



FVM Research Impact: Advancing Public Health through Regulatory Science

The **FVM Research Impact** Workgroup



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Vision

Protect consumers and promote the public health by safeguarding America's food supply and enabling consumers to choose healthy diets.

Mission

Secure high rates of compliance with science-based food safety and labeling standards by implementing integrated, prevention-oriented and risk-based programs to: Protect the safety and security of foods for humans and animals, Regulate the safety and effectiveness of animal drugs, and Ensure that food labels contain useful and reliable information.

"There has been heavy dependence on journal metrics [in the research community]. However, [journal] metrics show the reach of the research in terms of how widely it is disseminated and the uptake. But, journal metrics do not characterize the influence created, such as resulting actions or changes or the manner in which the research knowledge is used."

- Centers for Disease Control and Prevention

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FVM Research Impact Framework

If the Foods and Veterinary Medicine (FVM) research program is to contribute to FDA's public health mission, it is critical to understand how research leads to mission-relevant outcomes and public health improvements within a regulatory context.

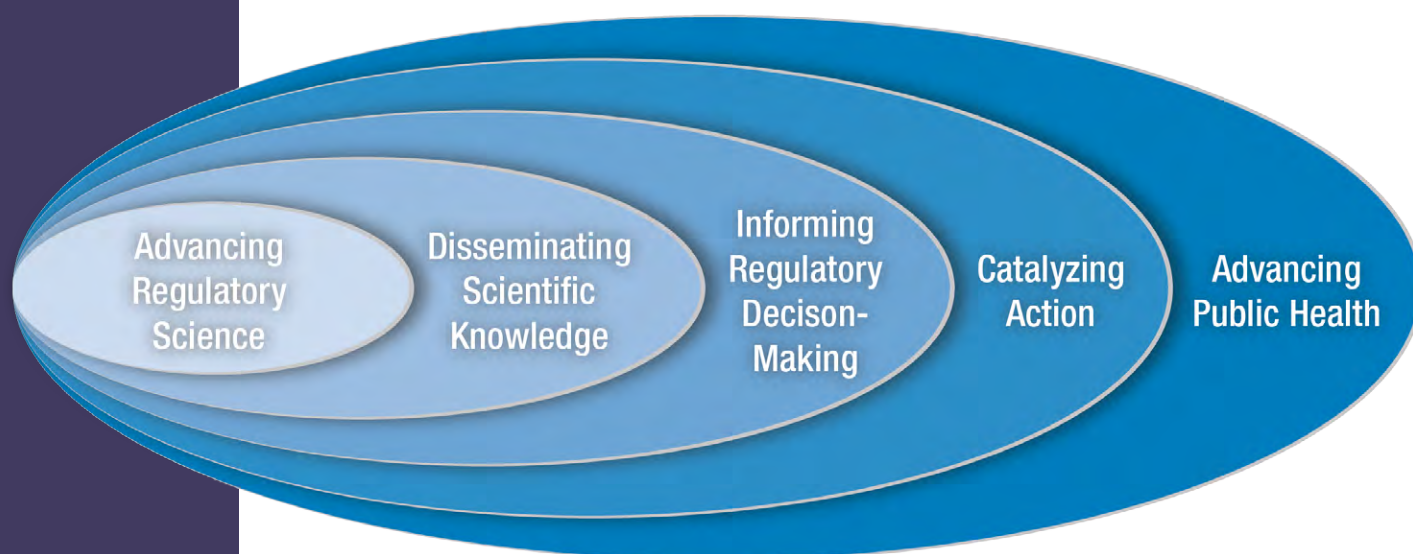
Specifically, there is a need to:

- Ensure that FVM research projects address critical public health issues,
- Ensure that the FVM's research investment yields the maximum return in relation to research priorities, and
- Ensure that the breadth and depth of FVM research progresses towards fulfilling Center-level and Agency missions.

