling for the 10 materials identified.
- Loading methods and performing calibration transfers on portable instruments.
- Sending instruments to designated field laboratories to begin collaborative studies for developed methods.
- Issuance of assignments and planning of work by CDER OC
- Regulatory support for any regulatory actions, import detentions, etc., resulting from negative findings or results.

**2010:**

- Complete collaborative studies for original 10 materials.
- Deploy methods in the field with chemist/inspector partnerships.
- Analysis of the effects and impact by CDER OC in conjunction with St. Louis Laboratory.
- Perform continuous quality improvement activities such as Plan-Study-Do-Act cycles to maximize feedback and learn from the initial development and deployment of rapid screening methods.
- Identify additional materials for rapid screening and begin cycle of development, validation, transfer, deployment.
- Identify new technologies that would be amenable to rapid screening in the field.

**2011:**

- Continue to expand the number of materials that can be screened by portable instrumentation with input from CDER OC and ORA.
- Consider placement of screening instrumentation in overseas FDA offices for use by inspectors on foreign inspections.
• Identify and implement new technologies to improve predictions of quality assessments of rapid screening instrumentation.
• Possible alerts and guidance from VDER OC based on findings/results.
Manufacturing Science

**Project Title:**
Implementation of Quality by Design Principles and Novel Process Analytical Technologies for Protein Therapeutic Manufacturing in the 21st Century

**Description:**
Biopharmaceuticals currently comprise about 30% of new molecular entities under review at CDER and, based on recent publications, this proportion will increase over time. The inherent complexity of glycoprotein biopharmaceuticals, such as enzymes and monoclonal antibodies, present significant challenges with respect to manufacturing and testing, as well as for subsequent formulation. Promotion of policies that ensure a complete understanding of these complex and heterogeneous products and processes would lead to (1) more efficient review and increased availability of safe and efficacious medications, (2) an enhanced ability to efficiently deal with product failures and (3) the ability to proactively address potential issues of follow-on protein products.

This program within the Office of Pharmaceutical Science (OPS) in CDER will allow us to build on the strengths of bioprocess knowledge and bioanalytical protein characterization from the Office of Biotechnology Products (OBP) and physical/chemical analytical approaches in the Office of Testing and Research (OTR). Our common goal is implementation of the FDA’s vision of cGMPs for the 21st Century for biopharmaceuticals. This proactive program will consist of four teams focused on interrelated areas of protein manufacturing science:

**Novel bioanalytical methodologies to determine protein structure and function.** This team will focus on attributes often essential to the potency of a biopharmaceutical: (i) post-translational modifications such as glycosylation and phosphorylation; (ii) three dimensional structure including oligomerized states and (iii) complex bioactivities such as activation of cellular responses, and delivery/enzymatic action at an intracellular site within a target cell. Relatively modest changes in fermentation, purification, scale or technology transfer can result in significant changes in the product critical quality attributes (CQAs). The development and/or evaluation of novel “state of the art” methods can be leveraged to examine how process changes impact product comparability, as it relates to clinical safety and efficacy.

**Real time process analytical technology (PAT) tools that can be used to improve control of the manufacturing process with a focus on cell culture.** This team will develop new strategies to monitor bioreactor processes and product CQAs at an on-line basis to speed biotechnology product development. The manufacturing of biopharmaceutical active pharmaceutical ingredients (APIs) is a complex process that can be affected by changes introduced in cell culture and/or purification units of production. In our experience, limited changes to the fermentation process have lead to profound changes in product attributes critical for clinical efficacy. PAT consists of a set of analytical tools combined with manufacturing process-control systems that allow real time product quality control. The goal is to implement corrective changes during fermentation to achieve a product with optimal structure and product quality attributes. No attempt to apply biopharmaceutical potency or structure measurements in a PAT setting has been made to date. This approach will minimize the need for extensive comparability assessment studies subsequent to production that rely on downstream physicochemical, animal and clinical studies.

**Physical and chemical stability of protein therapeutics.** A complete risk based understanding of the variables that cause instability in a protein product and strategies to detect
Instability signals is needed for biopharmaceuticals. Instability can be caused by a variety of product-specific compositional or process related factors of the API traceable to the bioreactor, down-stream processing or finished formulation stage. This team will perform comprehensive QbD studies to identify, characterize and ultimately mitigate these risks.

**Biopharmaceutical delivery and dosage forms.** A complete risk based understanding will be developed of variables stemming from the delivery form and the impact on biopharmaceuticals CQA’s. The unique nature of protein APIs combine with attributes of some complex delivery forms to pose unique challenges. This team will perform comprehensive QbD studies to identify and mitigate risks associated with individual dosage forms.

**Hypothesis:**
Focused on case studies of OBP products, we will establish a cutting-edge research program that will ultimately guide sponsors in the manufacturing of these complex proteins and provide improved clinical benefit to patients.

**Methods:**
We propose to create The Biopharmaceutical Manufacturing Science Research Group (BMSRG) under the overall leadership of an OBP/OTR steering committee. The BMSRG will consist of experts in the areas of protein chemistry, bioanalytics, enzymology, molecular & cellular biology, chemistry, pharmacology and chemical engineering who will integrate the biological and physical sciences with modern processing and process control equipment through an information technology infrastructure. We will also leverage expertise and on-going collaborations with the University of Maryland (Baltimore County), the National Institute for Bioprocessing Research and Training (NIBRT, Dublin Ireland), the US National Institute for Standards and Technology, the National Institute of Pharmaceutical Technology and Education (NIPTE) and an FDA CRADA with Novartis.

Two model manufacturing platforms will be established (a monoclonal antibody and a glycosylated enzyme) and apply innovative analytical techniques, PAT applications and physicochemical comparability assessment strategies to these platforms. An enzyme model will be chosen on the basis that enzymes used for replacement therapy in lysosomal storage diseases contain complex and often heterogeneous carbohydrate structures with varying levels of bis mannose-6-phosphate. Possession of the glycan structure with the correct level of bis mannose-6-phosphate is critical for delivery to the intracellular lysosomal compartment and subsequent product efficacy. A monoclonal antibody model will be chosen based on extensive use of this product class therapeutically for oncology, inflammatory and infectious diseases, and the sensitivity of their effector function, and thus product potency, to certain glycoform variants.

Specific work plans identified for this project are as follows:
- Development of a knowledge-base to support/direct the program; this information will be culled from OBP INDs and BLAs, as well as the scientific literature.
- Development of biopharmaceutical manufacturing process platforms for the two model proteins. This activity will span the breadth of a typical bioprocess; from cell lines and cell banks, to bioreactor cell culture and final protein purification and formulation.
- Novel “State of the Art” bioanalytical methods development focusing on protein capture methods for PAT and evaluation of glycoprotein structure, aggregation and potency.
- Application of the above bioanalytical methods in a PAT setting, leveraging existing at-line 2-dimensional HPLC methods, bioreactors, autosamplers, mass spectrometers, near-infrared and other online equipment as needed.
• Assessment of the “State of the Art” bioanalytical methods for utility in physicochemical comparability assessments. PAT tools will be bench-marked against data from these methods.
• Application of the novel bioanalytical methods in stability and formulation studies, focusing on CQA’s of concern for biopharmaceuticals
• Risk analysis and mitigation of protein:dosage form interactions via QbD studies focusing on stability, leachables and other analyses pertinent to biopharmaceuticals.

**Deliverables:**
1. Establishment of programmatic ability to handle major scientific issues concerning biopharmaceuticals.
2. Training and collaboration between scientists and reviewers to generate comprehensive QbD case studies and address real-world review problems encountered by biopharmaceutical reviewers.
3. Establishment of principles for risk analysis, risk prioritization, screening and optimization of variables, construction of design space, process verification, and real-time monitoring tailored to biopharmaceutical manufacture and testing.
4. Publication of illustrative examples of the application of PAT to bioreactor-based protein expression systems, manufacturing changes that may be encountered in follow-on protein products and physicochemical comparability exercises.
5. Guide elements and enhanced review practices for QbD applications in protein therapeutics as BLA, NDA, as well as potential follow-on protein products.
6. Publications in peer-reviewed journals and workshops with stakeholders within/outside FDA.
Project Title:
Inactivation Resistant Viral Contaminants: Risk of Human Transmission & Approaches for Elimination

Description:
Throughout history, human and animal derived pharmaceutical products have sometimes been the only therapeutic option. Inherent to the use of these biological products is the risk of infection from endogenous viral contaminants. Modern manufacturing techniques now result in the clearance or inactivation of most viruses, but there remain viruses that contaminate medically critical drug products that cannot be removed or inactivated by conventional methods without adversely affecting drug efficacy (Farshid et al., 2005). Such viruses are generally small capsid viruses (see ICH Q5A, Table 1). Two such examples are the human parvovirus B19 (B19) in human blood products and the porcine parvovirus (PPV) in pancreatic enzyme replacement products (PEP). B19, the virus responsible for a generally self-limited exanthema in humans, fifth disease, can be fatal if transmitted through blood products to a patient who is immunosuppressed or has sickle cell disease. It is not known whether PPV infects humans, but it has been reported to infect human cell lines in vitro. Because of the magnitude of the PEP doses used in clinical practice, their route of administration, and the need for life-long treatment, there is considerable concern that there is a tangible risk of swine-to-human viral transmission from this product. Parvoviruses infect a wide variety of species, evolve rapidly, have a high level of environmental resistance, shed high doses of virus, and are capable of interspecies transmission (feline to canine) with devastating consequences. While the human population at immediate risk is relatively small, the public health impact of zoonotic infection, if found to occur, is potentially enormous.

Because of the medical necessity of these products and the lack of viable alternatives, a certain level of viral contamination is deemed a necessary risk and control strategies have been developed to mitigate that risk. For B19, though the minimal infectious dose is not known, a limit of 10,000 infectious units (IU)/mL of pooled plasma has been established since case reports have documented B19 transmission by a plasma product with a viral load of 20,000 IU. For PPV, it remains to be established whether humans can be infected and correspondingly a minimal infectious dose is not known. In addition, porcine circovirus (PCV) types 1 & 2, as well as the porcine ortholog of human PARV4, are known to contaminate PEP and to infect human cell lines in vitro.

As with any product attribute, the level of viral control necessary should reflect the potential risk to product quality and safety. As a first step towards addressing the potential risk of such viruses to humans, we propose to establish sensitive and specific enzyme linked immunosorbent assays to detect human antibodies directed against these viruses as this is the most readily accessible indicator of human infection, or a history thereof, by these viruses. Such assays are already prescribed in ICH Q5A as evidence of infection in animal models.

As described above, small naked-capsid viruses are the most difficult to remove/inactivate from biopharmaceuticals (Farshid et al., 2005). Several technologies that easily clear larger viruses are ineffective (solvent/detergent, low pH) or inefficient (virus filters) at clearing parvo- and other small viruses. In addition, PEP products are crude, viscous mixtures containing numerous particles of various sizes; thus they are not amenable to viral-clearance process steps common for highly purified biotechnology products such as monoclonal antibodies (i.e. PEPs clog filters and columns). As, in the cases described above, when such viral contaminants are shown or
already known to infect humans (e.g. B19) it is imperative to the public health to introduce novel approaches (e.g. photo- and photochemical irradiation, UV-C irradiation, dry heat, spray-drying, PEN110) to the inactivation of these viruses. Given the capacity of paroviruses for mutation and interspecies transmission, optimally, these viruses should be inactivated whether or not there is current evidence that they are infectious to humans.

This project will leverage existing viral safety/clearance projects and expertise to this mission-critical issue for OBP/DTP. We envision a joint inter-Center proposal with CBER/OBRR and OBP/DMA, groups with solid track-records of productive viral safety/clearance projects and expertise. CBER/OBRR has extensive expertise in viral clearance strategies for their plasma-derived product portfolio. While this industry has developed effective strategies to mitigate risk from larger viruses (e.g. HIV, hepatitis virus), B19 contamination of human blood products is an on-going concern. OBP/DMA currently has projects evaluating the virus risk for the highly-purified monoclonal antibody product class. While, the virus contamination risk for this product class is very low, as reflected by the absence of any history of patient infections, OBP/DMA has developed several new process validation and risk-mitigation strategies to make this risk even smaller. The knowledge space and technical expertise from CBER/OBRR and OBP/DMA will be brought to bear on this OBP/DTP product class (PEPs) to first assess the parovirus risks, and then mitigate and reduce them. Recently, advanced methods have been developed to inactivate small viruses from plasma-derived and monoclonal antibody products. These include photo- and photochemical irradiation, dry heat, spray-drying, high-temperature/short time, UV-C irradiation, membrane adsorbers and PEN110. We envision a broad-based collaboration between CBER/OBRR and CDER/OBP evaluating multiple technologies for multiple product classes.

**Hypothesis**: Determination of the infectious (and zoonotic) potential of viruses that are currently not cleared or inactivated by standard manufacturing processes and validation of robust and scalable viral inactivation methods will result in biologic drug products with greater safety, efficacy, and public acceptability.

**Methods**: Enzyme linked immunosorbent assays (ELISA) currently used in animal husbandry will be adapted for use with human serum to detect human antibodies against contaminating viruses. Concurrently, in collaboration with CBER/OBRR and OBP/DMA, advanced methods will be assessed for their efficiency and robustness to inactivate such viruses from PEPs, plasma-products and monoclonal antibodies. Once established, unit operation validation strategies and design of experiment studies for these unit operations will be explored, providing industry and FDA with robust process design spaces for viral clearance and safety.

**Deliverables**:

2009:
Examine relevant human serum samples for presence of anti-PPV antibodies. Test at least one novel method for ability to inactivate PPV and B19. Conduct characterization studies on PPV and B19 to determine critical characteristics relevant to viral clearance (i.e. size by dynamic light-scattering, pl by chromatofocusing)

2010:
Examine relevant human serum samples for presence of anti-PCV antibodies. Test additional novel methods for ability to inactivate PPV, B19, and PCV. Conduct characterization studies on PCV.
Center for Devices and Radiological Health (CDRH)

Priority Areas:

Biomarkers
Clinical Trial Design and Analysis
Rapid Detection
CDRH Priority Area:

Biomarkers

Project title:
Computational Endpoints for Cardiovascular Device Evaluations

Description:
Computational modeling has the potential to provide new information for the evaluation of cardiovascular devices. It provides a more complete evaluation of potential risks with medical products and permits laboratory and clinical trials that are focused on critical questions. In many cases, the cost and difficulty of conducting reasonably-sized clinical studies inhibits the development of medical devices. This is especially true for niche populations (e.g. pediatrics) where clinical studies are difficult to design and the patient population is small, making exclusive use of clinical trials an impractical approach. Computational modeling approaches are advantageous since they can be used to simulate difficult or nearly impossible clinical trial situations.

Cardiovascular simulations are amongst the most complex computational models since they involve a variety of scientific and engineering disciplines. Cardiac models often need to simultaneously account for electrical, mechanical, chemical, physiological, and imaging issues. We propose to address the development of cardiovascular devices and the computational needs of other device types by establishing a multifaceted facility that will enable synergistic and efficient use of expertise, computing resources and model geometries across multiple scientific and engineering disciplines. This approach will allow us to access and develop “multi-physics” approaches that enable evaluation of issues across disciplines that may otherwise erroneously characterize device safety and performance using single discipline approaches. By consolidating our scientific knowledge base with a centralized-computational resource, we anticipate the development of models that accurately assess interactions between medical products and patients which will enhance the progress of new products through the critical path and enable more efficient review of submissions to FDA.

