ADVANCED TESTING METHODS TO ENHANCE PRODUCT SAFETY

GUIDANCE FOR THE U.S. DAIRY INDUSTRY

INNOVATION CENTER FOR U.S. DAIRY

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The Innovation Center for U.S. Dairy® (IC), formed in 2008, provides a forum for the dairy industry to work together pre-competitively. Collectively, the IC represents over 500 dairy manufacturers and over 80 percent of the U.S. milk supply. One important IC initiative is the Food Safety Team, which helps ensure dairy products are safe by providing resources and training in all facets of dairy manufacturing. The IC Food Safety Team is very active with over 100 experts from 50 organizations involved across multiple platforms. Learn more at: www.usdairy.com/foodsafety. If you have specific questions, please email innovationcenter@usdairy.com.

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To The Reader

For dairy manufacturers, a robust preventive controls program—supported by rapid, accurate pathogen detection—creates the operational discipline needed to hold product, investigate deviations, and implement corrective actions before issues escalate. Advances in testing technology have transformed this process. For example, comparative strain-typing now enables facilities, to differentiate transient contamination from resident strains, link isolates with far greater precision, and make faster, more confident decisions about product disposition. These capabilities reduce the volume of products placed on hold, minimize the cost of compromised inventory, and strengthen the defensibility of food safety decisions during regulatory or customer review.

Also, the FDA's food safety framework is built around a comprehensive, risk-based approach that aligns regulatory expectations with the realities of modern manufacturing. Under the Food Safety Modernization Act (FSMA), food facilities are required to implement preventive controls, conduct hazard analyses, and maintain documented programs that proactively manage risk across production, storage, and transportation. This shift toward prevention has elevated the

role of environmental monitoring, process control, and data driven decision-making across the industry.

This guidance document will cover what advanced testing methodologies are available for use, considerations for use to aid in the determination of what method or methods may work best within your facilities and how best to employ them during an investigation and CAPA process to ensure success. This guidance is offered by the Food Safety Operating Committee of the Innovation Center for U.S. Dairy. It is part of a broad set of food safety education initiatives designed to strengthen manufacturing practices in all dairy processing facilities with the goal of reducing food safety risks. Thank you for sharing in the industry's commitment to advancing food safety performance daily. Visit www.usdairy.com/foodsafety for more information.

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INTRODUCTION TO TESTING

If present, pathogens in manufacturing environments are often in low quantities. Their levels must be increased to allow detection by most testing methods. Therefore, a common way to analyze samples is through an enrichment and detection testing process, which must be validated for the product matrix being tested. The sample is added to enrichment media and incubated, which causes the target organism to increase in numbers, if present. The enrichment medium is often selective to allow the growth of the target organism and minimize growth of other types of bacteria. After the enrichment process is complete, a detection method is utilized to determine if the target organism is present in the enriched sample.

The detection method may be based on cultural techniques, or it may be based on more rapid testing technology. Some rapid detection methods, such as Enzyme-Linked Immunosorbent Assay (ELISA), Enzyme-Linked Fluorescent Assay (ELFA) (e.g., VITEK®) and Lateral Flow, look for the presence of specific proteins that are unique to the target organism. Other rapid methods, such as Polymerase Chain Reaction (PCR), Reverse Transcription (RT)-PCR, and Isothermal Amplification of DNA, look for the presence of nucleic acid fragments that are unique to the target organism.

If the result from a rapid detection method is positive, the food industry often considers this result "presumptive," as this indicates the presence of unique molecules of the target organism (e.g., proteins, nucleic acid) that can be associated with either viable or non-viable cells. These molecules can be present in the absence of viable cells for a variety of reasons, such as inactivation by a heat treatment or cleaning and sanitizing procedures.

After a presumptive positive result is obtained, confirmatory testing is often conducted to determine if there are viable cells of the target organism present or not. A confirmatory test generally involves further culturing of the enriched sample to isolate the target organism, if viable. These confirmatory tests also may begin to further characterize the organism that was detected, such as determining the species. Routine identification methods may be used during this confirmatory test process, such as Biochemical Testing with Profiling (e.g., API®, VITEK®), among others.

If no viable cells of the target organism are present, or a different organism was responsible for the presumptive positive result, the confirmatory test will yield a negative result. The interpretation of a result from a sample with a positive rapid test, but a negative confirmatory test should be further determined on a case-by-case basis. While the goal may be to achieve a negative test result, it may be important to consider why non-viable proteins or nucleic acid associated with the target organism may be present in the sample.

