



Learning About Kansas Department of Agriculture's Laboratory

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MPH Candidate

2023



- Started MPH program: Fall 2020
- Area of Emphasis: Food Safety and Biosecurity
- APE Site: Kansas Department of Agriculture's Laboratory (KDAL)
- Preceptor: Dr. Sally Flowers, Lab Director



Outline

- Overview of KDAL
- APE Objectives and Projects
- MPH Competencies
- FS&B Competencies
- Conclusion
- Acknowledgements



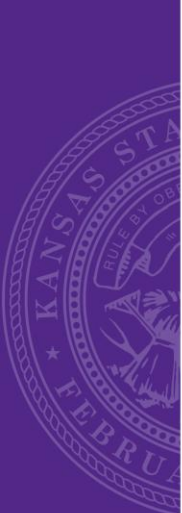
Overview of KDAL

- First state department of agriculture in the United States¹
- Services six of the eleven other programs and divisions of KDA
 - Dairy and Feed Safety
 - Food Safety and Lodging
 - Meat and Poultry
 - Pesticide and Fertilizer
 - Plant Protection and Weed Control
 - Weights and Measures Program

APE Objectives

- Obtain a better understanding of how a public health laboratory operates
- Become more familiar with basic microbiological and chemical techniques relating to food safety and quality





Projects

- Helped perform sample extractions for mycotoxin testing in animal feeds
- Assisted with method verification of a microbiological testing platform in their biosafety level-2 lab
- Learned about and applied their quality management system, ISO 17025:2017

Determination of Mycotoxins in Animal Feeds

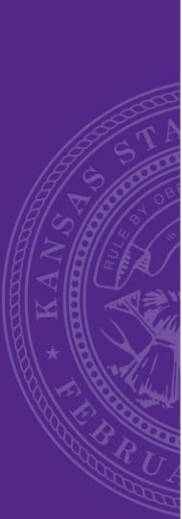


Feed and Fertilizer Lab

Determination of Mycotoxins in Animal Feeds

- Mycotoxins:
 - Produced by filamentous fungi
 - Typically from genera such as *Aspergillus*, *Penicillium*, and *Alternaria*²
 - KDAL tests for aflatoxins and deoxynivalenol
 - Can cause both chronic and acute health threats if inhaled and introduced to the lymphatic system and blood stream¹⁰





Determination of Mycotoxins in Animal Feeds

- Utilized high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS)³
- Sample preparation:
 - Grind feed samples
 - Weigh into 2 ± 0.02 g aliquots
 - Add extraction solution
 - Saturate sample powder in solution with wrist-action shaker for 30 min.
 - Centrifuge aliquots
 - Transfer 500 microliters of supernatant into new test tubes with equal parts of ammonium formate added and vortexed
 - Filter-syringe solution into autosampler vials
 - Load autosampler tray into for analysis

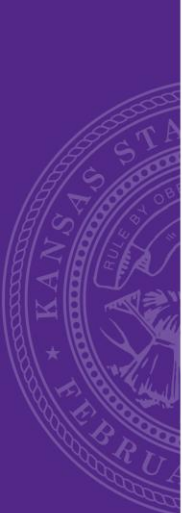
Determination of Mycotoxins in Animal Feeds



Wrist-Action Shaker



Auto-Sampler Tray



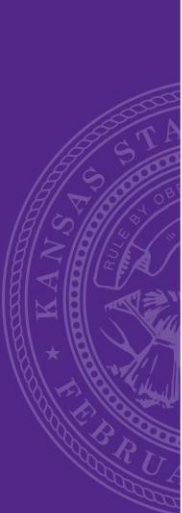
Determination of Mycotoxins in Animal Feeds

- Result analysis:
 - The software generates graphed peaks illustrating the concentration of analytes. The area under each peak is calculated and compared to the standard curve to quantify the amount of mycotoxins present.
 - The calculated levels of mycotoxins are then compared to regulations to determine acceptability.
- Public Health Implications
 - Concentrations outside of acceptable levels pose a health risk to animals and humans.
 - People consuming meat from infected animals causes indirect exposure of mycotoxicosis.

