Master of Public Health

Integrative Learning Experience Report

LEARNING ABOUT KANSAS DEPARTMENT OF AGRICULTURE'S LABORATORY

by

Micaela Lynn Freeman

MPH Candidate

submitted in partial fulfillment of the requirements for the degree

MASTER OF PUBLIC HEALTH

Graduate Committee:
Valentina Trinetta, PhD
Justin Kastner, PhD
Ellyn Mulcahy, PhD, MPH

Public Health Agency Site:
Kansas Department of Agriculture Laboratory
May 2023-June 2023

Site Preceptor:
Sally Flowers, PhD, MS – Laboratory Director at KDAL

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2023

Summary/Abstract

Food safety efforts must consist of multidisciplinary activites, and one key activity is laboratory testing. With sample submissions from different stakeholders, the Kansas Department of Agriculture Laboratory is equipped to perform various regulatory testing on the samples, contributing to the food safety surveillance of the food supply. With respect to animal feeds, the levels of mycotoxins present are analyzed to determine whether or not the product is safe for animal consumption. If animals consume unsafe amounts of mycotoxins, both animal and human health can be impacted. If that intoxicated animal was consumed, humans are at risk for indirect exposure. In light of human health, foodborne pathogens are of great concern as well. Testing methods are imperative in order to detect possible contaminants in Kansas' meat and poultry products. However, all testing and day-to-day practices would be meritless without some form of quality management system. Quality management systems, such as International Organization for Standardization/International Electrotechnical Commission 17025, ensure laboratories are performing competently and uniformly.

My main objectives were to obtain a better understanding of how a public health laboratory operates, as well as become more familiar with basic microbiological and chemical techniques related to food safety and quality. In attainment of my objectives, I helped perform sample extractions for mycotoxin testing of animal feeds, assisted with method verification of a microbiological testing platform in their Biosafety Level-2 lab, and learned about and applied their quality management system, International Organization for Standardization/International Electrotechnical Commission 17025:2017.

Subject Keywords: KDA; food safety; ISO 17025; mycotoxins; *Salmonella; Listeria monocytogenes*

Table of Contents

List of Figures

List of Tables

Chapter 1 - Overview of The Relevance of Microbiological/Chemical Testing and Adhering to ISO/IEC17025-2017

The Kansas Department of Agriculture (KDA) was the first state department of agriculture established in the United States.[1] The agency is "devoted to total support of agriculture in Kansas, working for the entire agriculture setting, including farmers, ranchers, food establishments, and agribusiness."[1] The KDA Laboratory (KDAL) is an integral program to the agency's mission. This state-of-the-art laboratory seeks to establish, maintain, and improve analytical laboratory services for many sectors, including: Dairy, Meat and Poultry, Pesticide and Fertilizer, and Food Safety and Lodging Inspection programs.[2] For my integrated learning experience, I assisted with method verification using the 3M™ Molecular Detection System (MDS) for *Salmonella* spp*.* and *Listeria monocytogenes* detection; testing of safety levels of mycotoxins in animal feed; and learned about the principles and applications of laboratory quality management systems, specifically ISO/IEC (International Organization for Standardization/International Electrotechnical Commission) 17025:2017, and its relevance to public health.

During the 3M™ MDS method verification, *Salmonella* spp*.* and *Listeria monocytogenes* were the two pathogens left to assist with verifying the method. Prior to my onboarding, the lab staff verified the detection methods for *Escherichia coli* O157:H7. The 3M™ molecular detection system is used for "rapid and specific detection of selected pathogens in enriched food and environmental samples."[5] The system operates by amplifying nucleic acid sequences using loop-mediated isothermal amplification and bioluminescence to identify the amplification.[5] This detection method

is important to ensure the public is consuming safe, non-contaminated food products. The Centers for Disease Prevention and Control (CDC) reports *Salmonella* bacteria are the cause of approximately 1.35 million infections annually, with food being the primary source.[7] *Salmonellae* are gram-negative, flagellated facultative anaerobes. If food contaminated with *Salmonella* is consumed, the bacteria are able to survive the acidic environment of the stomach and will infiltrate the mucosa of the intestines where endotoxins will be produced.[8] The body's inflammatory response to the invasion is what causes the typical symptoms of gastroenteritis. With *Listeria monocytogenes*, similar symptoms of foodborne illnesses are of concern but especially for immunocompromised or pregnant individuals. *Listeria monocytogenes* is a gram-positive, facultative intracellular rod.[10] Due to its psychrotrophic nature, *Listeria monocytogenes* is capable of replicating at refrigerated temperatures, making it difficult for the food industry to mitigate.[10] The detection of foodborne pathogens using the 3M™ MDS will aid KDAL with their surveillance efforts.

Another important topic for the safety of consumers and agriculture in Kansas is mycotoxins. Testing for mycotoxin levels in animal feeds is important for animal welfare, food safety, and public health. Exposure can occur in livestock by consumption of the infected feeds, and humans can have indirect exposure through consumption of infected animals.[9] With an ever-growing population, and demand for sustainable food sources, the livestock industry depends on feedstuffs such as wheat; corn; soybeans; and their products, all of which have the potential to harbor mycotoxins.[9] Mycotoxins are produced by filamentous fungi, typically those belonging to the genera *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium*.[3] Aflatoxins, fumonisins, ochratoxins, trichothecenes, and zearalenone

groups of mycotoxins are considered to be a safety and economic concern due to their high prevalence in feeds and known health effects on livestock.[3] Mycotoxicosis can occur through ingestion, inhalation, skin-to-skin contact, or via the lymphatic system. Health effects vary in severity depending on the type of mycotoxin. Symptoms of mycotoxicosis include: weakened immunity, identifiable health problems, death, and action as allergens or irritants.[9] Mycotoxins also pose health threats, both chronic and acute, if inhaled and introduced to the lymphatic system and blood stream.[9] To mitigate exposure, KDAL analyzes samples of all types of feedstuffs to ensure the products being fed to livestock and other animals are safe for consumption.

While performing all testing and analyses, KDAL adheres to accredited laboratory systems management, ISO/IEC 17025:2017. ISO standards are internationally agreed upon by experts. ISO/IEC 17025 provides standardization for testing and calibration in laboratories.[4] Under ISO/IEC 17025, KDAL is able to demonstrate that they operate competently and generate valid results.[4] This ensures consumer confidence in their work at the state and federal level and creates legally defensible test results. My preceptor, Dr. Flowers, describes ISO accreditation by the following: “Being accredited to ISO/IEC 17025 means that an independent expert has visited our lab, evaluated our work first-hand, and is vouching for our ability to test in a consistent, competent manner that is free from outside influences.”[6] ISO/IEC 17025 protocols are the backbone of sample analyses at KDAL.

Chapter 2 - Learning Objectives and Project Description

Throughout my time at KDAL, I participated in many activities, all promoting the attainment of my learning objectives. Depending on the day, I participated in general tasks such as attending section meetings, preparing microbiological media and chemical reagents, and contributing to the cleanliness of the laboratory sections. My main objective was to obtain a better understanding of how a public health laboratory operates and become more familiar with basic microbiological and chemical techniques for food safety and quality. Specific projects related to the achievement of the learning objective included:

- Performing sample extractions for mycotoxin testing of animal feeds;
- Assisting with method verification for food pathogen detection; and,
- Learning and applying principles of ISO/IEC 17025 quality management system.

During the determination of mycotoxins in animal feeds, I used high performance liquid chromatography tandem mass spectrometry (LC-MS/MS). To begin the procedure, the received samples were ground until a powder-like consistency was achieved in order to reduce particle size for better analysis of the sample. 2 ± 0.02 grams were then added to centrifuge tubes along with 10 milliliters of extraction solution and placed in a wrist action shaker for 30 minutes to ensure the ground sample was fully saturated.[11] After the shake cycle was complete, the samples were centrifuged; 500 microliters of the supernatant were transferred into separate test tubes. In the new test tubes, another 500 microliters of ammonium formate were added and then vortexed.[11] After the supernatant and ammonium formate were homogenized, the solution was poured into filtered syringes and dispensed into amber autosampler vials. The vials had pre-slit septum caps in order for the needle of the LC-MS/MS machine to extract the liquid.[11] The autosampler tray containing the vials were placed in the Waters Acquity H-Class Plus Sample Manager FTN-H for sample analysis.[11] This system detects aflatoxins and deoxynivalenol analytes. The results of each analyte are

calculated by the machine and illustrated graphically.[11] Figures 2.1 and 2.2 illustrate some of the steps involved in sample preparation.