After a confirmed positive result, you will typically have some basic information about the isolate (e.g., genus and sometimes species). ***At that point, more advanced testing methods can be utilized.*** For the purposes of this document, the term "advanced testing methods" refers to those testing methods that can yield in depth microorganism strain characterizations (e.g., strain, serotype) and/or comparisons. These methods can be utilized for both pathogens and non-pathogenic organisms (e.g., *Listeria innocua*) if their presence is a concern, although this document focuses on pathogens. The remainder of this document summarizes the common methods available for advanced isolate characterization and comparison, as well as considerations for their use.

CONSIDERATIONS FOR USE

Advanced testing methods can supplement an already established product and/or environmental testing program. It is, however, important to consider the range of possible results before utilizing these methods. It is best practice to consider all possible results/findings before the testing is initiated. This will help determine if this testing is appropriate, the true value of the testing, and to clearly state the ultimate goal. The following questions should be considered when determining the potential use of these advanced testing methods within your testing program. After each question we have added some information to consider.

Note: The development of a routine product or environmental monitoring testing program is not within the scope of this document. For more information on establishing a routine testing program see additional Food Safety Guidance Documents from the Innovation Center for U.S. Dairy.

Will advanced testing methods add value to my routine testing program?

The information gained from advanced testing methods can be useful, but the impact to food safety programs will be the highest in certain situations, such as the following:

a. When there is no further food safety mitigation step for the finished product (i.e., the product is ready to eat).
b. If the product is intended for a higher risk population (e.g., infants, elderly, immuno-compromised).
c. If the manufacturing plant has a history of positive finished product test results and/or has identified an increasing trend in environmental testing results. An increasing trend in environmental testing results could be a general increase in overall positive test results, and/or the potential presence of a resident pathogen (recurrence after wash cycles, non-consecutive positive results in the same area, etc.).
d. If the manufacturing plant has infrastructure/sanitary equipment design challenges in general, creating a higher risk during processing.

If advanced testing methods add value, when should I use them?

The use of these methods is situational, and they are not typically used in all parts of a routine testing program.

a. These methods can be valuable to investigate the root cause of a pathogen detected in a finished product.
b. The use of these methods after a pathogen is detected in the environment is situational but leveraging them to understand the baseline ecology of the plant environment can be very useful. For example, it may be more valuable to conduct this extra testing on samples originating from zones 1 and 2 vs. zones 3 and 4, unless the samples from zones 3 and 4 are part of an investigation into a larger issue.
c. Another strategy that can be employed is to save all isolates through freezing (this typically involves combining the bacterial suspension with a cryoprotectant, such as glycerol, and storing in a -80°C freezer). If an isolate is saved, it could be tested in the future if new information makes that data more critical. If this is done, a program should be established with input from a cross functional team, including those with legal and regulatory expertise.

Note: As always, implications to finished product should be considered when testing product isolates or isolates from zone 1 environmental locations.

d. These methods can be useful for all pathogens and may be especially valuable for pathogens that are more challenging to characterize with more traditional methods, such as *Clostridium* spp., *Bacillus* spp., and *Salmonella* spp.

e. The focus of these methods is typically for pathogens as part of the food safety program. They can also be utilized for non-pathogenic microorganisms of significance (e.g., *Listeria innocua*), "indicator" organisms (e.g., Enterobacteriaceae), and spoilage organisms (e.g., *Pseudomonas* spp. in fluid milk), if a root cause investigation is appropriate.

What specific method should I use?

It is important to focus on the desired outcome, not the specific method. You will need to understand what information is needed based on the problem you are trying to solve. For example, you may choose a different method if you are comparing isolates vs. if you are investigating whether certain pathogenic genes are present in an isolate, which some advanced testing methods are capable of, but not all. There are several things to consider when choosing the best method:

a. Do you have the infrastructure, budget, physical space, and resources to conduct the testing in-house or will you be using an external laboratory? If using an external laboratory, what testing methods are offered? Some external laboratories offer certain advanced testing methods, and if not, they may be able to send the sample(s) to another subcontracted laboratory that has the needed capabilities.
b. It is valuable to choose one method to be used for all samples from the same manufacturing facility to build a consistent database for future reference and comparisons.
c. What is the cost of the test? The cost of certain advanced testing methods can be significant and needs to be considered within the overall testing budget.
d. What is the turnaround time for results? Some of these methods have a longer turnaround time compared to standard microbiological tests, making them more useful as part of longer-term corrective actions vs developing immediate corrections to an issue.
e. How complex is the data analysis and interpretation? This may determine whether you need help from an external laboratory, or other experts in the area.

