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TENNESSEE STATE UNIVERSITY

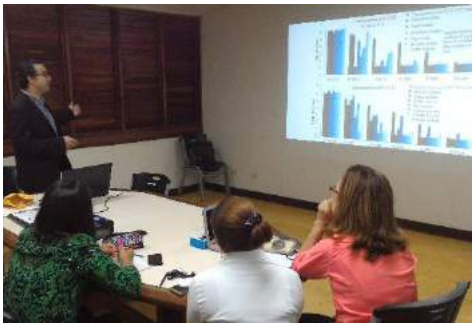
***Food Microbiology Outreach and Technical Assistance in Dominican Republic***

# Trip Report

*Aliyar Fouladkhah, PhD, MPH, CFS*

*Public Health Microbiology Research and Outreach Laboratory*

*Tennessee State University, Nashville, TN*



**October 12, 2017**

# Content

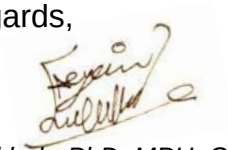
## (1). Executive Summary:

It was a great pleasure for me to serve as a Food Microbiologist volunteer for the USAID/F2F Program in the Dominican Republic. Am pleased by the progress and capacity building endeavors achieved during the two week assignment, and commend the enthusiasm, wiliness to absorb new curricula, and professionalism of the faculty and staff in ISA University and USAID/Partners of the Americas staff in Santo Domingo, DR.

Thanks to the progress made by the previous volunteers, enthusiasm of the faculty, and progress made during current assignment, I believe the ISA University now has enhanced capability to conduct microbiological analyses directly to assist stakeholders meeting regional and international standards as well as to conduct culture-dependent inoculation studies. I think the major curtailment of the program is availability of funding to invest in new infrastructure, equipment, supplies, and consumables. Availability of new and functional autoclaves, pipettes, biosafety cabinets, and culture-dependent media, just to name a few, could enhanced capability of program to great extent. Specific improvement in existing practices and operations could also assure enhanced success of ISA faculty to continue their critical mission, in assisting stakeholders, training future food microbiologists, and assuring safety of the island by reducing public health burden associated with consumption of raw agricultural commodities.

Am delineating progress and recommendations in further details in this trip report and recommendation form to the agency with the hope of further capacity building for the institution and to assure passing the baton to the next volunteer is “smooth sailing.” Once again, I thank the program administrators both in Santa Domingo and Washington, as well as colleagues in ISA University for their enthusiasm and hospitality.

Sincere regards,



*Aliyar Fouladkhah, PhD, MPH, CFS*

*Assistant Professor, Tennessee State University*

*Yale School of Public Health Alumnus*

## (2). Activities:

### (2).I. Activities on 8-14-2017:

Formal preparation for the trip began. Had a skype meeting with a USAID/Partners of America field officer (JA) from the capital of Domenica Republic (DR), Santo Domingo (Santo Domingo de Guzmán) and a faculty member (SCVB) from Universidad ISA from Santiago (Santiago de los Caballeros). During the meeting revised the drafted agenda (***annex 2***) based on the needs of stakeholders and ISA faculty and discussed the logistics and travel plans. The main request from the university was to develop training events that are a blend of lectures and hands-on activity. Development of training sessions on microbial food safety, inoculation studies, applied statistics for analyses of research data, sensory analysis, food safety modernization act, and microbiological methods from the US regulatory agencies were among the topics that were requested by the field officer and the faculty member based on the input from their institutions. This cornerstone meeting was a critical stage for development of the plan for the meeting, would strongly recommend similar endeavors to future volunteer to assure mutual understanding of the travel logistics and teaching curricula.

***Photo courtesy:*** *José Almodóvar, USAID Field Officers, Santa Domingo, DR (left) Sahira Vásquez Lecturer at ISA University, Santiago, DR (right).*

### (2).II. Activities on 8-20-2017:

Arrival to Santo Domingo. Thanks to the pre-arrangement during the above-mentioned meeting, it was relatively easy to locate a local volunteer who assisted in commuting to a local hotel. There are also rental cars and facilities in airport neighborhood so if the arrangement with a local volunteer is difficult for the future volunteer, rental car would be a feasible option as well since most companies in DR accept payment methods from the United States.

***Photo courtesy:*** *Department of Food Science building, ISA University, Santiago, DR.*

## (2).III. Activities on 8-21-2017:

Meeting with USAID/Partners of the Americas/Farmer-to-farmer program director and staff in Santo Domingo (Fundacion Quisqueya Por Las Americas, Inc.). The program director shared overview of the ongoing efforts in DR, delineating their institution is currently active in over 10 locations in the country and hosting several volunteers from US institutions per year for advancing their mission. I had also discussed briefly overview of our program in Nashville, and the two week curricula we had developed for ISA University faculty. Had a chance then to talk with field officers/technical translators of the program and share some overview of activities with them. There were also great discussions on the main industries of the island, recently overcome challenges such as fruit fly outbreak, and overall geography of the island and existing agricultural industries.


***Photo courtesy:*** *USAID, Partners of Americas, Farmers-to-farmers office, Santo Domingo, DR.*

## (2).IV. Activities on 8-22-2017:

Arrival to ISA University in Santiago. The drive from the capital (Santo Domingo) to Santiago was about 1.5 hours, on its own gave a snapshot of the agricultural industries in the island since most of the roads are passing across agricultural regions with many road-side vendors of raw agricultural commodities. Santiago is the second largest city in DR, the fourth largest city in the Caribbean, located in the north central region of the country, and home to several universities, including ISA University (Universidad ISA), the host institution of the program.


***Photo courtesy:*** *Entrance of ISA University, Santo Domingo, DR.*

Upon arrival met with department head of the food science program that currently is home to 8 lecturers/research advisers and approximately 275 students, around 10% of the population of the ISA University students. Timing of the program was commendable

since it was scheduled during the last two weeks prior to start of the semester were most faculty and research technicians were present on campus, in preparation for the new semester that enabled some to attend our training sessions. After the meeting with

department administrator had a chance to visit existing meat processing plant, poultry primary processing facility, fruit and vegetable processing area, and dairy/cheese plant of the department and meet some of the faculty/technician of each program. Requests were made to additionally discuss information on meat-borne pathogens and meat decontamination interventions as well as best practices and food safety management systems for meat processing.

Recommendation(s):

-Ultimately the purpose of a written proposal and project with USAID is to induce a lasting change in the home institutions, particularly those considered as emerging democracies and international allies of the United States. While the proposal was well-written in a critical area and arrangements were done well to meet the needs of the home institution in DR, more could be achieved if the budget of the proposal were more balanced. Particular a two week period could be shortened to perhaps one week or 10 days, and the saving from such reduction in period of the program could be awarded to host institution prior to event so they could purchase some consumables and material based on the input from the volunteer to be able to conduct the hands-on activities more efficiently. I would suggest allocating at least few hundred dollars of funding, derived from savings in the project, for the home institution per volunteer so they could seek input from the volunteer to purchase some of the consumables for the program.

## (2).V. Activities on 8-23-2017:

During the workshop (***annex 3***) information on inoculation studies were discussed for the attendees, particularly for choosing surrogate, attenuated, or indicator non-pathogenic inoculum and for conduct of microbiological validation studies in the university. After the training session, there were additional discussions on importance of

validating currently in place antimicrobial interventions in the island meat industry. There were also discussion of an existing thesis research project where the institution was trying to reduce the nitrate of fermented sausages by replacing some portion of the curing salts with natural and local ingredients such celery seed powder. There were also discussion on project of another graduate students where there was intended to compare the efficacy of chlorine dioxide and sodium hypochlorite. The research advisor expressed concern that so far they were not able to achieve the exact same concertation of the both chemicals, thus unable to compare the efficacy of the two. Rather than trying to achieve the exact same concertation for both chemicals, recommended to use each antimicrobial intervention at the highest level authorized by the regulatory agency and manufacturer(s), which would give an overview of maximum decontamination efficacy that could be achieved for each antimicrobial. Further studies could be designed to test the antimicrobial effectiveness at lower concertation *e.g.* 75%, 50%, or 25% of the maximum concentration proposed by the manufacturer(s).

After the lecture, activities, and discussions, spend time in poultry processing plant for observing the current practices. Information on the poultry processing practices are presented separately under the “poultry processing” section below. After hours, had a chance to spend time with a student and recent alumnus of ISA University assisting them for their graduate school application and GRE registration and preparation.

Recommendation(s)

- Many of the practices in the regional meat industries are solely adopted from the United States, those are validated based on the regulatory requirements and processing conditions in the States. To assure such interventions are efficacious in DR, they would require microbiological validation studies using locally isolated organisms from the island. Higher temperature, different altitude, and different processing practices could affect the efficacy of the antimicrobials that could be assessed and adjusted based on the knowledge gained during the workshop. Adjusting the exposure time, method of application, and concentration of lactic acid for decontamination of meat carcasses from Shiga Toxin-producing *Escherichia coli* were discussed and recommended for the ISA University stakeholders.

-For the ongoing above-referenced research project, for reducing nitrate of fermented sausages, microbiological safety evaluation of the re-formulated product is a critical stage before adoption of the practice by the private industry, particularly multiplication and survival of spore-forming organisms such as *Clostridium botulinum* and *Clostridium perfringens*. Since handing such pathogens and preparation of spore-suspension for the needed inoculation study is currently unavailable in the university, it was recommended to conduct a microbiological study using Aerobic Plate Count as an indicator of microbial proliferation during the shelf-life and disseminating the results with caution as an exploratory experiment that requires further validation studies for control of the above-mentioned spore-forming organisms. Regulatory information on the reductions could also be useful for the stakeholders, knowing that 33%, and 50% reduction of nitrate could qualify a producer for claims of reduced-nitrate, and low-nitrate, respectively, that could assist a producers in better marketing the product in the island.

-One major barrier for conduct of inoculation studies were limited availability of functional autoclaves that could pose a bio-hazard risk in case of growing and purifying microbial inoculum that would need to be addressed. To assure validity of the work conducted in the food safety and food microbiology programs, it is also vital to develop a plan for conducting and documentation of calibration of pipettes and balances to assure accuracy of the measurements during handling of solid and liquid material.

***Photo courtesy:*** *Training Program: [Instructor; Translator; Attendees; Date]*

*Lecture and Workshop: Microbial Challenge and Inoculation Studies using Planktonic and Biofilms of Foodborne Bacteria [A. Fouladkhah; José Almodóvar; Five Faculty members; August-23-2017].*

## (2).VI. Activities on 8-24-2017:

During the workshop (***annex 4***) information on recent transboundary diseases, sanitation standard operating procedures, good manufacturing practices, Hazard

Analysis and Critical Control Point (HACCP), and control of infectious diseases and peri- and post processing operations were discussed. After the meeting there were discussions on how producers and processors with limited resources could

comply with regulations such as HACCP, examples of existing exemptions in the US, existing resources on USDA small plant outreach website, and the role that academe could play in preparing stakeholders for regulatory requirements of the current food safety landscape. There were also discussions on importance of controlling zoonotic infectious diseases, particularly the 2012 Tuberculosis outbreak in their region that had still major negative consequences on the local economy. After the meeting there were one-by-one consultation with a research advisor for interpretation of the microbiological data provided by a third party laboratory of the island. In the results, the coliform counts of the same sample were considerably higher that the APC counts. Explained the Coliform is a laboratory term of at least 5 species that are considered as indicator for sanitary quality of food and water. Thus the Coliform count at most could be similar or lower than APC counts. Reviewing the data and sampling sources, it was recommended to request re-analyses of newly obtained sample by contacting the laboratory to assure the data provided are accurate and free of the above inconstancy. Had also spend additional time in the poultry processing area elaborated under “poultry processing” section and in observing the performance of the vegetable processing area.

***Photo courtesy:*** *Training Program: [Instructor; Translator; Attendees; Date]*

*Lecture and Hands-on Activity: Development of Food Safety “Best Practices” for Peri- and Post-Harvest Meat Processing Operations [A. Fouladkhah; José Almodóvar; Three Faculty members; August-24-2017].*

Recommendation(s)

-Considering the history of infectious and transboundary disease in the island, the local universities could impact the regional food security by preparing dissemination material for stakeholders implementing validated sanitation practices, good manufacturing efforts, and ultimately food safety management systems such as HACCP. Although the university engages in research and teaching endeavors for meeting the regional needs, overall very few outreach efforts currently exists unlike the land-grant institutions in the United States.

-Zoning in vegetable processing area could minimize the cross-contamination among fruits prior processing, keeping the perishable products away from the elevated temperatures of “danger zone” (4 to 60ºC), could also minimize the multiplication of unwanted bacteria. Cleaning the left over after processing would also minimize the chance of bacterial proliferation and biofilm formation on surfaces of processing area.

***Photo courtesy:*** *Fruit and vegetable processing plant.*

## (2).VII. Activities on 8-25-2017:

During the workshop (***annex 5***) information on conduct and analyses of sensory studies for trained and untrained panelists were provided. Discussions were made for using of an emerging method (Tetrad Test), importance of power analyses and sample size calculation for assuring the internal validity of the study, and using publically available software for such analyses. The importance of sensory analyses were also discussed as a low-cost extension for graduate students’ project and also for providing service to regional stakeholders that could improve the financial input of the university laboratories.

***Photo courtesy:*** *Training Program: [Instructor; Translator; Attendees; Date]*

*Lecture and Hands-on Activity: Sensory Analyses by Trained and Untrained Panelists: Discrimination and Hedonics Testing [A. Fouladkhah; José Almodóvar; Six Faculty members; August-25-2017].*

Recommendation(s)

-Incorporating sensory conduct and analysis to the list of services that the university provides to stakeholders to assist the regional industries and increases revenue of outreach laboratories.

## (2).VIII. Activities on 8-28-2017:

During the workshop information (***annex 6***) was presented for conduct of statistical analyses using open sourced software Open Epi and GInaFiT. Information on the sensory analyses was also reviewed for those faculty who did not attend the last session on Friday. Examples and exercises were provided based on discussed research projects. Each exercise was completed after on-by-one consultation with participants for assimilation of software options for data analyses. Sample size calculations, power analysis, conduct of T-test and ANOVA, and construction of confidence interval were exercised on topics associated with transboundary infectious diseases and decontamination of meat from bacterial pathogens.

***Photo courtesy:*** *Training Program: [Instructor; Translator; Attendees; Date]*

*Lecture and Workshop: Analyses of Research Data: Sample Size calculations, Power Analysis, T-Test, and ANOVA [A. Fouladkhah; Rafael Marte Aracena; Eight Faculty members; August-28-2017].*

## (2).IX. Activities on 8-29-2017:

During the workshop information (***annex 7***) was presented on use of recent analytical methods for calculation of microbial inactivation indices, validation of sanitation processing by using ATP-based assays and reviewing overview of challenge studies and demonstrations. Discussions were made on importance of record keeping, for Food Safety Modernization Act requirements for food processors, method for calibrating an ATP-based assay, and recent supplier verification requirements for export of product outside of the island.

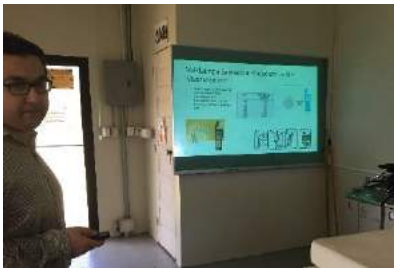


***Photo courtesy:*** *Training Program: [Instructor; Translator; Attendees; Date]*

*Lecture and Hands-on Activity: Validating Sanitation Procedure, Calculating Inactivation Indices & Overview of Challenge Studies [A. Fouladkhah; Rafael Marte Aracena; Four Faculty; August-29-2017].*

Recommendation(s)

-A record keeping plan for calibration of existing equipment's were recommended particularly thermometers, balances, and pipettes.

-Purchase of basic antimicrobials such as 70% ethanol, Quaternary Ammonium Compound or similar sanitizer to keep the laboratory environment prepared for microbiological studies.

## (2).X. Activities on 8-30-2017:

Per request of the faculty, during the workshop information (***annex 8***) additional analytical exercises were provided, through a one-by-one consultation with the faculty for utilization of the software capability. Examples include power analysis, sample size calculation, and conduct of inferential statistics for food safety and infectious diseases scenarios.

***Photo courtesy:*** *Training Program: [Instructor; Translator; Attendees; Date]*

*Lecture and Workshop: Analysis of Food Microbiology and Food Science Data: Modeling and Inactivation Indices [A. Fouladkhah; Rafael Marte Aracena; Three Faculty members; August-30-2017].*

## (2).XI. Activities on 8-31-2017:

Research consultation with a faculty member of the ISA for study on water activity and shelf-life of local dehydrated foods and preparation of an abbreviated recommendation list for the ISA University and USAID program. There were also brief discussions with faculty members on Food Safety Modernization Act requirements for producers and processors.

## (3). Evaluation of the University Poultry Processing:

Considering the popularity of poultry processing in the island and extend of the activities on campus of ISA University in regards to primary processing of poultry, I think a future volunteer could be recruited to further improve the existing practices. Just like any processing plant, some recommendations would require considerable additional

investments that could be unfeasible for the institution considering the limited financial resources available for the program. Vast majority of recommendations in this section are however practical interventions that could prevent, eliminate, or minimize the risk of foodborne disease during and after processing of the poultry. Suggested recommendations:

## (3).I. Avoiding time/temperature abuse during processing and storage:

Many of the pathogens of public health concern and spoilage organisms could proliferate extensively at temperatures beyond refrigeration. Pathogens of particular concern to poultry processing such as *Campylobacter* spp. could also proliferate extensively at temperatures above 30$^{o}$C.

*Recommendations:*

- Control of the temperature in the dump tank to avoid extensive multiplication of the pathogens. Use of ice water slurry and frequent substitution of tank/container water with clean water at cooler temperatures could minimize multiplication of the pathogen.

***Photo courtesy**: ISA University Poultry Processing.*

-Offal of the process, were particularly time/temperature abused on the day of the observation to great extent. They were stored at elevated temperature all day prior to being replaced in the cooler at the end of the shift. Storing such edible portions of process in smaller container in the processing plant would enable the workers to transfer the smaller containers to the cooler on hourly basis to avoid time/temperature abuse of such perishable edible products. Another alternative is covering the bottom of the container with ice and add several layers of ice during processing to assure control of temperature. Considering the existence of an ice making machine in the

processing plant, this recommendation appears to be an adoptable intervention for this specific plant.

***Photo courtesy**: ISA University Poultry Processing.*

-Ultimately conduct of primary processing in a room that has temperature control could be essential to assure microbiological safety of the products. This recommendation however appears to require initial capital investment that might not be feasible at this stage for the home institution.

***Photo courtesy**: ISA University Poultry Processing.*

-Final products are stored in a cooler that has a functional cooling system. The temperature of the coolers are not monitored that makes it difficult to assure microbiological safety of the products during storage. Some pathogens of public health concern could grow at temperatures slightly above 4°C, the reference temperature for industrial coolers. For examples, *Salmonella* serovars could proliferate at 5.2°C so daily monitoring of the temperature in refrigerators and have a calibrated thermometer inside each cooling unit could assure microbiological safety of the products. The plant workers/managers do take a detailed log for the weight of the processed poultry in the coolers, they could easily add a section for monitoring and documentation of the temperatures.

***Photo courtesy**: ISA University Poultry Processing.*

-Major concern was occurrence of cross-contamination from poultry products to prepared cheese products in the processing plant. It was observed (photo on the left) that a worker who had been handling live and processed poultry, without sanitization of hands, started to touch/move the blocks of prepared cheese to create additional space in the

refrigerators. Considering that the cheese could be consumed without any further treatment, cross-contamination episodes like this could easily lead to health concerns. It is strongly suggested to develop a protocol to have raw and processed produces separated from each other and to avoid any episode of cross-contamination similar to the above-mentioned incidence.

***Photo courtesy**: ISA University Poultry Processing.*

## (3).II. Improving the structure of the processing plant:

-As mentioned earlier, ultimately improvement in structure of the processing plant to control the temperature and creating a positive airflow could create an environment for even safer processing, although it requires considerable capital investment for renovation that might not be financially feasible for the program. Nevertheless, some adoptable and low-cost improvement could be implement to improve the safety of current practice.

***Photo courtesy**: ISA University Poultry Processing.*

*Recommendations:*

- Control of physical hazards in the plants by protecting the breakable items such as light bulbs could minimize the risk of physical hazards in processing area.

***Photo courtesy**: ISA University Poultry Processing.*

-Implementation of foot bath filled with sanitizers could eliminate introduction of contamination to the plant area or transfer of pathogens from processing area to other parts of the building. Such foot baths are available for less than $50 on popular on-line stores that have delivery service to the island and could be filled with low-cost sanitizers such as chlorine-based compounds or quaternary ammonium compound-based sanitizers.

-Flies were of abundance in the processing area, considering the climate and current structure of the building, complete elimination of this hazard is not practically feasible, but certain measure such as use of screens and washable curtains at entrances could minimize this condition and risk of vector-borne contamination of the products.

***Photo courtesy**: ISA University Poultry Processing.*

-Simple practices of having separate and distant zones for live and processed poultry could significantly reduce the chance of cross-contamination in the processing plant. Assigning workers to each area (*e.g.* "clean" and "dirty" zones) and requesting them to stay in their designated area could also help the processing plant minimizing the contamination of the final products prior to storage.

-Most of the equipment used in processing are made of stainless steel and are microbiological cleanable. However paddles used in the plant and the cutting boards are not industrial grade and are made of wood and plastic, respectively. These material could harbor microbial growth and bacterial biofilm that could hardly be removed by conventional sanitation. It is recommended to use only utensils and tools that are industrial grade and microbiologically cleanable.

***Photo courtesy**: ISA University Poultry Processing.*

-Processing of the waste water is also critical to avoid spread of the pathogens in the island environment, currently the water from the plant directly leaves the processing area without any further treatment.

***Photo courtesy**: ISA University Poultry Processing.*

-Mid-shift cleaning could also help the microbiological safety of the processing to great extent. At current times, after few hours of processing, floors are covers with products and by-products of the processing, consider the elevated temperature of the island and considering that most bacteria could multiply exponentially every 10 to 20 minutes in optimum temperatures, after few hours of processing floors could become a major source of contamination and cross-contaminations. Taking few minutes of time for cleaning and sanitizing the processing area after few hours of processing could minimize this risk.

***Photo courtesy**: ISA University Poultry Processing.*

-After slaughter, the birds would need to be further processed based on a “First in First Out” or FIFO rule to make sure they are not time and temperature abused. Current system of piling the birds in a basket, with adding newly processed bird on top and processing the products from the top first, create and environment that some products could remain on the bottom of the basket for potentially several hours that could become a breeding ground for extensive multiplication of the pathogenic and spoilage organisms.

***Photo courtesy**: ISA University Poultry Processing.*

-Although there are some cleaning programs in place, they are mainly designated to remove visual dirt prior to processing. Development of a sanitation standard operating procedure (SSOP) with use of validated cleaning and sanitation products could assist the processing managers reduce the final microbial loads of the products and assure microbiological safety of their operation.

***Photo courtesy**: ISA University Poultry Processing.*

## (3).III. Workers health and hygiene:

*Recommendations:*

- At lunch time, it was observed that workers, without washing hands started to have lunch in the processing area. The potential pathogens from the processing environment could easily cause health complications for the works and could be transferred to their living area via the utensils/containers they use for carrying their lunch. A designated area, far from the processing plant and close to a hand washing station could assist them having a safe break.

***Photo courtesy**: ISA University Poultry Processing.*

-Some basic protective gears could also increasingly protect the safety of the workers. Especially those who work with de-feathering machine and those who use large sharp cutting utensils, very close to other workers in a small working place.

***Photo courtesy**: ISA University Poultry Processing.*

-Personal belongings and clothing would also need to be stored in area away from production to avoid chance of transferring contamination from processing plant to outside area and workers home/vehicles/families.

***Photo courtesy**: ISA University Poultry Processing.*

## (3).IV. Animal welfare:

*Recommendations:*

-During the day of the visit, many of the birds were left on shanks awaiting the process for several hours. This “wait” time was particularly disconcerting during the lunch break. In addition to the welfare concern, this create a major quality issue during post-mortem.

***Photo courtesy**: ISA University Poultry Processing.*

-Although it requires capital investment that might not be feasible for the institution at current time, electrical stunning device could also extensively improve the animal welfare and quality of products during post-mortem.

## (4). Results and Recommendation:

List of all recommendations are summarized in **annex 1.**

## (5). Observations, Next Steps, and Future Volunteer Needs:

Completing the two week program in the island and assimilating the needs of the faculty and staff of the university, I think future activities in these areas could further assist the university and stakeholders.

- *Assuring microbiologically safe primary processing of poultry products* (improved zoning during the primary processing, minimizing time/temperature abuse prior to refrigeration, minimizing the risk of cross-contamination from processed poultry to RTE products, and reducing the microbial load of final products by incorporating processing best practices).
- *Developing validated Sanitation Standard Operating Procedures (SSOP) and Good Manufacturing Practices (GMP) for Poultry and Dairy Primary Processing,* to assure microbiological safety of products prepared on campus

and to provide additional hands-on learning opportunities for existing students in the area of food safety and quality control.

- *Building Capacity in Regards to Food Safety Modernization Act (FSMA)* for assisting the producers and processors maintaining access to US market by meeting and exceeding the new U.S. food safety requirements.
- *Train-the-Trainer FSMA workshop for ISA faculty,* to provide educational tools and certification for transfer of food safety regulatory knowledge to the island's stakeholders.
- *Observational Research Training and Epidemiological Tools,* to assist the ISA faculty executing observational studies to monitor prevalence of pathogens of public health concern for early detection of animal health concerns in the island's industries.

## (6). Personal Reflections and Acknowledgements:

Am pleased by the progress and capacity building endeavors achieved during the two week assignment, and commend the enthusiasm, wiliness to absorb new curricula, and professionalism of the faculty and staff in ISA University and USAID/Partners of the Americas staff in Santo Domingo. Am particularly thankful of the technical translation, photos, and great conversations with Mr. Jose Almodovar, Mr. Rafael Marte the USAID filed officers, and the orientation with Ms. Rosa Iris Almonte, director of the center. It is unequivocal for me that future of food security and public health in DR is even brighter with inspiring and career-oriented people like Rosa, Jose, and Rafael.

Am also thankful of the time and sincere care from Ms. Stephanie Verganza from Washington office for harmonizing the travel events and providing helpful information prior to the assignment. Special gratitude is also necessary for Ms. Sahira Vásquez lecturer and research adviser from ISA University for hosting the program, harmonizing the event scheduling, and her passion, energy, and enthusiasm. Am sincerely grateful of the students and research assistants in Public Health Microbiology Laboratory of TSU in Nashville (A. Allison, E. Troyanovskaya, S. Chowdhury, K. Sampson, A. Sumlin, and K. Day) who continued their research endeavors and successfully initiated their new semesters with dedication and professionalism, even though I was hundreds of miles

away from them serving on this program in DR. Am also thankful for assistance from Ms. Linda Buchanan in Nashville for her administrative direction for harmonizing this travel.

Finally, I would like to add that international outreach endeavors, such as current program are mutually beneficial endeavors for both nations. Progress and achievements of volunteers could enhance food security and economic and health equity in the host nation that shares the same values with people of the United States. On the other hand, learning more about diversity and culture of other nations is a much needed trait in the current US economic and political landscape, and could tremendously benefit career of a US faculty member, particularly those with limited exposure to international endeavors. In terms of economics and funding allocation, such programs are also mutually beneficial, a 2005 publication of New England Journal of Medicine as an examples, delineates that a $9.4M investment in public health infrastructure of the Dominican Republic and Haiti will lead to $20M saving in the United States over 20 years due to reduced tuberculosis-related morbidity and mortality in the United States.