Figure 2.1: Wrist Action Shaker Saturating the Ground Samples

Figure 2.2: Loading the Autosampler Tray into the LC-MS/MS Machine

With the second project, I assisted with verifying a detection method for *Salmonella* and *Listeria monocytogenes* in raw meat, poultry products, ready-to-eat (RTE) meat products, retail foods, outbreak samples, and environmental samples.[12] To begin the detection, I assisted with sample preparation and enrichment. We first tested RTE samples, so 25 ± 1 gram of the product was obtained. The samples typically arrive as intact product from the meat and poultry department, so the samples have to be blended into smaller sizes in order to obtain a representative sample.[12] The blended sample is then added to the appropriate enrichment media. For *Salmonella,* 225 ± 5 milliliters of Buffered Peptone Water (ISO) is added, and for *Listeria monocytogenes,*

Demi-Fraser Broth.[12] Next, the whirl-pak bag was placed in the stomacher for two minutes to wash any potential microbes into the enrichment broth.[12] The samples prepared for *Salmonella* detection were incubated at 37 ± 1 ºC for 18-26 hours. When testing for *Listeria monocytogenes,* the incubation temperature is still 37 ± 1 ºC but for 24-30 hours.[12] During incubation, a set of control broths were made as well to ensure quality standards were met. These sets include: a blank, to ensure the media is sterile; a negative control, to confirm specificity; and a positive control, to prove sensitivity. Specificity refers to the test results that are truly negative, and sensitivity refers to the test results that are truly positive.

Figure 2.3: Enriched Samples and QC Incubation

After incubation, 20 microliters are pipetted into the lysis tubes.[12] Proceeding completed transfers, the lysis tubes are heated at 100 ± 1 ºC for 15 ± 1 minute.[12] The solutions should turn from pink to yellow after the first heating point. Next, the lysis tubes are cooled on a chill block at 20-25 º for 5-10 minutes.[12] The lysis tubes then reverted back to their original yellow color. Figure 2.4 from the 3M™ protocol review illustrates the color change between heating and cooling.

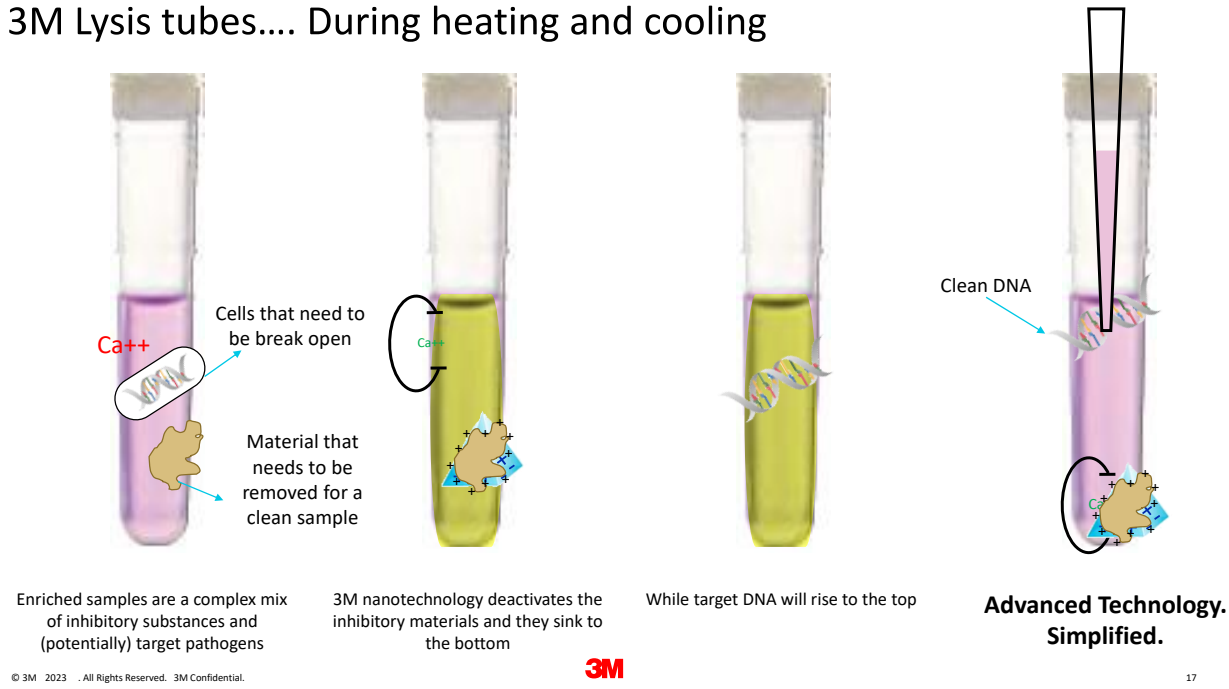


Figure 2.4: Lysis Tubes During Heating and Cooling[13]

After the lysis steps, I began the amplification process. To do so, 20 microliters of lysate were transferred into reagent tubes.[12] The reagent tubes were loaded into the speed loader tray and placed in the detection machine to begin the MDS assay. The results were automatically analyzed by the computer system, and each sample was reported as a positive or a negative.[12]