See Methods Summary on page 10 for more information on specific methods.

Are there any regulatory considerations?

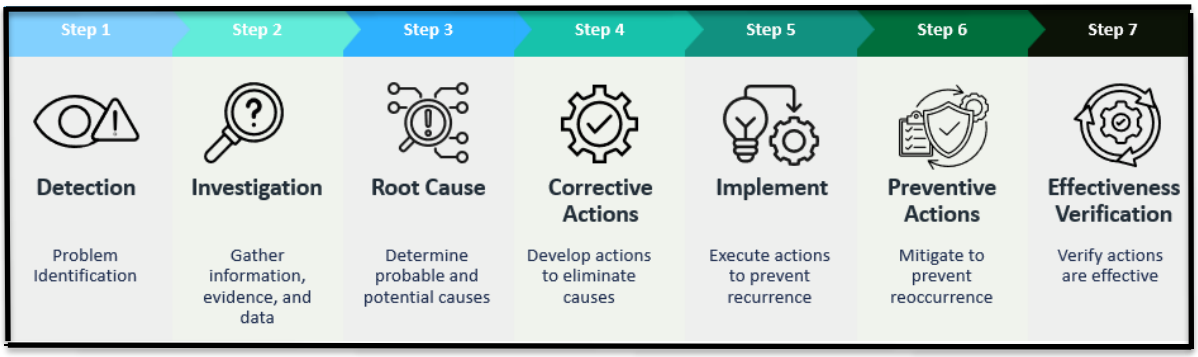
You need to understand where your results will be stored and who will have access to them. If external reference sources/databases are used, others could have access to that information and questions/liability concerns can arise about who controls that data and how it might be used by regulators or litigants. You may consider not storing information externally, such as in a public database, and only maintaining an internal database.

This topic has been a specific focus for whole genome sequencing (WGS) results because several regulatory agencies in the United States, including the Food and Drug Administration (FDA), the U.S. Department of Agriculture, and the Centers for Disease Control and Prevention, have embraced WGS as part of food safety surveillance, as a supplement to traditional epidemiology.

a. The FDA leads a program called GenomeTrakr, which is a network that uses WGS to link clinical, food, and environmental isolates, enabling faster and more accurate outbreak investigations.
b. There is a publicly accessible external database hosted by the National Center for Biotechnology Information, or NCBI, where companies and public health labs can upload whole genome sequences. These sequences are used to trace contamination sources, identify outbreak patterns, and support regulatory actions.
c. The FDA does not automatically have access to a company's WGS data, but there may be cases where this data is requested (e.g., a "for cause" inspection).
d. It is also important to understand that the WGS science is still evolving regarding what level of genomic relatedness results in a match since random genetic changes take place regularly. The amount of genetic change over time is dependent on many factors, including the specific organism and environmental conditions. This makes it difficult to determine a cutoff in the amount of genetic change that would still represent organisms from the same source.

Legal experts should be included in the decision of whether to use these methods, and the process for data collection and management.

USE WITHIN THE CORRECTIVE AND PREVENTIVE ACTION (CAPA) PROCESS



Introduction to CAPA

CAPA stands for Corrective and Preventive Actions. It is a structured system for identifying, investigating, and resolving problems related to food safety and quality. CAPA combines Corrective Actions, which are the steps to fix a problem, and Preventive Actions, which are the changes put in place to prevent recurrence.

The image above explains the CAPA process in 7 steps. The advanced testing methods described in this document can be valuable tools during this process, especially during Step 2 (Investigation) and Step 3 (Identify Root Cause), which are explained in more detail below.

a. It is important to note that the FDA has a focus in this area. There have been several warning letters published focusing on the lack of a thorough CAPA process that finds and addresses the root cause of an issue. The FDA has also specifically called out the use of these advanced testing methods as a critical tool that should be employed, when appropriate, to strengthen the root cause investigation and CAPA process overall.
b. It is important to note that even if a definitive root cause cannot be determined, it is important to correct any probable factors. If this is the case, these factors should be found, corrected, and documented.