As envisioned in our new impact paradigm, the continuous process by which past, present, and future FVM research is considered will provide a means for illustrating downstream relevance of prior efforts and for guiding the efficient utilization of FVM research in future activities.

To enable meaningful characterization of the breadth of research impact and to identify facilitators of impact, the **FVM Research Impact** Workgroup proposes a general framework. The framework is based on literature reviews, collaboration with other agencies and institutions, internal discussions, and case study analyses.

The Research Impact Workgroup is iteratively developing this framework to affirm that it is comprehensive and relevant to the research conducted within the FVM program. There are five general domains represented by the nested ovals. Within each domain are 3-8 indicators. The indicators are examples of impact: it is important to note that not all indicators are relevant to all research initiatives. The impact of research can look very different across the FVM research program due to the variety of needs and priorities within a regulatory setting. Furthermore, impact does not necessarily unfold in a linear or chronological manner. The framework is flexible enough to account for the range of time it might take for the impact of a study to be realized.



Within each domain are a variety of potential indicators of impact.

FVM Research Impact Framework

Preliminary Indicators of Research Impact

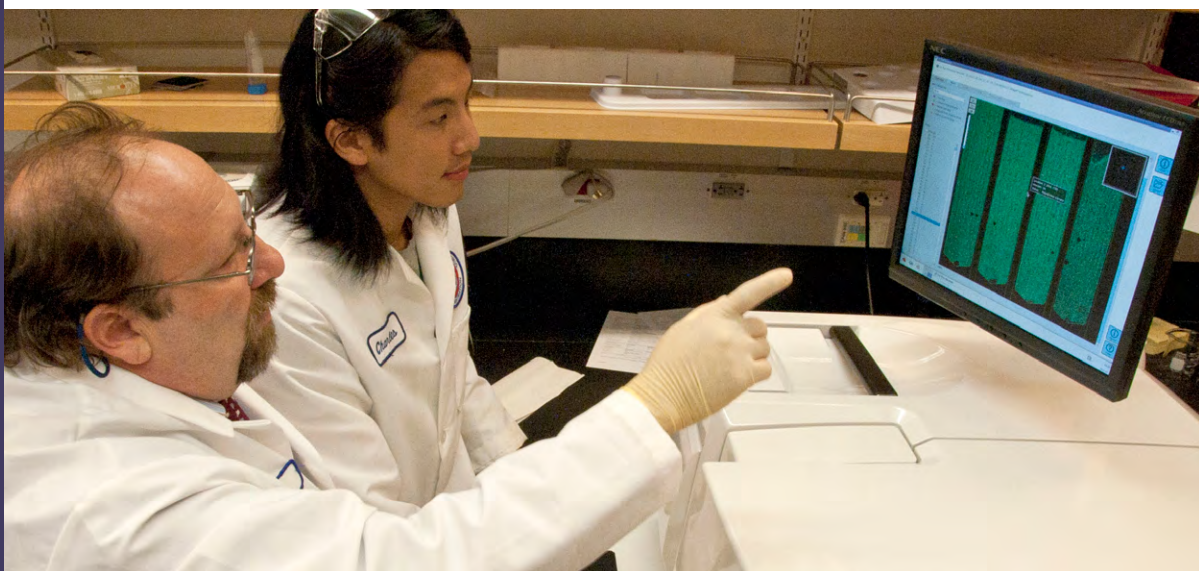
Advancing Regulatory Science	• Alignment with defined knowledge gap • Alignment with regulatory priorities • Adoption for the use in the field
Disseminating Scientific Knowledge	• Scientific publications • Impact factor of journals • Citations in scientific publications • Citations in grey literature • Presentations at trade meetings • Presentations at academic conferences • Incorporation into training or educational curriculum • Media coverage
Informing Regulatory Decision-making	• Development or change in surveillance strategies • Development or change in guidelines or regulations • Development or change in compliance and enforcement strategies • Development or change in inspection and sampling strategies • Development or change in external communication strategies • Policy decisions
Catalyzing Action	• Adoption and adaptation of research findings by government • Adoption and adaptation of research findings by industry • Technology transfer from FDA to stakeholder groups • Use of research findings in advocacy initiatives • Subject of professional society meeting • Creating or reorienting partnerships • Removal of hazardous commodity from the marketplace
Advancing Public Health	• Reduction in exposure time to hazardous commodities • Reduced frequency and severity of outbreak events • Improvements in consumer understanding of FVM-regulated products • Reduced economic burden of illness attributable to FVM-regulated products • Reduced morbidity and mortality attributable to FVM-regulated products