Methods:
This proposal will focus on two efforts. First, we will develop a high-capacity centralized computational resource that will facilitate collaboration and model development. Second, we will pursue the development of computational modeling in a number of specific areas that will utilize the computational resources and will contribute to the goal of multi-physics modeling.

High-Capacity Centralized Computational Resource: OSEL proposes to develop a modular high-performance computing solution. The first of three modules is a high-capacity (150-200 TB) data server that will allow scientists and engineers to securely share and permanently archive data on the OSEL research network. We will implement RAID 6 architecture across a network area storage (NAS) server which will allow for redundant data protection to prevent data loss for the sizable data sets that CDRH scientists have already developed. We will also implement a version control system over a portion of the NAS storage so that an index of available computational geometries can be stored and easily accessed by researchers for model development. We are planning to develop an off-site mirroring capability for data to further insure backup of the entire NAS server. The second module is a high-performance computing cluster (HPCC) that will be used to significant extend and expand the kinds of models that scientists can develop. OSEL proposes to purchase a high-capability, configurable SiCortex computing system for developing and testing computational modeling applications. This cross-office resource will allow scientists to port code currently running on small workstations to a
versatile 1134-core parallel computing environment to (a) accommodate models with increased complexity; and (b) reduce computational time. In addition, OSEL will purchase 4 smaller 72-core parallel computing platforms which will allow scientists to prototype their applications. Two of these smaller parallel computing platforms will be dedicated to applications engineers who will help scientists migrate their applications to the larger parallel computing environment. The third module is an application server that will allow OSEL to consolidate software licenses which will increase availability of software analysis tools to greater number of users while significantly reducing cost.

Specific Computational Modeling Efforts: To fully realize multi-physics computational solutions that can be applied to cardiovascular and other computational models, OSEL proposes supporting five projects under this proposal.

- **Prosthetic Pediatric Heart Values** -- OSEL proposes to work with the Office of Device Evaluation (ODE) to hold a public meeting to allow input from stakeholders regarding novel approaches for developing computational models that simulate pediatric heart valves. This project will identify the parameters and modeling approaches that need to be considered in developing clinically relevant models that support the regulatory evaluation of these devices.

- **Ultrasound HIFU Modeling** -- OSEL proposes to numerically simulate the propagation of high-intensity focused ultrasound (HIFU) beams through body tissues. This project will evaluate the amount of absorbed ultrasound energy in tissue and will determine the thermal and mechanical bioeffects resulting from this energy absorption. These simulations will assist in the safety and efficacy evaluation of a variety of new HIFU devices being reviewed by FDA, with medical applications in tumor ablation, vessel cauterization, clot lysis, and gene activation.

- **X-Ray Image Processing and Evaluation Modeling** – OSEL proposes to perform in-silico modeling of x-ray imaging devices and components, including the evaluation of competing image acquisition systems, imaging protocols, and display technologies. Models for the image capture process will incorporate all relevant x-ray, electron, and optical transport phenomena. This project will develop optimized patient-specific imaging protocols to improve task-based disease detection with the goal of reducing the need for clinical trial data for x-ray imaging devices.

- **Cardiovascular Finite Element Analysis** – OSEL proposes to develop advanced models for evaluating the effect of emerging tools in finite element analysis (FEA) such as algorithms for fluid-solid interactions and device-tissue interactions. The inclusion of these interactions will more comprehensively simulate the implanted environment and likely result in clearer determinations of stresses on the implant and the identification of new modes of failure. Finite element analysis is a computational method that is commonly used to predict the loads and deformations, i.e., the stress-strain distribution, in solid structures and has great utility in the medical device industry. This computational tool is used extensively by medical device manufacturers to determine the optimal design and safety of their implants.

- **Semantic Data Mining** – OSEL proposes to develop capability in semantic data mining which not only detects the meaning of words but attributes wording significance, its relationships with other words, and the context in which words are used. This effort has a plethora of applications for contextually mining data submitted through the Sentinel initiative, medical device adverse event reports (MDR), as well as through the pre- and post-market applications received by CDRH. These approaches can also be applied to
the genomic analysis in which gene sequences can be searched to define patterns of
do-location of gene groupings.

**Deliverables:** As a result of these efforts, OSEL will deliver the following

1. Workshop with internal and external partners focused on specific challenges regarding
   the development of computational models to simulate pediatric heart valves.

2. Increased analysis capability of regulatory issues through the development of high-
   performance computational models that aid in the regulatory review of medical devices.

3. Standardized library of high resolution anatomically correct body geometries that will be
   available throughout CDRH and to our collaborators

4. Guidelines and recommendations for patient-specific x-ray based imaging protocols for
   the critical tasks associated with coronary artery disease; modeling approaches for HIFU
   applications in body tissues; and predictive toxicology.

5. State-of-the-art approaches to contextual data mining that can be used in support of the
   Sentinel initiative, the analysis of medical device adverse event reports, and exploratory
   applications of genomic gene associations.

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CDRH Priority Area:
Clinical Trial Design and Analysis

Project Title:
Improving Clinical Trials for Imaging Devices

Description:
Imaging devices, computer-assist products, image analysis packages, and display devices represent a large regulatory portfolio in CDRH that is continuing to expand. A March 2008 CDRH panel meeting emphasized the need for new paradigms for the evaluation of these products using reader studies and standardized databases. These evaluations must be statistically interpretable, relevant for their intended use, and at reasonable cost.

We hypothesize that new, improved clinical trial designs, statistical methods, and data analysis tools can be developed and validated, leading to more powerful clinical studies of the efficacy of imaging devices for fewer resources. The knowledge gained in this project will be incorporated into new guidance to industry and training programs in CDRH, enabling effective products to get to market faster and at lower cost. In addition, the knowledge gained will support joint CDRH-CDER Critical Path initiatives to qualify imaging as a biomarker for treatment response.

This proposal would bring together a cadre of imaging physicists, mathematicians, clinicians, computational scientists, and statisticians, who would work together to develop new clinical trial methodologies appropriate for evaluation of imaging devices involving human readers. Working with outside experts and professional societies, we will develop approaches to quantitative image scoring that are acceptable by clinicians, relevant for clinical tasks, and appropriate for the intended use of the imaging device or application. These approaches will be evaluated through simulation, and subsequently validated in studies involving expert and novice readers. We will devise clinical reader training programs on quantitative image interpretation to reduce inter- and intra-reader variability that reduces power in clinical trials involving imaging. We will work with NIH, academia, and industry to develop image databases to cost-share the expense of device testing. Finally, we will provide training to FDA reviewers and external stakeholders on the resulting study design and data analysis tools, and disseminate statistical software for use by academia and industry.

Background:
Over the last 10 years, FDA has seen an increasing number of submissions for computer-aided diagnosis (CAD) devices to be used with radiological images. Such devices have been approved or cleared by CDRH in limited applications, and significant controversy has since arisen regarding the relationship between the data used to support those review decisions and subsequent reports in the literature suggesting lower CAD performance in larger clinical studies. Key questions regarding the size of the clinical studies used to support these approvals, the data analysis methods, and the relevance of the study conclusions to the expected use of the technologies remain. The purpose of this project is to develop more powerful clinical trial design and data analysis approaches to support the approval or clearance of imaging devices and related CAD software.

A large effort will be devoted to the development and validation of improved methods of evaluation based on receiver operating characteristic (ROC) analysis. The receiver operating
characteristic (ROC) curve is a curve displaying Se (sensitivity or true positive fraction (TPF)) as a function of Sp (specificity or false positive fraction (FPF)) when the threshold setting for the test is varied over the complete range of possible test scores over which a reader or machine algorithm may operate. A summary measure of the performance is derived by using the area under the ROC curve (AUC). AUC is the probability that given a random abnormal case and a random normal case, the physician or device can correctly diagnose which is which. Because AUC averages over a range of TPFs, it offers the potential for greater statistical power (i.e., tighter error bars) in an analysis than use of a single Se-Sp operating point. It can also be shown that the reliance of single operating points in the comparison of relative device performance can lead to ambiguity, and that different readers can operate at different operating points, leading to additional variability.

ROC analysis has become widely accepted as the method of choice for imaging system evaluation, but it makes a number of simplifying and limiting assumptions. As described below, we will develop validated evaluation methods and publicly available software packages for more clinically relevant study designs and reading protocols.

**ROC analysis, quantitative image scoring, and reader variability:** ROC analysis involves making measurements on observers of images and then analyzing the resulting data. ROC analysis requires readers to rate images according to their confidence that disease is present, and a number of measurement scales have been suggested for this purpose. An ROC curve is obtained by moving a threshold through the ratings – in the same manner as if the ratings were the output of a laboratory diagnostic test. Data analysis methods have been introduced to allow the interpolation and extrapolation of such data with smooth parametric fits that can be described by a small number of parameters. The use of a small number of rating categories leads to difficulties associated with convergence of such fits. Also, while coarse rating scales are easier for readers to use, they result in less information and greater variability in the summary figure of merit. A goal of this proposal is the development of quasi-continuous reading scales, reader training protocols, and data collection interfaces that greatly enhance the quality of the data collected in reader studies, leading to more powerful study conclusions.

In the last decade, one study after another has documented the existence of a wide range of levels of performance—i.e., reader skill—in studies of human observers using medical imaging systems. This additional variable complicates ROC analysis. To overcome this complication, multiple readers and cases (e.g., images) are “fully crossed”, that is, every reader reads every case. This design can be very efficient for detecting reading modality effects on performance. For this design, the statistical analysis needs to respect correlation among repeated readings of the same case, correlation among readings of cases by the same reader, and correlation among repeated observations per patient (e.g., two breasts per patient). Research under this proposal will investigate the suitability of particular methods of analysis of multi-reader multi-case ROC data acquired using the fully-crossed and alternative study designs, in terms of the bias and variance in the resulting reader performance measures. These results will provide CDRH with improved guidance on sample size requirements for reader studies that would provide a given desired effect size and acceptable probabilities of rejecting the null hypothesis when it is true and not rejecting the null hypothesis.

**Location-specific scoring:** Standard ROC methods rely on patient-based analyses; there is no accounting for the location of the disease. Free Response ROC (FROC) is similar to ROC while allowing for the possibility that each patient may be diagnosed at multiple anatomical locations. FROC does not assume that each patient is diagnosed entirely as normal or abnormal. For example, CAD devices typically present multiple marks per patient, where the majority of these
marks are typically false positives. A major concern for CDRH is that these false-positive marks can result in reduced reader performance and/or an increase in costly and unnecessary patient workups. While there is almost no limit on the number of false marks that could be generated by a CAD device, in general devices limit the number of marks per patient that are presented. This limit differs from device to device. Frequently, FROC analysis or comparisons of different devices using FROC requires some sort of artificial truncation of the data, e.g., to enable comparison over the same range of the number of false positives per case.

As with ROC, different metrics can be constructed using FROC data. No consensus exists in the image evaluation community regarding the appropriateness of these competing approaches. Research undertaken in this project will evaluate the problem of location-specific scorekeeping, including how methods for acquiring readers’ location score and location information pertaining to the reference standard impact the estimate of reader performance.

**Reference standards:** Per-patient ROC analysis requires a binary reference standard at the patient level. That is, all cases must be known to be either normal or abnormal (diseased). Per-lesion or per-location analysis requires a location-specific reference standard, which might be associated with a central x-y coordinate, a segmented image finding, or a bounding box. In actual clinical studies, every reference standard is imperfect, which leads to a number of study biases and sources of uncertainty. Evaluations in the absence of a reference standard, or with partial reference standard information, naturally suffer much greater uncertainty than assessment in the presence of a good reference standard which reduces statistical power for the comparison of interest in the study. Investigations will be pursued under this proposal to evaluate the impact of partial or imperfect reference standards on study conclusions, as a function of the degree of correlation between the test and reference modality, the sources of uncertainty in the data from each modality, and the sample sizes (numbers of readers and cases).

**Multilevel disease status:** For many applications the device output and/or the reference standard is multilevel, e.g., a CAD that color-codes areas of an image by estimated probability of malignancy, or a device that estimates progression-free survival, heart-ejection fraction, tumor size, or disease stage. It is important to note that binary performance metrics are often realized by binning the reference standard into two classes, so that the subsequent analysis follows standard ROC practice. However, this binning of data throws away information and statistical power, leading to larger data requirements. We will investigate and compare the binned binary approach to methods that utilize multi-level information such as regression models, correlation, and concordance. We will determine under what circumstances and degree to which these multi-level approaches have more statistical power.

Another direction of research will be the investigation of multi-level analysis methods for the examination of reader agreement. That is, instead of comparing reader scores to truth, one reader’s scores can be compared to another reader’s scores. We believe that increasing reader agreement, which can be interpreted as decreasing reader variability, is useful for comparing different reading methods and scoring systems. A practical convenience of this kind of comparison is that it does not require determination of the reference standard. We will investigate what new information reader agreement can provide to standard performance evaluation. We will generalize the variance estimates of these metrics for a single pair of readers to situations where agreement is averaged over a group of readers. We will investigate how image processing and automated feature extraction could be integrated into a reader interface and data collection protocol to improve agreement.
Up to this point, this proposal has focused on the evaluation of readers for the problems of disease detection or classification of patients into a small number of classes, and CAD devices that assist these tasks. Devices that estimate image-based measures of disease status are under development by academia and industry, and the validation of those estimates and their qualification for use as surrogate endpoints in clinical trials is an area of great interest to the scientific and clinical imaging communities. Under this proposal CDRH scientists will perform research on targeted imaging science questions relevant to current or expected knowledge gaps regarding emerging imaging modalities and image analysis approaches in MRI, optical, and CT imaging for use as endpoints for device evaluations and studies of drug efficacy. In particular, an intra-center collaboration will focus on specific applications for neurological and cardiac devices. These are two critical areas that challenge CDRH in clinical trials for stroke, epilepsy, traumatic brain injury, Parkinson’s disease, cardiac arrhythmias, vulnerable coronary plaque and heart failure. Trials for these chronic conditions often rely on patient reported outcomes and global measures that occur months after the treatment. As a result, FDA is encouraging the development of reliable and objective outcome markers.

Application to Brain Disorders: The intent for this specific application area is to evaluate how to incorporate objective imaging markers as outcome measures in clinical trials for brain disorders (stroke, Parkinson’s disease, epilepsy, dementia, traumatic brain injury). Presently, clinical disease scales without imaging are subject to variability and patient /rater expectations or other interventions. Inclusion criteria can encompass many underlying forms of the disease that manifest themselves similarly and thus confound a clinical device trial in which the intervention works well for limited underlying forms. Imaging features have the potential for refining inclusion criteria and improving trial specificity. Also, imaging data are being included as outcome measures for brain disorder trials, particularly through the use of diffusion tensor imaging (DTI) and functional magnetic resonance imaging (fMRI). Both of these modalities rely on the assessment of changes in brain physiology in the images via human or machine readers. We will develop improved methods for extracting image-based measures of disease status with reduced reader variability from DTI and fMRI images for brain disorders for use in clinical trials.