3M™ Molecular Detection System Method Verification

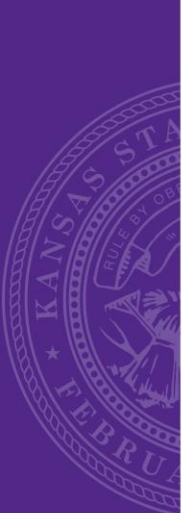


BSL-2 Lab



3M™ Molecular Detection System Method Verification

- Used for detection of foodborne pathogens
- *Salmonella* spp.:
 - CDC reports *Salmonella* contaminated foods are responsible for about 1.35 million infections/year.⁴
 - *Salmonella* symptoms: fever, stomach cramps, diarrhea (often bloody)
 - Capable of surviving stomach's acidic environment to infiltrate the mucosa of the intestines and produce toxins⁵
- *Listeria monocytogenes*:
 - CDC reports 1,600 listeriosis infections annually with about 260 deaths/year.⁴
 - Of great concern for immunocompromised, elderly, and pregnant individuals
 - Listeriosis symptoms: fever, nausea, muscle aches, vomiting, diarrhea
 - Psychrotroph⁶



3M™ Molecular Detection System Method Verification

- Operates by amplifying nucleic acid sequences⁷
 - loop-mediated isothermal amplification
 - Bioluminescence to identify the amplification
- Sample Preparation:
 - Blend 25 ± 1 gram of received sample
 - Add enrichment media to ground sample in sterile whirl-pak bag
 - *Salmonella*: Buffered Peptone Water (ISO)
 - *Listeria monocytogenes*: Demi-Fraser Broth
 - Stomach whirl-pak bag
 - Incubate at 37 ± 1 °C
 - *Salmonella*: 18-26 hrs
 - *Listeria monocytogenes*: 24-30 hrs
 - Control Broths made for each set of samples
 - Blank: ensure media sterility
 - Negative control: confirm specificity
 - Positive control: prove sensitivity

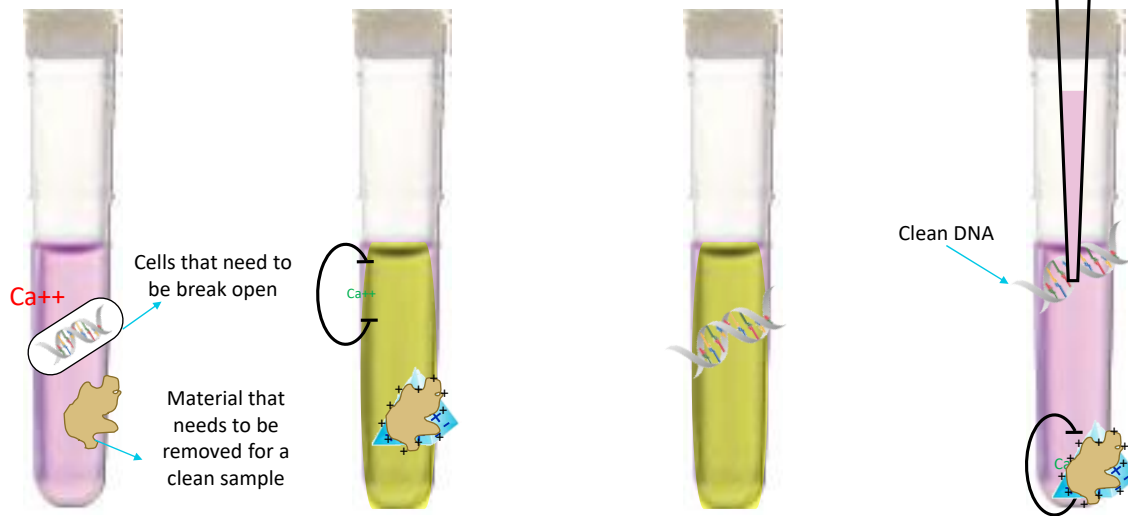
3M™ Molecular Detection System Method Verification

Incubation of
samples and QCs



3M™ Molecular Detection System Method Verification

3M Lysis tubes.... During heating and cooling⁸



Enriched samples are a complex mix of inhibitory substances and (potentially) target pathogens

3M nanotechnology deactivates the inhibitory materials and they sink to the bottom