CAPA Step 2 – Investigation

After the problem is identified during Step 1 (Detection), Step 2 (Investigation) should be started immediately. During the investigation step, the goal is to gather information related to the problem that has been identified. A cross-functional team should consider all potential causes as part of the investigation and then gather the related information.

Advanced testing methods can add significant value to this step, specifically through microbial isolate characterization and strain comparison.

a. Isolate characterization refers to providing an accurate, reliable identification. Understanding the identification of the organism is critical to determine the root cause of the problem and for determining preventive measures later in the CAPA process.
b. Strain comparisons can offer critical information as part of the investigation, and as you move into the root cause identification step.
    i. The current isolate(s) can be compared to all historical isolates, if there is an established database using the same advanced method.
    ii. Also, additional product and/or environmental testing is a common component of the investigation step related to a microbiological issue, and any new findings of the same organism can be compared to all previous isolates.
c. If these advanced testing methods are used during the CAPA process, consider the turnaround time.

d. Some advanced testing methods have a longer turnaround time (weeks vs days), but it is important that the timing for results does not slow down the CAPA process.
e. If isolate identification and comparison are an important part of the CAPA process, it may be beneficial to use faster, less discriminating methods to determine immediate actions, even if they are overly conservative. More discriminative advanced testing methods with a longer turnaround time can then be used to continue to build evidence of the true root cause.
f. These methods can also be helpful during an investigation into a testing laboratory. If there is any potential of cross contamination at the laboratory, these methods can be used for comparisons to the laboratory positive control strain and any other detections of the same organism in other samples.

CAPA Step 3 – Identify Root Cause

During the Root Cause Identification step, the goal is to use all the information available, including the information collected during the Investigation step, to determine the specific underlying reason(s) for the nonconforming product or environment. Understanding if the same organism from the same source, as determined by a match from an advanced method, has been found in different locations of the plant or at different times is critical.

a. If isolates are not the same species (even if they are the same genus), it is safe to assume they are NOT a match. This can be determined by standard microbiological testing.
b. If isolates are the same genus and species, then they MIGHT be a match and further testing with an advanced method is needed for more information.
c. If any advanced method indicates that the isolates are different, then they should be assumed as NOT matching.
d. If an advanced method indicates that isolates are a match, you could test again with a more discriminatory method, if available, for additional evidence.
e. This information can be used to trace the source of contamination and/or to understand how the organism is spreading to other areas in the plant. For example, tracking the same organism across multiple zones over time enables an investigation into possible contamination pathways (e.g., foot traffic, utensils). Understanding which organisms are related, and from the same source, will help to understand the scope of the issue, if it is new or ongoing, and where the focus should be to eliminate the source and prevent recurrence.
f. It is important to determine if the organism is resident or transient. A resident strain refers to an organism that has been detected multiple times in the same facility over a longer period of time and was never completely eliminated after previous findings. A

transient strain has only recently been introduced into the facility and is completely removed by cleaning or some other mitigation step.

i. Evidence of a resident strain is a concern as it shows that attempts to eliminate the source have been unsuccessful, and there may be an ongoing risk of product contamination. The FDA has suggested that resident strains may indicate insanitary conditions, especially if there is not a well-documented, effective CAPA.
ii. Using advanced testing methods with high discriminatory ability can be extremely valuable here because methods with lower discriminatory ability may conservatively show a match between strains, leading to a resident determination, when that might not actually be the case.

Methods Summary

The table below contains information about some of the common advanced testing methods that are available for microbial characterization and comparison. This is not a comprehensive list of all advanced testing methods that are available. Most of the methods below are utilized after a confirmed positive result is obtained by a detection method (e.g., cultural, ELISA, ELFA, lateral flow, biochemical, PCR, RT-PCR, isothermal amplification of DNA) and an isolate is available (Note: metagenomics is typically used for testing an entire microbial population, not an isolated organism). Most of the methods below are genomic-based, except for MALDI-TOF and FTIR, which use different target molecules as explained below. The table is formatted in general starting with methods of less discriminatory ability descending to methods with higher discrimination ability.