Application to Cardiac Disorders: DTI is becoming an important evaluation technique for heart diseases. In addition, there are new diagnostic optical imaging modalities for vulnerable plaque, a causal path marker for sudden coronary blockage. Here again, questions arise regarding the information contained in the images and its usefulness as clinical trial outcomes, and the impact of reader and/or algorithm variability in extracting the image information. For cardiac arrhythmia formation, new techniques that combine multi-site functional imaging through optical means with computer simulations to locate and evaluate arrhythmias are under investigation. Here reader variability has not been considered, but it should be built-in as the algorithms and software for this new imaging mode are developed and deployed.

Deliverables:
1. Workshops with internal and external partners focused on specific challenging clinical tasks (case studies in disease localization, stratification, staging, and quantitation, that include specific applications for DTI of brain)
2. Mathematical and simulation demonstrations of potential to increase statistical power with new clinical trial designs and analysis methods.
3. Reader training and data collection protocols
4. Application-specific software interfaces for image display and reader interpretation
5. Demonstration of ability to apply protocols and analyses with novice readers and simulated images to demonstrate proof-of-concept under a controlled setting
6. Reader studies with expert readers to tailor methods to the clinical environment, to include brain disorders and heart diseases
7. Vetted and qualified protocols and analyses for FDA guidance
8. Training programs in protocols and statistical tools for evaluating imaging devices
CDRH Priority Area:
Rapid detection:

High Throughput Methods for Detection and Identification of Contaminants and Emerging Diseases:

Project Title:
Assuring the Safety of Ophthalmic Medical Devices

Description:
In recent years, several ophthalmic devices have been associated with a number of devastating outbreaks that severely impact patients’ vision. Given U.S. estimates of 34 million contact lenses wearers and 3 million annual intraocular lens recipients, assuring the safety of ophthalmic devices is imperative to the public health, and two scenarios are of relevance. Toxic Anterior Segment Syndrome (TASS) is a severe intraocular inflammation caused by noninfectious substances that enter the anterior segment of the eye during intraocular lens (IOL) implantation. TASS is believed to be caused by contamination of the IOLs and/or surgical adjuncts by bacterial endotoxin, low molecular weight fragments, biological (DNA & RNA) contaminants, and/or inorganic compounds. Over the years, there have been cyclic occurrences of TASS that have seriously impacted patients. The etiology of many severe TASS outbreaks were not identified due to the following problematic areas that have hampered expeditious investigations of TASS outbreaks: 1) lack of established methodology for testing of suspected samples; 2) lack of knowledge of ocular reactivity to suspected contaminants; and, 3) lack of a reliable early warning signal of a TASS epidemic. This project is aimed at addressing these issues.

Acanthamoeba and Fusarium solani outbreaks occurred in the last 2 years in wearers of contact lenses. These outbreaks led us to question the adequacy of the premarket test methods that were used to demonstrate the disinfection efficacy of the contact lens care products. In both cases, the associated care products (contact lens solutions) exceeded the performance established by the premarket test methods. On June 10, 2008, the Ophthalmics Devices Advisory Panel met to discuss the issues and concerns raised by the outbreaks. The Panel strongly recommended that FDA revise and develop premarket microbiological test methods, including efficacy against Acanthamoeba, that would better represent “real world” and “worst case” consumer practices.

Due to the very large U.S. population affected by these contaminations / new diseases, recent outbreaks associated with IOL implantation and contact lens usage have received wide attention from professionals and the public. However, despite careful epidemiologic studies by the CDC and the affected industry, the causes of many outbreaks are still unknown.

Objectives:
The objectives of this proposal are to identify factors responsible for the recent outbreaks and to develop methodologies to aid in improving the safety of ophthalmic devices.
Deliverables:
TASS

1. Method Development
   Finding the precise etiology of a TASS outbreak is challenging due to the myriad of potential causes and lack of standardized methodology. Utilizing the expertise of research scientists such as a microbiologist and chemist, we will develop methods with sufficient detection levels to evaluate levels of endotoxins, metal contaminants, organic contaminants, foreign protein/nucleic acids, etc. associated with major ophthalmic products as outlined below.
   a. Analytical methods for endotoxin determination – Develop methods to assess endotoxin levels associated with ophthalmic devices. Endotoxins are a known cause of TASS and have been implicated in multiple TASS related recalls of ophthalmic devices.
   b. Analytical methods for inorganic contaminant determination – Develop methods to assess inorganic contamination of ophthalmic devices. Metal particulate contamination is a known cause of TASS and has been implicated in multiple TASS related recalls of ophthalmic devices.
   c. Analytical methods for organic contaminant determination – Develop methods to assess organic contamination of ophthalmic devices. Organic compounds, typically from the cleaning/sterilization process, have been implicated as one of the primary causes of TASS.
   d. Analytical methods for biological contaminant determination - Develop methods to assess biological contamination of ophthalmic devices. Foreign proteins/nucleic acids and low molecular weight breakdown products of ophthalmic viscosurgical devices (OVDs) have the potential to cause TASS.
   e. Analytical methods to characterize recalled OVD secondary to TASS – Develop methods to biologically and chemically characterize a recalled OVD which resulted in TASS. A full characterization of the device will be performed to determine what attributes of the device may have caused the TASS.

2. Intraocular animal studies
   We will undertake intraocular studies, utilizing the expertise of an ophthalmologist and research scientists, to assess the intraocular test methods and determine the dose response of organic and inorganic contaminants to cause inflammation. These will include:
   a. Evaluation of intravitreal injection compared to intracameral injection to assess intraocular inflammation in the rabbit;
   b. Inflammatory reactions caused by endotoxins in water and in sodium hyaluronate solutions;
   c. Inflammatory reactions caused by organic cleaning compounds used prior to sterilization;
   d. Inflammatory reactions caused by inorganic contaminants of ophthalmic devices;
   e. Inflammatory reactions caused by recalled OVD (provides correlation back to human experience)

3. Early Warning Signal Identification
   In order to identify a reliable early warning signal of a TASS epidemic, we established an extensive collaboration with professional organizations. FDA’s outreach to the leaders of American Society of Cataract and Refractive Surgeons (ASCRS) and American Academy of Ophthalmology (AAO) resulted in a joint FDA/ASCRS/AAO TASS Communication Task Force. This allowed FDA access to ASCRS TASS registry data.
The next steps, utilizing the expertise of an ophthalmologist and epidemiologist, are as follows:

a. Assess the data available in the ASCRS TASS registry;

b. Analyze current/future trends in the occurrence of TASS;

c. Identify factors that can serve as early warning of an epidemic and allow intervention to limit the impact of TASS on public health.

**Contact Lenses**

1. **Method Development**

   For contact lens outbreaks, it is believed that the disinfection efficacy of the contact lens care products may have been compromised by interactions between the lens and the care products. To address this issue, we will develop more robust analytical procedures to characterize the various components (preservatives, surfactants, additives) of the care product solutions utilizing the expertise of a microbiologist and chemist. In addition, we will categorize the numerous silicone hydrogels lens materials into fundamental groups based on how these materials interact with the components of the care product solutions. Furthermore, we will measure the relative tendency of various lens materials to cause adherence of bacteria in conjunction with various types of care products additives (moisture retention, comfort, lubrication). These efforts will result in test methods that predict the tendency of products to fail in disinfection performance under real world conditions. The specific studies are as follows:

   a. **Preservative depletion and efficacy study** - Perform *in vitro* study to evaluate effect of lens and lens case absorption of preservatives on antimicrobial activity of contact lens multipurpose solutions. Collected data will provide information on one of the most important factors that may result in reduced antimicrobial activity of contact lens solutions —uptake of antimicrobial agent by lenses and/or lens cases over time.

   b. **Analytical methods for preservatives development** - Develop improved analytical methods to characterize preservatives in care product solutions. During the *Fusarium* investigation, we found that the current analysis methods for preservatives in care product mixtures were less than robust. Improved analysis methods are critical to assuring safety of these products.

   c. **Films deposition, evaporation, and interference study** - Evaluate the tendency of various versions of commercial contact lens solutions to form films which can inhibit disinfection performance. This will identify the types of care product additives that may interfere with cleaning/disinfection performance.

   d. **Acanthamoeba test method development** - Develop a method for evaluating disinfection efficacy against *Acanthamoeba*. Most care product solutions are ineffective against *Acanthamoeba*, and an accepted disinfection test method is not available. This effort will lead to standardized comparisons of the efficacy of contact lens care products against this organism and will aid in the development of more efficacious products.

   e. **Silicone hydrogel sub-categorization** – This effort will update the current FDA lens groupings for silicone hydrogels and help predict lens-solution combinations which may compromise safety. The porous structure and charge distribution of the various versions of new silicon hydrogel and conventional lens technologies and the effect on interference with care product solution disinfection components will be quantified.

2. **Investigation of Disruption of Ocular Defense Mechanisms**

   We will utilize the expertise of an ophthalmologist to explore the impact of lens care solution characteristics on ocular defense mechanisms and its evaluation.

   a. Predicting disruption of natural defense mechanisms in the eye – assessing the effect of lens care characteristics on the ocular defense mechanisms such as tear antibacterial and cleaning functions.
b. **Corneal Staining** – assessing the viability of corneal staining to evaluate the effect of contact lens care products on functional status of ocular defense mechanisms.

This project will involve the following collaborators: ASCRS, CDRH/ODE, OSB, and OSEL; FDA/OR/WEAC; CDC; NIH; and universities. The resulting data from these studies will allow us to enhance pre-market review, update our guidance documents and make changes to current Standards for these ophthalmic devices. They will help to ensure a greater margin of safety for the millions of IOL recipients and contact lens wearers.
Rapid Detection:
High Throughput Methods for Detection and Identification of Contaminants and Emerging Diseases:

Project Title:
New Approaches to Analyzing Chemical and Biological Contamination at Medical Devices Surfaces

This is multidivisional program involving collaboration between the Divisions of Chemistry and Materials Science, Biology and Physics. We will develop new tools to assess the state of contamination of medical device surfaces with its goal being new technologies that can be used in the field (or perhaps at health care centers) to rapidly assess the presence and/or identification of contamination on device surfaces.

New spectroscopic methods including surface sampled mass spectroscopy (with and without laser induced ionization) and laser spectroscopy (non-linear, Raman, laser-induced fluorescence and infrared spectroscopies) will be used to detect the presence of small molecular contaminants (such as sterilant residues or manufacturing residuals), the presence of trace heavy metals (as recently seen in the dental crown situation) or complex biomolecules. The latter may be due to bacterial biofilms, pyrogens, or intentional contamination (as seen in the recent heparin contamination issue). To appropriately assess the sensitivity of these methods and the sensitivity of these methods for detecting these contaminants, we will use cell culture and bioassays to determine the effectiveness of the spectroscopic methods in assessing low levels of toxic contaminants and will address uncertainties in existing ISO and ASTM biocompatibility standards. In conjunction with the laser spectroscopic efforts, we will develop an enzyme-linked quantum dots assay to create a rapid screening technique analyze for the presence of heparin contamination, readily deployed for inspections.

Thrust Area A
Spectroscopic identification of contaminants at device surfaces

Description:
We will investigate the sensitivity and specificity of surface analytical techniques for determining the presence of contaminants on device surfaces. Specifically, we will use new methods of atmospheric pressure ionization mass spectrometry with “direct” sampling interfaces to determine the presence of small molecule contaminants on surfaces (solvents, sterilization residues, processing aids). We will couple this technique with laser ablation to analyze complex macromolecule contaminants (polysaccharides such as oversulfated glycosamineglycans, polypeptides such as prions, and lipopolysaccharides such as endotoxins). We will also utilize linear and non-linear optical spectroscopies in conjunction with the “sampling” laser to obtain another dimension of spectroscopic information. These technologies will exploit various advanced laser spectroscopy and fiber-optic sensing techniques which can be summarized in following three major groups: (1) linear laser spectroscopy, which will include applications of tunable laser absorption spectroscopy in a broad spectral range covering the ultraviolet, near-infrared and in particular, the mid-infrared range where the most identifiable molecules have specific absorption and radiation features that depend upon their chemical composition; (2) non-linear laser spectroscopy, which will involve various advanced laser spectroscopy techniques based on laser-induced non-linear optical frequency conversion effects such as Raman spectroscopy, surface-enhanced Raman spectroscopy (SERS), coherent anti-stokes Raman spectroscopy (CARS), fluorescence spectroscopy and multi-photon spectroscopy; and (3) fiber-optic based sensing techniques, which will combine the advance features of the broadband tunable laser spectroscopy approach with the high-resolution and effective sensing potential of various fiber-optic sensor designs such as smart fiber structures, nano-sized fiber probes, and
hollow-core sensing infrared fibers. Using these approaches, we anticipate developing methods for highly effective, accurate and repeatable sensing and analyzing chemical and biological contamination at medical device surfaces.

**Deliverables:**
- Assessment of the utility of multi-dimensional spectroscopic approaches to contaminant identification.
- Determination of the feasibility of developing a self-contained system to utilized the combined methods.

**Thrust Area B**
Analysis of chemical contamination of medical device surfaces using biological methods

**Description:**
This project will combine biological and analytical chemical approaches to assess the toxicity of chemical contaminants present at device surfaces (e.g., sterilants, disinfectants, residual solvents and monomers) and will address uncertainties in existing ISO and ASTM biocompatibility standards. The results will be used to address high profile issues in the Center, such as:
- the toxicity of alternatives to compounds like bisphenol A and DEHP in plastics
- the toxicity of antimicrobials in contact lens solution
- defining an acceptable level of cytotoxicity for surface contaminants on devices.

Further, this project will address fundamental issues relating to the clinical relevance of toxicity test methods for surface chemical contaminants in ISO and ASTM standards.

**Deliverables:**
- Cell-based systems to rapidly detect chemical contamination on device surfaces
- Use of these results to help revise ISO and ASTM standards and CDRH guidance documents

**Thrust Area C**
New quantum-dot based assays for rapid determination of heparin contamination

**Description:**
Oversulfated Chondroitin Sulfate (OSCS) contamination of heparin supplies, as demonstrated by recent events ([N. Engl. J. Med. 2008, 358, 2457](#)), can result in severe anaphylactoid reactions in exposed patients. Since heparin is routinely used in many FDA regulated products, a rapid, simple, quantitative, robust and field deployable high-throughput method of detecting OSCS would greatly aid in ensuring the safety of the heparin supply. The goal of this study is to investigate the use of a fluorescent resonance energy transfer (FRET)-based peptide substrate designed around a central quantum dot (QD) nanoparticle to measure OSCS induced kallikrein activity in human plasma. The resulting test offers the potential for rapid and sensitive quantification of kallikrein activation and hence heparin contamination. This assay is also amenable to incorporation in a high throughput format on field deployable devices.