While target DNA will rise to the top

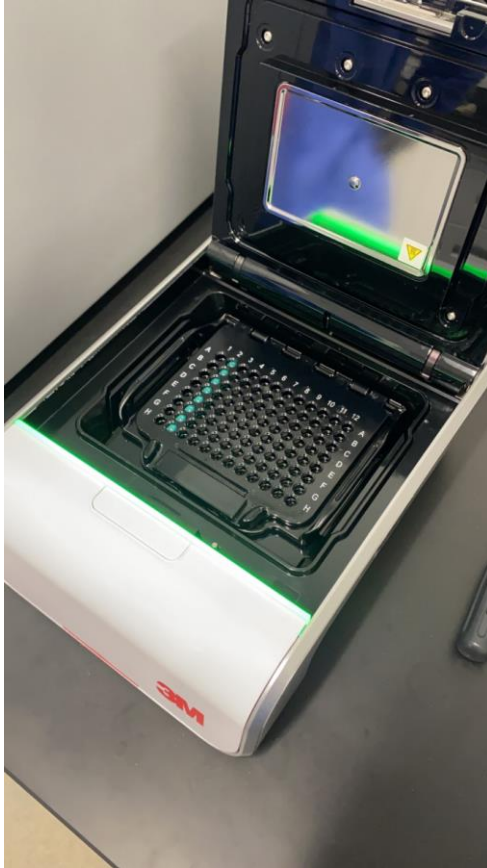
**Advanced Technology.
Simplified.**

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3M

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3M™ Molecular Detection System Method Verification



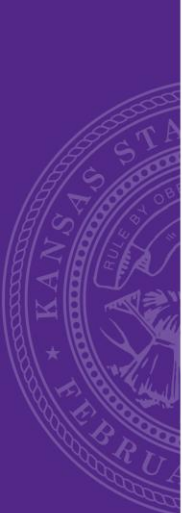
- Amplification preparation:
 - Lysate transferred into reagent tubes
 - Reagent tubes loaded into speed loader tray
 - Tray placed into detection machine to begin MDS assay¹²

3M™ Molecular Detection System Method Verification



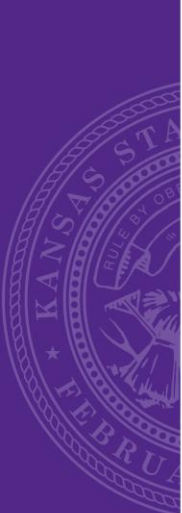
MDS Run Report

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 +



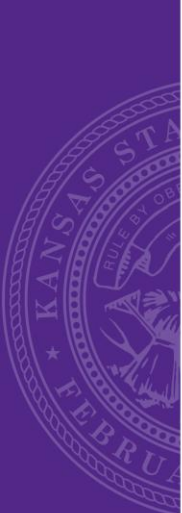
3M™ Molecular Detection System Method Verification

- Public Health Implications
 - Offers more rapid detection results than previously used BAX PCR
 - Meat and Poultry Program (M&P) could act on a nonconformance more quickly, possibly preventing a foodborne illness outbreak
 - Enables M&P to obtain smaller sample sizes from packing plants for testing
 - Routine surveillance of packing plant products further mitigates the chances of a foodborne illness outbreak



ISO 17025:2017 Principles & Applications

- General requirements for the competence of testing and calibration laboratories
- Applies to all processes and analyses at KDAL
- Agreed upon standards by experts internationally
- Enables KDAL to demonstrate they operate competently and generate valid results⁹
- “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.” – Dr. Sally Flowers



ISO 17025:2017 Principles & Applications

- Application:
 - Sample Receiving/Chain of Custody
 - Document exact minute a sample was transferred from program inspectors to laboratory analysts
 - Analysts' responsibility to ensure sample was not compromised while in custody
 - Chain of custody ends when sample is discarded

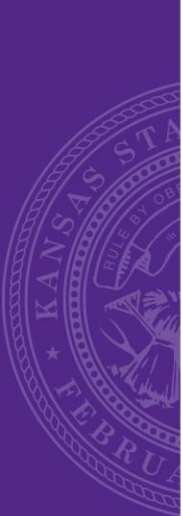
ISO 17025:2017 Principles & Applications

Sample Receiving Room



Meat and 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		
01 %		02 %		03 %		04 %		05 %		
☐ SALMONELLA SPP		☐ LISTERIA MONO		☐ E. COLI O157:H7		☐ STECs		☐ OTHER		
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					P. COLLECTED BY (PRINT AND SIGN)					Q. DATE COLLECTED
					R. ANALYST					S. DATE OF FINDINGS
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



ISO 17025:2017 Principles & Applications

- Applications:
 - Demonstration of Capability for analyses
 - Read SOP
 - Observe trained analyst
 - Perform analysis under supervision
 - Perform test independently successfully
 - Media Preparation Recipe Cards
 - Updated almost 60 recipe cards for the Dairy and Micro lab sections