# Annex 1
# Recommendation Form

PARTNERS *of the* AMERICAS
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# John Ogonowski and Doug Bereuter Farmer-to-Farmer Program

*Volunteer Recommendations Form*

Name of Volunteer: Aliyar Fouladkhah, PhD, MPH, CFS

Country of Service: Dominican Republic Dates of Trip: 8-20-2017 to 9-2-2017

| | | | | |
|---|---|---|---|---|
| # of Persons *Formally* Trained[1] – male: | 9 | | # of Persons Assisted in any way [2] – male: | 3 |
| # of Persons *Formally* Trained – female: | 19 | | # of Persons Assisted in any way – female: | 5 |
| # of Persons *Formally* Trained – total: | 28 | | # of Persons Assisted in any way – total: | 8 |

*****Please review footnotes for definitions of "persons trained" and "persons directly assisted"*****

**Recommendations Made by the Volunteer:**[3]

Please summarize the recommendations you made to the people/groups/organizations you assisted. Details of the recommendations should be included in the trip report – this is a summary table only.

| Recommendation | Category* | Person/group/organization |
|---|---|---|
| Ultimately the purpose of a written proposal and project with USAID is to induce a lasting change in the home institutions, particularly those considered as emerging democracies and international allies of the United States. While the proposal was well-written in a critical area and arrangements were done well to meet the needs of the home institution in DR, more could be achieved if the budget of the proposal were more balanced. Particular a two week period could be shortened to perhaps one week or 10 days, and the saving from such reduction in period of the program could be awarded to host institution prior to event so they could purchase some consumables and material based on the input from the volunteer to be able to conduct the hands-on activities more efficiently. I would suggest allocating at least few hundred dollars of funding, derived from savings in the project, for the home institution per volunteer so they could seek input from the volunteer to purchase some of the consumables for the program. | Financial | John Ogonowski and Doug Bereuter |
| Many of the practices in the regional meat industries are solely adopted from the United States, those are validated based on the regulatory requirements and processing conditions in the States. To assure such interventions are efficacious in DR, they would require microbiological validation studies using locally isolated organisms from the island. Higher temperature, different | Organizational | ISA University |

[1] **Persons Formally Trained:** number of persons who received technical/instructional training *in a "formal" setting:* classroom, workshop, institute/university or on-the-job setting with specific learning objectives and outcomes

[2] **Persons Assisted:** number of persons who receive **any type** of face-to-face or hands-on technical assistance, training or advice from the F2F volunteer, formal or informal, in any setting. (includes people counted as formally trained plus all others)

[3] **Recommendations Made by the Volunteer:** The definition of "recommendation" is quite subjective, but might include an improved procedure, a technological or management innovation, a useful product or marketing tool, etc. Volunteers might make numerous detailed recommendations to a variety of hosts. Recommendations should be written in a way that is clear and measurable. *Please try to limit recommendations to no more than six per host.*

| | | |
|---|---|---|
| altitude, and different processing practices could affect the efficacy of the antimicrobials that could be assessed and adjusted based on the knowledge gained during the workshop. Adjusting the exposure time, method of application, and concentration of lactic acid for decontamination of meat carcasses from Shiga Toxin-producing Escherichia coli were discussed and recommended for the ISA University stakeholders. | | |
| One major barrier for conduct of inoculation studies were limited availability of functional autoclaves that could pose a bio-hazard risk in case of growing and purifying microbial inoculum that would need to be addressed. To assure validity of the work conducted in the food safety and food microbiology programs, it is also vital to develop a plan for conducting and documentation of calibration of pipettes and balances to assure accuracy of the measurements during handling of solid and liquid material. | Organizational | ISA University |
| Considering the history of infectious and transboundary disease in the island, the local universities could impact the regional food security by preparing dissemination material for stakeholders implementing validated sanitation practices, good manufacturing efforts, and ultimately food safety management systems such as HACCP. Although the university engages in research and teaching endeavors for meeting the regional needs, overall very few outreach efforts currently exists unlike the land-grant institutions in the United States. | Economic | ISA University |
| Zoning in vegetable processing area could minimize the cross-contamination among fruits prior processing, keeping the perishable products away from the elevated temperatures of “danger zone” (4 to 60ºC), could also minimize the multiplication of unwanted bacteria. Cleaning the left over after processing would also minimize the chance of bacterial proliferation and biofilm formation on surfaces of processing area. | Environmental | ISA University |
| Incorporating sensory conduct and analysis to the list of services that the university provides to stakeholders to assist the regional industries and increases revenue of outreach laboratories. | Financial | ISA University |
| A record keeping plan for calibration of existing equipment’s were recommended particularly thermometers, balances, and pipettes. | Organizational | ISA University |
| Purchase of basic antimicrobials such as 70% ethanol, Quaternary Ammonium Compound or similar sanitizer to keep the laboratory environment prepared for microbiological studies. | Organizational | ISA University |
| Control of the temperature in the dump tank to avoid extensive multiplication of the pathogens. Use of ice water slurry and frequent substitution of tank/container water with clean water at cooler temperatures could minimize multiplication of the pathogen. | Organizational | ISA University- Poultry Processing |
| Offal of the process, were particularly time/temperature abused on the day of the observation to great extent. They were stored at elevated temperature all day prior to being replaced in the cooler at the end of the shift. Storing such edible portions of process in smaller container in the processing plant would enable the workers to transfer the smaller containers to the cooler | Organizational | ISA University- Poultry Processing |

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| on hourly basis to avoid time/temperature abuse of such perishable edible products. Another alternative is covering the bottom of the container with ice and add several layers of ice during processing to assure control of temperature. Considering the existence of an ice making machine in the processing plant, this recommendation appears to be an adoptable intervention for this specific plant. | | |
| Ultimately conduct of primary processing in a room that has temperature control could be essential to assure microbiological safety of the products. This recommendation however appears to require initial capital investment that might not be feasible at this stage for the home institution. | Organizational | ISA University- Poultry Processing |
| Final products are stored in a cooler that has a functional cooling system. The temperature of the coolers are not monitored that makes it difficult to assure microbiological safety of the products during storage. Some pathogens of public health concern could grow at temperatures slightly above 4°C, the reference temperature for industrial coolers. For examples, Salmonella serovars could proliferate at 5.2°C so daily monitoring of the temperature in refrigerators and have a calibrated thermometer inside each cooling unit could assure microbiological safety of the products. The plant workers/managers do take a detailed log for the weight of the processed poultry in the coolers, they could easily add a section for monitoring and documentation of the temperatures. | Organizational | ISA University- Poultry Processing |
| Major concern was occurrence of cross-contamination from poultry products to prepared cheese products in the processing plant. It was observed that a worker who had been handling live and processed poultry, without sanitization of hands, started to touch/move the blocks of prepared cheese to create additional space in the refrigerators. Considering that the cheese could be consumed without any further treatment, cross-contamination episodes like this could easily lead to health concerns. It is strongly suggested to develop a protocol to have raw and processed produces separated from each other and to avoid any episode of cross-contamination similar to the above-mentioned incidence. | Organizational | ISA University- Poultry Processing |
| Control of physical hazards in the plants by protecting the breakable items such as light bulbs could minimize the risk of physical hazards in processing area. | Organizational | ISA University- Poultry Processing |
| Implementation of foot bath filled with sanitizers could eliminate introduction of contamination to the plant area or transfer of pathogens from processing area to other parts of the building. Such foot baths are available for less than $50 on popular on-line stores that have delivery service to the island and could be filled with low-cost sanitizers such as chlorine-based compounds or quaternary ammonium compound-based sanitizers. | Organizational | ISA University- Poultry Processing |
| Flies were of abundance in the processing area, considering the climate and current structure of the building, complete elimination of this hazard is not practically feasible, but certain measure such as use of screens and washable curtains at entrances could minimize this condition and risk of vector-borne | Organizational | ISA University- Poultry Processing |

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| contamination of the products. | | |
| Simple practices of having separate and distant zones for live and processed poultry could significantly reduce the chance of cross-contamination in the processing plant. Assigning workers to each area (e.g. “clean” and “dirty” zones) and requesting them to stay in their designated area could also help the processing plant minimizing the contamination of the final products prior to storage. | Organizational | ISA University- Poultry Processing |
| Most of the equipment used in processing are made of stainless steel and are microbiological cleanable. However paddles used in the plant and the cutting boards are not industrial grade and are made of wood and plastic, respectively. These material could harbor microbial growth and bacterial biofilm that could hardly be removed by conventional sanitation. It is recommended to use only utensils and tools that are industrial grade and microbiologically cleanable. | Organizational | ISA University- Poultry Processing |
| Processing of the waste water is also critical to avoid spread of the pathogens in the island environment, currently the water from the plant directly leaves the processing area without any further treatment | Environmental | ISA University- Poultry Processing |
| Mid-shift cleaning could also help the microbiological safety of the processing to great extent. At current times, after few hours of processing, floors are covers with products and by-products of the processing, consider the elevated temperature of the island and considering that most bacteria could multiply exponentially every 10 to 20 minutes in optimum temperatures, after few hours of processing floors could become a major source of contamination and cross-contaminations. Taking few minutes of time for cleaning and sanitizing the processing area after few hours of processing could minimize this risk. | Environmental | ISA University- Poultry Processing |
| After slaughter, the birds would need to be further processed based on a “First in First Out” or FIFO rule to make sure they are not time and temperature abused. Current system of piling the birds in a basket, with adding newly processed bird on top and processing the products from the top first, create and environment that some products could remain on the bottom of the basket for potentially several hours that could become a breeding ground for extensive multiplication of the pathogenic and spoilage organisms. | Organizational | ISA University- Poultry Processing |
| Although there are some cleaning programs in place, they are mainly designated to remove visual dirt prior to processing. Development of a sanitation standard operating procedure (SSOP) with use of validated cleaning and sanitation products could assist the processing managers reduce the final microbial loads of the products and assure microbiological safety of their operation. | Organizational | ISA University- Poultry Processing |
| At lunch time, it was observed that workers, without washing hands started to have lunch in the processing area. The potential pathogens from the processing environment could easily cause health complications for the works and could be transferred to their living area via | Organizational | ISA University- Poultry Processing |

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| the utensils/containers they use for carrying their lunch. A designated area, far from the processing plant and close to a hand washing station could assist them having a safe break. | | |
| Some basic protective gears could also increasingly protect the safety of the workers. Especially those who work with de-feathering machine and those who use large sharp cutting utensils, very close to other workers in a small working place. | Organizational | ISA University- Poultry Processing |
| Personal belongings and clothing would also need to be stored in area away from production to avoid chance of transferring contamination from processing plant to outside area and workers home/vehicles/families. | Organizational | ISA University- Poultry Processing |
| During the day of the visit, many of the birds were left on shanks awaiting the process for several hours. This "wait" time was particularly disconcerting during the lunch break. In addition to the welfare concern, this create a major quality issue during post-mortem. | Organizational | ISA University- Poultry Processing |
| Although it requires capital investment that might not be feasible for the institution at current time, electrical stunning device could also extensively improve the animal welfare and quality of products during post-mortem. | Organizational | ISA University- Poultry Processing |

***** All recommendations should fall under one of four categories:

1. Economic: improvement of profitability of the farm, business, or enterprise
2. Organizational: improvement to organizational effectiveness, management, and sustainability
3. Environmental: improvement of environmental management and natural resource conservation
4. Financial: improvement in the provision of financial services

# Annex 2
# Teaching Agenda

**Department of Food Technology**

**Schedule volunteer 2017**

## F2F/USID Volunteer Instructor: A. Fouladkhah, Tennessee State University

| Date/hours | Place | Activity | Activities and Instructor's Lectures | Participants |
|---|---|---|---|---|
| 21/08/17<br>10:00 am -12:00 pm | Department of Food Technology | Welcome | 1. Meeting the head of the Food Tech Department<br>2. Visit the food microbiology lab<br>3. Visit the pilot meat processing plant | Buddys:<br>Sahira Vasquez<br>Ana Breton |
| 22/08/17<br>9:00-11:00 am | Food Microbiology Lab | Food microbiology analysis training | ***Lecture and Demonstration:***<br>Microbial Challenge and Inoculation Studies Using Planktonic and Biofilms of Foodborne Bacteria | Food Technologist teachers and employees of the food safety lab |
| 23/8/17<br>9:00-11:00 am | Food Microbiology Lab | Follow up food microbiology analysis training | ***Lecture and Hands on Activity on:***<br>1. Preparation of microbiology samples<br>2. Media and diagnostic kits<br>3. Microbiological analysis for:<br>• Heterotrophic plate counts<br>• *Staphylococcus*<br>• *E. coli* O157:H7 | |
| 24/8/17<br>10:00 am-12:00 pm | Meat Processing Plant | Development and installation of "Industry best practices" in the pilot Meat Processing Plant | ***Lecture and Hands on Activity on:***<br>Good Manufacturing Practices and Sanitation Standard Operation Procedures as Pre-requisite Programs of HACCP for assuring Safety of Meat Products | Buddy:<br>Sahira Vasquez<br>Ana Breton |
| 25/8/17<br>10:00 am-12:00 pm | Salon kellogs | Sensory panelists training | ***Lecture and Hands on Activity on:*** | Food Technologist |

| | | | | |
|---|---|---|---|---|
| | | | Sensory Analyses by Trained and Untrained Panelists:<br>identify saltiness, sourness, sweetness, and bitterness basic tastes and astringency, metallic, and umami sensations for hedonic rating of food products | teachers and employees of the food safety lab |
| 28/8/17<br>9:00 am-12:00 pm | Salon kellogs | Analyses of sensory data | ***Lecture:*** Randomized and Observation Research Methods<br><br>***Hands on Activity on:***<br>Data management in Excell, and Data Analyses Using Analysis ToolPack, GInaFiT, and OpenEpi | Food Technologist teachers and employees of the food safety lab |
| 29/8/17<br>9:00 am-12:00 pm | Food Microbiology Lab | Workshop<br><br>Foodborne pathogens identification and handling | ***Lecture:*** To know about conventional and contemporary methods for identifying and quantifying food-borne pathogens<br>Overview of Available Resources: Bacteriological Analytical Method (BAM) of US Food and Drug Administration and USDA Microbiology Laboratory Guidebook.<br><br>***Hands on Activity on:***<br>Access to available resources for microbiological analyses | Food Technologist teachers and employees of the food safety lab |
| 30/8/17<br>9:00 am-12:00 pm | Food Microbiology Lab | Follow up workshop foodborne pathogens identification and handling | ***Hands on Activity on:***<br>Microbial Challenge and Inoculation Studies Using Planktonic and Biofilms of Foodborne Bacteria- Review and Discussion | |

| | | | | |
|---|---|---|---|---|
| | | | Data management in Excell, and Data Analyses Using Analysis ToolPack, GInaFiT, and OpenEpi- Review and Discussion | |
| 31/8/17 | | Writing a report detailing the experience as a volunteer in the food tech department | | |
| 1/9/17 | Salon kellogs | Conference on Good Agricultural Practices (GAPs) | Microbial Food Safety for Current and Emerging Entrepreneurs | Open to public |

**Instructor:** Aliyar Fouladkhah, PhD, MPH, CFS, 2017 F2F/USAID Volunteer

Assistant Professor and Extension Specialist

Public Health Microbiology Laboratory

Tennessee State University

Lawson Hall 111.A and CARP Building Laboratories 112 & 114,

3500 John A Merritt Blvd, Nashville, TN 37209

Telephone: (970)690-7392| Lab: (615)963-1578

Email: afouladk@tnstate.edu or aliyar.fouladkhah@aya.yale.edu

# Annex 3

*Training Program: [Instructor; Translator; Attendees; Date]*

*[A. Fouladkhah; José Almodóvar; Five Faculty members; August-23-2017]*

## Lecture and Workshop: Microbial Challenge and Inoculation Studies using Planktonic and Biofilms of Foodborne Bacteria

# Microbial Challenge and Inoculation Studies using Planktonic and Biofilms of Foodborne Bacteria

***Aliyar Fouladkhah, PhD, MPH, CFS***
***Assistant Professor***
***Public Health Microbiology Laboratory***
***Tennessee State University***

***August 22 & 23, 2017***
***Universidad ISA, Santiago de los Caballeros,***
***Dominican Republic***

TENNESSEE STATE UNIVERSITY

- Food Safety Outreach, and Technical Assistance for Producers and Processors
- **Public Health Microbiology Laboratory**- A technically competent laboratory for testing and outreach validations


Colorado State University

Yale SCHOOL OF PUBLIC HEALTH



Microbac

RODELLE established 1976

boulder BRANDS

PUBLIC HEALTH MICROBIOLOGY LABORATORY
Aliyar Fouladkhah, PhD, MPH, CFS
Assistant Professor, College of Agriculture, Human, and Natural Sciences
(615)963-7496 | afouladk@tnstate.edu | 208 Farrell Westbrook Research Complex

TENNESSEE STATE UNIVERSITY

***Food Safety Regulatory Outreach***
***High Pressure Processing***
***CIFORE Targeted Epidemiological Questions***

CIFOR
www.cifor.us

***Shahid Chowdhury, MS***
***Laboratory Technician***

***Abimbola Allison, PhD Candidate***
***Former WHO Intern***

***Eleonora Troyanovskaya, PhD Candidiate***

# 1st Tennessean Growers' Scholarship of Raw Agricultural Commodities

2/6/2017 2017 Tennessee Growers' Scholarship

**2017 Tennessee Growers' Scholarship**
Fouladkhah, Aliyar (afouladk)
**Sent:** Monday, February 6, 2017 8:33 PM
**To:** Fouladkhah, Aliyar (afouladk); Aliyar.fouladkhah@aya.yale.edu
**Cc:** annettew@utk.edu; kkean@utk.edu; sdugger@utk.edu; Buchanan, Linda

Dear esteemed applicants,

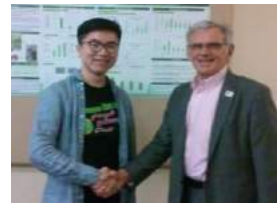
Thank you for your time and application. On behalf of the public health microbiology laboratory of Tennessee State University (project 2016-70020-25805), am pleased to announce that as a Tennessean grower of Raw Agricultural Commodities, you are selected as the recipient of 2017 Tennessee Growers' Scholarship to attend the Produce Safety Alliance training in Murfreesboro. This years' scholarship recipients are:

**-Linda Quillen**
**-Dollye Montogmery**
**-Cleophus Montogmery**
**-Laura Sleigh**
**-Billy Mc Crew**
**-John Erdmann**
**-Mike Minnis**
**-Karen Minnis**
**-Patricia Ann Brown**

# State-wide Research Competition: April 28 2017

*Vol. (Tennessee) section IFT and TSU Public Health Microbiology Laboratory*

1st place: Danielle Gunter-Ward



2nd place: Li Wang

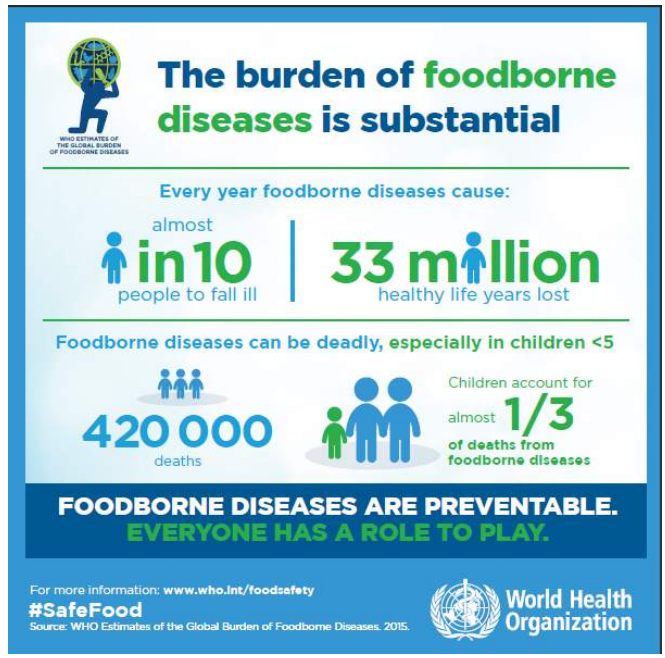
3rd place Shreya Singh Hamal

Volunteer Section
Institute of Food Technologis

Event Information:
*http://www.ift.org/meetings-and-events/calendar/events/2017/apr/ift-volunteer-section-2017-spring-meeting.aspx*

## Burden of Food Safety in the United States

- 47.8 million illnesses annually and…
- 127,839 hospitalizations
- 3,037 deaths in the United States.
- $77.7 billion for losses in productivity and economy
- Approximately 30% of population are especially “at risk” for foodborne diseases (The YOPI’s: The young, the old, Pregnant, and Immunocompromised)




# Emerging pathogens

*Vertical and Horizontal Gene Transfer and Emerging Pathogens*

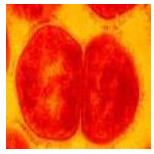
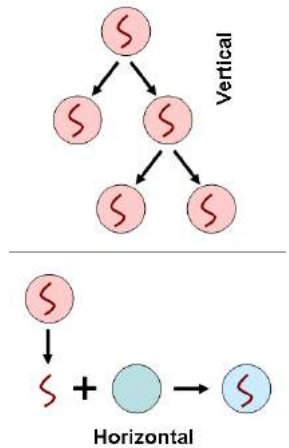
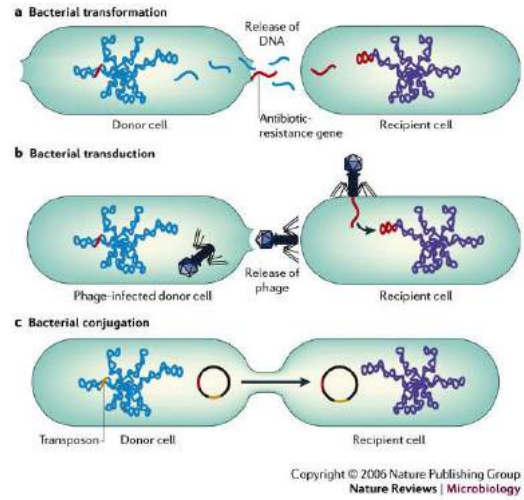


Photo Courtesy: http://www.daviddarling.info/encyclopedia/B/binary_fission.html

It is estimated only 1% of microbial community has been identified.



# Planktonic cells and Biofilm Communities

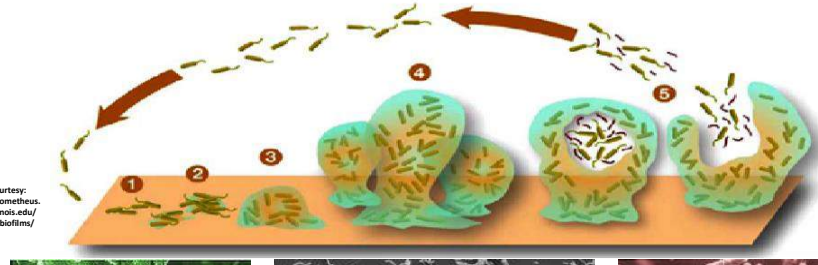



Photo Courtesy: http://prometheus.matse.illinois.edu/glossary/biofilms/

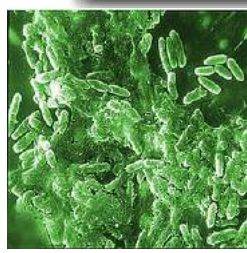


Photo Courtesy: http://micro-writers.egybio.net/blog/?tag=antibiotic-resistance

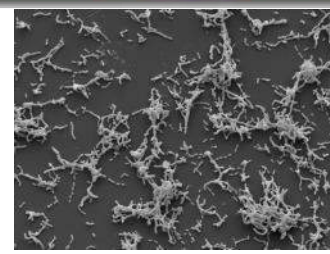


Photo Courtesy: http://www.ifenergy.com/S0226711/boosting_microbial_fuel_cells_with_biofilm.php

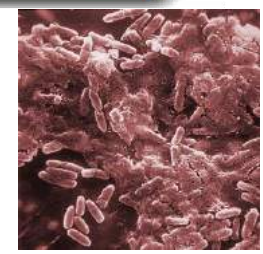
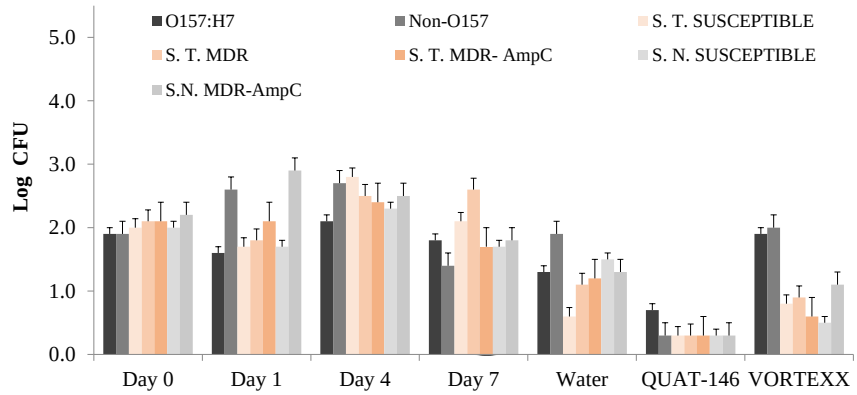


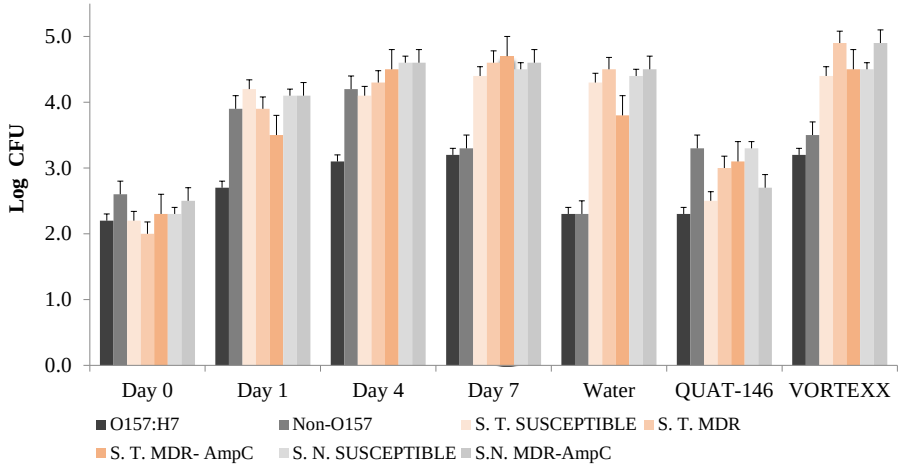
Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/

# Effect of Temperature on Microbial Growth and Sanitation Biofilm formation and decontamination at 4°C (40°F)




*Fouladkhah et al., 2013, J Food Science*

## Effect of Temperature on Microbial Growth and Sanitation Biofilm formation and decontamination at 25°C (77°F)




*Fouladkhah et al., 2013, J Food Science*

## Quorum Sensing and Biofilm formation

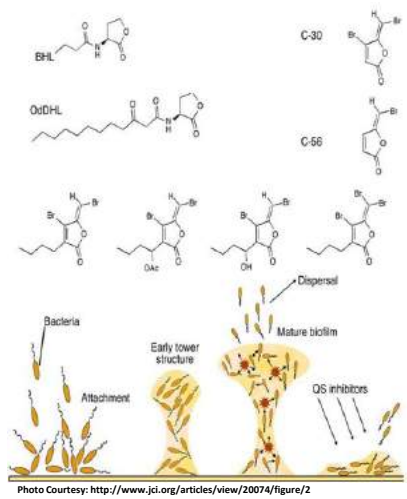



Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

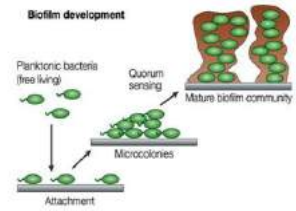



Photo Courtesy: http://labrat.fieldofscience.com/2010/07/quorum-sensing-and-biofilms.html

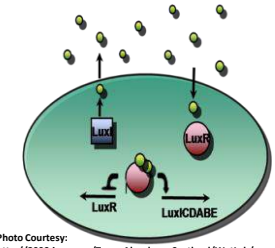
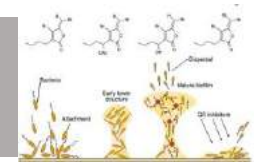



Photo Courtesy: http://2009.igem.org/Team:Aberdeen_Scotland/WetLab/quorumsensing

# Food Safety a Moving Target… Diversity, moving towards "*fitness*"



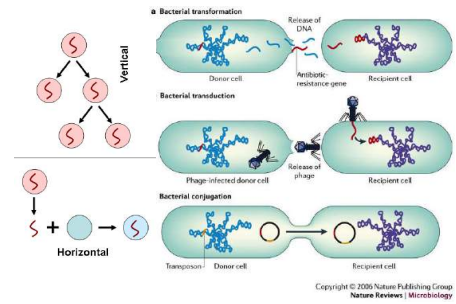
- It is estimated only 1% of microbial community has been identified.
- Currently etiological agent of 80.3% of foodborne illnesses, 56.2% of hospitalization, and 55.5% of deaths remain unknown.