**Deliverables:**
- A prototype assay for OSCS contamination in heparin based on QD-links enzymatic assays. (short term)
- A self contained device to accomplish this assay in the field. (long term)
Center for Food Safety and Applied Nutrition (CFSAN)

Priority Areas:

Rapid detection
Microbial Ecology and Contamination Mitigation Strategies
Manufacturing Science
CFSAN Priority Area:

Rapid Detection

High throughput methods for detection and identification of contaminants and emerging diseases

Project Title:
High Throughput Technology for Identification and Characterization of Microorganisms: Field trial of IBIS Biosensor

Description:
One of the high priority assignments for scientists developing methods to assist with outbreaks and trace-backs has been the evaluation of commercially available instrumentation that can be adapted to our regulatory mission. One of those instruments, the Ibis Biosensor System, has been purchased and evaluated in CFSAN’s research laboratories. The evaluation indicates that the technology has tremendous potential to improve sample throughput and can be further adapted to meet emerging needs. The instrumentation is laboratory based and provides scientists with broad-range and strain-specific identification of infectious organisms for multiple applications (clinical and environmental). The application does not require any prior knowledge of the sample identity and can simultaneously identify and characterize bacterial, viral, fungal, and other infectious organisms. A product of the Defense Agency Research Projects Agency (DARPA), the system is installed in various locations around the US, as rapid bio-threat detectors, including USAMRID and the FBI Academy Laboratories. The technology is extremely high throughput and can analyze thousands of samples a week. We propose the purchase of at least two of the systems for placement in ORA laboratories. A team of scientists from the Center, as we’ll as, ORA could then begin coordinated testing and refinement of the technology for FDA’s needs. The extremely high sample throughput, along with the high degree of specificity built into this technology has the potential to dramatically improve our response and trace-back capabilities.

Hypothesis:
The IBSI Biosensor System has extremely high sample throughput, along with a high degree of specificity, for clinical and environmental samples and may potentially be adapted to improve FDA’s response and trace-back capabilities in food borne outbreaks.

Methods:
The Ibis T5000 biosensor, marketed by Ibis Biosciences, is a high throughput, PCR/mass spectrometric based identification system capable of identifying bacterial, fungal, viral and protozoan pathogens as well as mutations in human mitochondrial DNA associated with disease and for forensic purposes. The principle behind this instrument is quite simple (Hofstadler et al 2005, Ecker, et al 2006). PCR is used to amplify small (80-150 bp) regions of DNA or cDNA that contain unique changes specific to a bacterial, fungal or viral species. The masses of these small fragments of DNA are then measured using electro-spray ionization time of flight mass spectrometry (ESI-TOF-MS). Since the mass accuracy of the mass spectrometer is so accurate the exact base composition (i.e. total number of A, G, T and C) can be calculated. These base counts are then compared to a library of known organisms to make a positive identification. The basic workflow of the T5000 from sample collection to data analysis includes nucleic acid extraction from sample. PCR amplification, PCR fragment clean-up (desalting), ESI-TOF-MS, and data analysis of the resulting mass spectra. This instrument is also capable of identifying several organisms in a mixed sample and is sensitive enough that pre-enrichment to increase the bacterial load is unnecessary. Quantification of the original sample is done through the addition of an internal synthetic DNA and PCRred calibrant. Thus in an unknown sample the
relative amount of each organism can be determined along with an accurate estimate of mass for each amplicon.

Depending on the question being asked, several assay kits are available to answer many common clinical questions with new assays in development for use in the areas of food borne pathogen detection, plant pathogen detection and human forensics and health (Hall et al 2005, Postnikova et al 2008). The broad bacterial surveillance kit can distinguish all major lineages of Eubacteria to the genus level and some to the species level (Hofstadler et al 2005). More specific genotyping can be achieved using specific genotyping kits, such as those for Acinetobacter, Bacillus anthracis, group A Streptococcus and Staphylococcus aureus (Ecker et al PNAS 2005, Ecker et al JCM 2006).

The power of this instrument comes from the bioinformatics that support its database. Most of the DNA changes that the T5000 is able to detect are single nucleotide polymorphisms. Many of the assays were developed based on prior multi-locus sequencing typing and analysis (MLST/MLSA) work done for each organism. Once unique SNPs are identified that delineated species or strains then primers are designed in the conservative regions that flank those variable sites and thus, informative sites can be assayed. However these primers must be positioned in such a way which allows base count differences to be seen and that do not interfere with the amplification and detection of the internal standard calibrant. Since mass spectrometry does not give sequence order, only base composition, certain changes in the DNA may not be visualized. For example, if two samples are identical in all bases except for a SNP change at position X that is an A to G change for sample one, but in sample two there is a similar SNP change (A to G ) but in position Y, the base counts for these two samples will be identical. Only when one sequences these amplicons will the differences be uncovered. This technology also is capable of using data from variable number tandem repeats (VNTRs, Van Ert et al, Biotechniques 2004) or any mutation for that matter as long as it does not exceed the 150 bp maximum for mass estimation. The accuracy of the mass spectrometer shows the differences in repeat units in a specified region and it reveals any SNPs that are present within a repeat. Our previous intensive bioinformatics MLSA work conducted on food borne pathogens of interest to the FDA and for food safety can now be transferred to this new technology. The hard work of determining the unique SNPs and VNTRs to specific lineages of microbial species make it possible now to design FDA specific assays for the T5000 system for the rapid identification of food borne pathogens.

Initial data collection will require DNA nucleic acid extraction from known Salmonella collections of import to the FDA, CFSAN mission and objectives. Once the DNA is isolated it will be amplified and sequenced for specific target genes that will be chosen to differentiate the Salmonella subspecies and serovars as well as the O and H antigen types. Much of this sequence is already collected and available. Once sequences are aligned in standard software packages such as ClustalX, then primers will be designed in conservative regions of the sequence so that they will bind to most Salmonella samples. Primer location will be chosen so that they bracket variable regions that house genetic variation that can differentiate the various Salmonella samples and are in a size range that is compatible with the Ibis MS technology. These PCR amplicons will be prepared for analysis on the Ibis ESI-TOF-MS system. Known samples (with known DNA sequences) will be validated to see that the mass spectra obtained fully correlate with their expected masses. Once primer systems are designed to differentiate the Salmonella samples found in our 4 objectives, we will both validate more known Salmonella collections as well as start collecting wild caught samples from the field. This proposal is specifically for Salmonella detection but the instrument will have many uses including the typing of E. coli and fin fish identification. Building an active team using Ibis technology will assist across all of these CFSAN projects.
Deliverables

2009:

- Purchase 2 instruments and supplies for ORA labs, train ORA scientists, and develop a working group specific to this application
- Begin field testing applications and validate with traditional approaches for species and sub-species level identification of *Salmonella*
- Contracts put in place to develop new, food pathogen specific targets including development of assay to differentiate the eight different subspecies (I,II, IIIa, IIIb, IV, V, VI and VII) of *Salmonella enterica* based on the sequences of the *mutS*, *gapA*, *recA*, *phoP*, and *Mdh* genes, that can be used to differentiate the eight subspecies. It may not be necessary to use all of the targets to differentiate these sub-species but some of these targets may be useful for our other objectives, below

2010:

- Increase the number of samples tested from prior year and develop modifications to testing protocols, including typing of the known SAR A, B and C reference collections.
- Develop a series of primers that will differentiating *Salmonella* based on known O-group types. Primer design will come from the assembled DNA sequences that code for known O-antigen types, using a subset of the *Salmonella enterica* reference set B (SAR-B) collection that is relevant to FDA food-related problems. The number of bacterial strains in the set used to develop the O-typing primers will be at least 30. All of the barcodes generated will be registered in the data base with the expected target bacteria, per the usual approach by Ibis.
- Develop *Salmonella enterica* primers to be able to distinguish between Newport and Saint Paul serovars. We will use the genomic software, tools, and data that are available to determine genetic variants that are specific to the common outbreak strains of Newport and Saint Paul and then we will develop a set of primers that will generate amplicons sufficient for typing these *Salmonella* serovars.

2011:

- Role out new testing applications based on performance in Field/Center lab, and data analysis
- Determine genetic variant SNPs that are specific to some of the other common outbreak strains of *Salmonella* and develop methods for their rapid identification.
- Validate new testing approaches and comparison with PFGE typing methods
- Populate the Ibis data-base with a large number of known samples as well as new field material that has been validated, for rapid field identification of pathogens.
Rapid Detection: High throughput methods for detection and identification of contaminants and emerging diseases

Project Title:
Rapid identification of food pathogens using high-throughput detection methods that target single-nucleotide polymorphisms (SNPs).

Description:
Recent outbreaks of Salmonella and Escherichia coli O157:H7 associated with fresh produce have underscored the need for rapid and high-throughput strain identification methods. Outbreak investigations typically involve screening of suspected produce sources for a single strain of a pathogen that are difficult to or impossible to distinguish using conventional typing and detection methods. This problem is due, in part, to the rapid diversification of the pathogens, allowing little to no time for phenotypic differences to develop between closely related strains and serovars. However, differences present in the genomes of these strains can be detected and used to identify and discriminate closely related isolates. Currently, we are exploring the application of two high-throughput technologies, the Bio-Plex (Luminex) SNP detection platform and a Pyrosequencing SNP detection system.

The Bioplex platform is based on the technology of flow cytometry, which in combination with fluorescent dye-incorporated polymeric beads is ideal for the design of biological multiplexed arrays. The application of this platform as a tool to probe both genomic and proteomic functions has clearly been demonstrated, and multiple assays have been described using this technology for gene expression profiling, HLA DNA typing, microbial detection, and SNP genotyping. SNPs, one of the most abundant forms of genomic variation, provide important biomarkers which can greatly facilitate molecular subtyping of microorganisms such as Salmonella. With the SNP data obtained from different sequencing strategies, one can use the Bioplex platform to design multiplexed SNP genotyping assays. A common approach for single nucleotide discrimination is to use direct hybridization format, that is, the hybridization of a labeled complementary sequence amplified by PCR with microsphere sets coupling oligonucleotide capture probes. This approach takes advantage of the fact that for oligonucleotides around 15 to 20 bases in length, the melting temperature for hybridization of a perfectly matched template compared to one with a single base mismatch can differ by several degrees.

Much like the Bioplex, pyrosequencing platforms use SNPs for high-throughput detection and identification. A series of SNPs which are evenly split between two bases in the population are useful in binning strains into groups, while SNPs which show rare variants are useful markers for identifying individual strains. Pyrosequencing is a rapid sequencing technology using the principle of “sequencing by synthesis”. A sequencing primer is annealed to single stranded PCR template and individual deoxynucleotide triphosphates (dNTPs) are added to the reaction. The sequential addition of dNTPs will read a short DNA sequence, including SNP positions. The SNP frequency in a pool of sample strains results in an estimation of how useful the SNP can be in dividing the population of strains. Once a series of validated SNPs is assembled, testing of individual strains with the panel of SNPs will give a profile for each strain, which can be used to cluster strains into groups or possibly identify individual strains depending on the level of discrimination of each SNP. Running the panel on a set of strains that represent the diversity of the group being studied will establish a reference database, and as new strains are encountered, such as strains isolated from an outbreak, profiles of the unknown strain can be compared to the reference database to find nearest neighbors.

As the microorganisms associated with recent foodborne outbreaks appear to be more aggressive than ever, the need for rapid detection, identification, and trace-back of the microorganism responsible any given outbreak is greater than ever. The information gained
from understanding the evolutionary relatedness of these foodborne pathogens is key to unraveling traits shared by the most infectious strains. Studies such as this grant us the opportunity to identify genetic markers which allow some *Salmonella* serovars to become pathogenic while others remain benevolent. Additionally, platforms like the Bioplex and Pyrosequencing allow for the accurate and timely analysis of many more samples than ever thought possible considering the means and personnel available to our ORA laboratories in this challenging economic period, making ‘high-throughput’ an attainable goal.

**Hypothesis:**
Molecular subtyping has become a critical component to the investigation and rapid identification of bacterial contamination of a food source by pathogenic species and strains. These rapid SNP-detection strategies will enhance the agency’s capabilities in responding to foodborne outbreaks involving foodborne pathogens by allowing for more rapid responses in real-time (i.e., allowing us to detect and match the specific genotypic patterns of an outbreak strain) and for deployment of high-throughput platforms, as described above, to allow for more comprehensive sampling. Additionally, the outcomes of these studies can provide insight into the evolution, population structure, and diversity of pathogens known to be endemic problems to the food supply.

**Methods:**
The Bio-Plex system has been deployed in more than 50 public health laboratories, including 8 ORA laboratories. Multiple applications of the Bio-Plex platform are being explored including: (i) validation of a rapid *Salmonella* serotyping technology that assigns serovar status based on the specific SNPs responsible for antigenic variation; (ii) development of probes for identification of specific *Salmonella* strains directly from food matrices; and (iii) rapid strain subtyping procedures for common *Salmonella* serovars including *S. Typhimurium*, *S. Enteritidis*, *S. Saintpaul*, and *S. Muenchen*. Pyrosequencing will be validated concordantly with Bio-Plex to determine the frequency and prevalence of SNP targets among various *Salmonella* serovars.

The requirements for experimental design of both Bioplex and Pyrosequencing platform assays are very similar as both technologies utilize genomic SNPs to differentiate among isolates. Initial data collection requires DNA nucleic acid extraction from food pathogens of importance to the center’s mission in protecting and promoting food safety and public health. The process of SNP harvesting begins by DNA sequence comparisons either over a whole genome or over individually sequenced genes. Sequences are obtained by either conventional Sanger sequencing, or now sequencing by 454 technology, which yields a whole genome sequence in a number of assembled contigs. From these sequences, or sequenced PCR products, alignments of genes matched by comparison methods will be made, allowing the detection of nucleotide base changes. Once regions of interest are determined, identification of the exact base position and the change from reference sequences must be made by short sequencing. At this point separate assays are designed for the Bioplex and Pyrosequencing platforms that will test the utility of the designated SNPs. Using the assay to test reference and predicted variant strains will determine if the SNP is truly present in variant strains or if the sequencing data was in error, a critical step in confirming the SNPs composing the validated detection assays.

**Deliverables**
2009:
- Field lab validation of Bio-Plex based rapid serotyping method for *Salmonella* in foods
- Expand SNP database based on traditional sequencing of genetic information. Validate this information using the pyrosequencing approach and add a greater number of food specific pathogens
2010:
- Begin developing/testing new SNP typing scheme for Bio-Plex platform based on pyrosequencing information and validation
- Expand use of Bio-Plex platform for *Salmonella* serotyping to other food pathogens as well as additional matrices

2011:
- Expand Bio-Plex platform to include serotyping and individual strain identification. Use of the technique in multiple ORA laboratories.
CFSAN Priority Area:

Microbial Ecology and Contamination Mitigation Strategies

Understanding Routes of Microbial Contamination and Ecology to Reduce or Eliminate Contamination

Project Title:
Ecology and Control of Salmonella on Tomatoes

Description:
An estimated 14% of all foodborne outbreaks reported in North America are attributed to the consumption of raw or minimally-processed fruits and vegetables. Consumption of fresh tomatoes has been linked recently to numerous foodborne outbreaks involving various serovars of Salmonella enterica. S. Newport, for example, has been linked recently with a series of recurring outbreaks of salmonellosis from ingestion of fresh-cut tomatoes produced on Virginia’s Eastern Shore. In 2002 (512 cases), 2005 (71 cases), and 2006 (107 cases), S. Newport was found to have sickened consumers who had eaten raw tomatoes from several Eastern shore farms. The epidemiological complexity of this pathogen’s association with tomato is manifold and includes numerous natural reservoirs of Salmonella in the ecosystem, a lack of suspect isolates actually found on tomato, and a widely-dispersed area of affected individuals to name but a few aggravating circumstances.