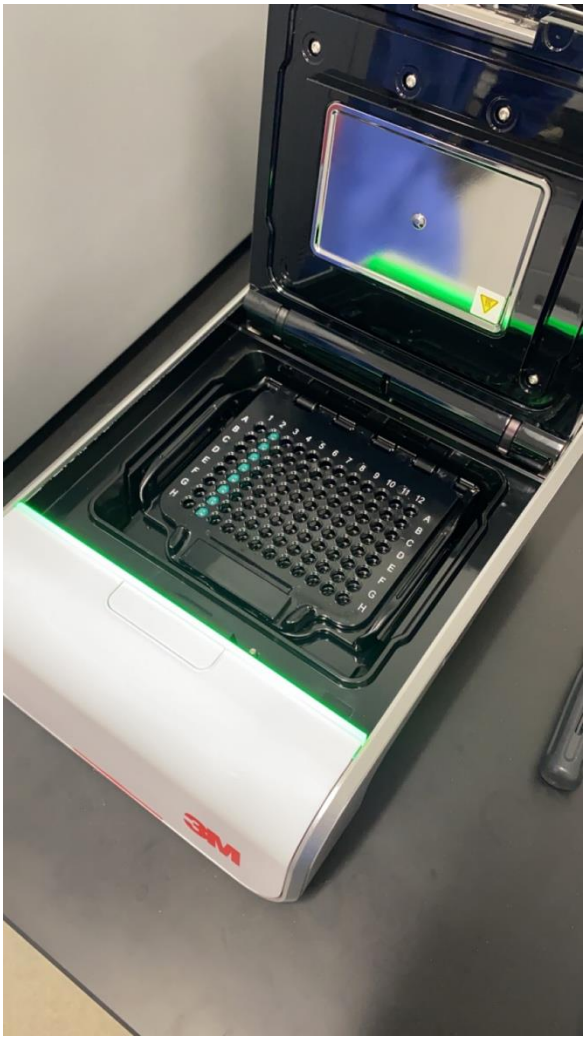


Figure 2.5: Reagent Tubes Loaded into MDS Machine

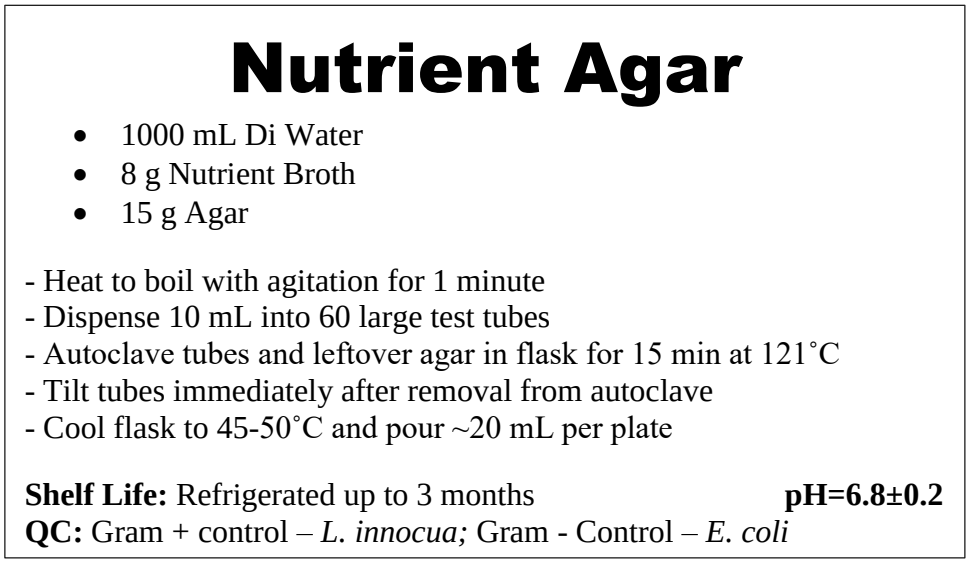
The third project was to learn about and apply KDAL's quality management system, ISO/IEC 17025. Dr. Flowers and I routinely met to discuss principles of ISO/IEC 17025. Within the before mentioned projects, there were steps I had to complete prior to performing the analyses, this was due to the compliance of ISO/IEC 17025 standards. The steps included: 1, read the SOP; 2, observe a trained analyst perform the test; 3, perform the test under supervision of a trained analyst; 4, perform the test successfully to demonstrate capability, which included a document signed by the trained analyst and laboratory director for approval. In addition to only trained personnel performing

analyses, the SOPs themselves were riddled with practices rooted in ISO/IEC standards. Another example, from the beginning of sample receiving to sample disposal, a chain of custody record was required for all samples needing tested. This process was as specific as documenting the exact minute a sample was transferred from the other program inspectors to the laboratory analysts. Once the sample was received, it became the analysts' responsibility to ensure that product was not compromised while at the laboratory. Their responsibility continues even after the Report of Analysis is reviewed and approved by a section supervisor and the laboratory director. That chain of custody ends when the sample is discarded, which can be retained for one month up to a year or more, depending on the type of sample or the severity of the case.

Another application of ISO/IEC 17025 principles I experienced during my APE was the preparation for an external audit to occur in September. Being accredited under ISO/IEC 17025, KDAL has annual audits performed by a third-party auditor to remove any possible bias. Through discussion with Dr. Flowers, I learned this third body is through ANAB (ANSI National Accreditation Board), who is recognized by the International Laboratory Accreditation Cooperation Mutual Recognition Arrangement (ILAC MRA). Dr. Flowers referred to this tiered system as a "chain of trust." She furthered explained the purpose of the audit is to verify KDAL is upholding the necessary practices within each laboratory section in order to maintain their accreditation.

I was able to apply the knowledge I gained about ISO/IEC 17025 principles by updating the microbiology and dairy section's media preparation cards. While attending

a quality assurance meeting, the analysts discovered the current media preparation cards were inaccurate, resulting in the issuing of a corrective action. Their solution was to revise the cards to reflect the most up-to-date information, and I volunteered to help with this task.



Nutrient Agar

- 1000 mL Di Water
- 8 g Nutrient Broth
- 15 g Agar

- Heat to boil with agitation for 1 minute
- Dispense 10 mL into 60 large test tubes
- Autoclave tubes and leftover agar in flask for 15 min at 121˚C
- Tilt tubes immediately after removal from autoclave
- Cool flask to 45-50˚C and pour ~20 mL per plate

Shelf Life: Refrigerated up to 3 months **pH=6.8±0.2**
QC: Gram + control – *L. innocua;* Gram - Control – *E. coli*

Figure 2.6: Updated Media Preparation Recipe Card

In total, there were about 60 recipe cards I reviewed to ensure the correct information was printed on the cards. By doing so, this assured the analysts the correct ratio of ingredients was being used to make their microbiological media, preventing further quality assurance issues to arise.

Chapter 3 - Discussion

My time spent at KDAL brought many lessons learned and a deeper appreciation for food safety surveillance. Regulatory laboratories are an invaluable link in the food safety chain. The other programs at the Kansas Department of Agriculture rely on KDAL to report accurate, reputable data in order to enforce food safety laws or react to a noncompliant finding.

The feed section of the laboratory offers this surveillance service for animal feedstuffs through many different analyses. The one test I was able to assist with was determining the levels of mycotoxins present in animal feeds. By performing this analysis, KDAL is able to determine which samples, if any, are out of compliance and unsafe for animal consumption. After reporting their findings to the necessary program, a “stop sale” is ordered to prevent animals from eating contaminated feeds. Should an animal intended for human consumption become intoxicated, it becomes a public health risk if that animal carcass enters the food supply.

Assisting with a method verification demonstrated KDAL’s dedication to continual improvement. Although their previous method detection system was adequate, they saw the benefits of updating their machine to promote efficiency and cost effectiveness. Now, they are able to report meat and poultry sample results faster. If a positive result was found, the KDA Meat and Poultry program would be able to act on the nonconformance much more quickly, possibly preventing a foodborne illness outbreak.

Overall, the reliability and credibility of their test results would not be possible without the implementation of a quality management system, in this case, ISO/IEC 17025. Their compliance to ISO/IEC 17025 principles enables them to maintain

accreditation, but most importantly assures their customers the data they produce are provided competently, confidently, and accurately. Food safety is a complex problem with multiple stakeholders and sources of threats. My integrated learning experience reinforced the concept that preventing foodborne illness is not a matter of one threat, but rather an effort from all invested to do their part towards mitigation.

Chapter 4 - Competencies

Student Attainment of MPH Foundational Competencies

Table 5.1 Summary of MPH Foundational Competencies

Number and Competency		Description
2	Evidence-based Approaches to Public Health	Select quantitative and qualitative data collection methods appropriate for a given public health context
3	Evidence-based Approaches to Public Health	Analyze quantitative and qualitative data using biostatistics, informatics, computer-based programming and software, as appropriate
4	Evidence-based Approaches to Public Health	Interpret results of data analysis for public health research, policy, or practice
12	Policy in Public Health	Discuss multiple dimensions of the policy-making process, including the roles of ethics and evidence
11	Planning and Management to Promote Health	Select methods to evaluate public health programs
21	Interprofessional Practice	Perform effectively on interprofessional teams

Competency 2 – Quantitative and qualitative data collection methods were selected based on the appropriateness for a given public health context. The selection process was dependent on which analysis was being performed. During the detection of mycotoxins in animal feed, data were collected using LC-MS/MS to produce results for the analyst to interpret. When assisting with the method verification for detection of *Salmonella* spp. and *Listeria monocytogenes* using 3M™ MDS, this method of data collection was selected because of its efficiency and affordability.

Competency 3 – After the data were collected, analyses were performed using software programs that incorporated informatics, as well as biostatistics. While determining the levels of mycotoxins in animal feed by LC-MS/MS, MassLynx™ software provided the analysis of the data collected from the feed samples. The method for detecting *Salmonella* spp*.* and *Listeria monocytogenes* used the 3M™ MDS program, whereas the old detection method utilized Hygiena's BAX System Q7.

Competency 4 – This competency was achieved by interpreting and reporting the analysis results to the other programs at KDA that requested the sample testing. After the results were reported, that program would take action when necessary to ensure consumer protection and public safety.

Competency 11 – Prior to purchasing a new PCR machine, KDAL used Hygiena's BAX System Q7. They discovered replacing their old method with a detection method for foodborne pathogens from 3M™ would offer more efficiency and affordability, so I assisted with verifying the 3M™ MDS method during my ILE. Selecting this new method allowed KDAL to better serve the other public health programs at KDA.