**"Emerging" Pathogens:**

- Vertical and horizontal gene transfer spores and biofilm formation
- Quorum sensing and cell to cell communication

"*It is the microbes who will have the last word.*"
*-Louis Pasteur*

Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2



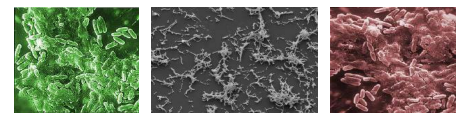



Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/
http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php
http://micro-writers.egybio.net/blog/?tag=antibiotic-resistance

# *Salmonella* serovars

- **Annual illness (death): 1,027,561 (378)**
- Infection causes nausea, vomiting, diarrhea, fever, headache
- Primary sources: Intestinal tract of people and animals
- Transmitted by meat, poultry, eggs, raw milk, unpasteurized juice, many other foods (nuts, spices, produce, chocolate, flour)
- Contributing factors: cross-contamination, undercooked food, poor agricultural practices

| Growth parameters | Minimum | Optimum | Maximum |
|---|---|---|---|
| Temperature | 41°F (5.2°C) | 95-109°F (35-43°C) | 115°F (46.2°C) |
| pH | 3.7 | 7-7.5 | 9.5 |
| $a_W$ | 0.94 | 0.99 | >0.99 |
| Other | Non-sporeformer | | |
| Atmosphere | Facultative - grows with or without oxygen | | |

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al., 2011, and FSPCA

# *Staphylococcus aureus*

- **Annual illness (death): 241,148 (6)**
- Produces heat stable toxins after extensive growth
- Primary sources: Boils, nasal passages and skin
- Transmitted by recontaminated cooked foods, and foods with high salt or high sugar
- Contributing factors: Recontamination and temperature abuse

| Growth parameters | Minimum | | Optimum | | Maximum | |
|---|---|---|---|---|---|---|
| | Growth | Toxin | Growth | Toxin | Growth | Toxin |
| Temperature | 45°F (7°C) | 50°F (10°C) | 99°F (37°C) | 104-113°F (40-45°C) | 122°F (50°C) | 118°F (48°C) |
| pH | 4 | 4 | 6-7 | 7-8 | 10 | 9.8 |
| $a_w$ | 0.83 | 0.85 | 0.98 | | >0.99 | |
| Other | Poor competitor, non-sporeformer | | | | | |
| Atmosphere | Facultative – grows with or without oxygen, but slower without | | | | | |

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al. 2011, and FSPCA

# *Campylobacter* spp.

- **Annual illness (death): 845,024(76)**
- Infection causes diarrhea, and potential nerve damage
- Primary sources: Intestinal tract of animals
- Transmitted by raw poultry, raw milk products, contaminated water
- Contributing factor: cross contamination and undercooking

| Growth parameters | Minimum | Optimum | Maximum |
|---|---|---|---|
| Temperature | 86°F (30°C) | 108-109°F (42-43°C) | 113°F (45°C) |
| pH | 4.9 | 6.5-7.5 | 9.5 |
| $a_w$ | >0.987 | 0.997 | - |
| Other | Non-sporeformer | | |
| Atmosphere | 3-5% oxygen optimum | | |

Sources: ICMSF 1995 and Bad Bug Book 2nd edition and FSPCA

# *Bacillus and Clostridium spp.*

- **Annual illness (death): 63,400 (0)**
- Produces toxins and extensive growth is required for illness
- Primary source: soil and GI track
- Transmitted by: rice and starchy foods, meats, vegetables, milk products, sauces
- Contributing factors: temperature abuse

| Growth parameters | Minimum | Optimum | Maximum |
|---|---|---|---|
| Temperature | 39°F (4°C) | 82-95° F (28-35°C) | 131°F (55°C) |
| pH | 4.3 | 6.0-7.0 | 9.3 |
| $a_w$ | 0.92 | - | - |
| Other | Sporeformer; one toxin is heat stable | | |
| Atmosphere | Facultative – grows with or without oxygen | | |

Sources: Seafood Hazards Guide, ICMSF 1995, Bad Bug Book, Scallan et al. 2011, and FSOCA

# Shiga-toxin Producing *Escherichia coli* (STEC)

- **Annual illness (death): 176,152 (20)**
- Infection causes bloody diarrhea, and sometimes kidney failure and death
- Primary sources: Intestinal tract of ruminant animals (e.g., cows, sheep)
- Transmitted by raw and undercooked beef, poultry, leafy greens, and unpasteurized milk and juices
- Contributing factors: poor GAP, inadequate heating, and person-to-person

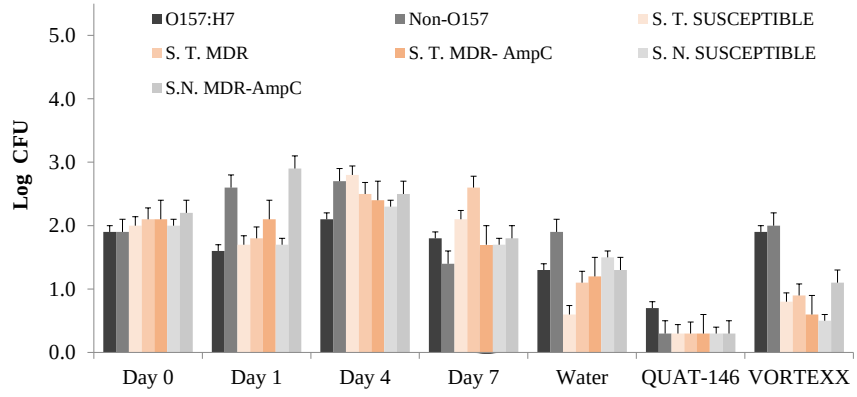
| Growth parameters | Minimum | Optimum | Maximum |
|---|---|---|---|
| Temperature | 44°F (6.5°C) | 95-104°F (35-40°C) | 121°F (49.4°C) |
| pH | 4 | 6-7 | 10 |
| $a_w$ | 0.95 | 0.995 | - |
| Other | Non-sporeforming | | |
| Atmosphere | Facultative - grows with or without oxygen | | |

Sources: ICMSF 1995 and Bad Bug Book 2nd edition,Scallan et al. 2011, and FSPCA

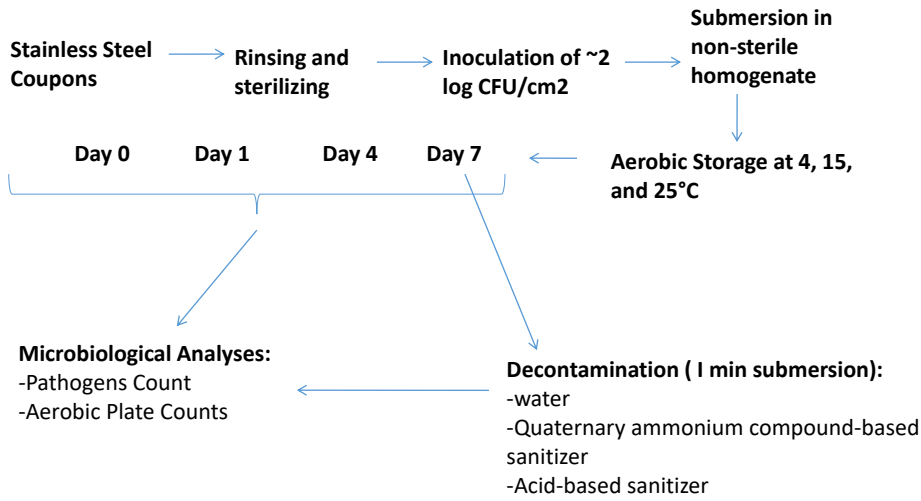
Challenge and Inoculation Studies

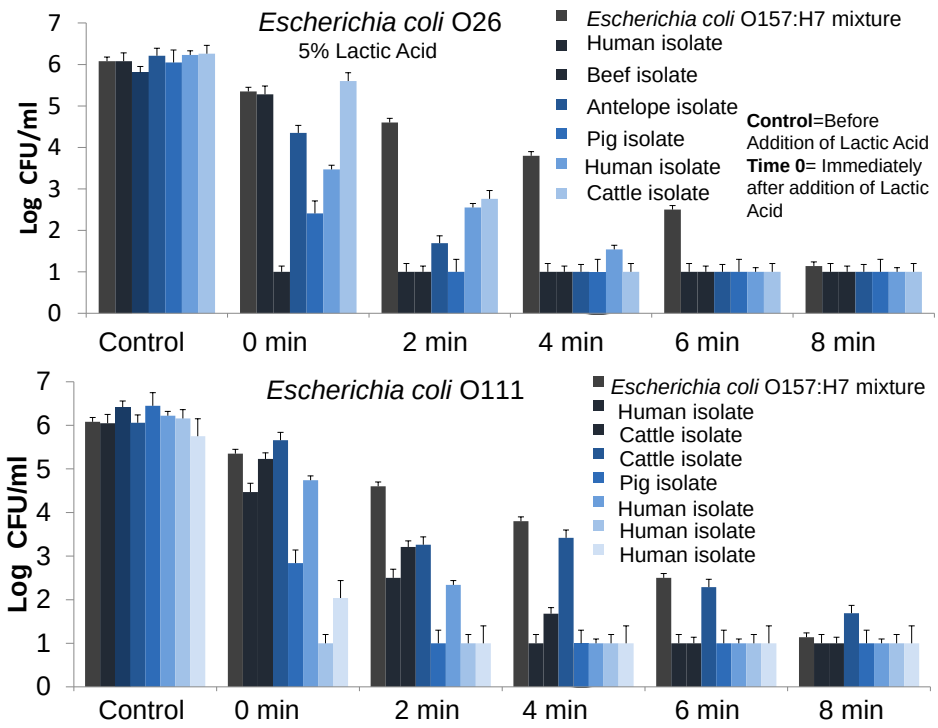
# Three Examples

## Biofilm formation and decontamination at 4°C

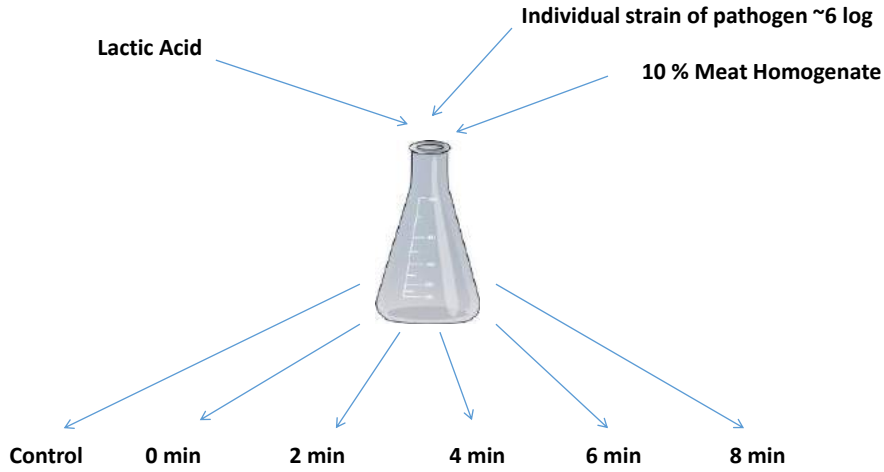


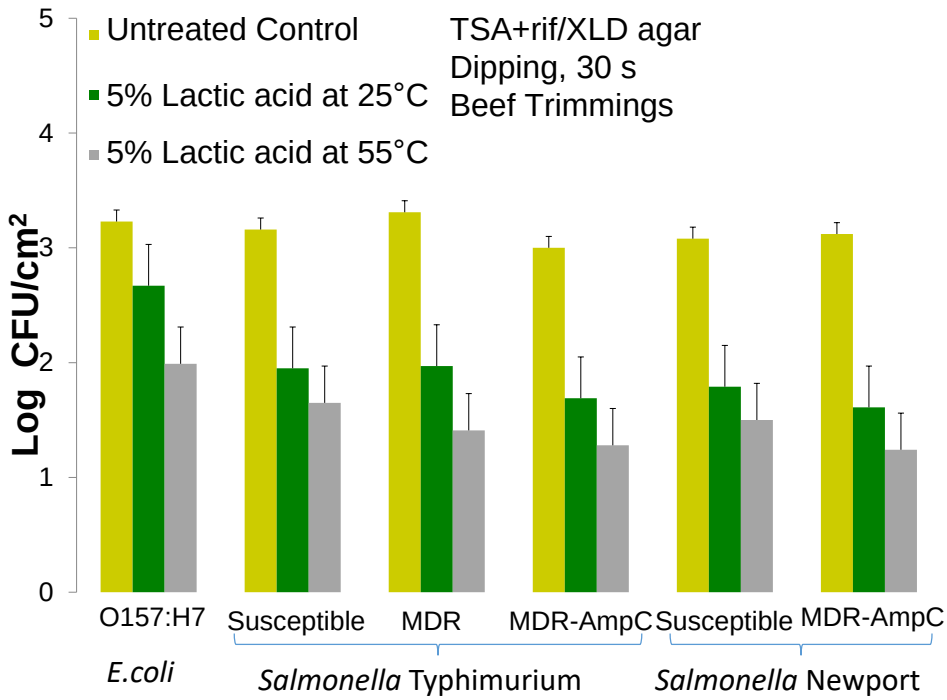
# Experimental Design



# Meat Homogenate model



# Beef Trimming Experiment

10 by 10 cm

*E. coli* O157:H7
*E. coli* O26
*E. coli* O45
*E. coli* O111
*E. Coli* O121
*E. coli* O145
*E. coli* O103
MDR S.N and S.T.
MDR-AmpC S.T.
Susceptible S.N and S.T.

Inoculation → Microbiological Analyses

Lactic Acid Treatment → Immersion for 30 seconds in:
a) LA at 25°C
b) LA at 55°C
Draining for 1 min

Storage for one hour → Microbiological Analyses

*Journal of Food Protection, Vol. 73, No. 1, 2010, Pages 140–202*

Supplement

## Parameters for Determining Inoculated Pack/Challenge Study Protocols†‡

ADOPTED 20 MARCH 2009, WASHINGTON, D.C.
NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS

*NACMCF Executive Secretariat,* U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Room 333 Aerospace Center, 1400 Independence Avenue S.W., Washington, D.C. 20250-3700, USA*

MS 09-287: Received 2 July 2009/Accepted 7 August 2009

**Laboratory Methods**

- CFSAN Laboratory Quality Assurance Manual
- Microbiological Methods & Bacteriological Analytical Manual (BAM)
- Drug & Chemical Residues Methods
- Elemental Analysis Manual (EAM) for Food and Related Products

## BAM: Food Sampling/Preparation of Sample Homogenate

SHARE TWEET LINKEDIN PIN IT EMAIL PRINT

April 2003

**Bacteriological Analytical Manual**
**Chapter 1**
**Food Sampling and Preparation of Sample Homogenate**

**Authors:** Wallace H. Andrews and Thomas S. Hammack

# Challenge and Inoculation Studies

1) Growing the cells planktonically: Overnight suspension, fecal sample, or highly contaminated food
2) Washing the cells for removing impurities and cell components
3) Preparation of microbial cocktail if more than one strain is in use
4) Habituation of the bacteria to "familiarize" the bacteria to the food environment
5) Preparation of Inoculum based on serial dilution
6) Inoculation of biotic or abiotic surface: inoculum load and attachment time
7) Aerobic or anaerobic storage for growth of the inoculum
8) Thermal and/or non-thermal antimicrobial interventions
9) Neutralization of samples: Ice slurry (for heat treatment) and DE broth (for antimicrobials)
10) Enumeration of survivors: YE and/or PY

# Challenge and Inoculation Studies

(1) Growing the cells planktonically: Overnight suspension, fecal sample, or highly contaminated food

- Pathogens: Purchased from ATCC (CDC Biosafety Level 1, Level 2, Level 3, Level 4)
- One alternative is use of bacteria isolated in lab: 80% glycerol stock at -80$^{o}$C.
- Attenuated Pathogens: Such LT2 *Salmonella*
- Surrogate Organism: generic *E. coli* K12 for E. coli O157:H7
- Fecal sample: 20% in PBS for field studies or when pathogens not available
- One alternative is to study the natural microflora without inoculation

- Temperature and incubation period choice based on study:

i.e. 22-24 at 37$^{o}$ C for *Salmonella* serovars

# Challenge and Inoculation Studies

2) Washing the cells for removing impurities and cell components

- Could vary based on the pathogen

**One protocol** (Fouladkhah et al., 2013):

- Centrifuging at 4,629 g for 15 min, discarding the "supernatant"
- Washing the "pellet" with 10 ml phosphate-buffered saline
- Centrifugation (4,629 g at 4°C for 15 min) again,
- Resuspension of the pellet in 10 ml PBS.

# Challenge and Inoculation Studies

3) Preparation of microbial cocktail if more than one strain is in use

4) Habituation of the bacteria to "familiarize" the bacteria to the food environment

- It is a common practice to combine a mixture of strains in a "cocktail" *i.e* five strain mixture of *salmonella*
- If a cocktail to be used each stain would need to be prepared (steps one and two) separately prior to mixing the strains
- Habituation allows a more realistic results

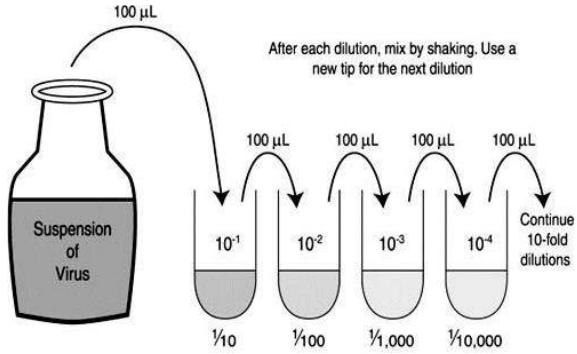
**One protocol** (Fouladkhah et al., 2011)

Strains of *L. monocytogenes* habituated for two days at 7°C in ham homogenate to allow acclimatization of *L. monocytogenes* cells to the food environment and low-temperature.

# Challenge and Inoculation Studies

5) Preparation of Inoculum based on serial dilution

Overnight Suspension of most bacteria around 9.5 log CFU/mL




# Challenge and Inoculation Studies

6) Inoculation of biotic or abiotic surface: inoculum load and attachment time

7) Aerobic or anaerobic storage for growth of the inoculum

- Inoculation could be based on **weight** (Log CFU/ g) or **volume** (Log CFU/ mL) of final products
- Could be **biotic** (e.g. leafy greens, beef trimming etc.) or **abiotic** (Stainless steel)
- Could be in **planktonic** stage (after inoculation) or **biofilm** stage (several days after inoculation).
- Samples could be stored **aerobically** or **anaerobically** prior to analysis
- **Attachment time** is a critical factor that would need to be extracted from literature.

One Protocol (Fouladkhah et al., 2013)

I hour attachment was recommended for adherence of *Escherichia coli* O157:H7 to stainless steel coupons

# Challenge and Inoculation Studies

8) Thermal and/or Non-thermal Antimicrobial Intervention

9) Neutralization of samples: Ice slurry (for heat treatment) and D/E broth (for antimicrobials)

- Exposure time and Concentration : would need to be pre-determined
- Samples would need to be plated immediately after treatment to minimize extra exposure time to antimicrobials
- Use of ice-water slurry for heat-treated samples to “stop” the thermal treatment
- Use of D/E Neutralizing broth for samples treated with antimicrobials to “stop” the antimicrobial treatment

# Challenge and Inoculation Studies

10) Enumeration of survivors: YE and/or PY

- Recovery of **Injured cells** after thermal or non-thermal treatments.
- New media would need to be formulated to enhance the growth of injured cells
- Yeast extract and Pyruvic acid are two common supplement to general purpose media

- **One Protocol** (Fouladkhah et al., 2017):

0.6% Yeast Extract added to Triptic Soy Agar could enhance recovery of injured cells

Also use of **Maximum Recovery Broth** instead of PBS for serial dilution could enhance the recover of injured cells.

# Additional Resources

*Journal of Food Protection*, Vol. 73, No. 1, 2010, Pages 140–202

Supplement

## Parameters for Determining Inoculated Pack/Challenge Study Protocols†‡

ADOPTED 20 MARCH 2009, WASHINGTON, D.C.
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## Contact FDA About FSMA

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The **FDA Food Safety Modernization Act (FSMA) Technical Assistance Network (TAN)** has been operational since September 9, 2015 and providing technical assistance to industry, regulators, academia, consumers and others regarding FSMA implementation. The TAN addresses questions related to the FSMA rules, FSMA programs, and implementation strategies after the rules are final.

***Note: For Food Safety Preventive Controls Alliance (FSPCA) training and scientific/technical questions, please contact the FSPCA Technical Assistance Network using their web form at http://www.iit.edu/ifsh/alliance.***

**Submit Inquiry**

**System Status Message:**
The FSMA TAN Knowledge Management System is fully operational.

### Mail an Inquiry

Inquiries may also be submitted by mail if the Internet is not available at the following address:

Food and Drug Administration
5100 Paint Branch Pkwy
Wiley Building, HFS-009
Attn: FSMA Outreach
College Park, MD 20740

# Additional Resources

USDA United States Department of Agriculture
Food Safety and Inspection Service

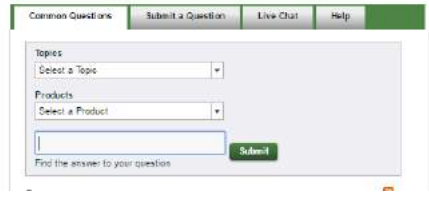
Ask Karen provides information for consumers about preventing foodborne illness, safe food handling and storage, and safe preparation of meat, poultry, and egg products.

For answers to questions about inspection-related policies, programs, systems and procedures, access askFSIS.

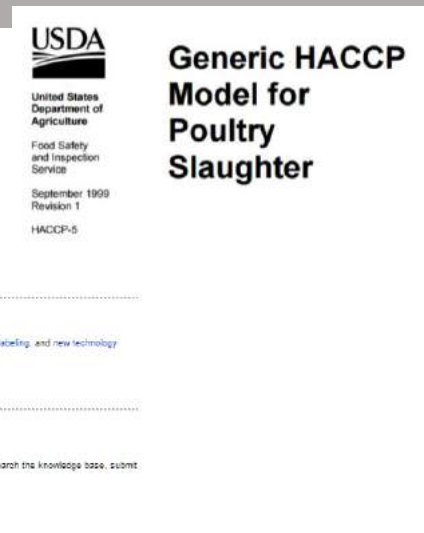
Want to know how long you can safely keep meat in the refrigerator? Or how long to boil an egg? How about whether it's better to use wooden or plastic cutting boards?

Just ask Karen, your guide to expert knowledge on handling and storing food safely and preventing food poisoning.

Use this page to search our knowledge base of common food safety questions (available 24/7). On your mobile phone access m.askkaren.gov | En Español



# Additional Resources


# Additional Resources


## Bad Bug Book

**Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins**

### Introduction

Food safety is a complex issue that has an impact on all segments of society, from the general public to government, industry, and academia. The second edition of the Bad Bug Book, published by the Center for Food Safety and Applied Nutrition, of the Food and Drug Administration (FDA), U.S. Department of Health and Human Services, provides current information about the major known agents that cause foodborne illness. The information provided in this handbook is abbreviated and general in nature, and is intended for practical use. It is not intended to be a comprehensive scientific or clinical reference.

Under the laws administered by FDA, a food is adulterated if it contains (1) a poisonous or otherwise harmful substance that is not an inherent natural constituent of the food itself, in an amount that poses *a reasonable possibility* of injury to health, or (2) a substance that is an inherent natural constituent of the food itself; is not the result of environmental, agricultural, industrial, or other contamination; and is present in an amount that *ordinarily* renders the food

# Thank you for your time

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**Yale School of Public Health Alumnus**
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**Email: afouladk@tnstate.edu**
**Telephone: (615)963-7496**
**Cellphone: (970)690-7392**

# Annex 4

*Training Program: [Instructor; Translator; Attendees; Date]*

*[A. Fouladkhah; José Almodóvar; Three Faculty members; August-24-2017]*

## Lecture and Hands-on Activity: Development of Food Safety “Best Practices” for Peri- and Post-Harvest Meat Processing Operations

# Development of Food Safety "Best Practices" for Peri- and Post-Harvest Meat Processing Operations

***Aliyar Fouladkhah, PhD, MPH, CFS***
***Assistant Professor***
***Public Health Microbiology Laboratory***
***Tennessee State University***

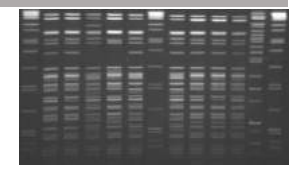
***August 24, 2017***
***Universidad ISA, Santiago de los Caballeros,***
***Dominican Republic***

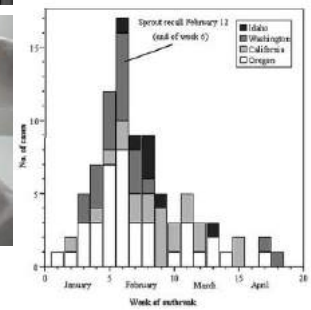
TENNESSEE STATE UNIVERSITY


- Investigation by FDA, the USDA FSIS, and the CDC with state and local health officials.
- *E. coli* O157:H7 infections in California, Colorado, Montana, Missouri, Utah, Virginia, and Washington.

# Foodborne Disease Laboratory and Epidemiology Interaction

- Reportable diseases from Patients
- Public Health Laboratories
  (Isolation, molecular subtyping and PFGE patterns)
- Nationwide Surveillance Networks
  (FoodNet, PulseNet, etc)
- Track back Epidemiology
  (Patients, Retailers, producers)
- Sampling from Retailer/Producers
  (Isolation and subtyping and PFGE)
- Subtyping, PFGE pattern, and DNA Sequencing






# Epidemiology of Foodborne Diseases

- **Based on data from 1990s:** *(Mead et al., 1999)*

76 million illnesses, 323,000 hospitalizations, 5,200 deaths in the United States.

- **More recent estimates show:** *(Scallan et al., 2011)*
- 47.8 million **illnesses**, 127,839 **hospitalizations**, and more than 3,037 **deaths** in the United States.
- 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths are cause by 31 known foodborne agents.
- In addition to consumer insecurity, foodborne diseases cause **around $77.7 billion for losses** in productivity and economical losses.
- Approximately 30% of population are especially “at risk” for foodborne diseases

## Significant foodborne pathogens…
*based on Mead et al., 1999 and Scallan et al., 2011 studies*

- **Leading etiological agents for illnesses**: *Norovirus* (58%), Nontyphoidal *Salmonella* serovars (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp (9%).
- **Leading etiological agents for hospitalization:** Nontyphoidal *Salmonella* serovars (35%), *Norovirus (26%), Campylobacter* spp (15%), and *Toxoplasma gondii* (8%).
- **Leading etiological agents for death:** Nontyphoidal *Salmonella* serovars (28%), *T. gondii* (24*%), Listeria monocytogenes* (19%), and *Norovirus (11%).*

## Signs and Symptoms of Foodborne Diseases

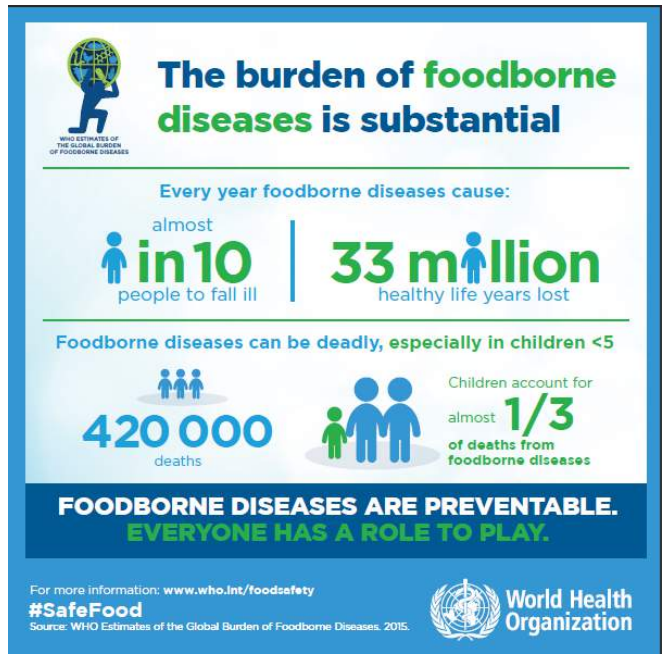
- Mild illness (no medical care sought)
- **Guillain–Barré syndrome** (*Campylobacter* and *Salmonella*)
- **Post-infectious irritable bowel syndrome** (*Campylobacter* and *Salmonella*)
- **Reactive arthritis** (*Campylobacter* and *Salmonella*)
- **Haemolytic uraemic syndrome** (*E. coli* O157)
- **End-stage renal disease** (*E. coli* O157)
- Death

# Significant foodborne pathogens…
*based on Scallan et al., 2015 study*

- **Disability adjusted life year** (DALY). *DALY: Loss of life and health due to illness compared with 'perfect' health*

- Non-typhoidal *Salmonella* (329000)
- Toxoplasma (32700)
- *Campylobacte*r (22500)
- Norovirus (9900)
- *Listeria monocytogenes* (8800)
- *Clostridium perfringens* (4000)
- *Escherichia coli* O157 (1200)

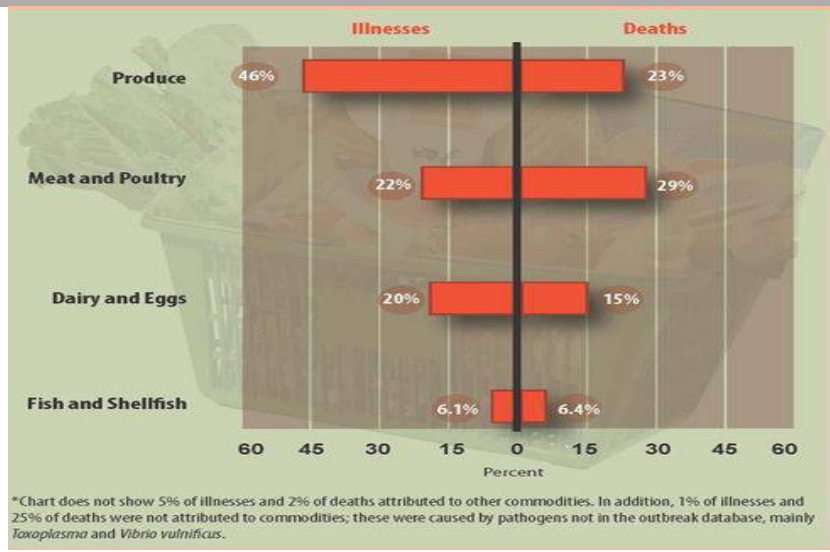
**62% bacterial agents; 29% parasitic agents; 9% viral agents**


WHO ESTIMATES OF THE GLOBAL BURDEN OF FOODBORNE DISEASES
The burden of foodborne diseases is substantial
Every year foodborne diseases cause:
almost
in 10
people to fall ill
33 million
healthy life years lost
Foodborne diseases can be deadly, especially in children <5
420 000
deaths
Children account for
almost 1/3
of deaths from foodborne diseases
FOODBORNE DISEASES ARE PREVENTABLE.
EVERYONE HAS A ROLE TO PLAY.
For more information: www.who.int/foodsafety
#SafeFood
Source: WHO Estimates of the Global Burden of Foodborne Diseases. 2015.
World Health Organization


WHO ESTIMATES OF THE GLOBAL BURDEN OF FOODBORNE DISEASES
Diarrhoeal diseases are the most common illnesses resulting from unsafe food
Diarrhoeal diseases are responsible for:
1/2
global burden of foodborne diseases caused by 31 hazards
Key global causes of diarrhoeal diseases:
Norovirus
E. coli
Campylobacter
Non-typhoidal Salmonella
550m
people falling ill
230 000
deaths
including
220m
children <5 falling ill
96 000
of whom die

# CDC Estimates of Food Safety Burden

http://www.cdc.gov/foodborneburden/attribution-image.html#foodborne-illnesses




# National-wide and Regional Foodborne Episodes

- Centers for Disease Control and Prevention: Foodborne diseases episodes 1998 to 2015.

| | Outbreaks | Illness | Hospitalization | Deaths |
|---|---|---|---|---|
| Nation-wide | 18,211* | 358,391 | 13,715 | 318 |
| Tennessee | 299 | 7,212 | 276 | 1 |

*Etiological agents for Tennessee episodes:

>200 species of bacteria, viruses, parasites, and chemical toxins.