S. Newport, in general, appears to be a prominent and rising Salmonella serotype associated with produce and increased foodborne illness. In addition, S. Newport poses a substantial health risk since numerous Newport strains appear to be resistant to at least one antibiotic with many strains displaying multi-drug resistant phenotypes. Many salmonellae seem to thrive in numerous unlikely environments. Several serovars has been shown to persist in certain niches for long periods of time. For example, recent reports have demonstrated long-term survival of this pathogen in the gut of free-living nematodes. Other studies have shown these salmonellae to be effective colonizers of living plant tissues which could help to account for the persistence of Salmonella on tomatoes and tomato plants. The ecological and microbiological factors allowing for persistence of Salmonella in these micro-environments remains largely unknown. Thus, studies focused on assessing the ecological, microbiological, and epidemiological nature of Salmonella and its associations with fresh-cut tomatoes are essential to mitigating current outbreaks in this food commodity. In addition, we hope to gain a better understanding of the environmental factors that contribute to or aggravate the colonization of tomato plants and/or fruit surfaces with this pathogen.

Microbial ecology has become a critical component to the investigation and understanding of bacterial contamination of a food source by pathogenic species and strains. Such studies hold the potential for elucidating bacterial outbreaks to their points of origin including unsuspected food commodities and unprocessed food constituents. The information gleaned from understanding the ecologic microcosms in which these pathogens persist is key to understanding the link between a pathogen and a food source as well as to developing stop gap measures to deter future outbreak events. Why Salmonella Newport predominates among salmonellae associated with Eastern shore tomatoes and persists over years in this microcosm remains unclear. One explanation is that Newport strains enjoy a genetic and/or ecological fitness advantage over other salmonellae in this environment. It maybe that Newport enjoys a growth advantage over other subtypes. The ecological studies proposed here can explore this hypothesis. Additionally, prevalence studies in the local growing areas will answer questions as to the distribution and prevalence of Salmonella in the U.S. tomato supply and may explain
infection rates in certain mid-Atlantic regions. Finally, research focused on a kill-step against *Salmonella* infestation of Eastern Shore tomatoes may lead to important intervention steps by exploiting naturally-occurring epiphytic microflora endemic to the tomato growing environment.

**Hypothesis:**
Understanding the microbial ecology and environmental factors which contribute to contamination of tomatoes with *Salmonella* will result in development of more effective prevention and intervention strategies as well as improve mitigation steps on the farm and in the ‘farm to fork’ tomato production continuum. Ultimately, this work will aid in reducing *Salmonella* contamination of tomatoes, and, by pinpointing important reservoirs, will reduce the time needed to conduct field investigations of outbreaks and resulting human foodborne illness associated with that contamination. We hypothesize that *Salmonella* colonization is endemic to the Eastern Shore tomato growing areas of the mid-Atlantic. It is likely that ecologically important characteristics will be ascribed to this environment that allow for an enhanced biological control of *Salmonella* on tomatoes *in situ*.

**Methods:**
*Microbial ecology.* Microbiological studies will be conducted related to understanding the growth and survival characteristics of *Salmonella* on tomatoes and in the tomato growing environment. Studies will include pH tolerance, desiccation and other stress responses, osmotic tolerances, and the potential for internalization and subsequent survival of *Salmonella* inside tomato fruits. Similar studies will be conducted on fresh tomatoes as well as in the dump tank and post harvest environments. The potential role of biofilm formation also will be investigated.

*Microbial interactions.* A broad survey of epiphytic microflora associated with Eastern shore tomatoes, tomato farms, and other local environmental microcosms where *Salmonella* is suspected to persist will be undertaken. This will include aerobic plate counts (APCs) of tomato surfaces followed by a characterization of the epiphytic inhabitants of native Eastern shore flora, drainage water, and actual tomato plant surfaces will be isolated on general media, taxonomically identified using 16S rDNA sequencing then screened for antagonistic properties against *Salmonella* using a rapid *in vitro* screening assay based onpour plate methodologies and growth curve analyses. Successful epiphytic species will be advanced to bactericidal studies *in vivo* directly on tomato surfaces. Optimal antagonistic strains will be examined in greater detail to ascertain MIC values for each antagonist to rate the potency and potential mechanisms for these microbes. Optimal antagonists will be entered into field trials and once a successful candidate is selected, growers can be provided with the strain as part of ongoing trials into the its effectiveness as a kill-step in the persistence of *Salmonella* on Eastern shore tomatoes.

*Endemic prevalence.* Using PCR and conventional culture/serological methods, we will probe several environments on the Eastern Shore and neighboring states in attempts to ascertain which tomato microcosms are most suitable for *Salmonella* to persist and/or which *Salmonella* serotypes are most dominant in various natural ecological microcosms. In order to ascertain the relative prevalence of various serovars in pond water, soil environments, barnyard waste, and other natural reservoirs, microbiological and PCR based surveys will be conducted to determine the relative prevalence of Salmonella in the specific niche or locale being studied. In each case, *Salmonella* will be universally enriched for from a specific niche sample, and the resultant colonies will be serotyped to determine the relative prevalence of individual salmonellae in the specific environment.

**Deliverables:**
2009-2010:
• Identification of various survival factors and conditions that may effect the growth and survival dynamics of *Salmonella* in the tomato growing environment including bacterial and environmental conditions that foster colonization and adherence to the tomato surface, the biodiversity and role of native flora in the colonization and persistence of *Salmonella* on the tomato surface, the likelihood of internalization of *Salmonella* into the tomato plant and fruit, and the survival of *Salmonella* in the dump tank environment during immediate post-harvest.

• Several suspected natural reservoirs of Salmonella will also be investigated from the tomato growing regions for endemic *Salmonella* prevalence including pond water, soil, local livestock, and domestic and feral avian sources.

2009-2010:

• Assessment of the ecological and allelopathic interactions of naturally-occurring microbes associated with tomato fruits, plants, and the surrounding growing environment and pathogenic *Salmonella* serovars associated with tomato-borne outbreaks of human disease. This outcome will foster development of effective and ecologically sound intervention strategies that reduce the persistence and contamination of *Salmonella* on fresh cut tomatoes.

2009-2011:

• Analysis of the endemic prevalence of epiphytic *Salmonella* on tomatoes grown on the Eastern shore of Virginia. This will include comparisons of red round, Roma, and cherry tomatoes. This survey will also include comparisons of organic and inorganically raised tomatoes. While focusing on the Eastern shore (i.e., Delmarva peninsula), tomatoes grown throughout the Southeastern US will be targeted in order to ascertain the endemic nature of *Salmonella*, including predominant serovars, on fresh grown tomatoes for human consumption. A key focus will be on tomatoes from distinct growing regions and during differing growing seasons.

• We will also continue to sample and develop a database of microorganisms that reduce *Salmonella* loads on fresh tomatoes. Additionally, using antagonistic strains, we will develop and test models of mitigation in the farm environment, in cooperation with the UMD's field test facilities
CFSAN Priority Area:

Manufacturing Science

Novel technologies in manufacturing science to enhance quality, efficiency and safety

Project Title:
High pressure processing as a new technology for producing safe shelf-stable foods

Description:
High pressure processing (HPP) is a new technology potentially capable of producing foods superior in quality (taste and appearance) to those produced by traditional heating alone. It also will allow for the production of shelf-stable foods that cannot be produced using traditional thermal processing (i.e., shelf-stable mash potatoes). High pressure processing works by applying extreme pressures to the packaged food product such that the microorganisms within the food product are inactivated. The pressures (up to 800 MPa) used during the high pressure processing are at levels where the transfer fluid, usually water, may decrease in volume by as much as 20%. At these pressures, even the metals used to makeup the vessel can be affected. During pressurization, the pressure transfer fluid and the food product rise in temperature due to adiabatic heating. The amount of adiabatic heating depends on the food composition, but often is over 20°C. The combination of adiabatic heating, initial temperature, maximum pressure, and treatment time all determine the extent of microbial destruction that takes place during a process.

The actual mechanism of microbial destruction is not known. However, it has been shown that there is a synergistic effect between the temperature of the food product and the maximum pressure of the process. Thus, an advantage of using high pressure in combination with heat is that the amount of heat needed to destroy potential pathogens within the food is less than that which would be required if the food was treated with heat alone. This reduced processing temperature as compared to heat alone results in food products of superior quality. We presently do not have a comprehensive understanding of the kinetics of inactivation of microorganisms by high pressure or the combination of high pressure and heat. In particular, we have limited understanding of the inactivation of Clostridium botulinum, a very important pathogen of concern for low-acid shelf-stable foods in hermetically sealed containers.

Due to the limited amount of information that is available on both the mechanism and reaction rate kinetics for the destruction of pathogens by HPP it is difficult for the FDA to evaluate the potential health risks of specific processing conditions used to generate shelf-stable HPP foods. A clear understanding of the critical factors that affect the lethal delivery of the process need to be understood. This includes processing factors such as product composition, initial temperature of the food product, final temperature of the product after pressurization, heat loss during processing, packaging, and process time.

Just as important as an understanding of how the process can be designed and controlled so that it delivers the necessary treatment needed to render the food safe is a procedure to be able to validate within a production facility that a specific processing procedure is able to deliver the necessary lethal dose. To this end a suitable surrogate for spores of Clostridium botulinum is needed. At present, it appears that spores of C. botulinum are among the most resistant to this type of process, making these processes difficult to validate within the processing plant. If the mechanism of destruction was known the process by which to identify a reasonable surrogate sporeformer should be simplified.
Hypothesis:
To date, there is very little published data on the resistance of *C. botulinum* spores to HPP. To a large degree this is due to the fact that there are limited laboratories that can conduct experiments with select agents such as *Clostridium botulinum*. In order to start developing an understanding of destruction kinetics for HPP treatments a sufficient amount of data must be collected for a large number of *C. botulinum* strains. Also, the relative resistance among the strains must be determined so that when establishing a process to create a shelf-stable food the proper safety factors are incorporated into the process to make sure that the most resistant *C. botulinum* spore is destroyed. Using an appropriate database of resistance data, current models used for thermal destruction can be modified to incorporate the effect of pressure, which will allow for the addition of statistical variability into the process design.

An understanding of the mechanism of microbial destruction during HPP will allow for the potential prediction of not only how *C. botulinum* will be affected by a given process, but also how other spore formers are affected. Using either DNA microarray's or pulse-field gel electrophoreses (PFGE) patterns, a correlation between the microarray or PFGE and spore resistance may be attainable. These correlations will help identify resistance factors and their importance in providing safe shelf-stable foods. This information should also provide the scientific basis for the industry and the agency to move forward with validated HPP for both shelf-stable and extended shelf-life refrigerated foods and thus ensure the safety of these foods.

Methods:
Resistance of *C. botulinum* spores and other sporeforming bacteria of public health and food spoilage significance to combinations of temperature and pressure will be evaluated using a PT-1 high pressure unit manufactured by Avure, Inc. The high pressure unit is capable of achieving a maximum pressure of 690 MPa. HPP is being evaluated for its ability to produce a shelf stable food free of the hazard of *C. botulinum*. To determine such a lethal treatment it is necessary to use the most resistant strain of spore known. Determining this strain will require screening multiple strains. Testing these strains requires growing spore stocks of each strain. Each spore stock will contain high numbers of spores with few vegetative cells. Spore stocks of thirty potentially resistant strains will be generated using a biphasic method. A known culture is streaked and a pure colony is selected, and inoculated into biphasic media and incubating from 10 to 30 days until the culture reaches 80% sporulation as judged by microscopic evaluation. At this level of sporulation the mixture is centrifuged and concentrated into a smaller, desired volume.

Preliminary high pressure experiments will be performed by exposing each of the thirty proteolytic *C. botulinum* strains to identical combinations of temperature and pressure. This data will show which strain(s) is the most resistant. Once the desired strain(s) is selected, a large spore crop of this proteolytic strain(s) will be grown for use in the kinetic destruction characterization studies.

In addition to the screening HPP tests, pulsed field gel electrophoresis (PFGE) will be performed on each of the thirty strains to help genetically characterize them. Once a PFGE pattern is obtained for each strain, the patterns will be compared to determine if there is any correlation between banding pattern and HPP resistance. If there is a correlation between PFGE patterns and HPP resistance, more cultures will be obtained and analysed using PFGE. DNA microarray’s might also be used to try and elucidate a correlation.

High Pressure Processing (HPP) experiments will be performed utilizing pressures between 600 and 690 MPa along with temperatures between 100 and 121°C. Known levels of spores will be aseptically placed into sample vials, placed inside the HPP chamber and treated. Survivors will
be enumerated using plating and/or MPN methods. PCR will be evaluated as a tool to determine if any spores of *C. botulinum* survive the treatment.

The lethality data at various pressure/temperature combinations will be analysed to derive a model based on processing pressure and temperature for predicting the lethality of *C. botulinum* spores under varying processing conditions. This model will be validated by inoculating spores of *C. botulinum* into real food matrices.

**Deliverables:**

- Grow spore crops of 30 proteolytic *C. botulinum* strains.
- Screen Proteolytic strains of *C. botulinum* to determine the most HPP resistant strain.
- Obtain PFGE patterns for the 30 strains.
- Determine if HPP resistance can be predicted using PFGE patterns.
- Obtain data on spore resistance for the time, temperature and pressures range potentially used by industry for the HHP treatment of extended shelf-life and shelf-stable foods.
- Develop a model for *C. botulinum* resistance to HPP that can be used to predict and evaluate a commercial HHP system that would ensure the food has been treated to prevent a potential public health hazard.
- Validate the model using real food matrices.
Center for Veterinary Medicine (CVM)

Priority Area:

Rapid Detection
CVM Priority Area:
Rapid Detection

Project Title:
Simultaneous Detection and Identification of Multiple Foodborne Bacterial Pathogens Isolated from Animals and Foods by Bio-Plex Technology and Microarray.

Description:
Understanding the ecology of foodborne bacteria and the genetic bases for virulence and antibiotic resistance are necessary for FDA to advance public health. Tools that can rapidly characterize bacteria from different environments are needed to help understand the environmental sources of foodborne disease and to inform mitigation strategies. This information will also help FDA develop hazard and risk assessment models needed to establish preventative food safety policies. In addition, rapid screening and typing methods greatly expedite outbreak investigations caused by foodborne bacteria, and have been shown to limit the duration and severity of outbreaks.

In general, the research proposed here will overcome the time and expense that has been needed to determine the microbiological status of foods in the past. For example, many types of virulent and avirulent *E. coli* may be present in foods. In the past, making this determination involved several days of testing, beginning with overnight culture of bacteria on artificial medium, followed by a battery of biochemical and antimicrobial susceptibility testing, and a stepwise determination of gene content (usually by PCR) to assess pathogenic potential. The method advancement proposed here will reduce this process to a few hours at lower cost, and provide much more detailed and definitive information.