FVM research is conducted by a productive and talented workforce of scientists across multiple Centers and Offices tackling diverse and challenging scientific issues. The impact of FVM research can be viewed through many different lenses with no one definition of impact conferring more value than another: the impact of a research project will be initially defined by its original goal and intention. This issue features four pilot case studies identified by the Research Impact Workgroup to highlight FVM research and to illustrate a breadth of regulatory research impact.

Whole Genome Sequencing and Foodborne Outbreaks

Over the past 10 years, advances in Next Generation Sequencing technology have reduced many of the hurdles required to perform such types of analysis on a routine basis. Current benchtop sequencers now allow standard microbiological laboratories to sequence entire bacterial genomes more easily, in a shorter amount of time and at a fraction of the cost. The Whole Genome Sequencing (WGS) program at FVM has embraced this technology and deployed it through its Genome Trakr Network: a network of public health and university sequencing laboratories. WGS “reveals the complete DNA make-up of an organism, enabling us to better understand variations both within and between species. This in turn allows us to differentiate between organisms with a precision that other technologies do not allow.” (FDA WGS Website) Drs. Marc Allard, Eric Brown, and Errol Strain are the lead investigators of the WGS program at the Center for Food Safety and Applied Nutrition (CFSAN), and are instrumental in the use of WGS technology to track foodborne outbreaks.

WGS has been a revolutionary resource for solving foodborne illness outbreaks with few clinical cases, and continues to be developed for use in prevention, control, and regulation. The WGS team has partnered with several entities to develop a streamlined genomic workflow. Within the FDA, these partners include Center for Veterinary Medicine (CVM) and Office of Regulatory Affairs (ORA). Outside the FDA, these partners include Centers for Disease Control and Prevention (CDC), United States Department of Agriculture (USDA), National Institutes of Health (NIH), and GMI (Global Microbial Identifier). See the table for how the WGS program has successfully demonstrated impact across all impact domains.



Indicators of Research Impact from the Whole Genome Sequencing case study

Advancing Regulatory Science	- In 2008, FDA began using genetic data from WGS to support internal research efforts. - In 2010, at CORE's request, CFSAN researchers used WGS to help identify the cause of a <i>Salmonella</i> outbreak that prior technology could not resolve. - In the current study, WGS applications were expanded from infectious disease research to applications in food safety and foodborne outbreaks. - WGS linked pathogens from foods to clinical isolates from patients to identify the specific organism causing the illness.
Disseminating Scientific Knowledge	- The CFSAN Communications team built a webpage dedicated to the Whole Genome Sequencing Program. - The CFSAN WGS team presented to at least 16 academic and trade conferences since Sept. 2013 and continues to participate in discussions on the value of WGS in food safety. - The CFSAN WGS team met with several internal (e.g. Compliance, CORE) and external "(e.g. National Center for Biotechnology Information (NCBI), CDC, trade groups) stakeholders.
Informing Regulatory Decision-making	- The detailed genetic data from WGS led FDA to stop using older methods which were often uninformative or ambiguous in many ways. - To continue these advances, Center management increased the resource allocation and leadership support for WGS. - In the "Index" case of 2014, FDA used WGS to inspect a food processing facility for microbial contamination and made a link between a <i>Salmonella</i> drain isolate from the facility and only a few illnesses scattered around the country.
Catalyzing Action	- A growing network of state, federal, academic, and international laboratories use and contribute to the WGS data network "GenomeTrakr." - The American Society for Microbiology is hosting the 1st Conference on Rapid Next Generation Sequencing and Bioinformatic Pipelines in 2015. - There is a growing awareness in industry that FDA's use of WGS has raised the bar for facility cleanliness.
Advancing Public Health	- The 2014 Index case led to a large national recall, voluntary shut down, infrastructure rebuild and clean-up of facility. - WGS enables faster, more specific, and geographically targeted identification of source contamination, which allows for earlier intervention and reduced illness. - WGS reduces the number of illnesses required to identify a potential foodborne illness outbreak, because intervention occurs in front of the outbreak curve.