*Fouladkhah et al., 2017 (in review publication); Data source: http://wwwn.cdc.gov/foodborneoutbreaks/*

# National-wide and Regional Foodborne Episodes Involving Poultry

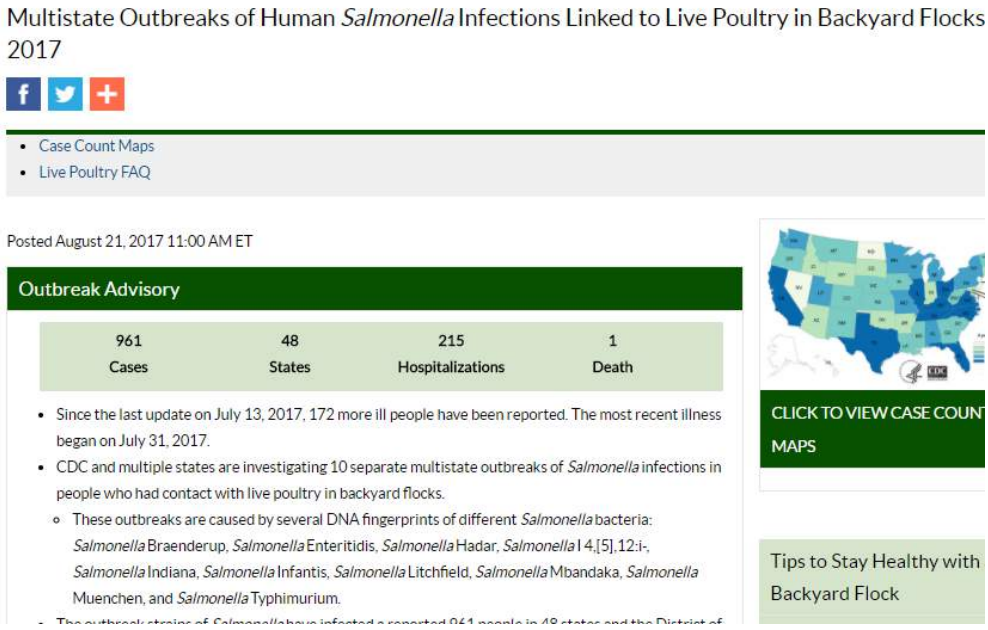
- Centers for Disease Control and Prevention: Foodborne diseases episodes 1998 to 2015.

| | Outbreaks | Illness | Hospitalization | Deaths |
|---|---|---|---|---|
| Nation-wide | 1,261 | 22,868 | 992 | 10 |
| Tennessee | 19* | 338 | 37 | 0 |

*Etiological agents for Tennessee episodes:

*Salmonella* serovars (5 episodes); *Staphylococcus aureus* (3 episodes); *Campylobacter* (2 episodes); *Clostridium perfringens* (2 episodes); *Bacillus cereus* ( 2 episodes); *Norovirus* (2 episodes); *Escherichia coli* ( 1 episode); *Giardia* (1 episodes)

*Fouladkhah et al., 2016 (in review publication); Data source: http://wwwn.cdc.gov/foodborneoutbreaks/*



# National-wide and Regional Foodborne Episodes Involving All Leafy Greens

- Centers for Disease Control and Prevention: Foodborne diseases episodes 1998 to 2015 (Outbreaks involving leafy greens and salads)

| | Outbreaks | Illness | Hospitalization | Deaths |
|---|---|---|---|---|
| Nation-wide | 449 | 11,278 | 286 | 3 |
| Tennessee | 23* | 1,147 | 27 | 0 |

*Etiological agents for Tennessee episodes:

Norovirus*, campylobacter, Salmonella, Bacillus cereus, Escherichia coli,* and parasites.

*Fouladkhah et al., 2016 (in review publication); Data source: http://wwwn.cdc.gov/foodborneoutbreaks/*

# National-wide and Regional Foodborne Episodes Involving Lettuce Only

- Centers for Disease Control and Prevention: Foodborne diseases episodes 1998 to 2015.

| | Outbreaks | Illness | Hospitalization | Deaths |
|---|---|---|---|---|
| Nation-wide | 149 | 3,758 | 200 | 3 |
| Tennessee | 10* | 130 | 14 | 0 |

*Etiological agents for Tennessee episodes:

*Salmonella, Bacillus cereus, and Norovirus.*

*Fouladkhah et al., 2016 (in review publication); Data source: http://wwwn.cdc.gov/foodborneoutbreaks/*

# National-wide and Regional Foodborne Episodes Involving Tomato Only

- Centers for Disease Control and Prevention: Foodborne diseases episodes 1998 to 2015.

| | Outbreaks | Illness | Hospitalization | Deaths |
|---|---|---|---|---|
| Nation-wide | 99 | 6,768 | 774 | 7 |
| Tennessee | 3* | 31 | 9 | 0 |

*Etiological agents for Tennessee episodes:

*Salmonella, Bacillus cereus, and Norovirus.*

*Fouladkhah et al., 2016 (in review publication); Data source: http://wwwn.cdc.gov/foodborneoutbreaks/*

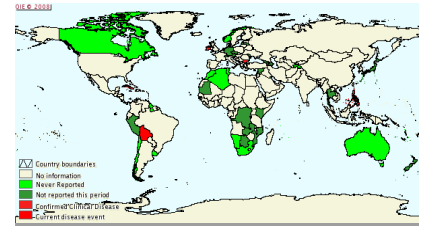
## Infectious and Transboundary Disease

# Anthrax

- Available in many parts of the world
- Spores highly infective
- Remain effective during aerosolization
- Low lethal dose
- High mortality
- Person-to-person transmission rare

# Pseudorabies

- Contagious viral diseases
- Primary concern in domesticated pigs and feral swine
- Other mammals
  - Reproductive
  - Nervous system
- Humans are not affected
- Could be a ubiquitous virus in some area
- Eradicated in most countries
  - Still occurs in parts of world
- Current USDA Surveillance to detect any potential case



# Hendra Virus

- Viral disease consider as emerging (first observed in Australia)
- Natural infections had been reported only in:
  - Horses
  - Humans
- Current transmission by:
  - Fruit bats
  - Bodily fluids and urine of those infected
- Clinical signs in Horses
  - Sudden respiratory signs
  - Nasal discharge
  - Fever
  - Encephalitis
  - Sudden death
- Clinical signs in Humans
  - Flu-like illness
  - respiratory complications
  - Highly fatal in human, could be as high as 2 in 3 cases

# Brucellosis and Tuberclusis

- Caused by bacteria (several species)
  - Highly infectious
  - Easily aerosolized
- Transmission:
  - Ingestion
  - Inhalation
  - Direct contact
- Signs in animal:
  - Reproductive complications
- Signs in humans:
  - Cyclic fever and
  - Flu-like symptoms

# Are these outbreaks associated with corporates and Lager manufactures?

## Prevalence of Pathogens in Medium-sized Poultry Operations

- 200–300 ft houses, 3000 to 5000 birds, conventional operation

(Alali et al., 2010)

| | *Salmonella* serovars |
|---|---|
| **Fecal samples (n=420)** | 38.8% |
| **Feed (n=140)** | 27.5% |

- Total of 135 sample from commercial free-range chicken producers

(Bailey et al., 2005)

| | *Salmonella* serovars |
|---|---|
| **Chicken Carcasses in Operation 1** | 64% |
| **Chicken Carcasses in Operation 2** | 31% |

*Alali et al., 2010, J Foodborne Pathogens and Diseases; Bailey et al., 2005, J Food Protection*

# Prevalence of Pathogens in Small Poultry Farms

- Study of 60 Small poultry slaughterhouses (fewer than 200 birds slaughtered per day)

| Sampling sites | *Salmonella* serovars (Albany, Hadar, Indiana, and Enteritidis sub-species) |
|---|---|
| **Carcasses after slaughter** | 42% |
| **Utensils** | 23.1% |
| **Storage freezers and refrigerators** | 71.4% |

- The Study concluded "*The widespread occurrence of Salmonella in small slaughterhouses reinforces the need for implementation of effective control measures…*"

*Terumi et al., 2000, Journal of Food Protection*

# Food Safety Considerations for Primary Processing of Poultry Products

## Avian Influenza in Western Hemisphere

- HPAI outbreak of **2014-2015, largest animal health emergency in history of the country**.
- >**48 million** birds had died or depopulated due to the diseases
- Diseases introduced by **wild migratory water fowl and** transmitted from farm to farm by personnel
- Pathogens can be secreted through feces, oral, and nasal secretions of the birds
- Prevention and biosecurity **to eliminate and minimize exposure**

## Birds Health and Prevention of Infectious Diseases

- **Stablished controlled access:** minimizing the exposure of potentially contaminated *personal* and *vehicles* to your back yard farm.
- **Structural barriers**: Keeping free flying birds and four-legged animal out
- **Securing the doors and entrances**.
- **Have separate clothing for farm activities.**
- **Designated foot baths** for each pen or a designated pair of shoes for each pen (disinfectant mats)
- **Protective clothing** for service personnel, veterinarians, and all visitors.


*Source: Special Needs Competitive Grant, U. Maryland/USDA*

## Pre-Harvest Prevention

- **Feed and water** are stored in covered area and free of contaminants to avoid wild fowl and pests (small batches)
- **Clean and disinfect** all equipment, farm equipment, egg baskets etc.
- **Wash hands and arms** before and after entering chicken houses.
- **Hunters** with exposure to wild fowl would need to change cloths and shower before re-entrance to chicken houses, specially after contact with water fowl that harbor avian influenza without signs of sickness.
- Keeping poultry away from natural or man made **ponds or rivers** would also reduce the exposure risk.

*Source: Special Needs Competitive Grant, U. Maryland/USDA*

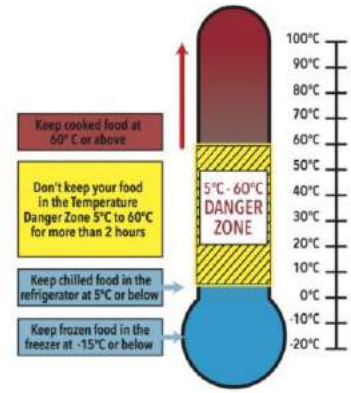
## Pre-Harvest Prevention

- **Separation** of younger birds from older and separation of chickens from different races would also reduce the likelihood of infectious disease spread (separation of chickens from turkeys). This may help to reduce *Salmonella* and *Campylobacter* colonization.
- Proper and timely **disposal** of dead birds, to avoid flies, pests, and scavengers.
- Contact **government laboratory** after observing sick or dying birds to identify and diagnose the infectious agents. In case of sudden spike in mortality (>50%).
- **Do not visit farms with high mortality** rate among their birds
- Other helpful strategies are use **of antibiotics, probiotics, prebiotics, botanical feed additives, and vaccines**.

*Source: Special Needs Competitive Grant, U. Maryland/USDA*

# Safe Harvest and Processing and Sanitation Basics

- **Material and equipment** that are microbiologically cleanable and in harmony with USDA regulations.
- **Standard Sanitation Operation Procedures** for cleaning and sanitizing.
- Use of **potable water or treated well water** could lead minimize the risk carcass contamination.
- Sanitary and frequent discharge of **water and waste water.**
- **Temperature control** can reduce multiplication of pathogens during processing.
- **Separation** of final products from lives birds could reduce the chance of cross contamination.
- **Time/Temperature** Control (i.e. FIFO to avoid time temperature absue)
- Ultimately a series of **prerequisite program(GMP's, SSOP's)** and prevention based food safety management system could lead to development of a HACCP-based Food Safety Plan.




# HACCP, and Best Practices

- Food Safety Management System
- Purpose is "prevention"…


**Table 1. HACCP Principles**

| | |
|---|---|
| **Principle 1** | Conduct a hazard analysis. |
| **Principle 2** | Determine the critical control points. |
| **Principle 3** | Establish critical limits. |
| **Principle 4** | Establish monitoring procedures. |
| **Principle 5** | Establish corrective actions. |
| **Principle 6** | Establish verification procedures. |
| **Principle 7** | Establish record-keeping and documentation procedures. |

# Good Manufacturing Practices (GMP)

**Personnel Education**

- Periodic training for all employees
- Disease control
- Open lesions or infected wounds
- Personal cleanliness in production
- Clean outer garments
- Wash hands
- Remove jewelry
- Storage of personal belongings
- Hair nets and beard nets

**Building and Facilities**

- Grounds and area outside
- Cafeteria or lunch area
- Entrances
- Hand washing stations

**Equipment and Utensils**

- Design and construction of equipment
- Instruments for control of temperature or pH must be accurate

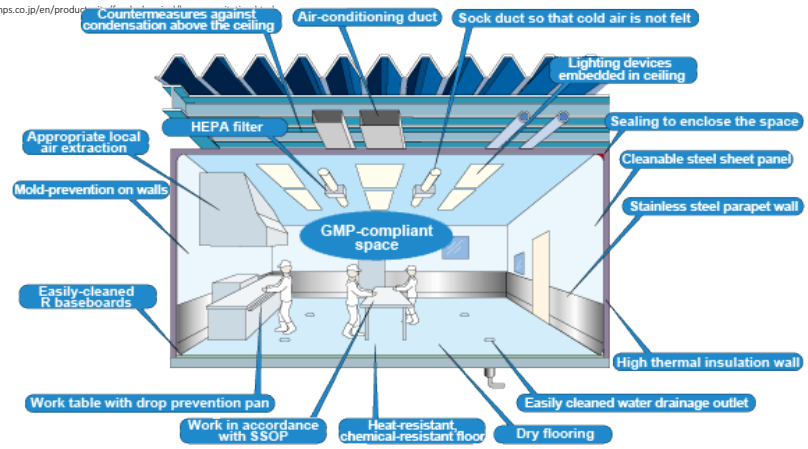
**Production and Process Controls**

- Raw materials (feed and water)
- Manufacturing operations

**Sanitation Standard Operating Procedures**

# GMP's in Manufacturing

source: http://www.hitachi-hps.co.jp/en/produc



# SSOP: Sanitation Standard Operating Procedure

**Developed by the facility/company**

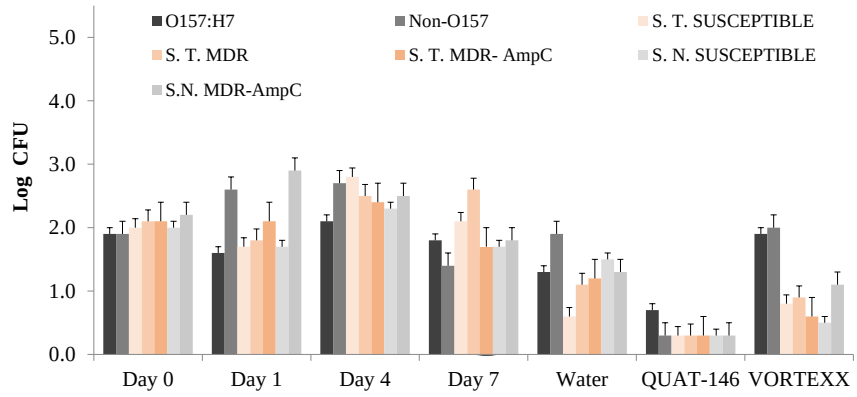
**Must address pre-operational sanitation and operational sanitation**

**Plant needs to update SSOPs to reflect changes in equipment and facilities**

- Cleaning
- Dissemble and more cleaning
- Sanitizing: Type of chemical exposure time would need to be **validated**
- Assembly and Final rinse

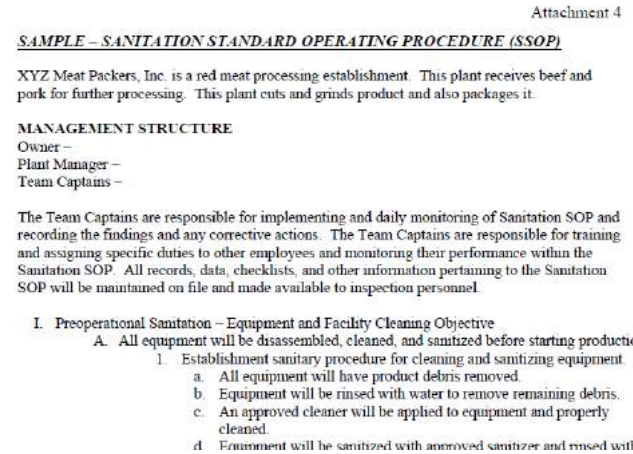
- **Pre-operational checklist:** inspection and regulatory compliance
- **Operational Sanitation:** Equipment cleaning during production
- **Post-operational sanitation:** Detailed descriptions of equipment disassembly and re-assembly

## Effect of Temperature on Microbial Growth and Sanitation
## Biofilm formation and decontamination at 4°C (40°F)




*Fouladkhah et al., 2013, J Food Science*

# A Sample of SSOP



Attachment 4

***SAMPLE – SANITATION STANDARD OPERATING PROCEDURE (SSOP)***

XYZ Meat Packers, Inc. is a red meat processing establishment. This plant receives beef and pork for further processing. This plant cuts and grinds product and also packages it.

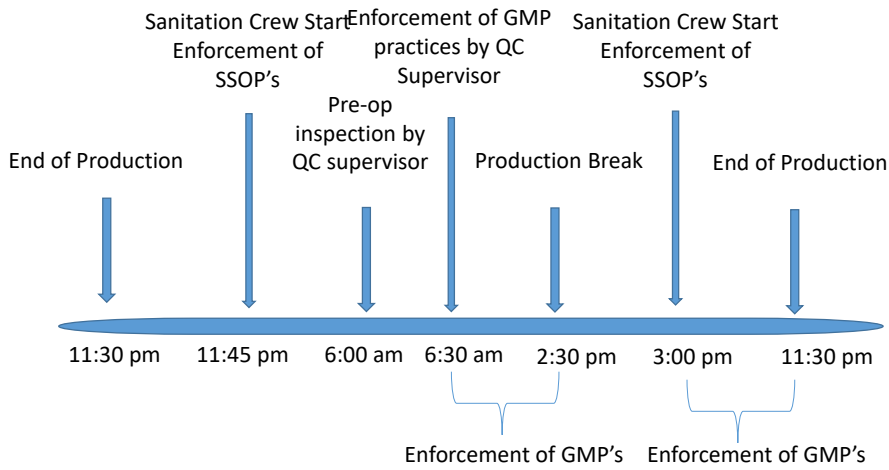
**MANAGEMENT STRUCTURE**
Owner –
Plant Manager –
Team Captains –

The Team Captains are responsible for implementing and daily monitoring of Sanitation SOP and recording the findings and any corrective actions. The Team Captains are responsible for training and assigning specific duties to other employees and monitoring their performance within the Sanitation SOP. All records, data, checklists, and other information pertaining to the Sanitation SOP will be maintained on file and made available to inspection personnel.

I. Preoperational Sanitation – Equipment and Facility Cleaning Objective
  A. All equipment will be disassembled, cleaned, and sanitized before starting production.
    1. Establishment sanitary procedure for cleaning and sanitizing equipment.
      a. All equipment will have product debris removed.
      b. Equipment will be rinsed with water to remove remaining debris.
      c. An approved cleaner will be applied to equipment and properly cleaned.
      d. Equipment will be sanitized with approved sanitizer and rinsed with

## An Operation with Food Safety Basics and Prerequisite Program

SSOP: Sanitation Standard Operating Procedure
GMP: Good Manufacturing Practices



# Pre-requisite Programs and HACCP

- Food Safety Management System
- Purpose is “prevention”…


**Table 1. HACCP Principles**

| | |
|---|---|
| **Principle 1** | Conduct a hazard analysis. |
| **Principle 2** | Determine the critical control points. |
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| **Principle 4** | Establish monitoring procedures. |
| **Principle 5** | Establish corrective actions. |
| **Principle 6** | Establish verification procedures. |
| **Principle 7** | Establish record-keeping and documentation procedures. |

# Initial validation

Initial validation is conducted **within the first weeks or months** of implementation of the HACCP plan. During initial validation, the team should aim to achieve the following:

• assure that the plan is valid for **controlling food safety hazards** associated with the ingredients, process, and product, and

• verify that the **plan can be implemented as written**. HACCP plan validation should include:

• a review of the **hazard analysis**,

• **CCP determination**,

• **justification for critical limits**, based for example on current good science and regulatory requirements, and

• determination of whether **monitoring activities**, **corrective actions**, **record keeping** procedures and verification activities are appropriate and adequate. I

f deficiencies are noted in any of these areas, the HACCP team **must revise the HACCP plan**. These changes must be implemented as rapidly as is practicable

# Revalidation, or reassessment

Revalidation , or reassessment, also is a required element of the HACCP system. It is necessary:

- after any **changes are made** that could affect the hazard analysis or the HACCP plan,
- when any changes are made to the HACCP system, and
- when **specifically required by regulatory authorities** or private standards bodies (e.g. in the United States, the federal regulatory authorities require annual reassessments of HACCP plans).

# Verification

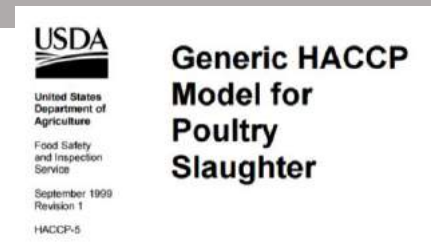
The second important element of verification procedures is to confirm that the HACCP system is **being implemented according to the written plan**. This is the part of HACCP plan verification that asks the question "**Am I actually doing what I say I should be doing**?" HACCP plan verification activities are designed to ensure that the HACCP plan is being implemented properly. This includes:

- **Verification of prerequisite programs**
- **Verification of CCPs**

Verification of the HACCP plan Verification activities can be carried out by individuals **within the company**, **third party experts**, and **regulatory agencies**. It is important that individuals conducting verification activities have appropriate technical expertise to perform this function.

# Additional Resources






# Additional Resources



## Bad Bug Book

**Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins**

### Introduction

Food safety is a complex issue that has an impact on all segments of society, from the general public to government, industry, and academia. The second edition of the Bad Bug Book, published by the Center for Food Safety and Applied Nutrition, of the Food and Drug Administration (FDA), U.S. Department of Health and Human Services, provides current information about the major known agents that cause foodborne illness. The information provided in this handbook is abbreviated and general in nature, and is intended for practical use. It is not intended to be a comprehensive scientific or clinical reference.

Under the laws administered by FDA, a food is adulterated if it contains (1) a poisonous or otherwise harmful substance that is not an inherent natural constituent of the food itself, in an amount that poses *a reasonable possibility* of injury to health, or (2) a substance that is an inherent natural constituent of the food itself, is not the result of environmental, agricultural, industrial, or other contamination; and is present in an amount that *ordinarily* renders the food

# Thank you for your time

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**Yale School of Public Health Alumnus**
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**Cellphone: (970)690-7392**

# Annex 5

*Training Program: [Instructor; Translator; Attendees; Date]*

*[A. Fouladkhah; José Almodóvar; Six Faculty members; August-25-2017]*

## Lecture and Hands-on Activity: Sensory Analyses by Trained and Untrained Panelists: Discrimination and Hedonics Testing

# Sensory Analyses by Trained and Untrained Panelists: Discrimination and Hedonics Testing

***Aliyar Fouladkhah, PhD, MPH, CFS***
***Assistant Professor***
***Public Health Microbiology Laboratory***
***Tennessee State University***

***August 25, 2017***
***Universidad ISA, Santiago de los Caballeros,***
***Dominican Republic***


## Discrimination Testing

- **Discrimination Testing** allows sensory specialists to determine if two product are different from each other.
- For **example**:

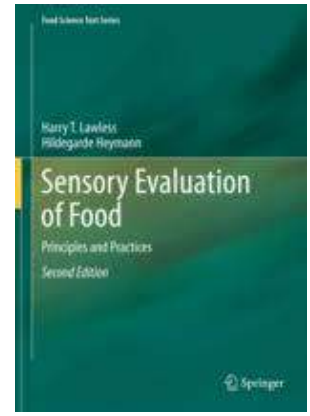
Comparison of original and low-sodium salad dressing

Comparison of ice-cream with natural and artificial vanilla flavor

- They are relatively i**nexpensive** to conduct and **do not require training of the panelists**
- All are design to answer the question:
- "Are these products perceived as different or are they similar enough to be used interchangeably?"

# Discrimination Testing

- Most Common Discrimination Tastings are:
  - Triangle
  - Duo-Trio
  - 2-AFC
  - 3-AFC
- Recently "**Tetrad Testing**" are gaining popularity since they require fewer panelist to obtain a satisfactory result.
- Recommend using the lawless text…




- **Triangle**: Which one is different?
- **Duo-Trio**: Which is the same as reference?
- **2-AFC**: Which one is more…?
- **3-AFC**: Which one is more…?

- **Triangle**: Which one is different?

Date _____
Name _____
Set _____

Rinse your mouth with water before beginning. Expectorate the water into the container provided. You received three coded samples. Two of these samples are the same and one is different. Please taste the samples in the order presented, from left to right. Circle the number of the sample that is different (odd). Rinse your mouth with water between samples and expectorate all samples and the water.

_____ _____ _____

- **Duo-Trio**: Which is the same as reference?


Date _____
Name _____

Before starting please rinse your mouth with water and expectorate. There are three samples in each of the two duo–trio sets for you to evaluate. In each set, one of the coded pairs is the same as the reference. For each set taste the reference first. Then taste each of the coded samples in the sequence presented, from left to right. Take the entire sample in your mouth. NO RETASTING. Circle the number of the sample which is most similar to the reference. Do not swallow any of the sample or the water. Expectorate into the container provided. Rinse your mouth with water between sets 1 and 2.

| Set | | | |
|---|---|---|---|
| 1 | Reference | _____ | _____ |
| 2 | Reference | _____ | _____ |

- **2-AFC**: Which one is more…?

Date _____
Name _____

Please rinse your mouth with water before starting. There are two samples in each of the two paired comparison sets for you to evaluate. Taste each of the coded samples in the set in the sequence presented, from left to right, beginning with Set 1. Take the entire sample in your mouth. NO RETASTING. Within each pair, circle the number of the sweeter sample. Rinse with water between samples and expectorate all samples and water. Then proceed to the next set and repeat the tasting sequence.

| Set | | |
|---|---|---|
| 1 | _____ | _____ |
| 2 | _____ | _____ |

- **3-AFC**: Which one is more…?

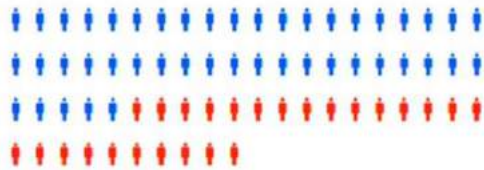
Date _____
Name _____

Please rinse your mouth with water before starting. There are two samples in each of the two paired comparison sets for you to evaluate. Taste each of the coded samples in the set in the sequence presented, from left to right, beginning with Set 1. Take the entire sample in your mouth. NO RETASTING. Within each pair, circle the number of the sweeter sample. Rinse with water between samples and expectorate all samples and water. Then proceed to the next set and repeat the tasting sequence.