Competency 12 – The ISO/IEC 17025 quality management system provided me with safety training necessary for each laboratory skill. Studying ISO/IEC 17025 allowed me the opportunity to practice safe laboratory techniques in order to promote accurate and credible test results.

Competency 21 – I was able to perform effectively on interprofessional teams in the day-to-day work environment at KDAL. More specifically, multiple analysts reference the same SOP during testing, and I was successful at adhering to the same SOP while working alongside other analysts to obtain the same test results.

Student Attainment of MPH Emphasis Area Competencies

Table 5.2 Summary of MPH Emphasis Area Competencies

MPH Emphasis Area: Food Safety and Biosecurity		
Number and Competency		**Description**
1	Food safety and biosecurity	Evaluate solutions appropriate for different food safety, biosecurity, and defense issues in the food production continuum.
2	Threats to the food system	Examine specific threats to the food system and scientifically investigate how each can be prevented, controlled, and/or mitigated in the food production system.
3	Food safety laws and regulations	Differentiate key U.S. food safety regulatory bodies and their unique legislative authorities, missions, and jurisdictions
4	Food safety policy and global food system	Analyze and distinguish how food safety and governmental biosecurity policies, globalization, and international trade cooperation influence public health.
5	Multidisciplinary leadership	Contrast the food safety and biosecurity technical needs of different stake holders and make judgements as to the appropriate methods of collaboration.

Competency 1 – Food Safety and Biosecurity – Through samples received by the different programs at KDA, the KDAL is the “investigative link” in the metaphorical regulatory chain for food safety and biosecurity. The testing performed on food products provides the other KDA programs (i.e. the Food Safety & Lodging or Meat & Poultry programs) with information necessary to promote food safety and better biosecurity. During my time in the laboratory, the Food Safety & Lodging (FS&L) program submitted environmental swabs from a food manufacturing company for detection of foodborne pathogens on food-contact surfaces. Through this surveillance testing, KDAL was able to report the results back to FS&L to evaluate appropriate solutions for food safety and biosecurity at the food manufacturer.

Competency 2 – Threats to the Food System – Although KDAL evaluates various types of threats to the food system, my focus during my ILE related to food system threats were foodborne pathogens and mycotoxin levels. The detection of foodborne pathogens in the food samples received provided information on whether or not a pathogen was present in samples from food production facilities. With these results, KDA is able to offer risk assessments and preventive actions to producers. When determining the levels of mycotoxins present, this information is useful for producers to evaluate where threats may exist in their production processes.

Competency 3 – Food Safety Laws and Regulations – Many U.S. food safety laws and regulations are abided by during analyses at KDAL. The overarching regulatory body for almost all tests performed at KDAL is their quality management system that complies with ISO/IEC 17025:2017. Through ISO/IEC 17025, the lab is able to carry out KDA's mission of ensuring a safe food supply and providing consumer protection by providing scientifically sound, high quality analytical services to ensure KDA meets the demands of its customers[14] For testing of foodborne pathogens, regulations from FDA and USDA are utilized depending on the product sample. Regulations from the National Grain and Feed Association under FDA are followed when determining mycotoxin levels, as well as guidelines from AAFCO for quality assurance and control and AOAC International.[11]

Competency 4 – Food Safety Policy and the Global Food System – This competency was achieved when learning and applying principles from ISO/IEC 17025-17. ISO/IEC accreditation enables KDAL to assure consumers they are producing consistent and unbiased data because the results provided are audited by a third-party.

KDAL is confident their efforts towards food safety and biosecurity are standardized on a global level since they are accredited by an international organization.

Competency 5 – Multidisciplinary Leadership – Collaboration with various stakeholders occurs continually at KDAL in order to communicate technical needs for promoting food safety and biosecurity. The other programs at KDA communicate often with the laboratory in order to address their needs. For example, the FS&L program will request specific tests on food samples depending on what food safety threat is of concern. The Dairy and Feed Safety program also communicates its testing needs to KDAL when feedstuffs or dairy products need analyzed for the sake of biosecurity and food safety. Prior to my onboarding, KDAL collaborated with the USDA to validate detection methods using the 3M™ MDS for *e. Coli* O157:H7 and STECs.

References

[1]*Kansas Department of Agriculture*. Kansas Department of Agriculture - Agency Information. (n.d.). Retrieved May 4, 2023, from https://agriculture.ks.gov/about-kda

[2]*Kansas Department of Agriculture*. Kansas Department of Agriculture - Agriculture Laboratory. (n.d.). Retrieved May 4, 2023, from https://agriculture.ks.gov/divisions-programs/ag-lab

[3]Santos Pereira, C., C Cunha, S., & Fernandes, J. O. (2019). Prevalent Mycotoxins in Animal Feed: Occurrence and Analytical Methods. *Toxins*, *11*(5), 290. https://doi.org/10.3390/toxins11050290

[4]*ISO/IEC 17025 - testing and Calibration Laboratories*. ISO. (2020, March 11). Retrieved May 4, 2023, from https://www.iso.org/ISO-IEC-17025-testing-and-calibration-laboratories.html

[5]*Molecular detection matrix control*. 3m.com. (n.d.). https://multimedia.3m.com/mws/media/772560O/3m-molecular-detection-matrix-control-product-instructions.pdf

[6]APHL. (n.d.). *Accreditation provides strong data to take action on contaminated pet food*. aphl.org. https://www.aphl.org/aboutAPHL/publications/Documents/FS-2019Jun-Raw-Pet-Food-Update.pdf

[7]Centers for Disease Control and Prevention. (2023, May 23). *Salmonella homepage*. Centers for Disease Control and Prevention. https://www.cdc.gov/salmonella/index.html

[8]Giannella, R. A. (1996). Chapter 21 Salmonella. In *Medical Microbiology* (4th ed.). essay. Retrieved May 24, 2023, from https://www.ncbi.nlm.nih.gov/books/NBK8435/.

[9]Awuchi, C. G., Ondari, E. N., Nwozo, S., Odongo, G. A., Eseoghene, I. J., Twinomuhwezi, H., Ogbonna, C. U., Upadhyay, A. K., Adeleye, A. O., & Okpala, C. O. R. (2022). Mycotoxins' Toxicological Mechanisms Involving Humans, Livestock and Their Associated Health Concerns: A Review. *Toxins*, *14*(3), 167. https://doi.org/10.3390/toxins14030167

[10]Rogalla D, Bomar PA. Listeria Monocytogenes. [Updated 2022 Jul 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534838/

[11]Document 2563: Determination of Mycotoxins in Animal Feeds by LC-MS/MS, Version #14, Effective 5/9/2023. Kansas Department of Agriculture Laboratory

[12]Document 588: Detection of *Salmonella* spp. Using the 3M™ Molecular Detection System, Version #11, Effective 6/5/23. Kansas Department of Agriculture Laboratory

[13]*3M™ MDS Lysis Tubes During Heating and Cooling*. (2019). 3M Science Applied to Life. Retrieved June 7, 2023, from https://multimedia.3m.com/mws/media/1083968O/3m-molecular-detection-assay-2-protocol-reference-guide.pdf.

[14]Document 708: ISO/IEC 17025 Quality Manual for the Kansas Department of Agriculture Laboratory, Version #11, Effective 8/10/2022. Kansas Department of Agriculture Laboratory

Appendix

Appendix 1: Determination of Mycotoxins in Animal Feed by LC-MS/MS KDA's SOP

KANSAS DEPARTMENT OF AGRICULTURE LABORATORY

DETERMINATION OF MYCOTOXINS IN ANIMAL FEED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Approvals

Editor: Amelia Hemmert **Date:** 5/5/2023 2:50:05 PM
Reviewed By: Sally Flowers **Date:** 5/9/2023 5:34:47 PM
Approved By: Sally Flowers Date: 5/9/2023 5:35:18 PM

All printed copies are considered uncontrolled.

Table of Contents

1. Purpose

   The purpose of this procedure is to describe the determination of Total Aflatoxins and Deoxynivalenol (DON) in complete animal feeds by high performance liquid chromatography tandem mass spectrometry (LC-MS/MS).