Identifying the Cause of Pine Mouth Syndrome

Imagine that one day, you begin to experience a bitter, metallic taste in your mouth. Your tongue feels odd and it is distracting and uncomfortable. Every time you eat or drink something, the feeling is worse. You have never experienced food allergies, are a non-smoker, and have not had recent trauma or sinus problems. What could be causing such symptoms?

You may have just experienced a case of dysgeusia after consuming pine nuts. The FDA received an increase of complaints from consumers who reported dysgeusia in 2009, and received 501 complaints from July 2008 to June 2012. At the time, “pine mouth syndrome” (the colloquial term for pine-nut associated dysgeusia) was recognized in the medical field, but little was known about the cause of the condition. FDA surveys and analysis of the complaint reports revealed no obvious demographic, social, or medical factors associated with the complaints. Fifteen consumer complaint samples (samples ranged from 50-150 seeds) were brought to the FDA and measured for pesticides, lipid oxidation, and fatty acid profiles. However, this conventional screening did not yield a cause. To address this knowledge gap, Dr. Sara Handy’s study aimed to determine the cause of pine mouth using a genetic method that identified pine nuts to the species level, primarily using the *ycf1* chloroplast gene.



Dr. Sara Handy pours pine nuts into a container for examination. From the FDA Flickr.

Twenty-seven authenticated species standards from the genus *Pinus* were used as a comparison group for the complaint samples. A small segment of seed was added to a microcentrifuge tube, and DNA was extracted using a DNeasy Blood & Tissue kit. The DNA was amplified using PCR, then purified and sequenced on an ABI 3730 instrument. The sequences were further edited, and there was post-processing of both authenticated specimens and complaint samples.

The study determined *P. armandii*, a species of pine nut that is considered “not fit for food,” was present in all 15 consumer complaint samples: some samples were purely *P. armandii*, and some were mixed with other pine nut species. This was the first application of DNA-sequencing for pine speciation, and Dr. Handy and her study team developed and published a detection method which could help prevent future incidences of pine mouth.

“To support its public health mission and minimize economic fraud, the FDA must verify the accurate labeling of products under its purview. This is difficult when the products are mixtures of different plant or animal species, such as the mixtures of edible and non-edible pine nuts... The FDA will develop next generation DNA sequencing and targeted PCR methods to identify plant species or plant mixtures in food products. These tools will give FDA the ability to characterize complex plant-based materials in trace back and complaint investigations, as well as better enforce labeling and other regulations.”

– Dr. Sara Handy, CARTS #IF01086



Dr. Sara Handy grinds up pine nuts for DNA analysis. From the FDA Flickr.

P. armandii is native to China and Taiwan, so the FDA communicated the findings of this study to Chinese officials via the FDA Office in China. They further communicated the findings to liaisons to the food producers and FDA Compliance and field staff. In 2011, the FDA issued a Safety Alert and Advisory that explained pine mouth syndrome and its association with pine nut consumption.

The number of consumer complaints for pine mouth syndrome decreased following the completion of this study. Further, subsequent studies were initiated to investigate the use of genetic identification of regulated plant and dietary supplements.