_____ _____ _____

# Tetrad Testing

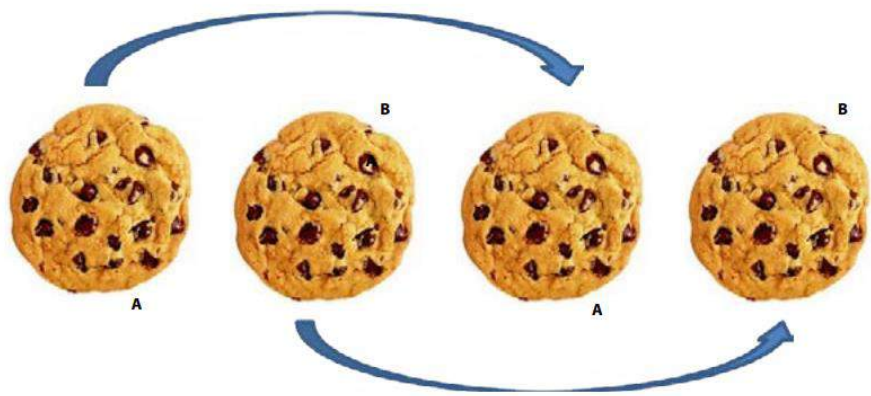
- Triangle test is the most popular sensory testing in manufacturing
- Tetrad test is gaining popularity since it allows similar results with smaller number of participants.
- As an example in a study of condensed sweetened milk:



Tetrad n=45

Triangle n=72

# Tetrad Testing



# Analysis of Discrimination Data

- Total number of panelists
- Number of Correct Judgements
- Significance level (usually chosen at 5%)

Three ways to analyze:

- **Traditional way** is using Tables from sensory text books (I would suggest: Lawless sensory book)
- Best method is to program a code in SAS or R
- Also **pre-programmed softwares** such Compusense and IFpress

# Using the table

**Table 4.3** Minimum numbers of correct judgments[a] to establish significance at probability levels of 5 and 1% for paired difference and duo–trio tests (one tailed, $p = 1/2$) and the triangle test (one tailed, $p = 1/3$)

| Paired difference and duo–trio tests | | | Triangle test | | |
|---|---|---|---|---|---|
| Number of trials ($n$) | Probability levels | | Number of trials ($n$) | Probability levels | |
| | 0.05 | 0.01 | | 0.05 | 0.01 |
| 5 | 5 | – | 3 | 3 | – |
| 6 | 6 | – | 4 | 4 | – |
| 7 | 7 | 7 | 5 | 4 | 5 |
| 8 | 7 | 8 | 6 | 5 | 6 |
| 9 | 8 | 9 | 7 | 5 | 6 |
| 10 | 9 | 10 | 8 | 6 | 7 |
| 11 | 9 | 10 | 9 | 6 | 7 |
| 12 | 10 | 11 | 10 | 7 | 8 |
| 13 | 10 | 12 | 11 | 7 | 8 |
| 14 | 11 | 12 | 12 | 8 | 9 |
| 15 | 12 | 13 | 13 | 8 | 9 |
| 16 | 12 | 14 | 14 | 9 | 10 |
| 17 | 13 | 14 | 15 | 9 | 10 |
| 18 | 13 | 15 | 16 | 9 | 11 |
| 19 | 14 | 15 | 17 | 10 | 11 |
| 20 | 15 | 16 | 18 | 10 | 12 |
| 21 | 15 | 17 | 19 | 11 | 12 |
| 22 | 16 | 17 | 20 | 11 | 13 |
| 23 | 16 | 18 | 21 | 12 | 13 |
| 24 | 17 | 19 | 22 | 12 | 14 |

# Using the table

Example:
Triangle Test
14 Participants
10 correct answer by panelists
Significance level 5%

Result:
Two products are statistically different from each other

**Table 4.3** Minimum numbers of correct judgments[a] to establish significance at probability levels of 5 and 1% for paired difference and duo–trio tests (one tailed, $p = 1/2$) and the triangle test (one tailed, $p = 1/3$)

| Paired difference and duo–trio tests | | | Triangle test | | |
|---|---|---|---|---|---|
| Number of trials ($n$) | Probability levels | | Number of trials ($n$) | Probability levels | |
| | 0.05 | 0.01 | | 0.05 | 0.01 |
| 5 | 5 | – | 3 | 3 | – |
| 6 | 6 | – | 4 | 4 | – |
| 7 | 7 | 7 | 5 | 4 | 5 |
| 8 | 7 | 8 | 6 | 5 | 6 |
| 9 | 8 | 9 | 7 | 5 | 6 |
| 10 | 9 | 10 | 8 | 6 | 7 |
| 11 | 9 | 10 | 9 | 6 | 7 |
| 12 | 10 | 11 | 10 | 7 | 8 |
| 13 | 10 | 12 | 11 | 7 | 8 |
| 14 | 11 | 12 | 12 | 8 | 9 |
| 15 | 12 | 13 | 13 | 8 | 9 |
| 16 | 12 | 14 | 14 | 9 | 10 |
| 17 | 13 | 14 | 15 | 9 | 10 |
| 18 | 13 | 15 | 16 | 9 | 11 |
| 19 | 14 | 15 | 17 | 10 | 11 |
| 20 | 15 | 16 | 18 | 10 | 12 |
| 21 | 15 | 17 | 19 | 11 | 12 |
| 22 | 16 | 17 | 20 | 11 | 13 |
| 23 | 16 | 18 | 21 | 12 | 13 |
| 24 | 17 | 19 | 22 | 12 | 14 |

# Using SAS or R

- Could be complicated at beginning to program, but researchers have more control over the analyses.
- Require some background training in programing
- SAS is relatively expensive, R is an open sourced software that is publically available.

```
libname dolphin "C:\Documents and Settings\aliyar fouladkhah\Desktop";
proc contents data= dolphin.diab2; run;
*****for table 1 :)****;
proc means data=dolphin.diab2 maxdec=1; var HSAGEIR; run;
proc freq data=dolphin.diab2;
tables DMARETHN FEMALE EDUC3 MARITAL4 INSULIN ORALMED SMOKEHX3 CCSUM3/ chisq;
run;
*****for table 2 :)****;
proc glm data=dolphin.diab2;
class DMARETHN;
model HSAGEIR=DMARETHN;
means DMARETHN/Tukey;
run;

proc freq data=dolphin.diab2;
tables FEMALE*DMARETHN / chisq;
tables EDUC3*DMARETHN / chisq;
tables MARITAL4*DMARETHN / chisq;
tables INSULIN*DMARETHN / chisq;
tables ORALMED*DMARETHN / chisq;
tables SMOKEHX3*DMARETHN / chisq;
tables CCSUM3*DMARETHN / chisq;
run;
```

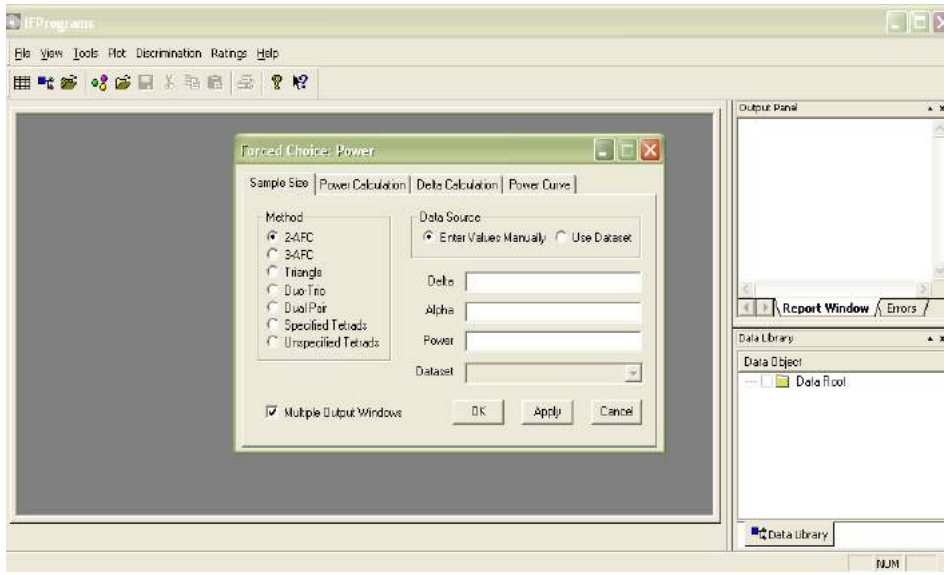
# Using Pre-Programmed Software

- Compusense, a Canadian software, very common in food manufacturing.
- Ifpress is relatively newer software with some advanced featured that is introduced by Institute for Sensory Perception in VA.

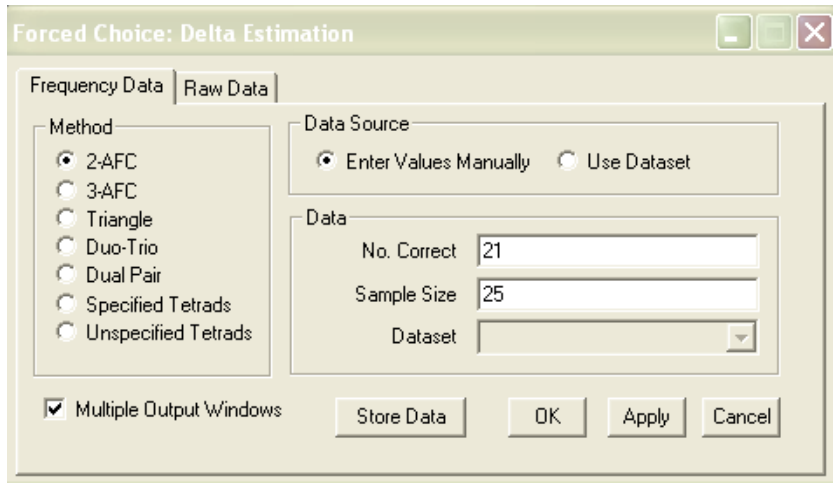
Compusense®

The Institute for Perception
Developing and Applying Advanced Research Tools for Human Perceptual Measurement

# Power and Sample Size in IFPrograms®



# 2-AFC by in IFpress®

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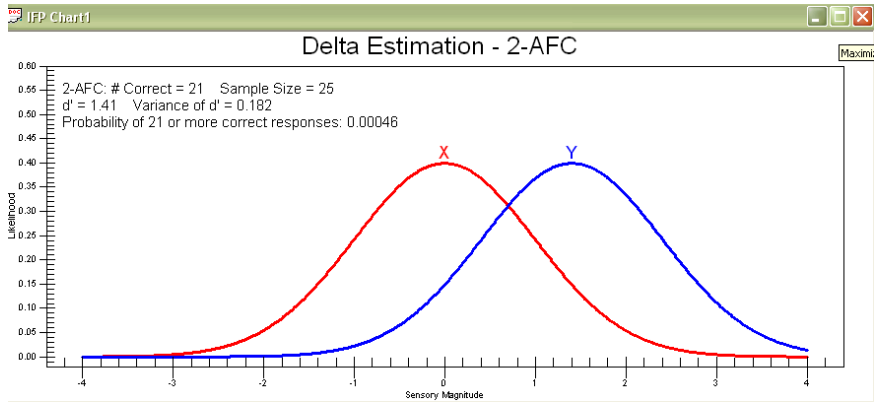
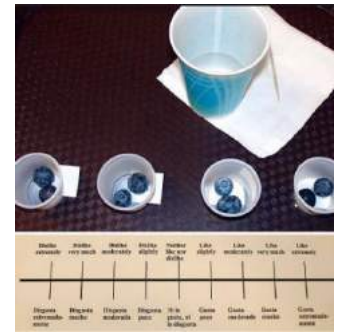


# Sensory Analysis Using Trained Panelist

- A sensory specialist would recruit and train a group of panelist to rate a specific attribute of a product.
- Usually a small group of panelists is required.
- In many case Hedonic Scales are used.



| DISLIKE EXTREMELY | DISLIKE VERY MUCH | DISLIKE MODERATELY | DISLIKE SLIGHTLY | NEITHER LIKE NOR DISLIKE | LIKE SLIGHTLY | LIKE MODERATELY | LIKE VERY MUCH | LIKE EXTREMELY |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

- Training of panelist
- Analyses of data

# An example: Sensory Analyses of Reduced Sodium Products: Back Ground Information

- Excess sodium intake is one of the main contributing factors to development of **hypertension** in adults.
- It is estimated that around **26% of people around the globe** are currently suffering from hypertension
- Hypertension is a leading cause of **Cardiovascular diseases**.
- It is estimated that, as high **16.7 million** individuals die every year around the world, including around 850,000 people in the United States, as the result of cardiovascular diseases
- It is estimated that our sodium intake is:
  - 70-75% of intake is associated with **processed foods**,
  - 10-15% to natural foods,
  - 10-15% to discretionary salt added to meals

# An example: Sensory Analyses of Reduced Sodium Products: Back Ground Information

- People in Western hemisphere are also suffering from **low potassium in diet**.
- As an example, it is estimated that **less than 1%** of American adults meet the joint dietary guidelines for sodium and potassium.
- **Potassium chloride** the main alternative for sodium chloride in low sodium, formulations.
- It has a **bitter and metallic aftertaste** so sensory analyses is need to make sure low-sodium formulations are acceptable by panelists.

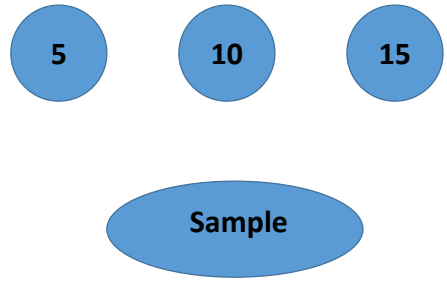
# Training of Panelist for low Sodium Products

| Ratings Level | Saltiness* (Sodium Chloride) | Sourness* (Citric Acid) | Sweetness* (Sucrose) | Bitterness* (Caffeine) | Umami* (MSG) | Astringency* (Aluminum Sulfate) | Metalicity* (Ferrous Sulfate) |
|---|---|---|---|---|---|---|---|
| 20 | 1.500 | 0.600 | 12.00 | 0.260 | 0.500 | 0.100 | 0.041 |
| 19 | 1.425 | 0.570 | 11.40 | 0.247 | 0.475 | 0.095 | 0.039 |
| 18 | 1.350 | 0.540 | 10.80 | 0.234 | 0.450 | 0.090 | 0.038 |
| 17 | 1.275 | 0.510 | 10.20 | 0.221 | 0.425 | 0.085 | 0.036 |
| 16 | 1.200 | 0.480 | 9.60 | 0.208 | 0.400 | 0.080 | 0.034 |
| 15 | 1.125 | 0.450 | 9.00 | 0.195 | 0.375 | 0.075 | 0.032 |
| 14 | 1.050 | 0.420 | 8.40 | 0.182 | 0.350 | 0.070 | 0.029 |
| 13 | 0.975 | 0.390 | 7.80 | 0.169 | 0.325 | 0.065 | 0.027 |
| 12 | 0.900 | 0.360 | 7.20 | 0.156 | 0.300 | 0.060 | 0.025 |
| 11 | 0.825 | 0.330 | 6.60 | 0.143 | 0.275 | 0.055 | 0.023 |
| 10 | 0.750 | 0.300 | 6.00 | 0.130 | 0.250 | 0.050 | 0.021 |
| 9 | 0.675 | 0.270 | 5.40 | 0.117 | 0.225 | 0.045 | 0.019 |
| 8 | 0.600 | 0.240 | 4.80 | 0.104 | 0.200 | 0.040 | 0.017 |
| 7 | 0.525 | 0.210 | 4.20 | 0.091 | 0.175 | 0.035 | 0.015 |
| 6 | 0.450 | 0.180 | 3.60 | 0.078 | 0.150 | 0.030 | 0.013 |
| 5 | 0.375 | 0.150 | 3.00 | 0.065 | 0.125 | 0.025 | 0.011 |
| 4 | 0.300 | 0.120 | 2.40 | 0.052 | 0.100 | 0.020 | 0.008 |
| 3 | 0.225 | 0.090 | 1.80 | 0.039 | 0.075 | 0.015 | 0.006 |
| 2 | 0.150 | 0.060 | 1.20 | 0.026 | 0.050 | 0.010 | 0.004 |
| 1 | 0.075 | 0.030 | 0.60 | 0.013 | 0.025 | 0.005 | 0.002 |

* % (w/v)

# Example of Training Session
## One sensation at the time: (Bitterness, Umami, etc)

- Three reference samples and one unknown to the panelist e.g. 12




# The combination of Sensations
## Metallic, Bitterness, and Astringency

Sensory Training Session #6: 4-02-2013 Panelist:

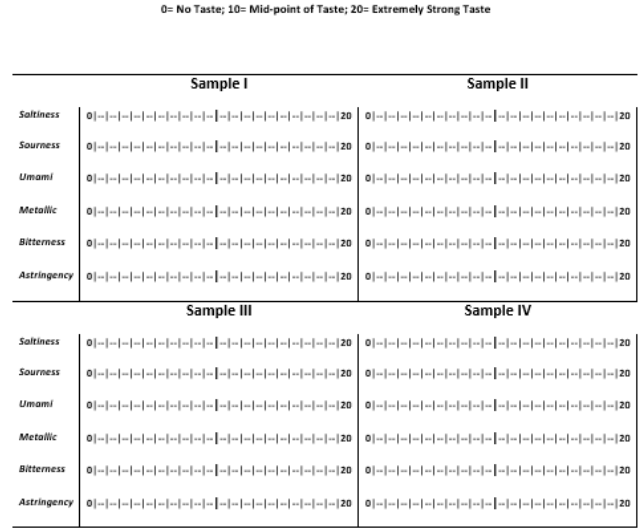
0= No Taste; 10= Mid-point of Taste; 20= Extremely Strong Taste

| Metallic Sample | Bitter Sample | Astringent Sample |
|---|---|---|
| 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 | 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 | 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 |

| | Sample I | Sample II |
|---|---|---|
| *Metallic* | 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 | 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 |
| *Bitterness* | 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 | 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 |
| *Astringency* | 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 | 0\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|--\|20 |

# Once you select you panelist: Main Trial for trained panelists

- **Seven sensations**.
- Initiated the training on **23 panel member**.
- Concluded the training with **16 panelists**.
- Their rating was used for comparing original and low-sodium products.
- **Usually takes few month**.



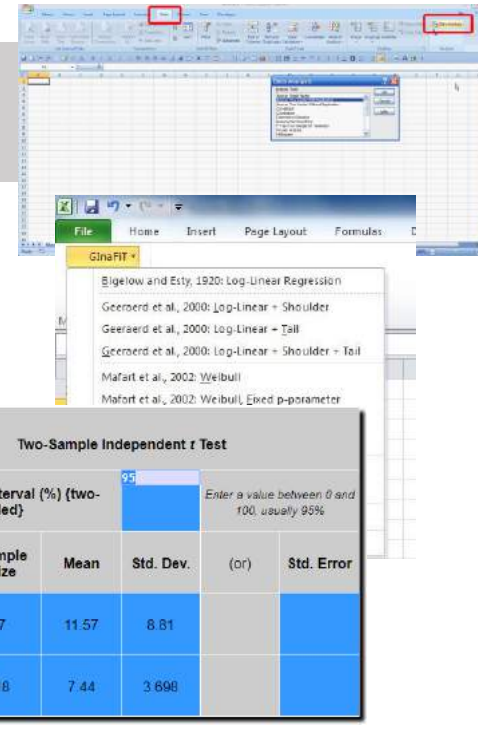

# Data Management in Excel

| | Sample I (Lo Salt Product) | | | | | | | Sample II (Origional Product) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | outlier | Saltiness | Sourness | Umami | Metallic | Bitterness | Astringency | Saltiness | Sourness | Umami | Metallic | Bitterness | Astringenc |
| Devon | 3 | 8 | 0 | 1 | 2 | 4 | 1 | 7 | 0 | 0 | 0 | 1 | 1 |
| Justin | 6 | 9 | 2 | 4 | 2 | 4 | 3 | 10 | 1 | 7 | 3 | 3 | 2 |
| Meismer | 5 | 7 | 0 | 3 | 3 | 9 | 1 | 8 | 0 | 7 | 4 | 1 | 1 |
| Julie | 5 | 4 | 4 | 7 | 4 | 3 | 2 | 7 | 3 | 3 | 2 | 2 | 2 |
| Mathew | 6 | 6 | 2 | 7 | 5 | 3 | 2 | 8 | 4 | 6 | 3 | 4 | 3 |
| Jim | 4 | 3 | 2 | 5 | 1 | 4 | 4 | 8 | 3 | 4 | 1 | 3 | 3 |
| Caryn | 2 | 4 | 1 | 4 | 2 | 1 | 3 | 5 | 0 | 4 | 1 | 1 | 4 |
| Bryan | 7 | 4 | 2 | 3 | 4 | 1 | 3 | 3 | 3 | 3 | 2 | 2 | 3 |
| Dan | 2 | 6 | 0 | 0 | 3 | 5 | 6 | 7 | 0 | 0 | 0 | 0 | 1 |
| James | 2 | 4 | 0 | 1 | 3 | 2 | 5 | 5 | 0 | 0 | 0 | 0 | 1 |
| Lisa | 4 | 4 | 2 | 3 | 0 | 0 | 0 | 8 | 1 | 3 | 2 | 0 | 0 |
| Hirn | 13 | 7 | 0 | 9 | 8 | 9 | 3 | 9 | 0 | 8 | 7 | 3 | 5 |
| Ryan R | 10 | 9 | 0 | 4 | 2 | 0 | 3 | 8 | 0 | 5 | 4 | 3 | 3 |
| Peters | 2 | 4 | 1 | 1 | 1 | 1 | 1 | 4 | 1 | 2 | 1 | 1 | 2 |
| Gina | 5 | 6 | 1 | 4 | 1 | 2 | 4 | 6 | 3 | 5 | 0 | 1 | 4 |
| Deb W | 5 | 5 | 0 | 1 | 4 | 2 | 1 | 10 | 0 | 0 | 0 | 4 | 2 |
| | | 5.6 | 1.1 | 3.6 | 2.8 | 3.1 | 2.6 | 7.1 | 1.2 | 3.6 | 1.9 | 1.8 | 2.3 |
| | | 1.9 | 1.2 | 2.5 | 1.9 | 2.7 | 1.6 | 2.0 | 1.5 | 2.7 | 2.0 | 1.4 | 1.4 |

# Data Analyses

- Comparing two products: T-test
- Comparing Multiple Products: ANOVA
- Calculation of Sample Size

- Free Software:
- Analysis Tool Pack (Add-on to Excel)
- OpenEpi
- GInaFiT (Add-on to Excel)




# Monday…

- A brief 10 minute lecture
- Then exercise to use the software for analysis
- We would need to have laptop and internet connection

# Final thoughts…
# Common Sensory Problems

- Would need to consider these in a design

- Positional Bias
- Memory Issue
- Sensory Fatigue


Thank you

# Annex 6

*Training Program: [Instructor; Translator; Attendees; Date]*

*[A. Fouladkhah; Rafael Marte Aracena; Eight Faculty members; August-28-2017]*

## Lecture and Workshop: Analyses of Research Data: Sample Size calculations, Power Analysis, T-Test, and ANOVA

# Analyses of Research Data: Sample Size calculations, Power Analysis, T-Test, and ANOVA

***Aliyar Fouladkhah, PhD, MPH, CFS***
***Assistant Professor***
***Public Health Microbiology Laboratory***
***Tennessee State University***

***August 28, 2017***
***Universidad ISA, Santiago de los Caballeros,***
***Dominican Republic***

TENNESSEE STATE UNIVERSITY

## *Using OpenEpi for Analyses of Research Data*

***Available at no cost: http://www.openepi.com/Menu/OE_Menu.htm***

# Exercise 1:
# Sample Size Calculation for Frequency in a Population

- A group of entomologists/Researchers would like to study **prevalence** of Wild-Type Fruit Flies in Agricultural setting in order to determine efficacy of sterilized male flies on eradication of the fly in an area.
- It is anticipated that there are close to **68,000 farming operations** in the area.
- Based on preliminary data, the anticipated **frequency is around 15%**
- For obtaining a **95% confidence limit**, how many insect traps (**sample size**) is needed for a random sampling?

# Exercise 1:
# Sample Size Calculation for Frequency in a Population

- Sample Size
  - Proportion
  - Unmatched CC
  - Cohort/RCT
  - Mean Difference

| Sample Size for % Frequency in a Population (Random Sample) | | |
|---|---|---|
| Population size | 68000 | If large, leave as one million |
| Anticipated % frequency(p) | 15 | Between 0 & 99.99. If unknown, use 50% |
| Confidence limits as +/- percent of 100 | 5 | Absolute precision % |
| Design effect (for complex sample surveys--DEFF) | 1 | 1.0 for random sample |

# Exercise 1:
# Sample Size Calculation for Frequency in a Population

| | |
|---|---|
| Population size(for finite population correction factor or fpc)(*N*): | 68000 |
| Hypothesized % frequency of outcome factor in the population (*p*): | 15%+/- |
| Confidence limits as % of 100(absolute +/- %)(*d*): | 5% |
| Design effect (for cluster surveys-*DEFF*): | 1 |

**Sample Size(*n*) for Various Confidence Levels**

| ConfidenceLevel(%) | Sample Size |
|---|---|
| 95% | 196 |
| 80% | 84 |
| 90% | 138 |
| 97% | 240 |
| 99% | 337 |
| 99.9% | 548 |
| 99.99% | 764 |

Equation

Sample size $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$

Results from OpenEpi, Version 3, open source calculator--SSPropor
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 2:
# Sample Size Calculation for Frequency in a Population

- An epidemiologists would like to study **prevalence** of Salmonella serovars in backyard poultries in a region.
- It is anticipated that there are more than 1m poultry in the area
- Based on preliminary data, the anticipated **frequency is around 65%**
- For obtaining a **95% confidence limit**, how many poultries (**sample size**) is needed for a random sampling?

# Exercise 2:
# Sample Size Calculation for Frequency in a Population

| Sample Size for % Frequency in a Population (Random Sample) | | |
|---|---|---|
| Population size | 1000000 | If large, leave as one million |
| Anticipated % frequency(p) | 65 | Between 0 & 99.99. If unknown, use 50% |
| Confidence limits as +/- percent of 100 | 5 | Absolute precision % |
| Design effect (for complex sample surveys--DEFF) | 1.0 | 1.0 for random sample |

# Exercise 2:
# Sample Size Calculation for Frequency in a Population

**Sample Size for Frequency in a Population**

Population size(for finite population correction factor or fpc)(*N*): 1000000
Hypothesized % frequency of outcome factor in the population (*p*): 65%+/-5
Confidence limits as % of 100(absolute +/- %)(*d*): 5%
Design effect (for cluster surveys-*DEFF*): 1

**Sample Size(*n*) for Various Confidence Levels**

| ConfidenceLevel(%) | Sample Size |
|---|---|
| 95% | 350 |
| 80% | 150 |
| 90% | 247 |
| 97% | 429 |
| 99% | 604 |
| 99.9% | 985 |
| 99.99% | 1377 |

Equation

Sample size $n = [DEFF^{*}Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}{}^{*}(N-1)+p^{*}(1-p)]$

Results from OpenEpi, Version 3, open source calculator--SSPropor
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 3:
# Sample Size Calculation for Comparing two means

- An sensory scientist had developed a low-sodium formulation of salad dressing.
- Based on a preliminary sensory trial the mean and Standard Deviation of saltiness associated with the products are:

  Original Product: Mean= 12.23; SD=1.72

  Low-Sodium Product: Mean= 10.17; SD=2.24

- At **confidence level of 95%,** and at **statistical power of 80%,** how many panelists (**sample size**) are needed for a sensory trial with equal panelists for each product?

# Exercise 3:
# Sample Size Calculation for Comparing two means

| Sample Size For Comparing Two Means | | | | | |
|---|---|---|---|---|---|
| Confidence Interval % (two-sided) | | | 95 | *Enter a value between 0 and 100, usually 95%* | |
| Power | | | 80 | *Enter a value between 0 and 100, usually 80%* | |
| Ratio of sample size (Group 2/Group 1) | | | 1 | | |
| | Group 1 | | Group 2 | Enter means OR difference on next line | |
| Mean | 12.23 | and | 10.17 | or Difference | |
| Std. Dev. | 1.72 | | 2.24 | *Enter Std. Deviation OR Variance of each group* | |
| Variance | | | | | |

# Exercise 3:
# Sample Size Calculation for Comparing two means

**Sample Size For Comparing Two Means**

**Input Data**

| | | | |
|---|---|---|---|
| Confidence Interval (2-sided) | 95% | | |
| Power | 80% | | |
| Ratio of sample size (Group 2/Group 1) | 1 | | |
| | **Group 1** | **Group 2** | **Difference*** |
| Mean | 12.23 | 10.17 | 2.06 |
| Standard deviation | 1.72 | 2.24 | |
| Variance | 2.9584 | 5.0176 | |
| Sample size of Group 1 | 15 | | |
| Sample size of Group 2 | 15 | | |
| Total sample size | 30 | | |

*Difference between the means

Results from OpenEpi, Version 3, open source calculator--SSMean
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 4:
# Power Analysis

- An food scientist had developed a low-nitrate formulation of fermented sausage.
- Based on a chemical analysis of 10 sample per product the mean and Standard Deviation of Nitrate associated with the products are:

  Original Product: Mean= 320.25 PPM; SD=55.36

  Low-Nitrate Product: Mean= 217.35 PPM; SD=75.65
- At **confidence level of 95%,** what is the **statistical power** of this trial?

# Exercise 4: Power Analysis

| Power For Comparing Two Means | | | | | |
|---|---|---|---|---|---|
| Confidence Interval(%) {two-sided} | | | 95 | Enter a value between 0 and 100, usually 95% | |
| | Group 1 | | Group 2 | Enter means or difference below | |
| Mean | 320.25 | and | 217.35 | Or Difference | |
| Sample size | 10 | | 10 | | |
| Std.Dev. | 55.36 | | 75.65 | Enter Std. Deviation OR Variance of each group | |
| Variance | | | | | |

# Exercise 4: Power Analysis

**Power For Comparing Two Means**

**Input Data**

| Two-sided 95% Confidence Interval | | | |
|---|---|---|---|
| | **Group 1** | **Group 2** | **Difference*** |
| Mean | 320.25 | 217.35 | 102.9 |
| Sample size | 10 | 10 | |
| Standard deviation | 55.36 | 75.65 | |
| Variance | 3064.73 | 5722.92 | |

**Power = 93.47%**
**by the normal approximation method**

* Mean difference= (Group 1 mean) - (Group 2 mean)

Results from OpenEpi, Version 3, open source calculator--PowerMean
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 5:
# Power Analysis for Cross-sectional Study

- An researcher would have studies the effects of vaccinations on prevalence Avian Influenza in domestic poultry.
- The prevalence of the disease is:

  Vaccinated (Exposed): 15%; Sample size=6

  Non-Vaccinated (Non-Exposed): 65%; Sample size=6
- At **confidence level of 95%,** what is the **statistical power** of this trial?

# Exercise 5:
# Power Analysis for Cross-sectional Study

| Power for Cross-Sectional Studies | | |
|---|---|---|
| Confidence Interval (%) {two-sided} | 95 | Enter between 0 and 100, |
| | **Exposed** | **Non-exposed** |
| Sample Size | 6 | 6 |
| Prevalence (or) Coverage (%) | 15 | 65 |

# Exercise 5:
# Power Analysis for Cross-sectional Study

**Power for Cross-Sectional Studies**

| | Input Data |
|---|---|
| **Two sided-confidence interval (%)** | 95 |
| **Number of Exposed** | 6 |
| **Prevalence/Coverage among Exposed (%)** | 15 |
| **Number of Non-exposed** | 6 |
| **Prevalence/Coverage among Non-exposed (%)** | 65 |
| **Prevalence/Coverage Ratio** | 0.23 |
| **Prevalence Difference (%)[1]** | -50 |
| **Power based on:** | |
| Normal approximation | 41.16% |
| Normal approximation with continuity correction | 13.71% |

[1] Prevalence Difference = Prevalence in Exposed - Prevalence in Non-exposed.

Results from OpenEpi, Version 3, open source calculator--PowerCros
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 6:
# T- Test

- An sensory scientist had developed a low-sodium formulation of salad dressing.
- Based on sensory trial the mean and Standard Deviation of saltiness associated with the products are:

  Original Product: Mean= 12.23; SD=1.72, Sample Size= 10

  Low-Sodium Product: Mean= 10.17; SD=2.24, Sample Size= 12

- At **confidence level of 95%,** are these products statistically different in saltiness perception?