ISO 17025:2017 Principles & Applications

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

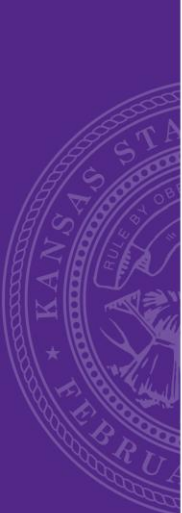
pH=7.2±0.2

QC: Pos. Control – *L. mono*; Neg. Control – *E. coli* 25922

MPH Competencies

Number and Competency		Description
2-4	<u>Evidence-Based Approaches to Public Health:</u> Select and analyze quantitative/qualitative data for a given public health context, and interpret results of data analysis for public health practice.	Appropriate data collection methods were selected depending on the test performed. Analyses were performed using informatics and computer-based programming and software. Results were interpreted and reported to their respective programs for necessary action related to public safety and consumer protection.
11	<u>Planning and Management to Promote Health:</u> Select methods to evaluate public health programs	Verified a new detection method for <i>Salmonella</i> spp. and <i>Listeria monocytogenes</i> in food products to increase the lab's efficiency

For information regarding the other competencies, see APE & ILE reports.

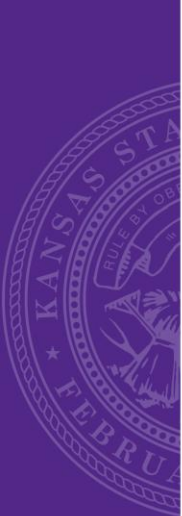


Food Safety & Biosecurity Competencies

MPH Emphasis Area: Food Safety and Biosecurity

Number and Competency		Description
3	<u>Food Safety Laws and Regulations:</u> Differentiate key U.S. food safety regulatory bodies and their unique legislative authorities, missions, and jurisdictions	ISO 17025 enables KDAL to ensure a safe food supply and provide consumer protection by producing scientifically sound, high quality analytical services. Regulations such as FDA and USDA are utilized for testing procedures of foodborne pathogens, National Grain and Feed Assoc. under FDA for mycotoxin testing
5	<u>Multidisciplinary leadership:</u> Contrast the food safety and biosecurity technical needs of different stake holders and make judgements as to the appropriate methods of collaboration.	Continual collaboration between KDAL and other KDA programs to support food safety and biosecurity. Prior to onboarding, collaboration with the USDA occurred in order to validate the 3M™ MDS method for detection of <i>E. coli</i> O157:H7 and STECs.

For information regarding the other competencies, see APE & ILE reports.



Conclusion

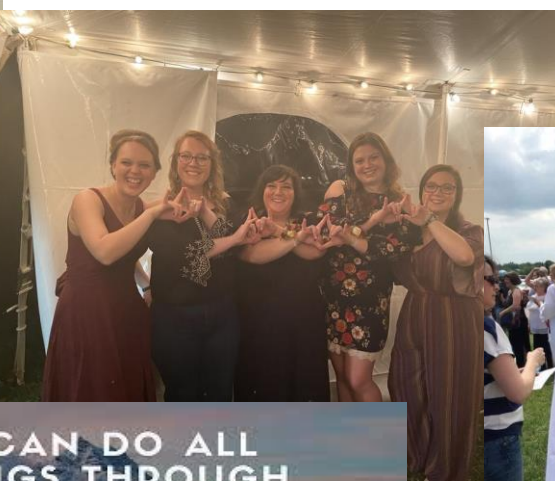
- Regulatory laboratories are an invaluable link in the food safety chain.
- Without testing food samples and reporting accurate, reputable data, the other programs at KDA would not be able to enforce food safety laws or react to noncompliant findings.



Acknowledgements

- MPH Committee
 - Dr. Trinetta, major professor
 - Dr. Kastner, committee member
 - Dr. Mulcahy, committee member
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 - Dr. Flowers, Laboratory Director
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 - Buudhika, Chemistry Section Supervisor
 - Amelia, Quality Assurance Manager
 - Katie, Jared, Michelle, Wan Jun, and Kallie – analysts, trainers, and friends

Thank You, All!



"I CAN DO ALL
THINGS THROUGH
CHRIST WHO
STRENGTHENS ME."

PHILIPPIANS 4:13

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