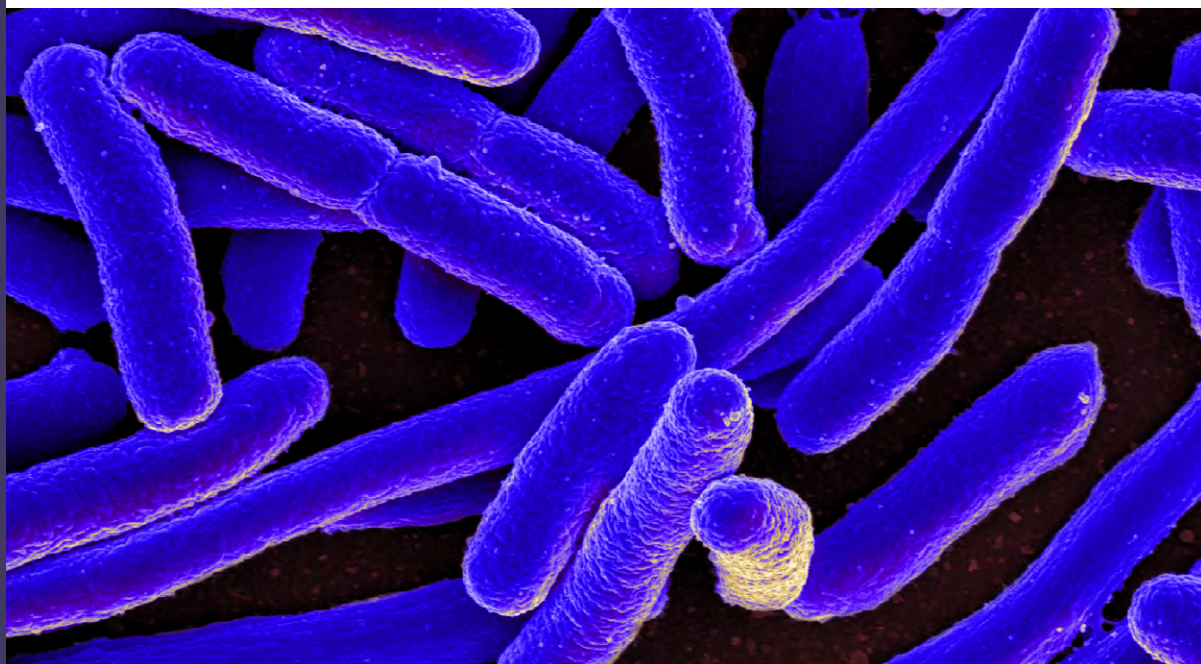
Molecular Serotyping of non-O157 Shiga toxin-producing *E. coli* (STEC)

The FDA is charged with protecting public health by ensuring the safety of the nation's food supply. To do so, the FDA needs laboratory methods to quickly identify pathogens that are likely to cause human illness and discriminate from those that do not pose a health threat. Before Dr. Andrew Lin's study, FDA labs tested for *E. coli* O157:H7, the most commonly identified STEC associated with illness in the United States. However, if there was a negative finding for *E. coli* O157:H7, it was then optional to test for other STECs. This subsequent testing was manual and time-consuming. Since it is estimated that non-O157 STECs are responsible for over 60% of STEC infections or an estimated 112,000 illnesses in the U.S. each year, it was critical to develop a way to quickly identify multiple STECs at the same time.

Dr. Andrew Lin of ORA recognized this knowledge gap and developed a method to detect and identify the 11 most clinically relevant O-serogroups in a single reaction. In collaboration with Dr. Julie Kase at CFSAN, this bead-based suspension array assay was validated in a multi-laboratory study and approved by the Bacteriological Analytical Manual (BAM) Council in 2013. It is now required, not optional, to test for non-O157 STECs.