# Exercise 6: T- Test

| Two-Sample Independent *t* Test | | | | | |
|---|---|---|---|---|---|
| Confidence Interval (%) {two-sided} | | | 95 | *Enter a value between 0 and 100, usually 95%* | |
| | Sample Size | Mean | Std. Dev. | (or) | Std. Error |
| Group 1 | 10 | 12.23 | 1.72 | | |
| Group 2 | 12 | 10.17 | 2.24 | | |

# Exercise 6: T- Test

**Two-Sample Independent *t* Test**

**Input Data**

Two-sided confidence interval 95%

| | Sample size | Mean | Std. Dev. | Std. Error |
|---|---|---|---|---|
| Group-1 | 10 | 12.23 | 1.72 | |
| Group-2 | 12 | 10.17 | 2.24 | |

| Result | *t* statistics | *df* | p-value[1] | Mean Difference | Lower Limit | Upper Limit |
|---|---|---|---|---|---|---|
| Equal variance | 2.37867 | 20 | 0.02745 | 2.06 | 0.2535 | 3.8665 |
| Unequal variance | 2.43796 | 20 | 0.02423 | 2.06 | 0.297433 | 3.82257 |

| | *F* statistics | *df*(numerator,denominator) | p-value[1] |
|---|---|---|---|
| Test for equality of variance[2] | 1.69605 | 11,9 | 0.4367 |

# Exercise 6: T- Test

- An sensory scientist had developed a low-sodium formulation of salad dressing.
- Based on sensory trial the mean and Standard Deviation of saltiness associated with the products are:

  Original Product: Mean= 12.23; SD=1.72, Sample Size= 10

  Low-Sodium Product: Mean= 10.17; SD=2.24, Sample Size= 12
- At **confidence level of 95%,** are these products statistically different in saltiness perception?

# Exercise 7: T-Test

- An food scientist had developed a low-nitrate formulation of fermented sausage.
- Based on a chemical analysis of 10 sample per product the mean and Standard Deviation of Nitrate associated with the products are:

  Original Product: Mean= 320.25 PPM; SD=55.36

  Low-Nitrate Product: Mean= 217.35 PPM; SD=23.65
- At **confidence level of 95%,** are these products statistically different from each other?

# Exercise 7: T-Test

| Two-Sample Independent *t* Test | | | | | |
|---|---|---|---|---|---|
| Confidence Interval (%) {two-sided} | | | 95 | *Enter a value between 0 and 100, usually 95%* | |
| | Sample Size | Mean | Std. Dev. | (or) | Std. Error |
| Group 1 | 10 | 320.25 | 55.36 | | |
| Group 2 | 10 | 217.35 | 23.65 | | |

# Exercise 7: T-Test

**Two-Sample Independent *t* Test**

**Input Data**

Two-sided confidence interval 95%

| | Sample size | Mean | Std. Dev. | Std. Error |
|---|---|---|---|---|
| Group-1 | 10 | 320.25 | 55.36 | |
| Group-2 | 10 | 217.35 | 23.65 | |

| Result | *t* statistics | *df* | p-value[1] | Mean Difference | Lower Limit | Upper Limit |
|---|---|---|---|---|---|---|
| Equal variance | 5.40528 | 18 | 0.00003895 | 102.9 | 62.9049 | 142.895 |
| Unequal variance | 5.40528 | 12 | 0.0001587 | 102.9 | 61.4224 | 144.378 |

| | *F* statistics | *df*(numerator,denominator) | p-value[1] |
|---|---|---|---|
| Test for equality of variance[2] | 5.47936 | 9,9 | 0.01846 |

[1] p-value (two-tailed)
[2] Hartley's *f* test for equality of variance

# Exercise 8: ANOVA

- A researcher wants to know the yield associated to three varieties of Guava.
- After a harvest season the yield associated to cultivars are:

  Variety 1: Mean= 83.9 kg; SD=6.5; Sample size=12.

  Variety 2: Mean= 45.7 kg; SD=7.6; Sample size=10.

  Variety 3: Mean= 93.2 kg; SD=10.4; Sample size=11.
- At **confidence level of 95%,** are these products statistically different from each other?

# Exercise 8: ANOVA

| Analysis of Variance (ANOVA) | | | | | |
|---|---|---|---|---|---|
| | N (counts) | Mean | Std. Dev. | (or) | Std. Error |
| Group 1 | 12 | 83.9 | 6.5 | | |
| Group 2 | 10 | 45.7 | 7.65 | | |
| Group 3 | 11 | 93.2 | 10.4 | | |
| Group 4 | | | | | |
| Group 5 | | | | | |
| Group 6 | | | | | |
| Group 7 | | | | | |
| Group 8 | | | | | |
| Group 9 | | | | | |
| Group 10 | | | | | |

Enter your summary data in any respective group, no need to be consecutive. (see the

# Exercise 8: ANOVA

**Analysis of Variance (ANOVA)**

**Input Data**

| Group | N (count) | Mean | Std. Dev. | Std. error |
|---|---|---|---|---|
| 1 | 12 | 83.9 | 6.5 | |
| 2 | 10 | 45.7 | 7.65 | |
| 3 | 11 | 93.2 | 10.4 | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |

**ANOVA Table**

| Source of variation | Sum of squares | d.f | Mean square | *F* statistics | p-value[1] |
|---|---|---|---|---|---|
| Between Groups | 13173.1 | 2 | 6586.56 | 95.3168 | 0.000000000000100402 |
| Within Groups | 2073.05 | 30 | 69.1018 | | |
| Total | 15246.2 | 32 | | | |

| | Chi square | d.f | p-value[1] |
|---|---|---|---|
| Test for equality of variance | 2.32291 | 2 | 0.31303 |

| | | 95% CI of individual sample mean | | 95% CI assuming equal variance | |
|---|---|---|---|---|---|
| Group | Mean | Lower Limit | Upper Limit | Lower Limit | Upper Limit |
| 1 | 83.9 | 79.7701 | 88.0299 | 78.6183 | 89.1817 |
| 2 | 45.7 | 40.2276 | 51.1724 | 39.7535 | 51.6465 |
| 3 | 93.2 | 86.2131 | 100.187 | 87.6154 | 98.7846 |

# Exercise 8: ANOVA- Paired Comparisons

| | 95% CI of individual sample mean | | | 95% CI assuming equal variance | |
|---|---|---|---|---|---|
| Group | Mean | Lower Limit | Upper Limit | Lower Limit | Upper Limit |
| 1 | 83.9 | 79.7701 | 88.0299 | 78.6183 | 89.1817 |
| 2 | 45.7 | 40.2276 | 51.1724 | 39.7535 | 51.6465 |
| 3 | 93.2 | 86.2131 | 100.187 | 87.6154 | 98.7846 |

# Exercise 9: ANOVA

- A researcher wants to know the effects of 5% lactic acid for decontamination of red meat from *E. coli* O157:H7 at 25 and 55 degree C.
- After a application of
  - Control: Mean= 7.5 log/cm2; SD=1.1; Sample size=6.
  - Trt @ 25 C: Mean= 5.8 log/cm2; SD=0.9; Sample size=6.
  - Trt @ 55 C: Mean= 3.4 log/cm2; SD=0.6; Sample size=6.
- At **confidence level of 95%,** are these treatments effective to control the bacterium?

# Exercise 9: ANOVA

Analysis of Variance (ANOVA)

| | N (counts) | Mean | Std. Dev. | (or) | Std. Error |
|---|---|---|---|---|---|
| Group 1 | 6 | 7.5 | 1.1 | | |
| Group 2 | 6 | 5.8 | 0.9 | | |
| Group 3 | 6 | 3.4 | 0.6 | | |
| Group 4 | | | | | |
| Group 5 | | | | | |
| Group 6 | | | | | |
| Group 7 | | | | | |
| Group 8 | | | | | |
| Group 9 | | | | | |
| Group 10 | | | | | |

Enter your summary data in any respective group, no need to be consecutive. (see the

# Exercise 9:
# ANOVA

**Analysis of Variance (ANOVA)**

**Input Data**

| Group | N (count) | Mean | Std. Dev. | Std. error |
|---|---|---|---|---|
| 1 | 6 | 7.5 | 1.1 | |
| 2 | 6 | 5.8 | 0.9 | |
| 3 | 6 | 3.4 | 0.6 | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |

**ANOVA Table**

| Source of variation | Sum of squares | d.f | Mean square | *F* statistics | p-value[1] |
|---|---|---|---|---|---|
| Between Groups | 50.92 | 2 | 25.46 | 32.0924 | 0.0000038095 |
| Within Groups | 11.9 | 15 | 0.793333 | | |
| Total | 62.82 | 17 | | | |

| | Chi square | d.f | p-value[1] |
|---|---|---|---|
| Test for equality of variance | 1.59436 | 2 | 0.450598 |

| | | 95% CI of individual sample mean | | 95% CI assuming equal varianc | |
|---|---|---|---|---|---|
| Group | Mean | Lower Limit | Upper Limit | Lower Limit | Upper Limit |
| 1 | 7.5 | 6.34562 | 8.65438 | 6.56527 | 8.43473 |
| 2 | 5.8 | 4.8555 | 6.7445 | 4.86527 | 6.73473 |
| 3 | 3.4 | 2.77034 | 4.02966 | 2.46527 | 4.33473 |

# Exercise 9:
# ANOVA- Paired Comparisons

| | | 95% CI of individual sample mean | | 95% CI assuming equal varianc | |
|---|---|---|---|---|---|
| Group | Mean | Lower Limit | Upper Limit | Lower Limit | Upper Limit |
| 1 | 7.5 | 6.34562 | 8.65438 | 6.56527 | 8.43473 |
| 2 | 5.8 | 4.8555 | 6.7445 | 4.86527 | 6.73473 |
| 3 | 3.4 | 2.77034 | 4.02966 | 2.46527 | 4.33473 |

# Exercise 10: Confidence Interval

- A farmer wants to know the average size of the king avocados grown in his operations.
- He has 123 trees each approximately yielding 50 avocados per year. (123*50= 6,150).
- He took 20 samples: Mean= 533 g; SD= 32
- At **confidence level of 95%,** what is the weight of the avocados from this farm?

# Exercise 10: Confidence Interval

**Confidence Intervals for a Sample Mean**

| | | | | | | |
|---|---|---|---|---|---|---|
| Sample Mean | 533 | | | | | |
| Sample Std. Deviation | 32 | or Std Error | | or Var-iance | | (Enter one) |
| Sample Size | 20 | | | | | |
| Population Size | 6150 | | | | | |
| Confidence Level (%) | 95 | | | | | |

# Exercise 10: Confidence Interval

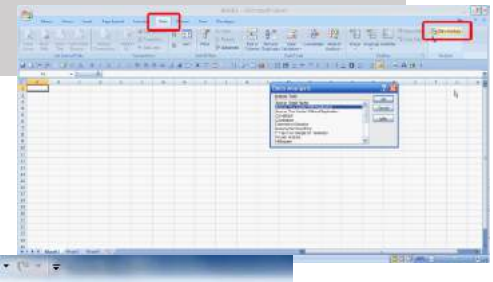
**Confidence Intervals for a Sample Mean**

| | Input Data | | | | |
|---|---|---|---|---|---|
| Sample Mean | 533 | | | | |
| Sample Std. Deviation | 32 | Std. Error | 7.15542 | Variance | 1024 |
| Sample size | 20 | | | | |
| Population size | 6150 | | | | |
| Confidence Interval | 95% (100) | | | | |

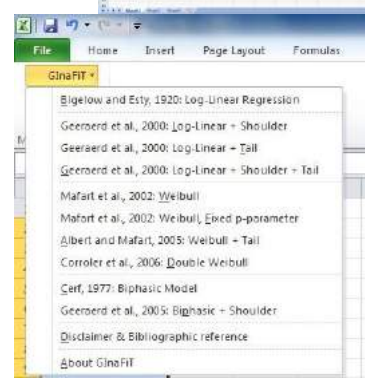
**95% Confidence Limits for the Mean of 533**

| Based on: | Lower Limit | Upper Limit |
|---|---|---|
| z-test | 518.998 | 547.002 |
| t-test | 518.047 | 547.953 |

## Other Options…



- Comparing two products: T-test
- Comparing Multiple Products: ANOVA
- Calculation of Sample Size




- Free Software:
- Analysis Tool Pack (Add-in to Excel)
- GInaFiT (Add-on to Excel)

# Annex 7

*Training Program: [Instructor; Translator; Attendees; Date]*

*[A. Fouladkhah; Rafael Marte Aracena; Four Faculty; August-29-2017]*

## Lecture and Hands-on Activity: Validating Sanitation Procedure, Calculating Inactivation Indices & Overview of Challenge Studies

# Validating Sanitation Procedure, Calculating Inactivation Indices and Overview of Challenge Studies

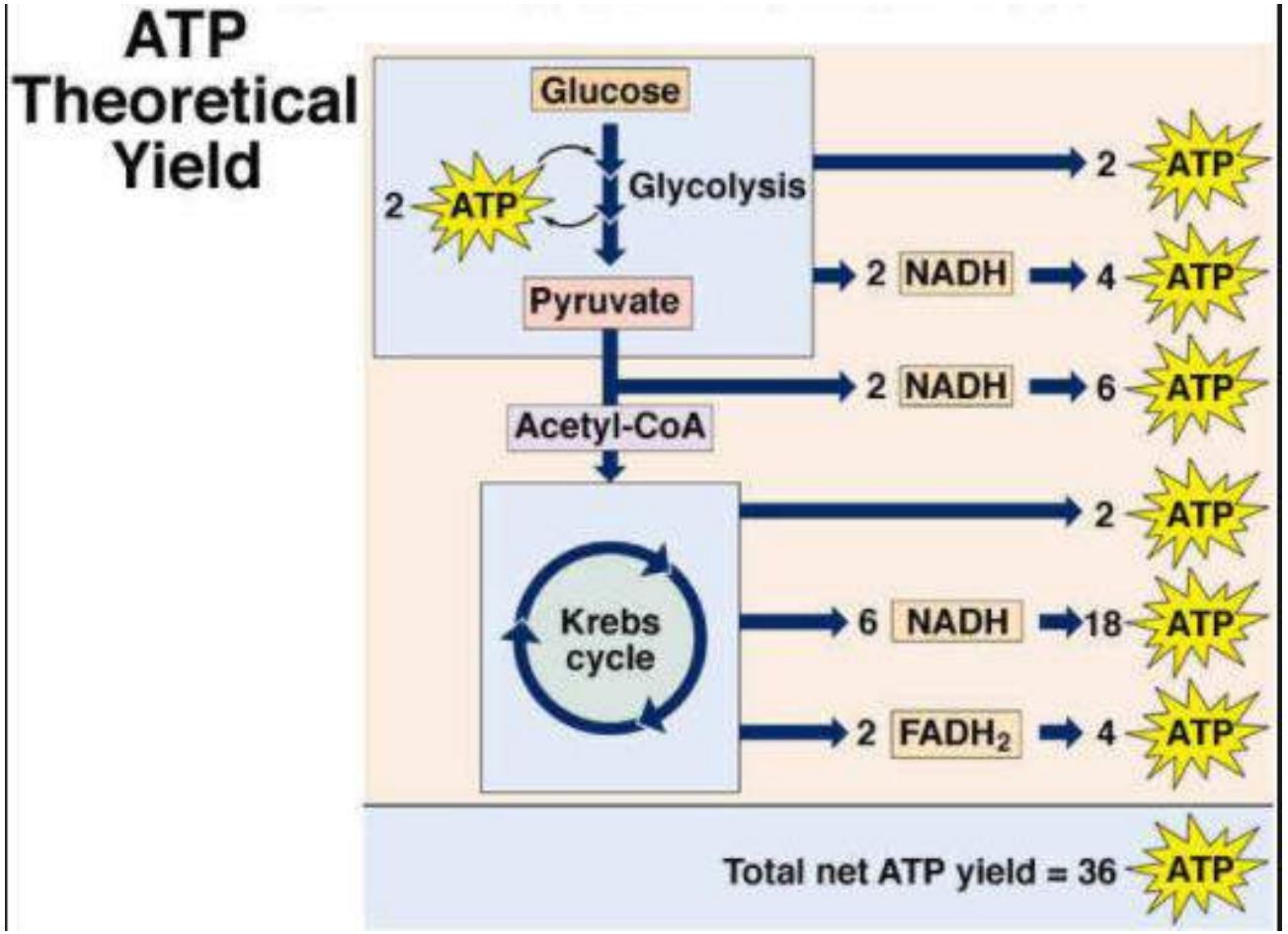
***Aliyar Fouladkhah, PhD, MPH, CFS***
***Assistant Professor***
***Public Health Microbiology Laboratory***
***Tennessee State University***

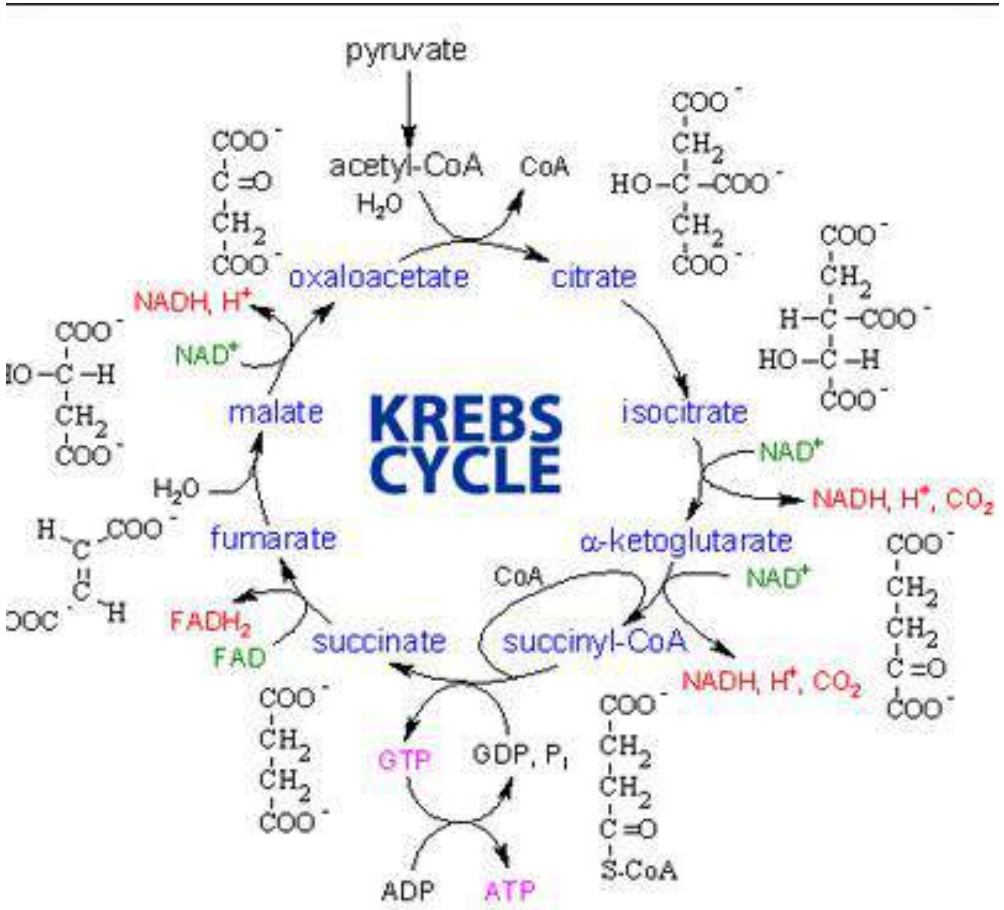
***August 29, 2017***
***Universidad ISA, Santiago de los Caballeros,***
***Dominican Republic***

- A practical tool for validation of sanitation before operation
- Using on-line software for calculation of inactivation indices
- FDA BAM and USDA Microbiology Laboratory Guidebook Overview
- Summary of inoculation procedure

# A practical tool for validation of sanitation before operation

# Krebs Cycle and ATP

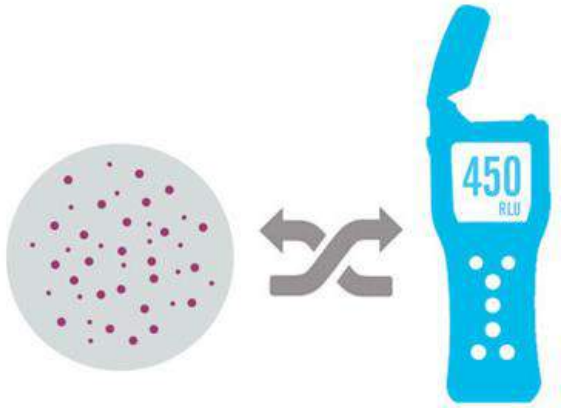


# Validating a Sanitation Procedure by ATP Measurement

- Relative Light Unit is measured as indicator of ATP and microbial presence
- New unit are cable of *E. coli*, Coliform, and *Listeria* testing as well

| CFU | Equivalent RLU (EnSURE) |
|---|---|
| ≤40 | ≤10 |
| 125 | 30 |
| 300 | 100 |
| 800 | 300 |
| 2,000 | 1,000 |
| 5,500 | 3,000 |
| 15,000 | 10,000 |




# Using on-line software for calculation of inactivation indices

# Using an on-line software for calculation of inactivation indices

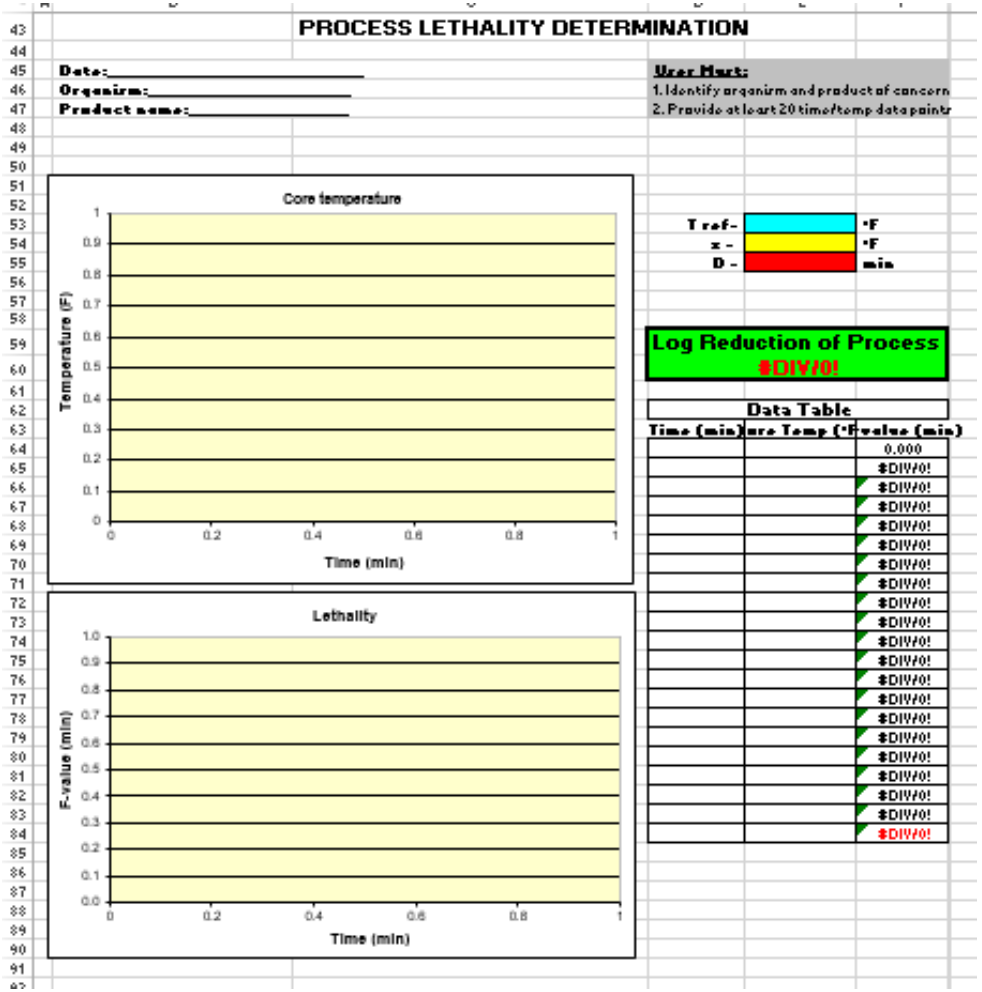
- Inactivation indices were calculated based on linear and non-linear models.

- **Linear Index** of D-value was obtain using American Meat Institute Foundation Process Lethality Spreadsheet*
- **D-value:** decimal reduction time and required at a given condition to kill 90% (or 1 log) of the exposed microorganisms

- **Non-Linear Index** of K max were obtain using the **GInaFit software**** based on best fitted non-linear model . Maximum $R^2$ was chosen as goodness-of-fit criterion.
- **$K_{max}$ value:** Number of log reductions per unit of time

  - *Available at: http://meatpoultryfoundation.org/content/process-lethality-spreadsheet
  - ** https://www.ncbi.nlm.nih.gov/pubmed/15893399

# American Meat Institute Foundation Process Lethality Spreadsheet




*Available at: http://meatpoultryfoundation.org/content/process-lethality-spreadsheet

# GInaFiT Software

Bigelow and Esty, 1920: Log-Linear Regression

Geeraerd et al., 2000: Log-Linear + Shoulder

Geeraerd et al., 2000: Log-Linear + Tail

Geeraerd et al., 2000: Log-Linear + Shoulder + Tail

Mafart et al., 2002: Weibull

Mafart et al., 2002: Weibull, Fixed p-parameter

Albert and Mafart, 2005: Weibull + Tail

Coroller et al., 2006: Double Weibull

Cerf, 1977: Biphasic Model

Geeraerd et al., 2005: Biphasic + Shoulder

| | | | | |
|---|---|---|---|---|
| f | 0.9997 | 0.00033 | **Mean Sum of Squared Error** | 0.2816 |
| kmax1 | 3.34 | 0.38 | **Root Mean Sum of Squared Error** | 0.5307 |
| kmax2 | 0.41 | 0.12 | **R-Square** | 0.9349 |
| LOG10(N0) | 6.35 | 0.20 | **R-Square adjusted** | 0.9288 |
| | | | **4D reduction is reached at** | ±3.4 |

| **Parameters** | **Paramete** | **Standard Error** | | |
|---|---|---|---|---|
| kmax | 0.52 | 0.09 | **Mean Sum of Squared Error** | 0.6893 |
| LOG10(N0) | 5.76 | 0.22 | **Root Mean Sum of Squared Error** | 0.8303 |
| | | | **R-Square** | 0.5257 |
| | | | **R-Square adjusted** | 0.5104 |