2. Scope

   This procedure is used to detect and quantify Total Aflatoxins and Deoxynivalenol in animal feed matrices by LC-MS/MS. This method will not be performed on molasses products, samples containing a moisture content of greater than or equal to 20 % moisture and greater than or equal to 5.5 % of calcium.

3. Responsibility

   It is the responsibility of the analyst to perform this procedure.

4. Frequency

   This procedure is performed on samples as needed.

5. References

   5.1 Food and Drug Administration (FDA), Determination of Mycotoxins in Corn, Peanut Butter, and Wheat Flour Using Stable Isotope Dilution Assay (SIDA) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), Version 1.0 (2017).
   5.2 Waters Corporation MassLynx Online Information System Acquity, 2011-2016.
   5.3 National Grain and Feed Association (NGFA), Food and Drug Administration (FDA) Mycotoxin Regulatory Guideline, A Guide for Grain Elevators, Feed Manufacturers, Grain Processors and Exporters, August 2011.
   5.4 AAFCO Quality Assurance/Quality Control Guidelines for Feed Laboratories with ISO/IEC 17025:2005, 2014.
   5.5 International Standard ISO/IEC 17025:2017, General requirements for the competence of testing and calibration laboratories, Third edition 2017-11.
   5.6 AOAC INTERNATIONAL Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food, Dietary Supplements, and Pharmaceuticals. An Aid to Interpretation of ISO/IEC 17025:2017, Revised August 2018.

6. Safety and Hazardous Waste

   6.1 Always wear Personal Protective Equipment (PPE) pertaining to the type of testing being performed. Handling of concentrated acids should be performed under a safety fume hood.
   6.2 Place all solid waste exposed to Mycotoxins (e.g., instrument vials, disposable pipettes, PTFE filters, glass test tubes, pipette tips) in the appropriate hazardous waste receptacle.
   6.3 Waste from the instrument should be disposed of as non-halogenated waste. Follow KDAL Document No. 704, Hazardous Waste.

7. Supplies, Reagents, Equipment, and Standards

   7.1 Supplies
      7.1.1 Lab coat or apron
      7.1.2 Safety glasses or face shield
      7.1.3 Disposable gloves
      7.1.4 Compressed Argon (Ar) – collision (COL) gas
      7.1.5 Nitrogen (N) – atmospheric pressure ionization (API) gas
      7.1.6 Weigh paper or boats – various sizes
      7.1.7 Weigh utensils
      7.1.8 Disposable transfer pipettes
      7.1.9 Glass reagent bottles – various sizes
      7.1.10 Pipettes – various sizes
      7.1.11 Pipette tips – various sizes
      7.1.12 Class A amber volumetric flasks – various sizes
      7.1.13 Class A graduated cylinders – various sizes
      7.1.14 Falcon centrifuge tubes – 15 mL
      7.1.15 Disposable glass test tubes – 10 mL
      7.1.16 Amber glass autosampler vials with pre-slit septum – 2 mL
      7.1.17 Luer-lock syringes – 3 mL
      7.1.18 PTFE luer-lock filters – 13 mm x 0.2 µm
      7.1.19 Permanent marker or labeling marker
      7.1.20 Autosampler tray
   7.2 Reagents
      7.2.1 Acetonitrile (ACN), Optima®
      7.2.2 Ammonium formate, Optima®
      7.2.3 Methanol (MeOH), Optima®
      7.2.4 Formic acid, Optima®
      7.2.5 Millipore water
      7.2.6 **Mobile Phase A** – 10 mM ammonium formate with 0.1 % formic acid in Millipore water. Expires after one month. Volume may be adjusted if the final concentration remains the same.

7.2.7.1 Weigh 0.63±0.02 g of ammonium formate into a 1 L volumetric flask. Add approximately 500 mL of Millipore water and swirl until solid has dissolved. Pipette 1 mL of formic acid then bring to volume with Millipore water and mix well. Transfer to a 1 L reagent bottle.

7.2.7 **Mobile Phase B** – 10 mM ammonium formate with 0.1 % formic acid in MeOH. Expires after three months. Volume may be adjusted if the final concentration remains the same.

7.2.8.1 Weigh 0.63±0.02 g of ammonium formate into a 1 L volumetric flask. Add approximately 500 mL of MeOH and swirl until solid has dissolved. Pipette 1 mL of formic acid then bring to volume with MeOH and mix well. Transfer to a 1 L reagent bottle.

7.2.8 **20 mM ammonium formate** – Weigh 0.63±0.02 g of ammonium formate into a 500 mL reagent bottle and add 500 mL of Millipore water. Mix until solid has dissolved. Expires after three months. Volume may be adjusted if the final concentration remains the same.

7.2.9 **Needle wash (35:35:30 MeOH:ACN:H_2O)** – Combine 350 mL of MeOH, 350 mL of ACN and 300 mL of Millipore water in a 1 L graduated cylinder. Transfer to a 1 L reagent bottle and mix well. Expires after three months. Volume may be adjusted if the solvent ratios remain the same.

7.2.10 **Seal wash (50:50 ACN:H_2O)** – Combine 250 mL of MeOH and 250 mL Millipore water. Transfer to a 500 mL reagent bottle and mix well. Expires after one month. Volume may be adjusted if the solvent ratios remain the same.

7.2.11 **Extraction solution (85:15 ACN:H_2O)** – Combine 850 mL of ACN and 150 mL of Millipore water in a 1 L graduated cylinder. Transfer to a 1 L reagent bottle and mix well. Expires after 3 months. Volume may be adjusted if the solvent ratios remain the same.

7.2.12 **Blank fluid (50:50 ACN:H_2O)** – Combine 100 mL of Millipore water and 100 mL of ACN. Transfer to a threaded 250 mL E-flask and mix well. Expires after three months. Volume may be adjusted if the solvent ratios remain the same.

7.3 Equipment

7.3.1 Waters Acquity H-Class UPLC Plus Sample Manager FTN-H

7.3.2 Waters Acquity H-Class UPLC Plus Quaternary Solvent Manager

7.3.3 Waters Acquity H-Class UPLC Plus Tandem Quadrupole Detector

7.3.4 Waters Acquity H-Class UPLC Plus Controlled Column Compartment

7.3.5 Waters Acquity UPLC Column – BEH C18 1.7 µm, 2.1 x 50mm
7.3.6 Waters Acquity Vanguard Pre-Column – BEH C18 1.7 µm, 2.1 x 5mm
7.3.7 Computer with MassLynx software
7.3.8 Wrist action shaker
7.3.9 Centrifuge
7.3.10 Vortex mixer
7.3.11 Analytical balance
7.3.12 Fume hood

7.4 Standards

NOTE: The Certificate of Analysis (COA) form for each standard must be retained and readily available. The certified concentration of the stock standards and expiration dates can be found on the COA forms. Use the certified values when calculating the final concentrations.

7.4.1 **Total Aflatoxins (1 µg/mL)** – Romer Labs Mix 9 (Aflatoxins), catalog number 10002879, 5 mL. Store refrigerated between 2-8 °C or in a freezer at ≤ -15 °C. Allow to warm to room temperature before use.

7.4.2 **Deoxynivalenol (100 µg/mL)** – Romer Labs Deoxynivalenol in Acetonitrile, catalog number 10000334, 5 mL. Store in a freezer at ≤ -15 °C. Allow to warm to room temperature before use.

7.4.3 **Working Standard (100 ng/mL Total Aflatoxins and 2,500 ng/mL Deoxynivalenol)** – Pipette 1000 µL of Mix 9 Total Aflatoxins standard and 250 µL of Deoxynivalenol standard into a 10 mL amber volumetric flask. Bring to volume with ACN and mix well. Expires after one month. Store in a freezer at ≤ -15 °C. Allow to warm to room temperature before use.

7.4.4 **5-point calibration curve** – Prepare curve from the working standard with blank fluid into separately labeled 2mL amber autosampler vials. Prepare fresh on day of use. Curve may be prepared as follows:

7.4.4.1 50 ng/mL Total Aflatoxins and 1,250 ng/mL Deoxynivalenol - 500 µL of working standard diluted with 500 µL of blank fluid. Total volume: 1.0 mL. Mix well.