Bio-Plex and microarray methods represent the latest advances in molecular technology for rapid sample testing. Microarray methods offer a discriminatory, highly informative, high-throughput alternative for the rapid detection of many genes of interest simultaneously and at relatively low cost. By imprinting short DNA (oligonucleotide) probes on a glass slide or chip, researchers are able to interrogate a sample of unknown composition for thousands of genetic markers of epidemiological importance. In addition, microarrays are exceptionally versatile. The technology allows genetic probes imprinted on the glass microarray chip to be customized in any orientation for any group of genes of interest; and therefore can be used to rapidly screen diverse sample types in an information dense manner. The technology is flexible and can be customized to suit different and changing public health research needs.

The Bio-Plex (Luminex) technology is similar to a microarray in that DNA targets are attached to a solid surface and can be used to probe samples of interest. It too is flexible and can be tailored to specific needs. The Bio-Plex platform is a bead-based technology that uses fluorescently labeled DNA probes, protein, or antibody particles conjugated to a colored bead. This is the platform developed at the CDC for molecular serotyping of *Salmonella*, and is becoming widely available in many FDA and research labs. While microarrays are able to test for many gene targets, it is neither practical nor cost effective to use microarray on many thousands of microorganisms. The advantage of the Bio-Plex technology is the ability to screen large numbers of samples for a smaller subset of genes in a rapid and cost effective manner. This method can detect up to 100 genes in a single sample in a quantitative manner in a few minutes. In addition, Bio-Plex is capable of detecting single nucleotide changes, allowing detailed analysis of mutations (such as those associated with antibiotic resistance or other traits) and the disposition of genetic alleles among populations. Allelic distribution is a promising approach for assessing strain relatedness and source attribution for several
pathogens. For these reasons, Bio-Plex is an ideal technology to use in conjunction with microarray for the rapid and comprehensive analysis of bacteria.

We propose to use these two technologies in a multistep method development plan. Based on antibiotic resistance information and pulsed-field gel electrophoresis data, we will screen representative strains of foodborne bacterial pathogens in order to catalog the array of resistance, virulence and other genes present in foodborne bacteria from different sources. Based on this information and what others have reported, we will identify distinguishing traits of epidemiological relevance. We will then employ the Bio-Plex technology to examine strains acquired from national food surveillance systems and those obtained in foodborne disease outbreaks. In a third phase of development, we will employ the Bio-Plex to test food samples directly for the presence of foodborne pathogens and their genes of interest, thereby establishing a rapid and definitive test without having to first culture the bacteria on artificial media. Ultimately, we intend to provide a valuable method that can be used by ORA laboratories and others in need of a very fast and accurate detection tool for foods that can be customized for a variety of specific needs.

In addition to the rapid detection of genetic determinants and transmissible genetic elements (plasmids, transposons, integrons, etc), microarray methods have many research applications relevant to FDA research needs. It can be used to quantify changes in gene expression in response to a particular stimulus, such as antibiotic exposure. This is a valuable research tool for furthering our understanding of the biology of foodborne pathogens. It permits the identification of genetic determinants involved in effecting resistance in an organism, and can be used to investigate dose-response relationships for various pathogens and drugs. This information can be used to evaluate the parameters of safe drug use as it relates to antimicrobial resistance development. The phenomenon of co-selection of distinct resistance and virulence genes on mobile DNA elements is largely unexplored, but is essential to developing interventions to limit resistance development in food animals and to understanding links between drug use and virulence. The application potential for each of these platforms spreads across most sectors of the life sciences, including environmental microbiology and microbial ecology; human, veterinary, food and plant diagnostics; water quality control, and industrial microbiology. The technology has been employed successfully for drug discovery, drug evaluation, cancer research, microbial pathogenicity, mechanisms of antimicrobial resistance, as well as genomic “fingerprinting” and detecting genetic polymorphisms of microorganisms.

**Hypothesis/Objective:**
The objective is to generate comprehensive genetic data on foodborne pathogens using high-density microarrays; and to use this information to identify bacterial traits that can be rapidly assayed in many strains using the Bio-Plex platform. This will permit the FDA to rapidly generate data on specific bacterial characteristics important in surveillance programs and outbreak investigations.

**Methods:**
We propose establishing a microarray platform and rapid detection method based on the bio-Plex platform. We will develop DNA probes, perform pilot testing for assay methods that would be applied to screening food and feed samples for foodborne pathogens. We will work to provide: 1) A single comprehensive microarray platform that can be used to identify multiple pathogens and their epidemiologically important traits; 2) A Bio-Plex based method for rapidly screening many samples of bacteria or foods; 3) A reference set of strains fully characterized genetically that can be use by others to validate methods.
Deliverables:
Enhanced food safety by reducing the time to test samples and characterize pathogens for traits associated with an increased public health threat. Improve collaborations with CFSAN and ORA with regards to pursuing research to address urgent food safety priorities.
National Center for Toxicological Research (NCTR)

Priority Areas:

- Rapid detection
- Biomarkers
- Personalized Medicine and Nutrition
NCTR Priority Area:
Rapid Detection of Bacteria

Project title:
Validation of Advanced Technologies for Rapid Detection of Bacterial Contaminants

Description:
Bacterial contamination of foods historically is a common cause of illness that ranges from mild discomfort to life-threatening conditions and even death. The modern global food distribution network and advanced agricultural practices have dramatically increased nutritional status in the world and unintentionally provided the opportunity for more rapid spread of bacterial contamination and resulting sickness. The complex and varied nature of food matrices complicate detection and intervention against contaminations. Intervention strategies seek more effective surveillance of potential threat and rapid response to find and contain the source of contamination in food illness outbreaks. Innovative technologies and strategies for rapid (a few minutes) detection of bacterial pathogens based upon flow cytometry were developed for detection of bacteria on environmental surfaces and in complex food matrices. Generally, the need for rapid technologies can be separated into two operational functions: a) rapid, mobile, field-rugged test kits for general screening for viable bacterial pathogens in numerous matrices at the numerous outlet food sources; and screening for suspect-target organisms during contamination outbreaks in the same arenas; b) rapid, high-throughput laboratory techniques for both validation of field screens and positive identification/characterization of organisms. Rapid-B technologies have been developed with CRADA partner, LITMUS LLC, for field and laboratory use in rapid screening for bacterial contaminants commonly encountered in the causation of food illness; e.g., Salmonella enterica enterica strains, Listeria monocytogenes, Listeria spp., generic E. coli, and E. coli O157:H7. Detection times have been reduced from several hours to days to a few minutes to a few hours depending on the level of contamination and or the complexity of the food matrices. Detection limits have been observed as low as one viable cell. Validation studies will be designed and conducted for detection of commonly encountered food contamination pathogens by independent laboratory assessment to characterize the ruggedness of the systems for regulatory field use.

Hypothesis:
Implementation of validated advanced technology will increase surveillance resources of the Office of Regulatory Affairs to detect bacterial pathogens in food matrices and on environmental surfaces.

Objectives & Methods:
Validation studies will characterize the ability of the Rapid-B technologies to perform a common microbiological test for detection total viable bacteria in a handling period of only five minutes. Detection of Salmonella enterica enterica strains in tomatoes has been selected for the initial validation studies under field “unknown” conditions; parallel analysis using the established FDA BAM procedure will be run for comparison. Thirty Salmonella serovars responsible for the majority of food outbreaks will be tested for inclusivity in the assay. Fifteen additional species to include Citrobacter, E. coli, Listeria, Pseudomonas, Staphlococcus, Enterobacter and Bacillus will be tested for exclusivity. Additional validation trials can be scheduled for Listeria monocytogenes, Listeria spp., generic E. coli and E. coli O157:H7 in varied food matrices: e.g., raw and cooked chicken, ice cream, milk, spinach, and peanut butter. Provide general proof of concept of additional technologies for rapid screening of food or drugs for general chemical or species specific microbiological contamnation utilizing advance mass spectrometry combined with advanced algorithms compensating for experimental (hardware/environment) variability and pattern recognition.
Deliverables:
This project is being conducted in three phases. Phase I developed the basic science technologies and feasibility of the approach with the LITMUS, LLC under the CRADA and Litmus found and refined the instrumentation and reagents for Rapid-B technologies.

Phase II will examine the utility of the systems under field and laboratory conditions encountered in the regulatory environment.

Phase III will examine the utility of mass spectrometry technologies for high-throughput validation of screening methods developed under Phase II.

Phase II data will be used to:

1. Establish the utility to obtain real-time (<5 min.) total plate count of general bacterial contamination.

2. Establish the utility of the methodology for mobil, field-rugged rapid detection without enrichment for specific pathogens in selected difficult food matrices.

3. Establish and or develop/standardize protocols for rapid (2-3h), general purpose microbiological enrichment procedures for rapid detection of targeted micro-organisms.

4. Provide an assessment of systems operability to include: (1) Training Analysis, (2) Failure Mode Effects Analysis, and (3) Workload Analysis.
NCTR Priority Area:  
Biomarkers

Project title:  
Development of a non-invasive and translatable biomarker using PET imaging

Description:  
Advances in pediatric and obstetric surgery have resulted in an increase in the complexity, duration and number of anesthetic procedures. To minimize risks to children resulting from the use of anesthesia, it is necessary to understand the effects of anesthetic drugs on the developing central nervous system (CNS). However, adverse effects related to CNS structure/function in pediatric populations are difficult, if not impossible, to study and detect.

High resolution dedicated positron emission tomography (microPET) is a non-invasive technology of nuclear medicine that has the sensitivity to trace low picomolar concentrations of radio-labeled molecules, which is useful for studying animal models of human diseases. Early in the apoptotic process, the characteristic cell surface phospholipid asymmetry is disrupted. This leads to the exposure of phosphatidylserine (PS) on the outer leaflet of the cell membrane. Annexin V is an anticoagulant protein that preferentially binds PS and, when conjugated with a reporter molecule, can be used as an indicator of apoptotic cell death. Thus, functional imaging of Annexin V may provide powerful tools for the diagnosis and monitoring disease progression including pathophysiological components of anesthetic-induced brain damage (apoptosis).

Pediatric anesthesia is well established. However, recent studies have found that general anesthetic agents, such as ketamine and gaseous anesthetics, may cause dose-dependent and widespread apoptotic neurodegeneration in the immature rat brain. It is postulated that, in rodents, excessive suppression of neuronal activity by ketamine or gaseous anesthetics during the brain growth spurt triggers neurons to commit "suicide" via apoptosis. L-carnitine (a mitochondrial protectant), melatonin and hypothermia have been reported to attenuate neurological brain injury associated with degenerative effects induced by ketamine or gaseous anesthetics. The issue of whether anesthetic-induced brain damage seen in rodents is relevant to children would be informed if specific biomarkers (tracer agents) of the pathophysiology associated with the use of anesthetic agents could be developed. This would allow for the determination of whether similar effects also occur in the developing nonhuman primate.

Relevance to FDA and Public Health Impact: This study in rodent and non-human primate models will provide critical information for understanding the risks associated with the pediatric use of anesthetic drugs. This study will also provide insight into sensitive periods and patterns of anesthetic-induced neuronal damage and could have direct scientific impact on the regulatory responsibilities of FDA. This project may ultimately provide new strategies for anesthetic drug assessment and neuroprotection in the pediatric population. Once developed, this non-invasive, translational biomarker may be useful as a universal indicator of brain cell death and have a significant impact on the practice and safety of pediatric anesthesia.

Hypothesis:  
PET imaging of Annexin V (a non-invasive and translatable biomarker) can be used to indicate brain cell death following pediatric anesthesia

Objectives & Methods:  
This study is designed to: 1) verify that Annexin V will be useful as a biomarker to provide information on the toxicological phenomena (apoptosis) associated with the pediatric use of anesthetic agents; 2) determine, using non-invasive microPET imaging, if a relatively high dose
or prolonged exposure to ketamine will induce long-lasting pathological changes; 3) identify, using neuroprotective compounds with known mechanisms of action, potential underlying mechanisms that could link alteration of mitochondrial function and elevation of reactive oxygen species (ROS) to ketamine-induced cell death; and 4) compare the robustness of ketamine-induced neurotoxicity between the developing rat and non-human primate.

All PET scans will be performed using a micro-PET Focus 220 animal scanner. The experimental rats and monkeys will be anesthetized and an emission scan will be acquired for 120 min in a 3D list mode with an energy window of 350-750 keV, immediately after the intravenous injection of F18-Annexin V. All list-mode data will be sorted as 3D sinograms, which will then be Fourier re-binned into two-dimensional sinograms.

**Rodent:** For the total 48 PND-7 rat pups, there will be 6 animals (both sexes combined) in each of eight treatment groups [controls (2 and 6 injections of saline at 2-h intervals); ketamine (2 and 6 injections of ketamine at 20 mg/kg at 2-h intervals); L-carnitine alone (2 and 6 injections of 300 mg/kg at 2-h interval); and ketamine plus L-carnitine (2 and 6 injections given at 2-h intervals)].

**Non-human Primate:** For a total of 12, 6-day old monkeys, two experimental groups of 6 animals each (both sexes combined) will be studied: control (saline) and ketamine treated (ketamine intravenous infusion for 24 hours at a rate of 20-50 mg/kg/hour, enough to maintain a stable anesthetic state).

**Feasibility & Time Frame:** A micro-PET Focus 220 animal scanner (Siemens Medical Solutions USA, Knoxville, TN) designed for rodents and small nonhuman primates has been installed in the Division of Neurotoxicology at NCTR. In collaboration with the University of Arkansas for Medical Sciences, the biomarker of F18-Annexin V, specific for identifying the early phases of apoptosis, has been developed and tested in the developing rodent.

Year 1: Static and dynamic imaging of developing rodents on PND-14 and at 1 and 6 months of age after exposures on PND-7.

Year 1 – 2.5: Monkeys will be scanned on PND-7 and 1, 6, 12 and 24 months of age.

**Deliverables:**
Our previous morphological and behavioral data have indicated that ketamine administration results in a dose and exposure-duration dependent increase in neuronal cell death in developing brains (perinatal), as well as long-term behavioral deficits. We expect that long-lasting pathological changes (from PND-14 to 45 in the rat; and/or from PND-7 to 24 months in the monkey) will be detected via microPET imaging of F18-Annexin V, after a prolonged exposure of the developing brain to ketamine. We also anticipate that co-administration of L-carnitine will attenuate the neurological brain injury induced by ketamine.
**Biomarkers**

**Project title:**
Evaluation of biological impact of nanoscale materials and development of biomarkers of exposure and biomarkers of impact.

**Description:**
Nanotechnology is the manipulation of atoms or material at dimensions between 1 and 100 nm (0.001 to 0.1 micrometer). The recent advances in material science, chemistry and physics that have allowed the ability to visualize matter at these dimensions (e.g. atomic force microscopy, electron microscopy) have also enabled scientists to manipulate and control of matter at these scales. The FDA has already reviewed and approved some nanotechnology based products, and expects a significant increase in the use of nanoscale materials in drugs, devices, biologics, cosmetics, and food. Understanding the biological impact of nanoscale materials will enhance the FDA’s ability to make informed science-based regulatory decisions regarding the safety of nanotechnology-based products.