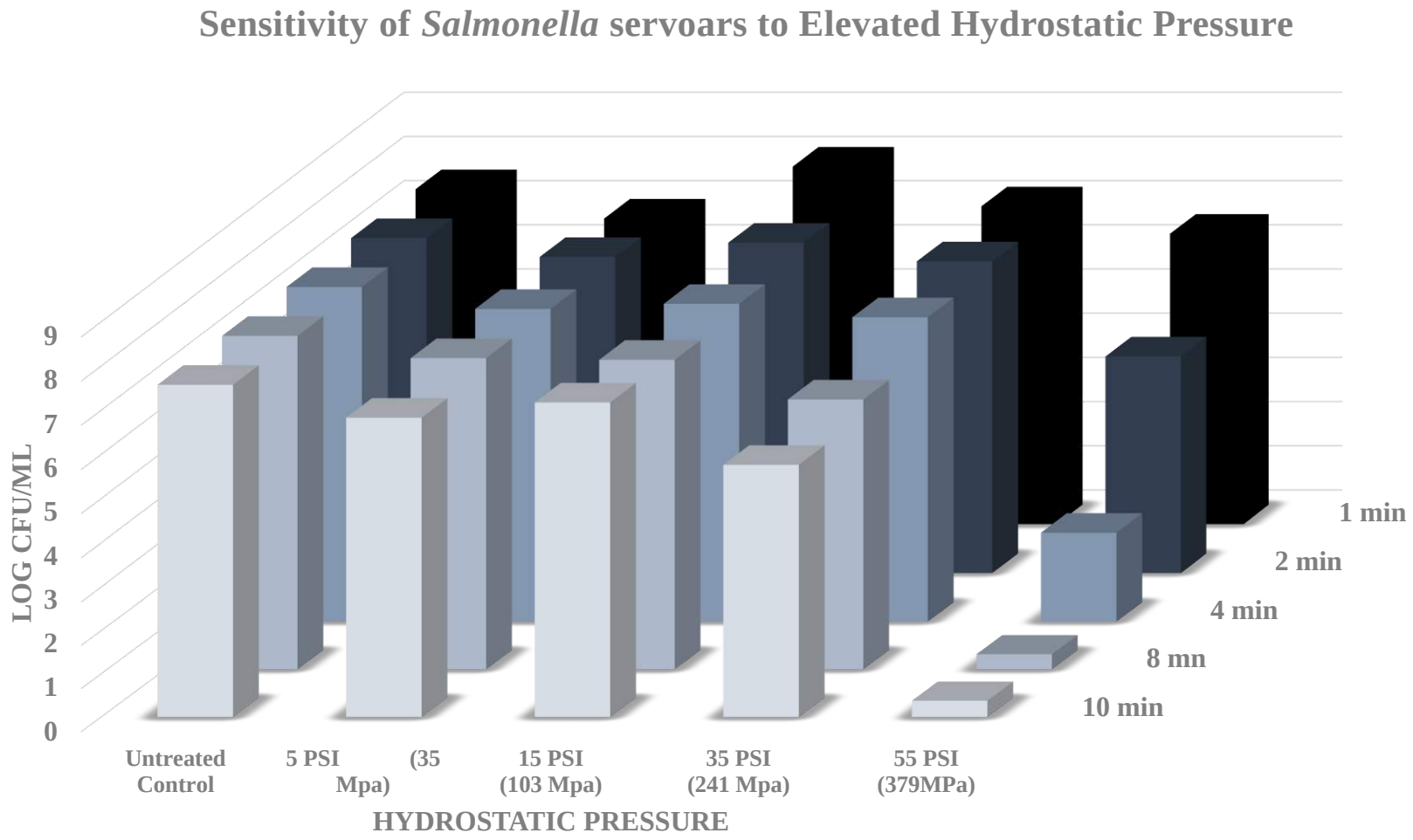
**Inactivation model identified**

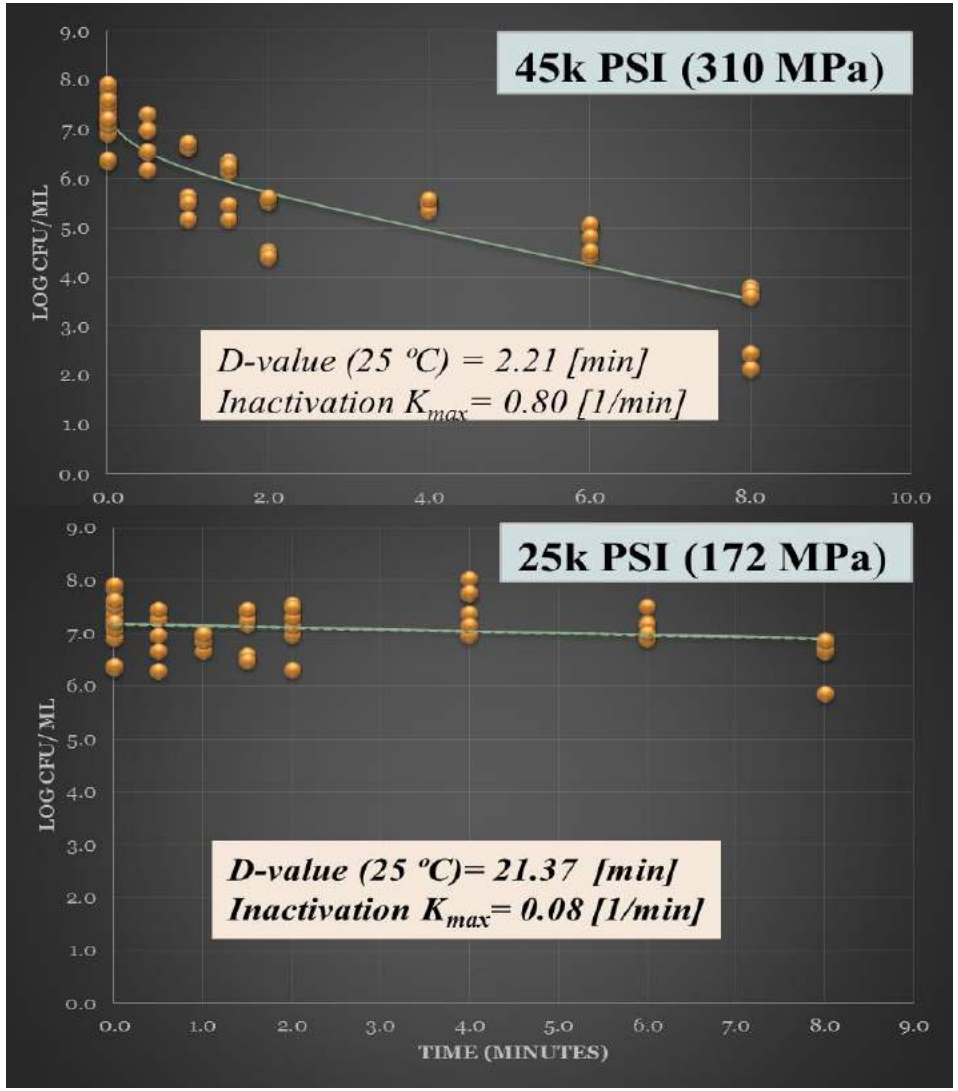
N= N0 * exp(-kmax * t)

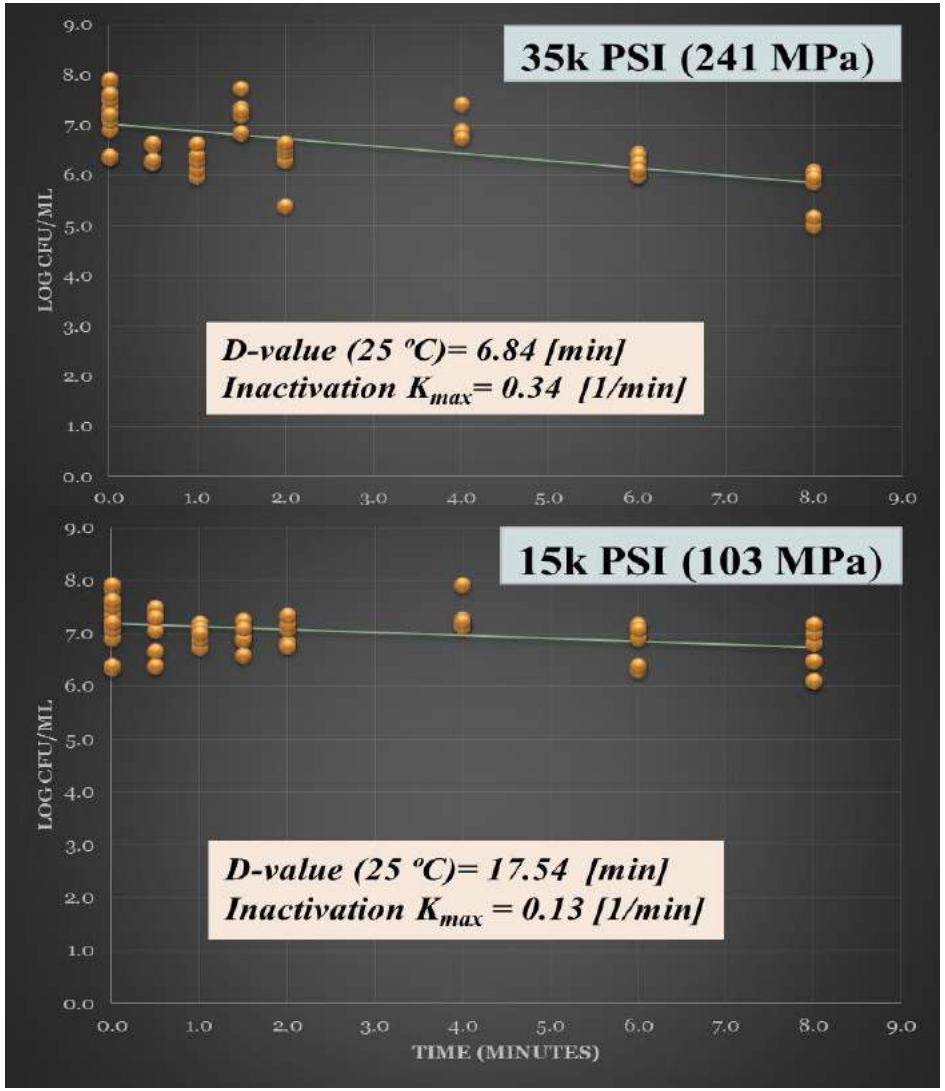
**For identification purposes reformulated as**

| **Parameters** | **Paramet** | **Standard Error** | | |
|---|---|---|---|---|
| kmax | 0.21 | 0.08 | **Mean Sum of Squared Error** | 0.6169 |
| LOG10(N0) | 6.37 | 0.20 | **Root Mean Sum of Squared Error** | 0.7855 |
| | | | **R-Square** | 0.1604 |
| | | | **R-Square adjusted** | 0.1350 |

# Calculation of Inactivation Indices- An Example

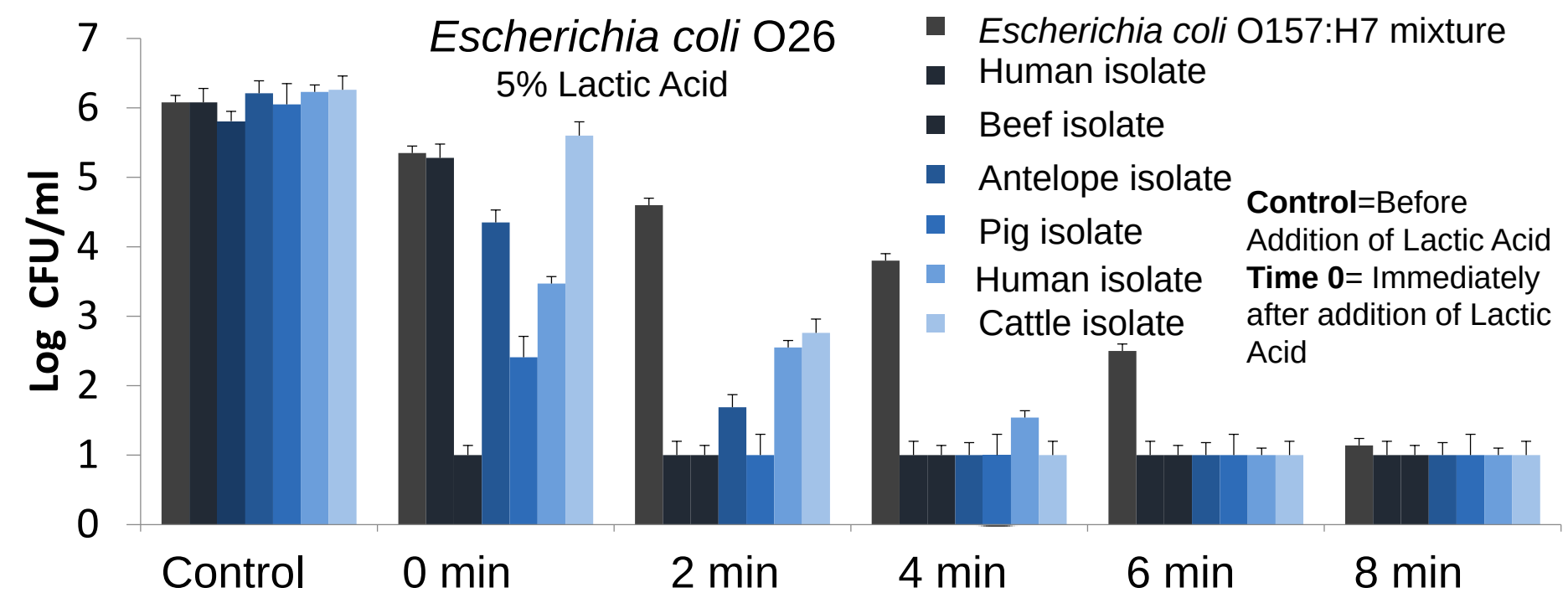


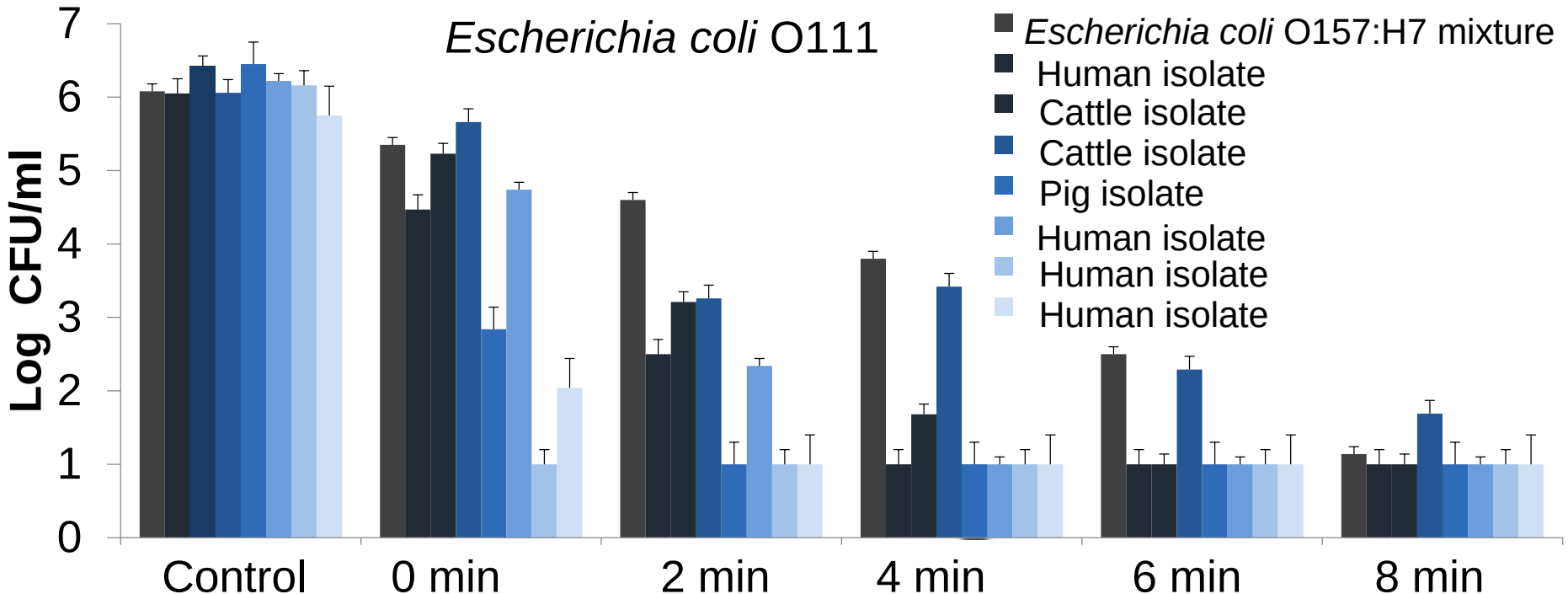

45k PSI (310 MPa)
LOG CFU/ML
D-value (25 ºC) = 2.21 [min]
Inactivation $K_{max}$= 0.80 [1/min]


35k PSI (241 MPa)
LOG CFU/ML
D-value (25 ºC)= 6.84 [min]
Inactivation $K_{max}$= 0.34 [1/min]


25k PSI (172 MPa)
LOG CFU/ML
D-value (25 ºC)= 21.37 [min]
Inactivation $K_{max}$= 0.08 [1/min]
TIME (MINUTES)


15k PSI (103 MPa)
LOG CFU/ML
D-value (25 ºC)= 17.54 [min]
Inactivation $K_{max}$ = 0.13 [1/min]
TIME (MINUTES)


Escherichia coli O26
5% Lactic Acid
Log CFU/ml
0
1
2
3
4
5
6
7
Control
0 min
2 min
4 min
6 min
8 min
Escherichia coli O157:H7 mixture
Human isolate
Beef isolate
Antelope isolate
Pig isolate
Human isolate
Cattle isolate
Control=Before Addition of Lactic Acid
Time 0= Immediately after addition of Lactic Acid


Escherichia coli O111
Log CFU/ml
0
1
2
3
4
5
6
7
Control
0 min
2 min
4 min
6 min
8 min
Escherichia coli O157:H7 mixture
Human isolate
Cattle isolate
Cattle isolate
Pig isolate
Human isolate
Human isolate
Human isolate

| Serotype/serogroup | Number of strains | Phenotype | $K_{max1}$ (1/min) | $K_{max2}$ (1/min) | 4D reduction (min) |
|---|---|---|---|---|---|
| *E. coli* O157:H7[a] | 5-strain mixture | Parent | 1.34 ± 0.36a | 1.34 ± 0.10a | 7.20 |
| *E. coli* O157:H7 | 5-strain mixture | Rifampicin-resistant | 2.32 ± 0.75a | 0.22 ± 0.40b | 6.64 |
| *E. coli* O26 | 7 single strains | Parent | 3.47 ± 0.30a* | 0.03 ± 0.29a* | 2.72 |
| *E. coli* O26 | 7 single strains | Rifampicin-resistant | 4.19 ± 0.29a^ | 0.11 ± 0.26a | 2.24 |
| *E. coli* O45 | 4 single strains | Parent | 5.47 ± 0.30a* | 0.22 ± 0.14a* | 2.16 |
| *E. coli* O45 | 4 single strains | Rifampicin-resistant | 4.34 ± 0.39a^ | 0.36 ± 0.13a | 2.34 |
| *E. coli* O103 | 7 single strains | Parent | 3.49 ± 0.50a* | 0.23 ± 0.27a* | 2.72 |
| *E. coli* O103 | 7 single strains | Rifampicin-resistant | 5.23 ± 0.60a^ | 0.47 ± 0.09a | 2.26 |
| *E. coli* O111 | 7 single strains | Parent | 4.70 ± 0.08a* | 0.39 ± 0.08a* | 2.40 |
| *E. coli* O111 | 7 single strains | Rifampicin-resistant | 4.32 ± 0.40a^ | 0.16 ± 0.18a | 2.16 |
| *E. coli* O121 | 5 single strains | Parent | 4.93 ± 0.09a* | 0.55 ± 0.09a* | 3.02 |
| *E. coli* O121 | 5 single strains | Rifampicin-resistant | 5.10 ± 0.08a^ | 0.36 ± 0.08a | 2.64 |
| *E. coli* O145 | 5 single strains | Parent | 4.37 ± 0.14a* | 0.42 ± 0.14a* | 2.14 |
| *E. coli* O145 | 5 single strains | Rifampicin-resistant | 3.87 ± 0.26a^ | 0.00 ± 0.32a | 2.40 |

Within each *E. coli* serotype/serogroup (i.e. O157:H7 mixture, O26, O45, O103, O111, O121, and O145) $K_{max}$ values followed by the same lowercase letter are not significantly different (student-based *t*-test, $P \geq 0.05$).

[a] $K_{max}$ values (parameter ± SE, [1/min]) of parent cells of the six serogroups of non-O157 *E. coli* followed by * are significantly (student-based *t*-test, $P < 0.05$) different than the $K_{max}$ value of parent cells of the *E. coli* O157:H7 mixture, and $K_{max}$ values of rifampicin-resistant variants of the six serogroups of non-O157 *E. coli* followed by ^ are significantly (student-based *t*-test, $P < 0.05$) different than the $K_{max}$ value of the rifampicin-resistant *E. coli* O157:H7 mixture.

# FDA BAM and USDA Microbiology Laboratory Guidebook Overview

**Laboratory Methods**

- CFSAN Laboratory Quality Assurance Manual
- Microbiological Methods & Bacteriological Analytical Manual (BAM)
- Drug & Chemical Residues Methods
- Elemental Analysis Manual (EAM) for Food and Related Products

## BAM: Food Sampling/Preparation of Sample Homogenate

SHARE | TWEET | LINKEDIN | PIN IT | EMAIL | PRINT

April 2003

### Bacteriological Analytical Manual
### Chapter 1
### Food Sampling and Preparation of Sample Homogenate

**Authors:** Wallace H. Andrews and Thomas S. Hammack

FDA U.S. FOOD & DRUG ADMINISTRATION

USDA United States Department of Agriculture
Food Safety and Inspection Service

### Microbiology Laboratory Guidebook

The Guidebook contains current protocols for analytical tests required by FSIS regulatory activities on meat, poultry and egg products. [1] Specifically, microbiological methods are presented for sample preparation, isolation and identification of the major foodborne pathogenic microorganisms and their toxins, meat tissue species identification, and the detection of antimicrobial residues. Appendices include a pathogen method summary chart, method flow charts, Media and Reagent formulations, and Most Probable Number Tables.

All methods are offered as PDF documents. If you require an alternative format, please contact:

# Bacteriological Analytical Method
# An Example: Aerobic Plate Count

A. **Equipment and materials**

1. Work area, level table with ample surface in room that is clean, well-lighted (100 foot-candles at working surface) and well-ventilated, and reasonably free of dust and drafts. The microbial density of air in working area, measured in fallout pour plates taken during plating, should not exceed 15 colonies/plate during 15 min exposure.
2. Storage space, free of dust and insects and adequate for protection of equipment and supplies
3. Petri dishes, glass or plastic (at least 15 × 90 mm)
4. Pipets with pipet aids (no mouth pipetting) or pipettors, 1, 5, and 10 ml, graduated in 0.1 ml units
5. Dilution bottles, 6 oz (160 ml), borosilicate-resistant glass, with rubber stoppers or plastic screw caps
6. Pipet and petri dish containers, adequate for protection
7. Circulating water bath, for tempering agar, thermostatically controlled to 45 ± 1°C
8. Incubator, 35 ± 1°C; milk, 32 ± 1°C
9. Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate
10. Tally register
11. Dilution blanks, 90 ± 1 ml Butterfield's phosphate-buffered dilution water (R11); milk, 99 ± 2 ml
12. Plate count agar (standard methods) (M124)
13. Refrigerator, to cool and maintain samples at 0-5°C; milk, 0-4.4°C
14. Freezer, to maintain frozen samples from -15 to -20°C
15. Thermometers (mercury) appropriate range; accuracy checked with a thermometer certified by the National Institute of Standards and Technology (NIST)



## BAM: Aerobic Plate Count



January 2001

### Bacteriological Analytical Manual Chapter 3 Aerobic Plate Count

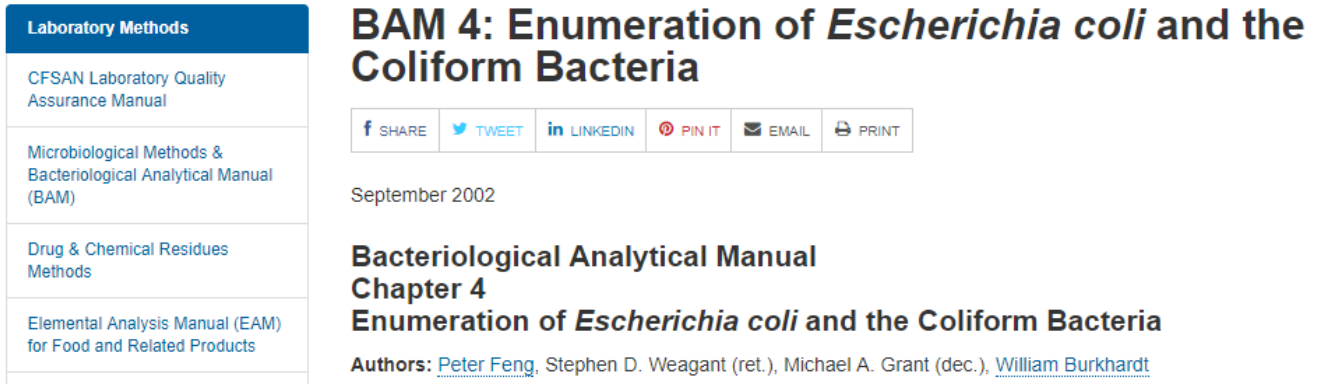

**Authors:** Larry Maturin (ret.) and James T. Peeler (ret)

For additional information, contact Guodong Zhang.

# Bacteriological Analytical Method
# An Example: Aerobic Plate Count

- **Normal plates (25-250).** Select spreader-free plate(s). Count all colony forming units (CFU), including those of pinpoint size, on selected plate(s). Record dilution(s) used and total number of colonies counted.
- **Plates with more than 250 colonies.** When number of CFU per plate exceeds 250, for all dilutions, record the counts as too numerous to count (TNTC) for all but the plate closest to 250, and count CFU in those portions of plate that are representative of colony distribution. See ref. 2 for detailed guidelines. Mark calculated APC with EAPC to denote that it was estimated from counts outside 25-250 per plate range (*see* D-3).
- **Spreaders.** Spreading colonies are usually of 3 distinct types: 1) a chain of colonies, not too distinctly separated, that appears to be caused by disintegration of a bacterial clump; 2) one that develops in film of water between agar and bottom of dish; and 3) one that forms in film of water at edge or on surface of agar. If plates prepared from sample have excessive spreader growth so that (a) area covered by spreaders, including total area of repressed growth, exceeds 50% of plate area, or (b) area of repressed growth exceeds 25% of plate area, report plates as spreaders. When it is necessary to count plates containing spreaders not eliminated by (a) or (b) above, count each of the 3 distinct spreader types as one source. For the first type, if only one chain exists, count it as a single colony. If one or more chains appear to originate from separate sources, count each source as one colony. Do not count each individual growth in such chains as a separate colony. Types 2 and 3 usually result in distinct colonies and are counted as such. Combine the spreader count and the colony count to compute the APC.
- **Plates with no CFU.** When plates from all dilutions have no colonies, report APC as less than 1 times the corresponding lowest dilution used. Mark calculated APC with asterisk to denote that it was estimated from counts outside the 25-250 per plate range. When plate(s) from a sample are known to be contaminated or otherwise unsatisfactory, record the result(s) as laboratory accident (LA)

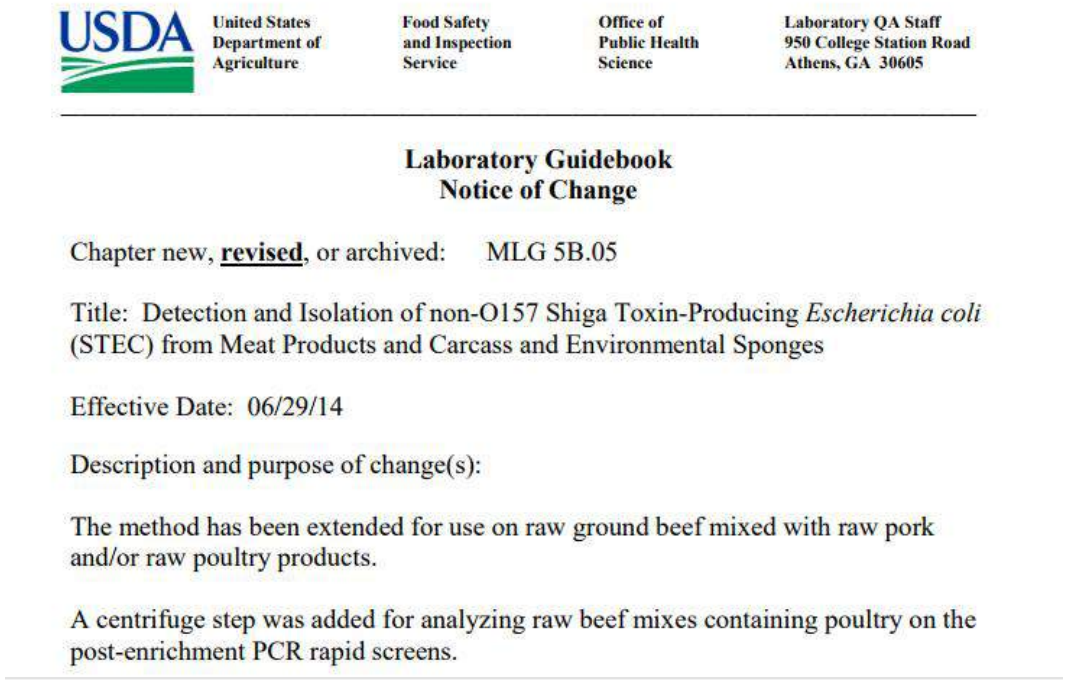
# Bacteriological Analytical Method Another Example: *E. coli* and Coliform




- Conventional Method for Determining Coliforms and *E. coli*
- LST-MUG Method for Detecting *E. coli* in Chilled or Frozen Foods Exclusive of Bivalve Molluscan Shellfish
- Bottled Water
- Examination of Shellfish and Shellfish Meats
- Analysis for *E. coli* in citrus juices
- Other Methods for Enumerating Coliforms and *E. coli*

# USDA Microbiology Guidebook Examples: Sample Preparation and Shiga-Toxin Producing Es*cherichia coli* detection

- Primarily for Meat, Poultry, and Eggs



USDA United States Department of Agriculture | Food Safety and Inspection Service | Office of Public Health Science | Laboratory QA Staff 950 College Station Road Athens, GA 30605

**Laboratory Guidebook**
**Notice of Change**

Chapter new, **revised**, or archived: MLG 5B.05

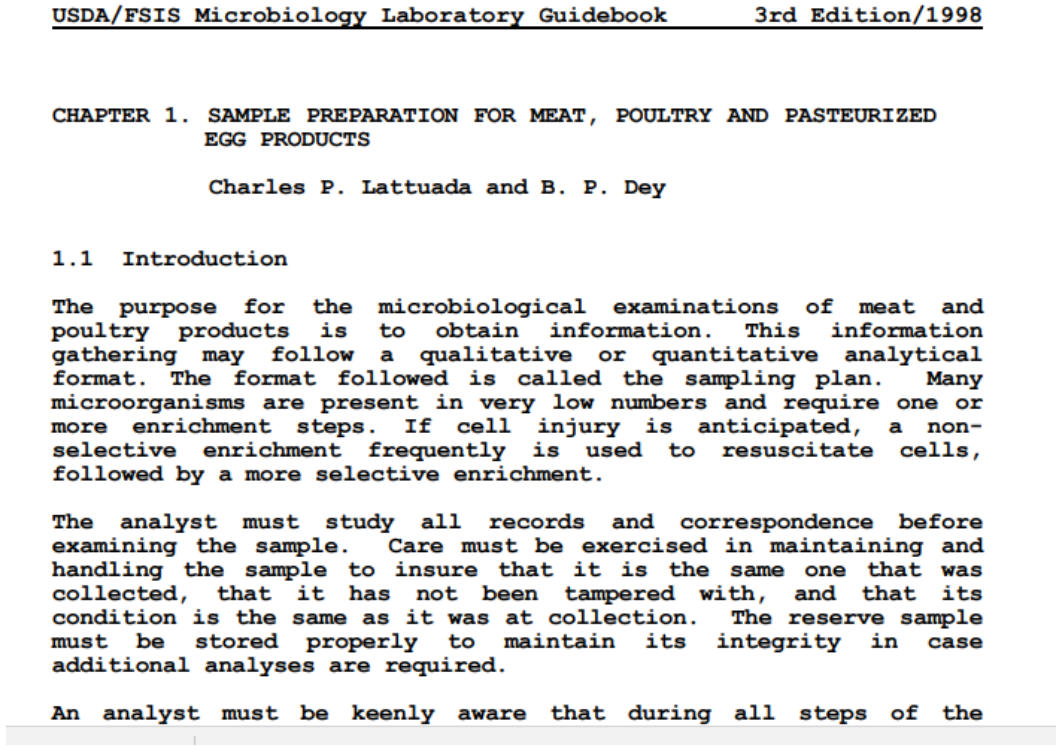
Title: Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges

Effective Date: 06/29/14

Description and purpose of change(s):

The method has been extended for use on raw ground beef mixed with raw pork and/or raw poultry products.