7.4.4.2 25 ng/mL Total Aflatoxins and 625 ng/mL Deoxynivalenol - 250 µL of working standard diluted with 750 µL of blank fluid. Total volume: 1.0 mL. Mix well.

7.4.4.3 10 ng/mL Total Aflatoxins and 250 ng/mL Deoxynivalenol – 100 µL of working standard diluted with 900 µL of blank fluid. Total volume: 1.0 mL. Mix well.

7.4.4.4 5 ng/mL Total Aflatoxins and 125 ng/mL Deoxynivalenol - 50 µL of working standard diluted with 950 µL of blank fluid. Total volume: 1.0 mL. Mix well.

7.4.4.5 1 ng/mL Total Aflatoxins and 25 ng/mL Deoxynivalenol – 10 µL of working standard diluted with 990 µL of blank fluid. Total volume: 1.0 mL. Mix well.

8. Outline of Procedure

8.1 Sample preparation
8.2 Dilute and filter samples
8.3 Instrument conditions
8.4 Compound channels
8.5 Instrument preparation and sample analysis
8.6 Data integration, processing and reporting

9. Specific Procedure

9.1 Prior to analysis, ensure there are adequate amounts of reagents and standards.
9.1.1 Track reagents, standards and expiration dates on KDAL Document No. 2592, Mycotoxins Trace Worksheet.
9.2 Sample preparation
NOTE: Label 15 mL centrifuge tubes and autosampler vials with the corresponding calibration curve concentration or sample ID.
9.2.1 With each batch include Quality Control (QC) samples; a reagent blank, a matrix blank, laboratory control sample (LCS), matrix spike (MS) and a duplicate.
9.2.1.1 Reagent blank – add 10 mL of extraction solution.
9.2.1.2 Matrix blank – weigh 2±0.02 g of a chosen AAFCO sample containing no robust mean for Total Aflatoxins or DON.
9.2.1.3 LCS – weigh 2±0.02 g of a chosen AAFCO sample containing a robust mean for Total Aflatoxins and DON.
9.2.1.4 Matrix spike – weigh 2±0.02 g of the AAFCO sample used as the matrix blank. Add 500 µL of the working standard to the centrifuge tube followed by 9.5 mL of extraction solution.
9.2.1.5 Duplicate – weigh 2±0.02 g of a chosen sample from the sample set.
9.2.2 Sample(s) – weigh 2±0.02 g of ground sample into the corresponding 15 mL centrifuge tube.
9.2.3 Using a graduated cylinder add 10 mL of extraction solution to QC samples and each sample, except for the MS (see 9.2.1.4). Vortex for approximately 15 seconds until the matrix is completely saturated with the extraction solution.
9.2.4 Place samples on a wrist action shaker for approximately 30 minutes.
9.2.5 After shaking, centrifuge samples for 10 minutes at approximately 4000

RPM.

9.2.6 Using care to not disturb the solid sample portion, transfer samples to a fume hood and pipette 500 µL of the supernatant from each sample into separate disposable glass test tubes.

9.2.7 Pipette 500 µL of 20 mM ammonium formate to each test tube.

9.2.8 Vortex each test tube for approximately 15 seconds.

9.2.9 Attach a PTFE filter to a 3 mL syringe and remove the plunger from the syringe. Carefully pour in the test tube contents and reinsert the plunger to filter the sample through the syringe and into the corresponding amber autosampler vial.

9.2.10 Screw on the pre-slit septum cap and place the vial in the autosampler tray. Repeat for each sample test tube.

9.2.11 Transfer the autosampler tray from the fume hood to the Waters Acquity H-Class Plus Sample Manager FTN-H for sample analysis.

9.3 Instrument conditions

9.3.1 Run time: 5.0 minutes (min)

9.3.2 Flow rate: 0.7 µL/min

9.3.3 Injection volume: 2.0 µL

9.3.4 Source voltages

9.3.4.1 Capillary (kV): 0.50

9.3.4.2 Cone (V): 27

9.3.5 Temperatures

9.3.5.1 Column: 50 °C

9.3.5.2 Desolvation Temp: 500 °C

9.3.5.3 Source Temp: 150 °C

9.3.5.4 Sample: 10 °C

9.3.6 Inlet gradient

Time (min)	Solvent B (%)
0.0	10.0
0.5	10.0
2.0	90.0
4.0	10.0

9.3.7 Source gas flow

9.3.7.1 Desolvation (L//Hr): 1000

9.3.7.2 Cone (L/Hr): 0

9.3.8 Analyzer

9.3.8.1 LM Resolution 1: 12.6

9.3.8.2 HM Resolution 1: 14.4

9.3.8.3 Ion Energy 1: -0.3

9.3.8.4 LM Resolution 2: 12.5

9.3.8.5 HM Resolution 2: 14.4

9.3.8.6 Ion Energy 2: 0.2

9.4 Compound channels

9.4.1 Dwell (s): 0.030

Compound	Parent mass	MRM Transition	Cone (V)	Collision (eV)	RT (min)
Deoxynivalenol	297.0700	231.2000	22	13	1.04±0.25
Deoxynivalenol	297.0700	249.0300	22	10	1.04±0.25
Aflatoxin B1	312.9827	241.0169	56	38	2.075±0.385
Aflatoxin B1	312.9827	284.8084	56	24	2.075±0.385
Aflatoxin B2	315.0042	258.9811	54	30	1.96±0.29
Aflatoxin B2	315.0042	286.9590	54	26	1.96±0.29
Aflatoxin G1	329.0042	199.7198	54	42	1.98±0.32
Aflatoxin G1	329.0042	242.9906	54	26	1.98±0.32
Aflatoxin G2	331.0042	244.9973	56	30	1.985±0.585
Aflatoxin G2	331.0042	313.0000	56	21	1.985±0.585

9.5 Instrument set-up and sample analysis

9.5.1 Open the MassLynx software. In the toolbar select ‘File’ → ‘Open Project.’ A window will appear warning the user about changing projects, select ‘Yes’ to indicate the user is allowing change of the project. Select ‘Mycotoxins’ and click ‘OK.’

9.5.2 With the Mycotoxins project open, select ‘File’ followed by ‘Save as….’ Name the file as date_initials analyte (e.g., 08302025_JD Aflatoxins). The ‘File Name’ of each sample will reflect the project name followed by the sample analysis number.

9.5.3 Add each sample by right clicking the project window and selecting ‘Add.’ In the ‘add’ window type the number of samples to be analyzed including the 5-point calibration curve and QC samples.

NOTE: Analyze a continuing calibration verification (CCV) and a continuing calibration blank (CCB) every ten samples and at the end of the sample set. Perform a column cleanup with the inlet file “Mycotoxins Cleanup” at the end of the sample set.

9.5.4 Enter each ‘Sample ID’, ‘Sample Type’, ‘Conc A’ and ‘Conc B’ (concentration). The remaining columns will reflect the following:

9.5.4.1 ‘MS File’: 26OCT2020_Mycotoxins

9.5.4.2 ‘MS Tune’: DON_Tune 27OCT2020

9.5.4.3 ‘Inlet File’: 26OCT2020_Mycotoxins Inlet

9.5.4.4 ‘Inject Volume’: 2.0

9.5.4.5 ‘User Factor 2’: 0.0 (calibration curve and blank), 10.0 (extracted samples only)

9.5.4.6 ‘User Divisor 1’: 0.0 (calibration curve and blank), 2.0 (extracted samples only)

9.5.4.7 ‘User Factor 1’: 0.0 (calibration curve and blank), 2.0 (extracted samples only)

9.5.5 Once all necessary data has been entered, select ‘File’ then ‘Save.’

9.5.6 Select the ‘Instrument’ tab on the top left corner of the window → ‘MS Console.’

9.5.7 In the 'MS Console' window select 'System'. Ensure the 'API' and 'Collision' are on and select 'Operate'. When each are green, indicating operation, select 'Control' → 'Start up system….'