NCTR is currently conducting studies to understand the toxicity and biological impact of nanoscale titanium dioxide (TiO2) and silver (Ag) in animal systems with the support of the FDA and U.S. National Toxicology Program. It is fully expected that the Office of Regulatory Affairs Arkansas Regional Laboratory (ARL-ORA) will be a focal laboratory for monitoring FDA regulated products for the presence and concentration of nanoscale materials.

The NCTR and ARL-ORA have created the Jefferson Laboratories Nanotechnology Core Facility with the purpose to support nanotechnology toxicity studies, develop the analytical tools to quantify nanomaterials in complex matrices, develop standard operating procedures for nanomaterial characterization in FDA regulated materials such as food, and support toxicological studies that will quantify the biological impact of nanoscale materials. The NCTR, ARL, and U.S. National Toxicology Program have provided the funds required for the purchase of equipment for the Nanotechnology Core Facility.

Nanoscale zinc is being used in many cosmetic products in the US and worldwide. There is a paucity of data regarding the penetration of skin or the biological impact of nanoscale zinc in biological systems. The Organization for Economic Cooperation and Development (OECD) is organizing multinational efforts to determine the risks associated with use and exposure to nanoscale materials (14 materials including zinc oxide, carbon nanotubes, fullerenes, titanium dioxide, nanosilver, etc.). The United States is a treaty member of this organization and the US delegation has asked the FDA to participate in this remarkable undertaking. All nanomaterials will be fully characterized and tested for environmental disposition and toxicity, and tested for toxicity using in vitro and in vivo methods. The US FDA has been asked to participate in determining the mammalian toxicity of nanoscale ZnO.

The benefits of participating in this OECD effort on nanoscale ZnO are: (1) take advantage of the tremendous characterization and toxicity information that will be obtained for the nanoscale ZnO test material, (2) nanoscale ZnO is present in many FDA-regulated products thereby we will be generating data that will be directly useful for FDA regulatory decisions, and (3) participating in this program will fulfill the FDA participation role in the OECD.

**Hypothesis:**
1. Nanoscale zinc will have toxicological impact on animal systems and biomarkers of toxicity can be developed for translational monitoring in humans;

2. Methods can be developed for the detection of nanoscale ZnO, TiO2 and silver in FDA regulated products including foods, food packaging and dietary supplements.
Objectives/Methods:
- Develop methodologies for the detection of nanoscale ZnO in biological matrices;
- Develop methods for the detection of nanoscale materials (ZnO, TiO2, Ag) in food and other FDA-regulated products;
- Determine the skin penetration of nanoscale ZnO in an animal model;
- Determine the toxicity of nanoscale ZnO (28- and 90-day mammalian toxicity studies);
- Identify biomarkers of toxicity of nanoscale ZnO (panomics);
- Develop enhanced bioinformatics tools for use in quantifying and interpreting panomic data from a variety of biological tissue samples.

The methods and information generated by this research program will also support indirectly the specific aims and goals of other ongoing protocols on the:
1. Determination of skin penetration and biological impact of nanoscale TiO2 and quantum dots (E2156);
2. Assessment of the distribution and effect of nanotubes on the nervous system;
3. Development of assessment tools for genotoxicity of nanoscale materials;
4. Biodistribution and toxicity of nanoscale silver;
5. Migration of nanoscale silver into foods from contact surfaces and packaging;
6. Biodistribution and toxicity of nanoscale gold.

 Deliverables:
- Quantitative data on the dermal penetration of nanoscale ZnO in mammalian systems;
- Quantitative data on the mammalian toxicity of nanoscale ZnO in support of the US contribution to the OECD efforts to understand the overall toxicity and environmental impact of nanoscale materials (ZnO);
- Quantitative data on the identity and predictivity of biomarkers of toxicity of nanoscale ZnO;
- Methodology development for detection and verification of nanoscale materials (ZnO, Ag, TiO2) in FDA-regulated products including food;
Biomarkers

Project title:
Investigation of molecular markers in the mitochondria using toxicogenomic, proteomic and metabolomic technologies to predict early events of drug-induced cardiotoxicity

Description:
The clinical use of a wide-range of therapeutic drugs, in particular, chemotherapeutic drugs, has been limited due to a risk of severe cardiotoxicity. Currently, cardiac troponin-T and/or -I are considered clinical specific biomarkers of cardiac injury. A quantitative correlation appears to exist between the levels of circulating troponins and the magnitude of myocardial cell injury. However, troponins identify cardiac toxicity only after tissue damage has occurred. To control or prevent irreversible injury to the heart tissue during drug therapy, prediction of cardiac tissue damage is crucial. Myocardial activity is dependent on the energy produced by mitochondrial function. Thus, any damage to mitochondria is likely to impose adverse effects on the function of the heart. Understanding the role of mitochondria during drug exposures is the key to the identification of early molecular events associated with drug-related cardiac failure.

Doxorubicin (DOX), a chemotherapeutic drug commonly used to treat a number of cancers causes dose-related cumulative and irreversible cardiomyopathy that may lead to congestive heart failure in patients. The anti-neoplastic action of DOX is due to its direct binding to DNA by intercalation between base pairs, thereby inhibiting DNA and RNA synthesis. It also interacts with topoisomerase II to form DNA-cleavable complexes. DOX is also a powerful iron chelator that forms an iron-DOX complex capable of binding to DNA and generating free radicals. Although iron-mediated free radicals may partly contribute to the therapeutic action of DOX by causing DNA strand breaks and membrane damage in cancer cells, these free radicals play a significant role in the toxicity of DOX. In addition, one of the key factors implicated in DOX-induced cardiotoxicity is impaired cardiac energetics, which ultimately leads to cell death by cell apoptosis or necrosis. Peroxidative damage to mitochondrial membrane lipids leading to impaired respiratory function has been suggested during DOX toxicity. Also, disruption of the electron transport system, as well as hydroxyl radical generation, in cardiac mitochondria, have been indicated in the drug-induced cardiotoxicity. High levels of mitochondrial DNA adducts resulting in altered expression of genes associated with the electron transport system have also been reported in heart mitochondria in rats treated with DOX. Altogether, this information greatly emphasizes the importance of evaluating mitochondrial activity at the molecular level in the heart during DOX exposure to develop predictive biomarkers of drug-induced cardiac toxicity. Translation of such biomarkers to the clinic would be useful in monitoring patients receiving DOX therapy so that optimal dosing may be achieved without DOX-associated cardiac injury. The correlation of organ damage with changes in peripheral blood mononuclear cells (PBMC) holds great promise in developing biomarkers in easily accessible tissue.

Hypothesis:
Understanding mitochondrial dysfunction at the molecular level in the heart during exposure to cardiotoxic agents, such as doxorubicin, will identify biomarkers of early events in drug-induced cardiotoxicity.

Objectives & Methods:
The goal of the project is to find molecular biomarkers that can be useful in preclinical, as well as clinical, studies to predict harmful effects of drugs on the heart. DOX will be used to develop a drug-induced cardiac toxicity model in B6C3F1 mice. A cumulative dose of 12-16 mg/kg DOX has been shown to cause cytoplasmic vacuolation, as well as mitochondrial and myofibrillar damage in cardiac myocytes in rats (4). Therefore, in the proposed study, animals will receive a weekly intra-peritoneal injection of 1 mg/kg DOX for 4, 8, 10, 12, and 14 weeks. Control animals will receive vehicle (saline solution). Non-invasive measurements of cardiovascular ECG variables such as QT and QA intervals, and waveform amplitudes and durations will be monitored in mice with a remote telemetry system (Data sciences, Inc.) that will detect cardiac abnormalities before acute and chronic myocardial infarctions occur. Animals will be sacrificed 3 weeks following the last injection. Blood will be collected for serum to measure levels of troponin and creatine kinase-MB, cardiac-specific isoenzyme of lactate dehydrogenase, and various metabolites, including cardiolipin, as markers of cardiac tissue injury. Gene expression analysis of mitochondria-related genes will be carried out in peripheral blood mononuclear cells. Hearts will be removed immediately and a small piece (left ventricular wall) will be stored in 10% neutral buffered formalin for light microscopy and another small piece will be stored in Karnovsky’s buffer for electron microscopy to determine DOX-related tissue damage. The rest of the heart will be flash frozen in liquid N2 for transcriptomic, proteomic, and metabolomic analyses. Transcriptomic analysis of mitochondria-related genes using mitochondria-specific gene array (MitoChip)\(^6\), protein profiling by 2D-HPLC/MS/MS, and profiling of metabolites by NMR and mass spectrometry will be carried out on the heart. Integration of the toxicogenomic, proteomic, and metabolomic data will define the molecular basis of drug-induced toxicity in the heart and lead to candidate predictive biomarkers. Correlation between the heart and PBMC data may identify biomarkers in an easily accessible tissue.

Deliverables:
- Identify candidate heart toxicity biomarkers and associated algorithms for use in preclinical drug development that will predict the probability of occurrence of heart injury.
- Integrate toxicogenomic, proteomic, and metabolomic data to define molecular basis of drug-induced toxicity in the heart and correlate -omics data generated in the heart to genomic findings obtained in peripheral blood mononuclear cells (PBMCs).
- Results will be shared with FDA product centers through seminars, manuscripts, and other means.
- Results will be published in the open peer-reviewed literature.

Biomarkers

Project title: Liver Toxicity Biomarkers Study

Description:
Drug-induced liver injury (DILI) is the primary adverse event that results in withdrawal of drugs from the market and a frequent reason for the failure of drug candidates in development. The Liver Toxicity Biomarker Study is an innovative approach to investigate DILI because it compares molecular events produced in vivo by compound pairs that a) are similar in structure and mechanism of action, b) are associated with few or no signs of liver toxicity in preclinical studies, and c) show marked differences in hepatotoxic potential. The Liver Toxicity Biomarker Study is a collaborative preclinical research effort in molecular systems toxicology between the National Center for Toxicological Research (NCTR), FDA and BG Medicine, Inc., and is supported through a CRADA by seven pharmaceutical companies and three technology providers.

Hypothesis:
Despite the absence of conventional indicators of liver toxicity in preclinical studies, there exist biochemical signals (molecular biomarkers) in liver or body fluids that can be used to distinguish between a drug candidate that has the potential to cause DILI in susceptible patients and drugs that do not have this potential.

Objectives & Methods:
The goal of this project is to create new tools, termed molecular biomarkers, that can be used in preclinical and clinical studies to predict potentially harmful effects of drugs in humans. This will be accomplished by treating rats orally for 28 days with a pair of similar drugs, one of which was discovered to cause liver toxicity only after being approved for use in humans, while the other has not shown any indications of causing liver toxicity. Urine will be collected for metabolomic analysis by NMR and mass spectrometry, blood will be collected for clinical chemistry, proteomic, metabolomic, and gene expression analyses, and liver samples will be collected for histopathology, proteomic, metabolomic, and gene expression analyses. If successful, the molecular biomarkers developed during the course of this study can then be applied during drug development to prevent the inadvertent administration of drugs that cause liver toxicity to humans.

Deliverables:
This project is being conducted in two phases. Phase I, which examined a single pair of drugs (tolcapone and entacapone), demonstrated the feasibility of the approach (i.e., that collaboration between the NCTR/FDA and BG Medicine under the CRADA worked very well and that very high quality bioanalytical datasets could be generated).

Phase II will examine four addition pairs of drugs, using the bioanalytical platforms that provided the most useful information in Phase I.

These data will be used to:
- Establish liver toxicity biomarkers and associated algorithms for use in preclinical drug development that will predict the probability of occurrence of hepatocellular injury at any subsequent phase of drug development or following approval of the drug for marketing.
- Establish an understanding of the biochemical mechanisms that underlie the liver toxicity biomarkers in order to create screening assays, enable early detection in drug discovery of potentially problematic compounds, and/or aid the discovery and development of therapeutic strategies for ameliorating drug-induced liver toxicity.
• Provide minimally-invasive liver toxicity biomarkers (i.e., biomarkers measurable in blood, plasma, or urine) that are associated with potentially injurious biochemical events in liver to enable drug development decisions to be made with minimal animal sacrifice.

• Provide minimally-invasive liver toxicity biomarkers that are associated with potentially injurious biochemical events in the liver for more sensitive and specific monitoring of liver function in subjects and patients in clinical trials.

The results will be furnished to the sponsors in the form of final reports and also published in the open peer-reviewed literature.
NCTR Priority Area:
Personalized Medicine and Nutrition

Project title:
NCTR Healthy Challenge

Description:
Health is often considered the absence of disease markers above some threshold. Omic technologies for identifying new biomarker profiles or patterns among metabolites, proteins and transcripts were applied to the resting (e.g., fasted) state. Hence, the results primarily provided measures of the robustness of homeostasis rather than predictors of disease initiation or progression. However, maintaining optimal health may differ from disease because: (i) processes involved in disease and disease progression are not necessarily the same as those involved in health optimization or disease prevention, (ii) homeostasis acts to maintain levels of many functional biomarkers within a limited range, masking early effects or predispositions under “normal” or “resting” conditions, and (iii) large inter-individual differences in “normal” values exist. Since diet, activity, psychological stress, and immune functions are known to alter physiological variables involved in disease progression, determining optimal health may be done by measuring physiological responses to acute challenges of these systems. For example, metabolic health may be determined by assessing metabolite fluctuations in body fluids or genetic repair capacity in response to challenges to homeostatic systems. Measuring glucose and other metabolites (e.g., amino acids) following an oral glucose tolerance test challenges carbohydrate homeostasis. Subsets of the measured variables are likely to provide information (i.e., biomarkers) of individual health status but also susceptibility to chronic diseases.

Hypothesis:
Acute but safe challenges to metabolic, drug responsiveness, immune, mental, and other homeostatic systems measured by a variety of omic and imaging technologies will identify biomarkers of health. A corollary hypothesis is that high dimensional datasets generated before, during, and after the challenges may identify patterns of responses that portend susceptibility to chronic diseases.

Objectives & Methods:
1. Develop an NCTR employee cohort of ~100 participants who will aid in the development of a research program designed for assessing personal health, responsiveness to an 18 month weight management program, and biomarker development for health, nutrient needs, and susceptibility to metabolic diseases. Study participants will be given the option to participate in a weight management program consisting of individually-designed diets and activity interventions. A registered dietitian and a physical trainer will be hired for initial and ongoing individual consultations. A recent survey indicated that ~80 individuals at NCTR would be willing to participate in the research and weight management program. However, we expect ~100 to join as full descriptions of the program become available. Partners: NCTR Environmental Health and Program Assurance

2. Comprehensive resting state baseline assessments will analyze physiology of each individual with standard clinical tests and measurements of immune function metabolites, serum and urine metabolites, microbiome determination, cardiovascular fitness, body composition by DEXA or CT scans (if funds are available), genetic repair capacity, gene expression analyses of lymphocyte, adipose, and muscle tissue, and oxidative stress. Obtaining resting-state data is critical for validation and qualification of novel omic biomarkers as well as for assessing cost-effectiveness of new diagnostic procedures.
3. Energy balance requires assessing calorie intake and expenditure. Lifestyle measurements will include food intake measures (such as food frequency questionnaires, food diaries, 24 hr recall methods) and physical activity questionnaires and measurements. Physical activity will be assessed by extended (up to 5 days) heart rate and other cardiovascular measures in response to aerobic and normal lifestyle activities. Heart rate variability is emerging as a key indicator of health and disease processes. Data from omic technologies (objectives 3, 6,7) will be associated with measures of lifestyle before, during, and at the end of the intervention. Partners: USDA – ARS (Little Rock); UAMS Family Medical Center. UC Davis Medical Informatics Program.