To facilitate implementation for regulatory use, a Food Emergency Response Network (FERN) web-based lecture was conducted in 2014 and a CFSAN serotyping workshop was held in 2012. To further disseminate the research and method beyond FDA, a team of scientists headed by Dr. Lin and Dr. Kase published a number of peer-reviewed articles and presented at various conferences such as the International Association for Food Protection (IAFP) General Meeting. Further, industry, specifically Luminex, is now making the test kit commercially available for use by state and industry stakeholders.

While it is still too early to determine the full extent of this new technology in the regulatory lab, the ultimate goal is for Dr. Lin's method to contribute to the reduced prevalence of non-O157 STEC infections in the United States.



Colorized scanning electron micrograph of *Escherichia coli*, grown in culture and adhered to a cover slip. From the NIAID Flickr, part of the National Institutes of Health.

DNA Barcoding and Seafood Identification

Dr. Deeds was hired by FDA in 2003 to help research the cause of several foodborne illness outbreaks that were linked to a domestic puffer fish species. In 2005-2006, Dr. Deeds was contacted by the Office of Import Operations in Los Angeles requesting assistance in identifying what was believed to be illegally imported Asian puffer fish (a.k.a fugu or blowfish products). The FDA had the Regulatory Fish Encyclopedia (RFE) as a reference, which used the only validated method for fish species identification then available, protein profiling (IEF), but no FDA lab was still running this method. Dr. Deeds contacted fish taxonomists at the Smithsonian Institution for assistance but the products were processed and visual species identification was not possible. At this time, Dr. Deeds was first introduced to Dr. Haile Yancy from the CVM who was working with collaborators at the University of Guelph in Ontario, Canada on a new DNA-based species identification technique called DNA Barcoding. Dr. Yancy had recently sent tissue samples from the RFE to the University of Guelph, first showing that DNA Barcoding could be a viable alternative to IEF for seafood product identification. Working with Dr. Yancy and his collaborators in Canada, these suspect products were confirmed using DNA Barcoding as puffer fish, in violation of FDA import restrictions, and these firms were placed on Import Alert.



A small sampling of the fish in the world's largest collection. An FDA project aimed at preventing mislabeling of fish starts in FDA labs, where individual species are identified and a bar code is created. From the FDA Flickr.

In 2007, the Chicago Department of Public Health reached out to the FDA for assistance in the analysis of homemade soup believed to contain toxic pufferfish. DNA Barcoding was used to determine that the product was in fact *Lagocephalus lunaris*, one of the only species of puffer fish in the world with high concentrations of toxin naturally in the meat. Using DNA testing, bits of fish from the implicated soup were further linked to a large shipment of product that was responsible for several restaurant-related illnesses in California and New Jersey. Since this new tool had proven useful both in an illness outbreak investigation and a case of intentional product misbranding to avoid an import restriction, it was decided to standardize, validate, and implement this method for the species identification of seafood products across the agency. The definitive gap in the field for seafood identification to prevent species substitution and potential illness helped facilitate the outcomes and impacts of this research.



*"Public health impact of research means everything to me."
– Dr. Jonathan Deeds, CFSAN*

Along with a diverse and collaborative team, including representatives from Office of Food Safety (OFS), Office of the Commissioner (OC), and ORA, and external collaborators, the Project Fish SCALE team has worked to compile the protocols and DNA barcoding data into a public online database to facilitate uptake of the methodology outside of the FDA. The team has provided protocols and training to its counterparts in seafood regulation at National Oceanic and Atmospheric Administration (NOAA), USDA, and U.S. Customs and Border Protection (CBP). In order to help industry and state regulators ensure proper seafood labeling, the FDA developed a series of training modules that are also accessible on the FDA website. The FDA posted a Consumer Update about seafood identification and substitution in 2014, with specific reference to the methodology and process FDA uses now as a result of this work. Further, the DNA Barcoding work has informed updates to FDA guidance document(s).