9.5.8 Within the 'System Startup' window select the tab 'SM.' The Sample Manager FTN will reflect the following:

9.5.8.1 Wash solvent: 10 sec

9.5.8.2 Purge solvent: 5 cycles

9.5.9 Select the 'QSM' tab. The Quaternary Solvent Manager will have 'A', 'B', 'C', 'D' and 'Seal wash' check marked. The 'Duration of prime' is 2.0 min.

NOTE: The 'Duration of prime' is 5.0 min if an instrument reagent has been replaced with fresh reagent.

9.5.10 When the startup of the system has finished, select 'Inlet Method' in the project window.

9.5.11 In the 'Inlet Method' window select 'LC' → 'Load Method.'

9.5.12 Select 'MS Console' → 'Quaternary Solvent Manager.' Locate the 'Delta' value and assure the delta value is less than 50 psi prior to starting analysis.

9.5.13 Open the project window and highlight all samples by selecting the first sample number and dragging to the last sample. To begin analysis, select 'Run' within the toolbar then click 'Start' from the dropdown menu.

9.6 Integrate and Process Results

9.6.1 Highlight all samples, select the tab 'TargetLynx XS' on the left-hand side of the project window, ensure 'NOV2020_Mycotoxins' is selected as the 'Method', then click 'Process Samples.'

9.6.2 Results are isolated by analyte. Review and integrate each sample for each analyte. The ion ratio for the QC samples and each sample must be within a range of ±20% of the average ion ratio of the 5-point standard curve. Additionally, the peak area shall be larger than the peak area of the lowest standard. If sample chromatographic data does not meet these criteria the peak will be neglected, this can be done by selecting any highlighted peak and pressing the 'delete' key.

NOTE: For further instruction see KDAL Document No. 4548, Work Instruction – Reprocessing Chromatographic Data.

9.6.3 Transfer results to the corresponding data worksheet. Report each Aflatoxin analyte in ng/mL and each Deoxynivalenol analyte in µg/mL.

9.6.3.1 Results are calculated in ng/mL by the instrument. Convert Deoxynivalenol results from ng/mL to µg/mL. See Calculation 10.3.

9.6.4 Update the corresponding control charts for each Aflatoxin analyte and DON LCS, duplicate, MS, reagent blank, CCV and CCB results. Refer to the Quality Assurance section for acceptability criteria.

9.6.5 If the QC results are acceptable, report the data to USAPlants or as

necessary.

9.6.6 Properly record the use of the instrument in the corresponding LC/MS/MS Equipment Logbook located near the instrument. Refer to Method Note 12.1.

10. Calculations

10.1 All samples are diluted by a user factor of 10.0 during extraction.
10.2 TargetLynx XS quantifies samples. The samples results are in ng/mL (PPB).
10.3 Divide ng/mL (parts per billion, PPB) by 1,000 in order to convert to µg/mL (parts per million, PPM), 1 PPM = 1000 PPB.

11. Quality Assurance

11.1 A run is a set of samples analyzed daily with corresponding quality control samples and may contain one or more batches. A *batch* is a collection of lab samples with corresponding required quality control. The maximum number of samples analyzed per batch is *35*.
11.2 An initial calibration verification (ICV) is not analyzed in this method due to the availability of a second source reference standard.
11.3 A Laboratory Control Sample (LCS) will be analyzed with each batch. The LCS results are monitored by creating accuracy control charts plotting Relative Standard Deviation (RSD). LCS value must be no more than ±3 standard deviations from the certified AAFCO value. If the LCS is unacceptable, all sample results are considered unreliable and cannot be reported.
11.4 A Laboratory Reagent Blank will be analyzed with every run. The blank recoveries are monitored by creating a recovery control chart plotting the percent found.
11.5 A Matrix Spike (MS) will be analyzed with each run. Matrix spike recoveries are monitored by creating a recovery control chart plotting percent recovered.
11.6 A Duplicate will be analyzed with each batch. Duplicate analysis results are monitored by creating precision control charts plotting Relative Percent Difference (RPD). The duplicate values should be ≤10 % RPD. If the duplicate is unacceptable, the sample used for the duplicate needs to be verified by a rerun. Further investigation needs to be performed to determine reliability of the run.
11.7 A Continuing Calibration Verification (CCV) and Continuing Calibration Blank (CCB) will be analyzed every 10 samples and at the end of each run. The CCV value(s) should be ±30 % of the true value. If the CCV and/or CCB is unacceptable, the samples after the last passing CCV and/or CCB result are considered unreliable and cannot be reported.

11.8 All unacceptable quality control (QC) results must be investigated and documented in the control chart comment section with a root cause analysis.

11.9 If a sample contains a level of Aflatoxins or DON past the action levels provided by the FDA, the sample is in violation. If the sample is a violation, the sample will be reground and rerun in duplicate to confirm results. The Midpoint value of the three results will then be reported.

11.9.1 If a sample is reground, a note must be made in USAPlants for feed samples. The note must include the date of when the sample was reground and the initials of who reground the sample.

11.10 Possible interferences may be present (e.g., matrix interference or carryover). Selectivity analysis has been conducted within the method validation study to identify interferences and method optimization has been conducted, as needed, to separate the target analyte from the interference. Glassware being used for chromatographical analysis will be properly cleaned.

11.11 Record solution and reagent numbers and expiration dates on KDAL Document No. 2592, Mycotoxins Trace Worksheet.

11.12 For clarification of terms or quality control requirements, see AAFCO QA/QC Guidelines for Feed Laboratories.

12. Method Notes

12.1 A day of use or equipment logbook for the specific equipment must be maintained and readily available. See KDAL Document No. 994, Calibration, Verification and Maintenance of Critical Equipment for details.

12.1.1 The logbook should include:

12.1.1.1 The date of analysis, instrument maintenance, or preventative maintenance by a technician or analyst.

12.1.1.2 Initials of analyst.

12.2 Instrument downtime, including downtime due to annual preventative maintenance, will be documented in the Feed Metrics excel document.

Appendix 2: Media Recipe Cards

mBPWp

- **500 mL DI Water**
- **21.05 g BPW w/Pyruvate**

- Mix to dissolve (heat if necessary)
- Dispense 225 mL into 500 mL bottles
- Autoclave 15 min at 121 ˚C
- Cool to 45-50°C
- Aseptically add 1 vial of the rehydrated ACV Supplement

Shelf Life: Refrigerated up to 3 months **pH=7.2±0.2**
QC: Pos. Control – *E. coli* w/ACV supp.; Neg. Control – *E. coli O157:H7* w/AVC supp.