4. DNA of each individual will be analyzed on the Illumina whole genome scan platform to assess genetic ancestry and candidate gene SNPs. Some candidate genes involved in metabolic responses will be re-sequenced to ensure capture presence of minor sequence variants. These genetic analyses are designed to develop a means to associate an individual’s resting state physiology and response to homeostatic challenges with their genetic make-up. Partners: NCTR Division of Personalized Nutrition and Medicine (DPNM)

5. Homeostatic challenge tests will be conducted prior to and at regular intervals of an 18 month nutritional and physical activity intervention. Collaborators will develop homeostatic challenges consisting of the oral glucose tolerance test, lipid challenge, responsiveness to a nonprescription drug (e.g., acetaminophen), immune function (lipopolysaccharide exposure), oxidative stress and aerobic capacity (exercise), and mental acuity (through challenge tests). Each person will serve as their own control since the concept of this protocol is to evaluate individuals, not groups. Partners: CDER, NCTR’s Division of Systems Toxicology (DST); Division of Genetic Toxicology (DGT); Division of Neurotoxicology (DNT). National Center for Food Safety and Technology.

6. Omic technologies (metabolomics, proteomics, transcriptomics) will be used to analyze responses to homeostatic challenges. The key concept of this objective is that multiple parameters will be measured before, during and after the challenge tests. For example, glucose and its catabolic products are substrates for amino acid metabolism and fluctuations in amino acid concentrations during the oral glucose tolerance test (OGTT) may reveal novel metabolites for health or disease diagnosis.

7. For the OGTT and the lipid challenge, an emerging method called metabolic flux analyses will be employed to assess the response to carbohydrate and lipid (i.e., fat) challenges. Metabolic flux analysis contributes knowledge of concentrations, and the rates at which metabolites are formed. Such data provides a more complete profile of an individual’s metabolic capability and function. Partner: SidMap, Inc; NCTR DST.

8. Analyze individual microbiomes before, during, and after weight management programs. The human GI tract is predominantly a bacterial ecosystem consisting of 8 common bacterial groups and several rare groups. Recent studies have shown that the composition of gut microbiota depend on genetic selection by host – bacteria interactions, are altered by physiology (obese vs lean) and diet changes. Partner: NCTR Division of Microbiology (DM)

9. Develop novel classification algorithms for high dimensional data generated from omic and other technologies. Results of these intensive physiological and subsequent statistical analyses may be considered training sets to create “metabolic response groups.” While each individual is unique and the human population has continuous traits (think height or skin color), statistical methods are used in biomedical research to create “bins” of individuals who are similar. The path to personalized healthcare may proceed through the identification of such metabolically similar groups. Partner: NCTR DPNM

Deliverables:
1. Pilot data for identifying the subset of variables that may be developed as biomarkers for the healthy state and susceptibility to disease
2. Dimensionality reduction and classification algorithms for personalized nutrition and medicine
3. Publications.
Office of Regulatory Affairs (ORA)

Priority Area:

Rapid detection
ORA Priority Area:

Rapid Detection

Project Title:
Enhanced Preventive Analytical Capabilities

Description:
Current regulatory testing and method development is directed at known or emerging public health threats and rarely stray beyond the scope or confines of compliance programs. Enhanced preventative capabilities seek to move the ORA field laboratories (the first line regulators) beyond the scope of the current center programs while expanding the use of new and emerging analytical technologies. ORA labs are now responsible for products originating from a global marketplace. Public health responsibilities have changed and ORA labs will be expected to perform compendial "targeted" analysis in addition to analysis of products for unknown contaminants. In order for FDA to provide an accurate assessment of the threats posed by purposeful or accidental chemical contamination, effective analytical methods to detect these toxins and selected chemicals of interest in foods are critical. Analytical methods to quantify specific toxins and chemicals in foods are also needed to evaluate risk and understand the factors that control the persistence of toxins in foodstuff.

Events in recent history have included the adulteration of protein concentrates with melamine and analogs, the adulteration of pharmaceuticals using diethylene glycol, and the adulteration of natural products or dietary supplements with prescription pharmaceuticals. Global demands for product/food safety will require ORA field labs to increase their capacity and problem solving capabilities in response to emerging issues and emergency public health situations.

Hypothesis:
Enhanced analytical capabilities with increased capacity to identify previously unanticipated contaminants and enhanced investigatory analytical processes will significantly increase our ability to protect public health. Early detection techniques will facilitate our ability to develop effective intervention strategies. New analytical technologies will combat deliberate and increasing sophistication of product adulterations. Additional equipment resources will provide the ability to perform in-depth investigatory laboratory analysis and perform complex analyses with state of the art instrumentations. To support additional forensic capabilities, the development of an additional laboratory with dedicated, advanced, analytical capabilities with expert level staff with commensurate skill sets will provide increased capacity and capabilities to 1) detect unknown toxic chemicals/toxins in FDA regulated products, 2) provide specialized analytical services including method development, troubleshooting, and critical evaluation of results related to product tampering, counterfeiting, terrorism, chemical contamination and product composition profiles, and 3) rapidly apply broad-based chemical and biological screens to consumer complaint samples will allow the agency to identify emerging product issues and potential public health threats. The addition of capabilities, similar to those existing in the Forensic Chemistry Center (FCC), to develop and maintain the capability to respond immediately to incidents involving adulterated products and illegal or counterfeit products, within another field laboratory would permit the organization to address additional situations in a timely manner. Addition of personnel with a problem driven as opposed to product driven mindset and the proper equipment to an existing laboratory will produce enhanced forensic and problem solving capabilities which will allow the agency to respond more effectively to emerging public health situations.
Methods:
1. Expand the range of current program testing which will include a review of current program testing and target methods to provide comprehensive analyses in addition to addressing current compliance program needs. The comprehensive testing need not be limited to specific contaminants but provide a broad spectrum coverage of potential contaminants. The LCMS for pesticides will serve as a reference for this program testing enhancement. In collaboration with FDA Centers, the following program testing/technologies/methods will be evaluated, assessed and implemented:
   a. Elemental Analysis Upgrades - ICP/MS, XRF
   b. Poison Screen – LCMS
   c. Dietary Supplements – LC/High Resolution
   d. Microbiological Enhanced BSL-3 Labs
   e. Drug Analysis Investigatory – LCMS
   f. All Programs Investigatory - Raman/FTIR, MALDI/TOF, LC/DART/High Resolution MS, NMR
2. Product characterizations will be introduced in all programs that test manufactured products. Product characterization, especially for bulk ingredients and product formulations that are predominant in a single ingredient may be directed at gross comparisons (to a control) or highly specific product markers to provide early warning when product substitutions or other adulterations are present.
3. Enhanced preventative capabilities will include technologies that permit faster, highly specific testing to permit rapid identification and confirmation of contaminants/adulterants. The technologies identified provide rapid identification and offer complementary, highly specific analysis to facilitate confirmation of identity. The testing conducted with these technologies may be as a follow up to the testing described in 1 and 2 above. They are generally not for routine analyses but this does not mean the use of these technologies is limited or used infrequently. The technologies can be applied to the investigation of unknown analytical responses detected through any analysis in any program (see #1)
4. In collaboration with FDA centers, ORA Science will establish challenge initiatives for preventive capabilities and prioritize activities through ORA’s Method Development and Validation Program (MDVP). The challenge initiatives and MDVP will be used to identify and support the developmental activities required to establish the enhanced preventative capabilities described in 1, 2, and 3.
5. Establish a satellite component within an existing ORA laboratory. FCC would be involved in the structure of this operation.

Staffing should include a sufficient number of personnel with an interest in forensics and problem solving to provide for good exchange of information and intellectual synergism. At a minimum the staff should include the following:
   • Supervisors/team leaders (1-2). These are operational as well as supervisory positions at an advanced technical level.
   • Analytical chemists (8-12, including several at an advanced technical level). Expertise would be required in the following areas: mass spectrometry, gas and liquid chromatography, vibrational spectroscopy, microscopy, nuclear magnetic spectroscopy, elemental analysis, etc.

Required equipment should be recommended in collaboration with FCC, reflect that used within the FCC, and allow the laboratory to address the full spectrum of potentially harmful unknown materials.

Deliverables:
   • Enhanced preventive capabilities in major program areas including metals, dietary supplements, pesticides, drug analyses, poison screening, and select agent detection. State-of-the art equipment/technologies in support of preventive capabilities for ORA field laboratories.
• Development of new methodologies/technologies, analytical approaches/algorithms in collaboration with FDA centers.
• The complete integration of food defense examinations with food safety assessments to fully leverage the enhanced capabilities in ORA field laboratories.
• Enhanced forensic and problem solving capabilities which allow the agency to respond more effectively to emerging public health situations associated with FDA regulated products.
Rapid Detection

Project Title:
ORA Science Leveraging/Collaborations

Description:
Our traditional approach to food safety and our concepts of potential contaminants are changing with the globalization of our food supply. Our food ingredients and finished products frequently are produced in countries with vastly different food safety regulatory structures and oversight. Our food or food ingredients may be produced in facilities where basic sanitation and process control systems lack the necessary safe guards that we would expect in this country. Substances or ingredients may be used in production of food that may not be acceptable in this country and there is increased potential for contamination with emerging pathogens or terrorist contamination. As a National Laboratory Resource, ORA field laboratories will need to be equipped with new technologies/equipment, rapid screening and confirmatory methodologies for targeted and comprehensive food testing. It is imperative that FDA leverage with federal and state regulatory counterparts in an integrated food safety/defense network with advanced analytical tools and methodologies.

Events such as the adulteration of human foods (including infant formula) with melamine have highlighted the importance of maintaining a state-of-the art emergency response network that can quickly identify and reliably quantify unknown contaminants in food in a timely manner. Partners in food protection are a critical element in a national food safety strategy. For example, the initial core of chemistry and radiological cooperative agreement program (CAP) labs were a significant complement to the Food Emergency Response Network (FERN) which when combined with FDA resources becomes a major national public health asset.

Their participation in food defense assignments and national food surveillance programs provide major enhancements in our overall food safety analytical capacities. However, their member numbers and sampling capacity is insufficient to support the needs of the present and future needs of the FERN and the Agency and highlights the need to expand the FERN CAP initiative for chemistry and radiology.

Hypothesis:
Collaboration with internal and external organizations will enhance the overall science base of the agency/ora and provide opportunities to develop quality science for targeted agency needs through leveraging our individual contributions. Specifically, partnerships/collaborations with internal (Centers) and external organizations (Lawrence Livermore National Laboratory), academia, and Centers of Excellence (i.e. UC Davis) will enhance science at ORA. Collaboration on methods development, the assessment and implementation of new technologies/equipment will support mobile laboratory functionality and the Analytical Tool Initiative (ATI). State-of-the art equipment such as LC/MS, GC/MS, ICP/MS, NMR, Raman/FTIR Spectrometers, and MALDI-TOF/MS, coupled with the expertise to support these systems, will tremendously increase the versatility of the chemistry laboratories and provide them with the tools necessary to rapidly identify unknown contaminants. Additionally, laboratories will be better equipped to develop and test protocols to quantify contaminants as part of emergency and/or surveillance assignment. Likewise, equipping radiological laboratories with sensitive instrumentation e.g. - and -spectrometers and liquid scintillation counters would give them the capability to swiftly identify harmful radiation in food/water samples. The addition of personnel with expertise in detection of specific radionuclides e.g. strontium and radon would enhance methods development to address emergencies involving unique radionuclides. Collectively, state laboratories will provide surge capacity in support of local/regional and national emergencies/outbreaks.
Methods:

- Further support the Analytical Tools Initiative (ATI) which has engaged subject matter experts (SME's) to assist in the development, review and implementation of new technologies/methods for use by ORA’s analytical and investigatory field force. The SME's represent CVM, CFSAN, CDER, CDRH and NCTR and directly utilize ORA's Method Development and Validation Program to facilitate implementation for field use.
- Continue the partnership between the Forensic Science Center and Lawrence Livermore National Laboratory by providing funding for a postdoctoral fellow to specifically develop methodology for the detection and quantification of chemical warfare agents and their degradation products in foods. Leverage mobile laboratory capabilities for detecting select agents and identification of unknowns.
- Establish collaborations with the Western Institute for Food Safety and Security and UC Davis to leverage their expertise in adapting and expanding multiplex assays for biological agents and method development for detection of chemical unknowns in foods and other matrices.
- Re-establish ORA/WEAC leveraging/collaborations with MIT and Harvard previously under the critical path initiative, specifically focusing on medical devices – failure, reliability and robustness.
- Establish support for two post-doctoral assistants, in conjunction with the University of Massachusetts Lowell working at WEAC on methods development for fast screening radionuclide protocols and University of New Hampshire working on FERN BSL-3 method development projects which include the isolation of Yersinia pestis and Francisella tularensis from food matrices.
- Leverage with NCTR Nanotechnology Core Facility by combining efforts of the research at NCTR with the ORA field application for determining the identity and amount of nano materials in FDA regulated products.
- Expansion of FERN Chemistry, Radiological, and Microbiological Cooperative Agreements
  - Selected laboratories will be outfitted with the proposed instruments – where there is a choice for the type of equipment, laboratories will make their own selection. FERN National Program Office (NPO) will make sure that the laboratories select different instruments to diversify FERN network analysis capability.
  - Selected labs, in coordination with the FERN NPO will recruit expert staff.
  - FERN NPO will work with CAP labs to implement any necessary IT upgrades.

Deliverables

1. Analytical Tool Initiative
   - Identification, assessment and implementation of new technologies/methods in support of mobile laboratory capabilities, food defense/food safety activities
   - Enhanced collaboration with internal (FDA) and external (industry, academia) stakeholders
   - Increased ability to screen FDA regulated products at ports/borders of interest
   - Directly supports Food Protection Plan, Import Safety Action Plan
2. Establishment of external networks to support and provide critical expertise, collaborations in support of mission critical objectives.
3. Expansion of agency science base with increased analytical capabilities.
4. FERN Cooperative Agreements
   - 3 new CAP chemistry labs equipped with 2 interfaced mass spectrometers (choice of LC/MS, GC/MS, ICP/MS, MALDI-TOF/MS), NMR, Raman and FTIR spectrometers.
   - 3 new radiological CAP labs equipped with alpha- and beta-spectrometers and liquid scintillation counters. The addition of 3 chemistry and 3 radiological CAP laboratories
to the existing FERN program would increase the chemistry testing capacity by 150 samples/week and the radiological testing capacity by 100 samples/week.

- Expert staff in each laboratory with the collective expertise covering a diverse area of small-molecule detection and radionuclide detection.
- Methodologies developed by the laboratories for specific detection systems
- Implementation of effective laboratory-information-management (LIM) among all the FERN laboratories for fast, secure, electronic, real-time data sharing.
- Establishment of QC/instrument maintenance staff and program at each laboratory to ensure instrument calibration and proper maintenance.