A centrifuge step was added for analyzing raw beef mixes containing poultry on the post-enrichment PCR rapid screens.



USDA/FSIS Microbiology Laboratory Guidebook 3rd Edition/1998

CHAPTER 1. SAMPLE PREPARATION FOR MEAT, POULTRY AND PASTEURIZED EGG PRODUCTS

Charles P. Lattuada and B. P. Dey

1.1 Introduction

The purpose for the microbiological examinations of meat and poultry products is to obtain information. This information gathering may follow a qualitative or quantitative analytical format. The format followed is called the sampling plan. Many microorganisms are present in very low numbers and require one or more enrichment steps. If cell injury is anticipated, a non-selective enrichment frequently is used to resuscitate cells, followed by a more selective enrichment.

The analyst must study all records and correspondence before examining the sample. Care must be exercised in maintaining and handling the sample to insure that it is the same one that was collected, that it has not been tampered with, and that its condition is the same as it was at collection. The reserve sample must be stored properly to maintain its integrity in case additional analyses are required.

An analyst must be keenly aware that during all steps of the

# Summary of inoculation procedure

*Journal of Food Protection, Vol. 73, No. 1, 2010, Pages 140–202*

**Supplement**

## Parameters for Determining Inoculated Pack/Challenge Study Protocols†‡

**ADOPTED 20 MARCH 2009, WASHINGTON, D.C.**
**NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS**

*NACMCF Executive Secretariat,* U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Room 333 Aerospace Center, 1400 Independence Avenue S.W., Washington, D.C. 20250-3700, USA*

MS 09-287: Received 2 July 2009/Accepted 7 August 2009

# Challenge and Inoculation Studies

1) Growing the cells planktonically: Overnight suspension, fecal sample, or highly contaminated food
2) Washing the cells for removing impurities and cell components
3) Preparation of microbial cocktail if more than one strain is in use
4) Habituation of the bacteria to “familiarize” the bacteria to the food environment
5) Preparation of Inoculum based on serial dilution
6) Inoculation of biotic or abiotic surface: inoculum load and attachment time
7) Aerobic or anaerobic storage for growth of the inoculum
8) Thermal and/or non-thermal antimicrobial interventions
9) Neutralization of samples: Ice slurry (for heat treatment) and DE broth (for antimicrobials)
10) Enumeration of survivors: YE and/or PY

# Challenge and Inoculation Studies

(1) Growing the cells planktonically: Overnight suspension, fecal sample, or highly contaminated food

- Pathogens: Purchased from ATCC (CDC Biosafety Level 1, Level 2, Level 3, Level 4)
- One alternative is use of bacteria isolated in lab: 80% glycerol stock at -80$^{o}$C.
- Attenuated Pathogens: Such LT2 *Salmonella*
- Surrogate Organism: generic *E. coli* K12 for E. coli O157:H7
- Fecal sample: 20% in PBS for field studies or when pathogens not available
- One alternative is to study the natural microflora without inoculation

- Temperature and incubation period choice based on study:

i.e. 22-24 at 37$^{o}$ C for *Salmonella* serovars

# Challenge and Inoculation Studies

2) Washing the cells for removing impurities and cell components

- Could vary based on the pathogen

**One protocol** (Fouladkhah et al., 2013):

- Centrifuging at 4,629 g for 15 min, discarding the “supernatant”
- Washing the “pellet” with 10 ml phosphate-buffered saline
- Centrifugation (4,629 g at 4°C for 15 min) again,
- Resuspension of the pellet in 10 ml PBS.

# Challenge and Inoculation Studies

3) Preparation of microbial cocktail if more than one strain is in use

4) Habituation of the bacteria to “familiarize” the bacteria to the food environment

- It is a common practice to combine a mixture of strains in a “cocktail” *i.e* five strain mixture of *salmonella*
- If a cocktail to be used each stain would need to be prepared (steps one and two) separately prior to mixing the strains
- Habituation allows a more realistic results

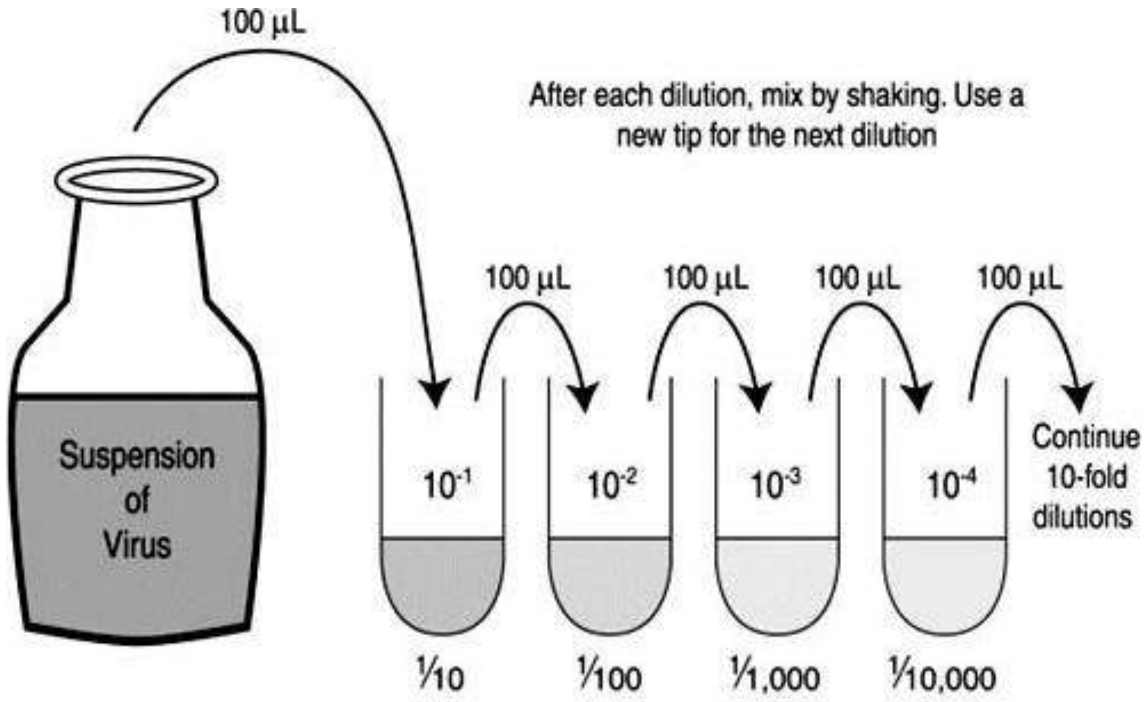
**One protocol** (Fouladkhah et al., 2011)

Strains of *L. monocytogenes* habituated for two days at 7°C in ham homogenate to allow acclimatization of *L. monocytogenes* cells to the food environment and low-temperature.

# Challenge and Inoculation Studies

5) Preparation of Inoculum based on serial dilution

Overnight Suspension of most bacteria around 9.5 log CFU/mL



# Challenge and Inoculation Studies

6) Inoculation of biotic or abiotic surface: inoculum load and attachment time

7) Aerobic or anaerobic storage for growth of the inoculum

- Inoculation could be based on **weight** (Log CFU/ g) or **volume** (Log CFU/ mL) of final products
- Could be **biotic** (e.g. leafy greens, beef trimming etc.) or **abiotic** (Stainless steel)
- Could be in **planktonic** stage (after inoculation) or **biofilm** stage (several days after inoculation).
- Samples could be stored **aerobically** or **anaerobically** prior to analysis
- **Attachment time** is a critical factor that would need to be extracted from literature.

One Protocol (Fouladkhah et al., 2013)

I hour attachment was recommended for adherence of *Escherichia coli* O157:H7 to stainless steel coupons

# Challenge and Inoculation Studies

8) Thermal and/or Non-thermal Antimicrobial Intervention

9) Neutralization of samples: Ice slurry (for heat treatment) and D/E broth (for antimicrobials)

- Exposure time and Concentration : would need to be pre-determined
- Samples would need to be plated immediately after treatment to minimize extra exposure time to antimicrobials
- Use of ice-water slurry for heat-treated samples to “stop” the thermal treatment
- Use of D/E Neutralizing broth for samples treated with antimicrobials to “stop” the antimicrobial treatment

# Challenge and Inoculation Studies

10) Enumeration of survivors: YE and/or PY

- Recovery of **Injured cells** after thermal or non-thermal treatments.
- New media would need to be formulated to enhance the growth of injured cells
- Yeast extract and Pyruvic acid are two common supplement to general purpose media

- **One Protocol** (Fouladkhah et al., 2017):

0.6% Yeast Extract added to Triptic Soy Agar could enhance recovery of injured cells

Also use of **Maximum Recovery Broth** instead of PBS for serial dilution could enhance the recover of injured cells.

# Thank you for your time

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**Cellphone: (970)690-7392**

# Annex 8

*Training Program: [Instructor; Translator; Attendees; Date]*

*[A. Fouladkhah; Rafael Marte Aracena; Three Faculty members; August-30-2017]*

## Lecture and Workshop: Analysis of Food Microbiology and Food Science Data: Modeling and Inactivation Indices

# Analysis of Food Microbiology and Food Science Data: Modeling and Inactivation Indices

***Aliyar Fouladkhah, PhD, MPH, CFS***
***Assistant Professor***
***Public Health Microbiology Laboratory***
***Tennessee State University***

***August 30, 2017***
***Universidad ISA, Santiago de los Caballeros,***
***Dominican Republic***

TENNESSEE STATE UNIVERSITY

## *Using OpenEpi for Analyses of Research Data*

***Available at no cost: http://www.openepi.com/Menu/OE_Menu.htm***

# Exercise 6:
# Sample Size Calculation for Frequency in a Population

- A group of Researchers would like to study **prevalence** of *Campylobacter* in a swine operation.
- There are close to **3,800** live animal in the operation.
- Based on preliminary data, the anticipated **frequency is around 20%.**
- For obtaining a **95% confidence limit**, how many live animal (**sample size**) is needed for a **random sampling**?

# Exercise 6:
# Sample Size Calculation for Frequency in a Population

| Sample Size for % Frequency in a Population (Random Sample) | | |
|---|---|---|
| Population size | 3800 | If large, leave as one million |
| Anticipated % frequency(p) | 20 | Between 0 & 99.99. If unknown, use 50% |
| Confidence limits as +/- percent of 100 | 5 | Absolute precision % |
| Design effect (for complex sample surveys--DEFF) | 1.0 | 1.0 for random sample |

# Exercise 6:
# Sample Size Calculation for Frequency in a Population

**Sample Size for Frequency in a Population**

| | |
|---|---|
| Population size(for finite population correction factor or fpc)(*N*): | 3800 |
| Hypothesized % frequency of outcome factor in the population (*p*): | 20%+/-5 |
| Confidence limits as % of 100(absolute +/- %)(*d*): | 5% |
| Design effect (for cluster surveys-*DEFF*): | 1 |

**Sample Size(*n*) for Various Confidence Levels**

| ConfidenceLevel(%) | Sample Size |
|---|---|
| 95% | 231 |
| 80% | 103 |
| 90% | 166 |
| 97% | 280 |
| 99% | 383 |
| 99.9% | 587 |
| 99.99% | 773 |

Equation

Sample size $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$

Results from OpenEpi, Version 3, open source calculator--SSPropor
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 7:
# Sample Size Calculation for Comparing two means

- An product development scientist had developed a low-cost formulation of vanilla mixture for baking products.
- Based on a preliminary sensory trial the mean and Standard Deviation of vanilla flavor associated with the products are:

  Original Product: Mean= 16.34; SD=2.73

  Low-Cost Product: Mean= 14.16; SD=2.94
- At **confidence level of 95%,** and at **statistical power of 90%,** how many panelists (**sample size**) are needed for a sensory trial with **equal panelists** for each product?

# Exercise 7:
# Sample Size Calculation for Comparing two means

| Sample Size For Comparing Two Means | | | | |
|---|---|---|---|---|
| Confidence Interval % (two-sided) | | | 95 | *Enter a value between 0 and 100, usually 95%* |
| Power | | | 90 | *Enter a value between 0 and 100, usually 80%* |
| Ratio of sample size (Group 2/Group 1) | | | 1 | |
| | Group 1 | | Group 2 | Enter means OR difference on next line |
| Mean | 16.34 | and | 14.16 | or Difference |
| Std. Dev. | 2.73 | | 2.94 | *Enter Std. Deviation OR Variance of each group* |
| Variance | | | | |

# Exercise 7:
# Sample Size Calculation for Comparing two means

**Sample Size For Comparing Two Means**

**Input Data**

| | |
|---|---|
| Confidence Interval (2-sided) | 95% |
| Power | 90% |
| Ratio of sample size (Group 2/Group 1) | 1 |

| | Group 1 | Group 2 | Difference* |
|---|---|---|---|
| Mean | 16.34 | 14.16 | 2.18 |
| Standard deviation | 2.73 | 2.94 | |
| Variance | 7.4529 | 8.6436 | |

| | |
|---|---|
| Sample size of Group 1 | 36 |
| Sample size of Group 2 | 36 |
| Total sample size | 72 |

*Difference between the means

Results from OpenEpi, Version 3, open source calculator--SSMean
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 8: Power Analysis

- An plant breeder had developed a high yield eggplant cultivar.
- Based on analysis of 5 “Tarea” per cultivar the Mean and Standard Deviation of yield associated with the cultivars are:

  Original Cultivar: Mean= 450.25 Ib/Tarea; SD=35.36

  High Yield Cultivar: Mean= 530.35 Ib/Tarea; SD=75.65
- At **confidence level of 95%,** what is the **statistical power** of this trial?

# Exercise 8: Power Analysis

| Power For Comparing Two Means | | | | | |
|---|---|---|---|---|---|
| Confidence Interval(%) {two-sided} | | | 95 | Enter a value between 0 and 100, usually 95% | |
| | Group 1 | | Group 2 | Enter means or difference below | |
| Mean | 450.25 | and | 530.35 | Or Difference | |
| Sample size | 5 | | 5 | | |
| Std.Dev. | 35.36 | | 75.65 | Enter Std. Deviation OR Variance of each group | |
| Variance | | | | | |

# Exercise 8: Power Analysis

**Power For Comparing Two Means**

**Input Data**

Two-sided 95% Confidence Interval

| | Group 1 | Group 2 | Difference* |
|---|---|---|---|
| Mean | 450.25 | 530.35 | -80.1 |
| Sample size | 5 | 5 | |
| Standard deviation | 35.36 | 75.65 | |
| Variance | 1250.33 | 5722.92 | |

**Power = 57.34%**
**by the normal approximation method**

* Mean difference= (Group 1 mean) - (Group 2 mean)

Results from OpenEpi, Version 3, open source calculator--PowerMean
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 9: Sample Size

- A food microbiologist is indenting to study effects of High Pressure Processing on inactivation of wild-type and rifampicin-resistant *Cronobacter Sakazakii.*
- At **Confidence level of 99%**, he would like to see **difference in means of 0.5** log/ml as statistically significant.
- **SD** associated with the wild-type and rifampicin-resistant pathogen are **0.9, and 0.7**, respectively based on a preliminary trial.
- For obtaining a **statistical power of 80%** what **sample size** is needed?

# Exercise 9: Sample Size

| Sample Size For Comparing Two Means | | | | | |
|---|---|---|---|---|---|
| Confidence Interval % (two-sided) | | | 99 | *Enter a value between 0 and 100, usually 95%* | |
| Power | | | 80 | *Enter a value between 0 and 100, usually 80%* | |
| Ratio of sample size (Group 2/Group 1) | | | 1 | | |
| | Group 1 | | Group 2 | Enter means OR difference on next line | |
| Mean | | and | | or Difference | 0.5 |
| Std. Dev. | 0.1 | | 0.2 | *Enter Std. Deviation OR Variance of each group* | |
| Variance | | | | | |

# Exercise 9: Sample Size

**Sample Size For Comparing Two Means**

**Input Data**

| | | |
|---|---|---|
| Confidence Interval (2-sided) | 99% | |
| Power | 80% | |
| Ratio of sample size (Group 2/Group 1) | 1 | |
| | **Group 1** | **Group 2** |
| Mean | | |
| Standard deviation | 0.1 | 0.2 |
| Variance | 0.01 | 0.04 |

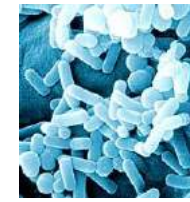
**Difference***
0.5

| | |
|---|---|
| Sample size of Group 1 | 3 |
| Sample size of Group 2 | 3 |
| Total sample size | 6 |

*Difference between the means

Results from OpenEpi, Version 3, open source calculator--SSMean
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

# Exercise 9: Sample Size



- A food microbiologist is indenting to study effects of High Pressure Processing on inactivation of wild-type and rifampicin-resistant *Cronobacter Sakazakii.*
- At **Confidence level of 90%**, he would like to see **difference in means of 0.5** log/ml as statistically significant.
- **SD** associated with the wild-type and rifampicin-resistant pathogen are **0.9, and 0.7**, respectively based on a preliminary trial.
- For obtaining a **statistical power of 80%** what **sample size** is needed?

# Exercise 10: ANOVA

- A researcher wants to know the effects of 3% acetic acid for decontamination of chicken carcasses from *Salmonella* serovars at 5 and 35 degree C.
- After a application of
  - Control: Mean= 8.4 log/g; SD=1.1; Sample size=5.
  - Trt @ 5 C: Mean= 6.1 log/g; SD=0.9; Sample size=5.
  - Trt @ 35 C: Mean= 3.7 log/g; SD=0.8; Sample size=5.
- At **confidence level of 95%,** are these treatments effective to control the bacterium?

# Exercise 10: ANOVA

| Analysis of Variance (ANOVA) | | | | | |
|---|---|---|---|---|---|
| | N (counts) | Mean | Std. Dev. | (or) | Std. Error |
| Group 1 | 5 | 8.4 | 1.1 | | |
| Group 2 | 5 | 6.1 | 0.9 | | |
| Group 3 | 5 | 3.7 | 0.8 | | |
| Group 4 | | | | | |
| Group 5 | | | | | |
| Group 6 | | | | | |
| Group 7 | | | | | |
| Group 8 | | | | | |
| Group 9 | | | | | |
| Group 10 | | | | | |
| Enter your summary data in any respective group, no need to be consecutive. (see the | | | | | |

# Exercise 10: ANOVA

| Group | N (count) | Mean | Std. Dev. | Std. error |
|---|---|---|---|---|
| 1 | 5 | 8.4 | 1.1 | |
| 2 | 5 | 6.1 | 0.9 | |
| 3 | 5 | 3.7 | 0.8 | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |

**ANOVA Table**

| Source of variation | Sum of squares | d.f | Mean square | *F* statistics | p-value[3] |
|---|---|---|---|---|---|
| Between Groups | 55.2333 | 2 | 27.6167 | 31.1466 | 0.000017757 |
| Within Groups | 10.64 | 12 | 0.886667 | | |
| Total | 65.8733 | 14 | | | |

| | Chi square | d.f | p-value[3] |
|---|---|---|---|
| Test for equality of variance | 0.379905 | 2 | 0.826998 |

| | | 95% CI of individual sample mean | | 95% CI assuming equal varia | |
|---|---|---|---|---|---|
| Group | Mean | Lower Limit | Upper Limit | Lower Limit | Upper Limi |
| 1 | 8.4 | 7.03417 | 9.76583 | 7.23081 | 9.56919 |
| 2 | 6.1 | 4.9825 | 7.2175 | 4.93081 | 7.26919 |
| 3 | 3.7 | 2.70667 | 4.69333 | 2.53081 | 4.86919 |

# Exercise 10: ANOVA

| | | 95% CI of individual sample mean | | 95% CI assuming equal variance | |
|---|---|---|---|---|---|
| Group | Mean | Lower Limit | Upper Limit | Lower Limit | Upper Limit |
| 1 | 8.4 | 7.03417 | 9.76583 | 7.23081 | 9.56919 |
| 2 | 6.1 | 4.9825 | 7.2175 | 4.93081 | 7.26919 |
| 3 | 3.7 | 2.70667 | 4.69333 | 2.53081 | 4.86919 |

# Calculation of inactivation indices

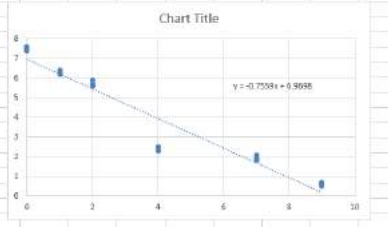
- Inactivation indices were calculated based on linear and non-linear models.
- **Linear Index** of D-value was obtain using American Meat Institute Foundation Process Lethality Spreadsheet*
- **D-value:** decimal reduction time and required at a given condition to kill 90% (or 1 log) of the exposed microorganisms
- **Non-Linear Index** of K max were obtain using the **GInaFit software**** based on best fitted non-linear model . Maximum $R^2$ was chosen as goodness-of-fit criterion.
- **$K_{max}$ value:** Number of log reductions per unit of time

  - *Available at: http://meatpoultryfoundation.org/content/process-lethality-spreadsheet
  - ** https://www.ncbi.nlm.nih.gov/pubmed/15893399

# Exercise 11:
# D and 4D value based on Linear Model

What is the D and 4D values based on linear Model?

| | |
|---|---|
| D-Value | 1.322926 |
| 4D | 5.291705 |




| Time | Log Reduction |
|---|---|
| 0 | 7.5 |
| 0 | 7.6 |
| 0 | 7.4 |
| 1 | 6.3 |
| 1 | 6.2 |
| 1 | 6.4 |
| 2 | 5.7 |
| 2 | 5.6 |
| 2 | 5.9 |
| 4 | 2.3 |
| 4 | 2.5 |
| 4 | 2.3 |
| 7 | 1.9 |
| 7 | 1.8 |
| 7 | 2.1 |
| 9 | 0.7 |
| 9 | 0.5 |
| 9 | 0.6 |

# Exercise 12:
# 4D and Kmax values in GInaFiT-Linear Model

| Time | LOG10(N) | LOG10(N) | Squared difference | Parameters | Paramete | Standard Error |
|---|---|---|---|---|---|---|
| 0.00 | 7.50 | 6.97 | 0.28 | kmax | 1.74 | 0.13 |
| 0.00 | 7.60 | 6.97 | 0.40 | LOG10(N0) | 6.97 | 0.28 |
| 0.00 | 7.40 | 6.97 | 0.19 | | | |
| 1.00 | 6.30 | 6.21 | 0.01 | | | |
| 1.00 | 6.20 | 6.21 | 0.00 | | | |
| 1.00 | 6.40 | 6.21 | 0.03 | | | |
| 2.00 | 5.70 | 5.46 | 0.06 | | | |
| 2.00 | 5.60 | 5.46 | 0.02 | | | |
| 2.00 | 5.90 | 5.46 | 0.20 | | | |
| 4.00 | 2.30 | 3.95 | 2.71 | | | |
| 4.00 | 2.50 | 3.95 | 2.09 | | | |
| 4.00 | 2.30 | 3.95 | 2.71 | | | |
| 7.00 | 1.90 | 1.68 | 0.05 | | | |
| 7.00 | 1.80 | 1.68 | 0.01 | | | |
| 7.00 | 2.10 | 1.68 | 0.18 | | | |
| 9.00 | 0.70 | 0.17 | 0.28 | | | |
| 9.00 | 0.50 | 0.17 | 0.11 | | | |
| 9.00 | 0.60 | 0.17 | 0.19 | | | |
| Least Sum of Squared Error | | | 9.52 | | | |

| | | |
|---|---|---|
| Mean Sum of Squared Error | 0.5947 | |
| Root Mean Sum of Squared Error | 0.7712 | |
| R-Square | 0.9188 | |
| R-Square adjusted | 0.9137 | |
| 4D reduction is reached at | ±5.31 | units of tim |

Inactivation model identified

N= N(
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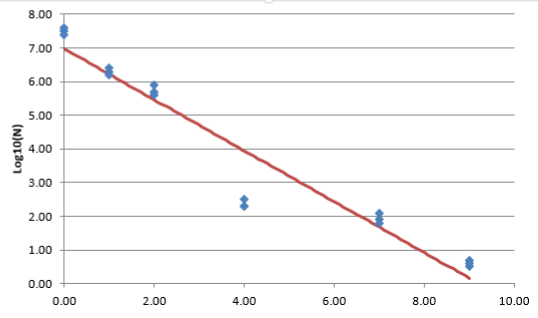
therm




| Time | Log Reduction |
|---|---|
| 0 | 7.5 |
| 0 | 7.6 |
| 0 | 7.4 |
| 1 | 6.3 |
| 1 | 6.2 |
| 1 | 6.4 |
| 2 | 5.7 |
| 2 | 5.6 |
| 2 | 5.9 |
| 4 | 2.3 |
| 4 | 2.5 |
| 4 | 2.3 |
| 7 | 1.9 |
| 7 | 1.8 |
| 7 | 2.1 |
| 9 | 0.7 |
| 9 | 0.5 |
| 9 | 0.6 |

# Exercise 13:
## 4D and Kmax values in GInaFiT Biphasic Curves

| Time | LOG10(N) | LOG10(N) | Squared difference |
|---|---|---|---|
| 0.00 | 7.50 | 7.70 | 0.04 |
| 0.00 | 7.60 | 7.70 | 0.01 |
| 0.00 | 7.40 | 7.70 | 0.09 |
| 1.00 | 6.30 | 6.42 | 0.01 |
| 1.00 | 6.20 | 6.42 | 0.05 |
| 1.00 | 6.40 | 6.42 | 0.00 |
| 2.00 | 5.70 | 5.15 | 0.31 |
| 2.00 | 5.60 | 5.15 | 0.21 |
| 2.00 | 5.90 | 5.15 | 0.57 |
| 4.00 | 2.30 | 2.81 | 0.26 |
| 4.00 | 2.50 | 2.81 | 0.10 |
| 4.00 | 2.30 | 2.81 | 0.26 |
| 7.00 | 1.90 | 1.49 | 0.17 |
| 7.00 | 1.80 | 1.49 | 0.10 |
| 7.00 | 2.10 | 1.49 | 0.37 |
| 9.00 | 0.70 | 0.86 | 0.03 |
| 9.00 | 0.50 | 0.86 | 0.13 |
| 9.00 | 0.60 | 0.86 | 0.07 |
| .east Sum of Squared Error | | | 2.78 |

| Parameters | Paramete | Standard Error |
|---|---|---|
| f | 0.9999 | 0.00025 |
| kmax1 | 2.95 | 0.33 |
| kmax2 | 0.72 | 0.35 |
| LOG10(N0) | 7.70 | 0.22 |

| | | |
|---|---|---|
| Mean Sum of Squared Error | 0.1985 | |
| Root Mean Sum of Squared Error | 0.4455 | |
| R-Square | 0.9763 | |
| R-Square adjusted | 0.9712 | |
| 4D reduction is reached at | ±3.24 | units of time |

Inactivation model identified

log1
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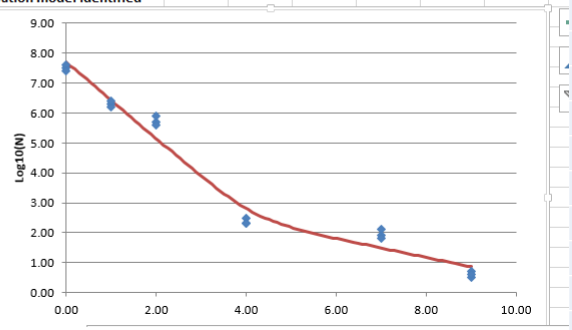


| Time | Log Reduction |
|---|---|
| 0 | 7.5 |
| 0 | 7.6 |
| 0 | 7.4 |
| 1 | 6.3 |
| 1 | 6.2 |
| 1 | 6.4 |
| 2 | 5.7 |
| 2 | 5.6 |
| 2 | 5.9 |
| 4 | 2.3 |
| 4 | 2.5 |
| 4 | 2.3 |
| 7 | 1.9 |
| 7 | 1.8 |
| 7 | 2.1 |
| 9 | 0.7 |
| 9 | 0.5 |
| 9 | 0.6 |

# Exercise 14:
## Which Model is a Better Match for Data? Biphasic Curves or Linear Model?

Linear Model

| | | |
|---|---|---|
| Mean Sum of Squared Error | 0.5947 | |
| Root Mean Sum of Squared Error | 0.7712 | |
| R-Square | 0.9188 | |
| R-Square adjusted | 0.9137 | |
| 4D reduction is reached at | ±5.31 | units of tim |

Biphasic Curve

| | | |
|---|---|---|
| Mean Sum of Squared Error | 0.1985 | |
| Root Mean Sum of Squared Error | 0.4455 | |
| R-Square | 0.9763 | |
| R-Square adjusted | 0.9712 | |
| 4D reduction is reached at | ±3.24 | units of time |

| Time | Log Reduction |
|---|---|
| 0 | 7.5 |
| 0 | 7.6 |
| 0 | 7.4 |
| 1 | 6.3 |
| 1 | 6.2 |
| 1 | 6.4 |
| 2 | 5.7 |
| 2 | 5.6 |
| 2 | 5.9 |
| 4 | 2.3 |
| 4 | 2.5 |
| 4 | 2.3 |
| 7 | 1.9 |
| 7 | 1.8 |
| 7 | 2.1 |
| 9 | 0.7 |
| 9 | 0.5 |
| 9 | 0.6 |