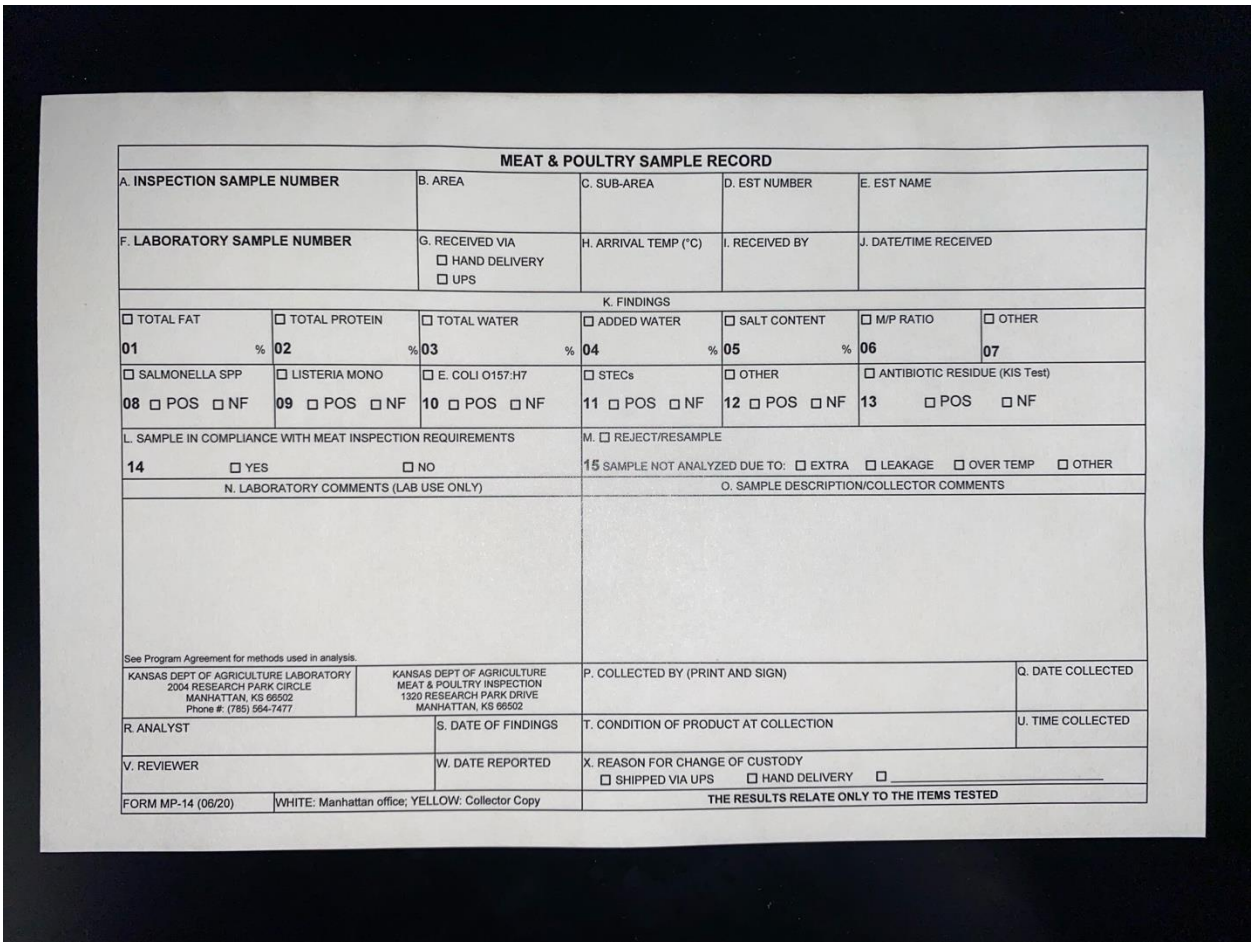
Demi-Fraser Broth

- 1000 mL DI Water
- 55 g Demi-Fraser Broth Base

- Mix to dissolve
- Dispense 225 mL into bottles
- Autoclave 15 min at 121˚C
- Cool to room temperature
- Aseptically add 2.25 mL Fraser Broth Supplement (Ref: BP0220010) to each bottle

Shelf Life: Refrigerated up to 3 months **pH=7.2±0.2**
QC: Pos. Control – *L. mono*; Neg. Control – *E. coli* 25922

Appendix 3: Meat & Poultry Sample Record



MEAT & POULTRY SAMPLE RECORD

A. **INSPECTION SAMPLE NUMBER**	B. AREA	C. SUB-AREA	D. EST NUMBER	E. EST NAME
F. **LABORATORY SAMPLE NUMBER**	G. RECEIVED VIA ☐ HAND DELIVERY ☐ UPS	H. ARRIVAL TEMP (°C)	I. RECEIVED BY	J. DATE/TIME RECEIVED

K. FINDINGS

☐ TOTAL FAT	☐ TOTAL PROTEIN	☐ TOTAL WATER	☐ ADDED WATER	☐ SALT CONTENT	☐ M/P RATIO	☐ OTHER
01 %	02 %	03 %	04 %	05 %	06	07
☐ SALMONELLA SPP	☐ LISTERIA MONO	☐ E. COLI O157:H7	☐ STECs	☐ OTHER	☐ ANTIBIOTIC RESIDUE (KIS Test)	
08 ☐ POS ☐ NF	09 ☐ POS ☐ NF	10 ☐ POS ☐ NF	11 ☐ POS ☐ NF	12 ☐ POS ☐ NF	13 ☐ POS ☐ NF	

L. SAMPLE IN COMPLIANCE WITH MEAT INSPECTION REQUIREMENTS	M. ☐ REJECT/RESAMPLE
14 ☐ YES ☐ NO	15 SAMPLE NOT ANALYZED DUE TO: ☐ EXTRA ☐ LEAKAGE ☐ OVER TEMP ☐ OTHER
N. LABORATORY COMMENTS (LAB USE ONLY)	O. SAMPLE DESCRIPTION/COLLECTOR COMMENTS
See Program Agreement for methods used in analysis.	

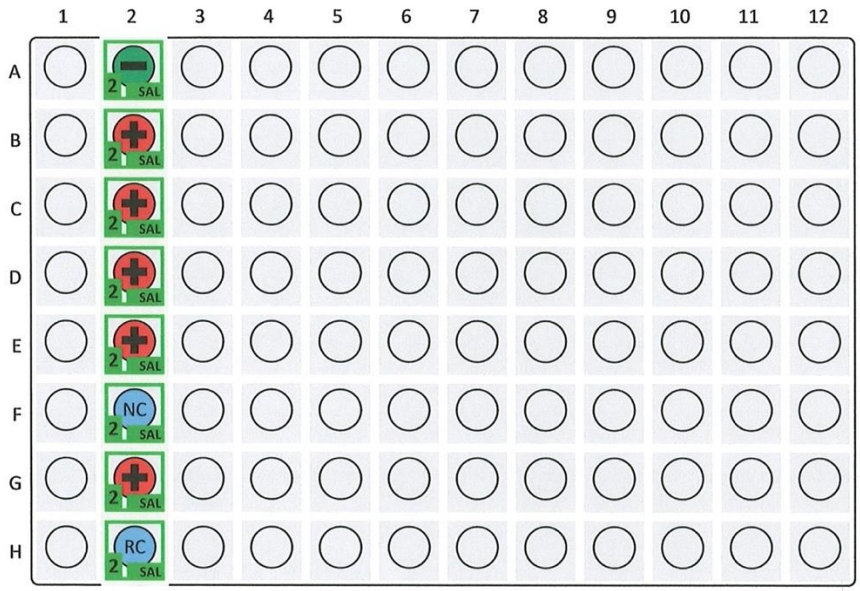
KANSAS DEPT OF AGRICULTURE LABORATORY 2004 RESEARCH PARK CIRCLE MANHATTAN, KS 66502 Phone #: (785) 564-7477	KANSAS DEPT OF AGRICULTURE MEAT & POULTRY INSPECTION 1320 RESEARCH PARK DRIVE MANHATTAN, KS 66502	P. COLLECTED BY (PRINT AND SIGN)	Q. DATE COLLECTED
R. ANALYST	S. DATE OF FINDINGS	T. CONDITION OF PRODUCT AT COLLECTION	U. TIME COLLECTED
V. REVIEWER	W. DATE REPORTED	X. REASON FOR CHANGE OF CUSTODY ☐ SHIPPED VIA UPS ☐ HAND DELIVERY ☐ _____	
FORM MP-14 (06/20)	WHITE: Manhattan office; YELLOW: Collector Copy	**THE RESULTS RELATE ONLY TO THE ITEMS TESTED**	

Appendix 4: 3M™ MDS Run Report for *Salmonella* spp. Method Verification

3M Molecular Detection Software

Run Report **Current Date Format** MM/dd/yyyy

Run ID	06232023(1)	**Run Date**	06/23/2023 09:59:11 AM
Run Status	Completed	**User**	Sally Flowers
Technician	Sally Flowers	**Report By**	Sally Flowers
Run Comment		**Instrument**	0221040033:221040033



Well ID	Sample ID	Assay Type	Gene Target	Well Type	Kit Lot Number	Result	Final Result	Comment
A2	S-B	Salmonella-2		Sample	9976014	Negative		
B2	S-1	Salmonella-2		Sample	9976014	Positive		
C2	S-2	Salmonella-2		Sample	9976014	Positive		
D2	S-3	Salmonella-2		Sample	9976014	Positive		
E2	S-4	Salmonella-2		Sample	9976014	Positive		
F2		Salmonella-2		Negative Control	9976014	Valid		Blank
G2	H2S -	Salmonella-2		Sample	9976014	Positive		
H2		Salmonella-2		Reagent Control	9976014	Valid		H2S +

Print Date : 06/23/2023 10:59:46 AM 1 of 3