Partnerships and Collaborations

- Center for Veterinary Medicine, FDA
- Office of Regulatory Affairs, FDA
- Smithsonian Institution National Museum of Natural History, USA
- Food Safety Inspection Service, USDA
- National Seafood Inspection Laboratory, NOAA
- Florida Department of Agriculture
- U.S. Customs and Border Protection
- University of Guelph, Ontario, Canada
- University of Louisiana at Lafayette, USA

Where to go for more information?

If you would like to see where we got some of our information:

BAM Online, <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm114664.htm>

Banzi, R., Moja, L., Pistotti, V., Facchini, A., & Liberati, A. (2011). Conceptual frameworks and empirical approaches used to assess the impact of health research: an overview of reviews. *Health Res Policy Syst*, 9(1), 26.

British Dental Journal 210, 151 (2011) <http://www.nature.com/bdj/journal/v210/n4/full/sj.bdj.2011.102.html#B1>

Centers for Disease Control and Prevention, E.coli: <http://www.cdc.gov/ecoli/general/>

Centers for Disease Control and Prevention, Science Impact Project.
<http://www.cdc.gov/od/science/impact/>

Food and Drug Administration, DNA-based Seafood Identification.

<http://www.fda.gov/Food/FoodScienceResearch/DNASeafoodIdentification/default.htm>

Food and Drug Administration, Whole Genome Sequencing (WGS) Program.

<http://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/>

Fardin-Kia, A. R., Handy, S. M., & Rader, J. I. (2012). Characterization of pine nuts in the US market, including those associated with “pine mouth”, by GC-FID. *Journal of agricultural and food chemistry*, 60(10), 2701-2711.

Handy, SM, Deeds, JR, Ivanova, NV, Hebert, PDN, Hanner, R., Ormos, A, Weigt, L.A., Moore, M.M., Yancy, H.F. (2011). A single laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. *J. AOAC* 94(1): 201-210.

For more information on the methods, check out these publications:

Handy, S. M., Parks, M. B., Deeds, J. R., Liston, A., De Jager, L. S., Luccioli, S., ... & Diachenko, G. W. (2011). Use of the chloroplast gene *ycf1* for the genetic differentiation of pine nuts obtained from consumers experiencing dysgeusia. *Journal of agricultural and food chemistry*, 59(20), 10995-11002.

Handy, S. M., Timme, R. E., Jacob, S. M., & Deeds, J. R. (2013). Development of a locked nucleic acid real-time polymerase chain reaction assay for the detection of *Pinus armandii* in mixed species pine nut samples associated with dysgeusia. *Journal of agricultural and food chemistry*, 61(5), 1060-1066.

Kwegyir-Afful, E.; DeJager, L.; Handy, S. M.; Wong, J.; Begley, T.; Luccioli, S. An investigational report into the incidence and causes of pine mouth syndrome events in U.S. consumers. Unpublished work.

Munk, M. D. (2010). “Pine mouth” syndrome: cacogeusia following ingestion of pine nuts (genus: *Pinus*). An emerging problem?. *Journal of Medical Toxicology*, 6(2), 158-159.

STEC Molecular Serotyping Protocol: <http://www.fda.gov/downloads/Food/FoodScienceResearch/LaboratoryMethods/UCM360921.pdf>

Want to chat with the PI's highlighted in the case studies?

Whole Genome Sequencing: Marc Allard, Eric Brown, Errol Strain (Marc.Allard@fda.hhs.gov, Eric.Brown@fda.hhs.gov, Errol.Strain@fda.hhs.gov)

Seafood and DNA Barcoding: Jonathan Deeds (Jonathan.Deeds@fda.hhs.gov)

Pine Mouth: Sara Handy (Sara.Handy@fda.hhs.gov)

STEC Serotyping: Andrew Lin (Andrew.Lin@fda.hhs.gov)

For questions or pithy discussion with the research impact workgroup, please contact:

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FVM Research Impact Intranet Site: <http://inside.fda.gov:9003/OC/OfficeofFoods/ucm463490.htm>