Method	Description	Pros	Cons	Use Details		
				Cost per Test	Turn Around Time	Complexity
16S Sequencing	Sequences the portion of DNA related to the 16S ribosomal RNA gene	Accurate genus identification Possible species identification (dependent on the platform used) Identification can be made	Does not discriminate at the species level for some organisms Not used for strain comparisons	$	Medium	Low

		through an established reference database				
MALDI-TOF Mass Spectroscopy *(Matrix-Assisted Laser Desorption/Ionization-Time of Flight)*	Generates a protein spectrum through mass spectroscopy	Accurate genus identification Accurate species identification in most cases Identification can be made through an established reference database	Does not discriminate at the species level for some organisms, such as *Bacillus* spp. and *Cronobacter* spp. Not used for strain comparisons	$	Fast	Low
FTIR Spectroscopy *(Fourier Transform Infrared)*	Generates an infrared spectrum based on the presence of certain biomolecules	Can be used for strain comparisons Identification can be made through an established reference database, including the serogroup for *Salmonella* spp. and *Listeria* spp.	Preparation workflow is critical as age and temperature of the culture can impact result	$	Fast	Low
Ribotyping	Characterizes portions of DNA related to the genes for ribosomal RNA through gel electro phoresis	Can be used for strain comparisons Accurate genus identification Identification can be made through a database supplied by the manufacturer	Does not discriminate at the species level for some organisms, such as *Bacillus* spp. and *Salmonella* spp. More useful for strain comparison than identification	$$	Fast	Medium

			Important Note: A large provider of this technology is phasing it out from their portfolio			
PFGE *(Pulse Field Gel Electrophoresis)*	Characterizes fragments of DNA by applying alternating electric pulses during gel electro phoresis	Can be used for strain comparisons Had been considered the "gold standard" for strain comparisons before methods with higher resolution became more common	Not used for organism identification Difficulty in differentiatin g certain types of *Salmonella* spp. Time consuming and can be sensitive to errors	$$	Long	Medium
MLST *(Multilocus Sequence Typing)*	Sequences portions of the genome Characterizes strains by the unique allelic profile of specific "housekeeping genes"	Can be used for strain comparisons	In general, not used for organism identification	$$$	Long	Medium
cgMLST *(Core Genome Multilocus Sequence Typing)*	Sequences the entire genome Characterizes strains by the unique allelic profile of their "core genes"	Can be used for strain comparison	In general, not used for organism identification High level of training and data storage required for data analysis if test is conducted in-house	$$$	Long	High
wgMLST *(Whole Genome Multilocus Sequence Typing)*	Sequences the entire genome	Can be used for strain comparison	High level of training and data storage required for	$$$	Long	High

		Characterizes strains by the unique allelic profiles of most genes	Highly accurate identification	data analysis if test is conducted in-house			
WGS *(Whole Genome Sequencing*)	**Next Generation** *(Short-read sequencing: Illumina, Ion Torrent)*	Sequences and analyzes the entire genome Revolutionary sequencing technique introduced in the 1990s Fragments the DNA, amplifies all fragments at the same time, and then uses bioinformatics to determine the order of the fragments Sequences the genome in short fragments, typically 50-300 base pairs	Highly accurate for both identifications and strain comparison Can be used to analyze genes of interest to predict behavior (e.g., virulence, resistance to compounds)	Can be less accurate compared to First Generation Sequencing (Sanger) Some difficulty in accurately sequencing genomes with repetitive fragments High level of training and data storage required for data analysis if test is conducted in-house	$$$	Long	High
	Third Generation *(Long-read sequencing: PacBio SMRT, Oxford Nanopore)*	Sequences and analyzes the entire genome Sequences the genome in large fragments without the need for amplification, typically >10,000 base pairs	Accurate for identification and strain comparisons Can reliably assemble complex genomes Can be used to analyze genes of interest to predict behavior (e.g., virulence,	Higher error rates compared to both First Generation and Next Generation, although this technology is improving High level of training and data storage required for data analysis if test is conducted in-house	$$$	Long	High

			resistance to compounds)				
Metagenomics	**Targeted**	Typically sequences the portion of DNA related to the 16S ribosomal RNA gene for an entire microbial population Can also target other genes	Provides the identification of all organisms present in a sample, along with their relative abundance	Does not discriminate at the species level for most organisms when 16S ribosomal RNA genes are used as targets May not detect organisms present at very low levels Not used for strain comparison	$$$	Long	High
	Shotgun	Sequences and analyzes the entire DNA present in a sample	Provides the identification of all organisms present in a sample to the species level Highly accurate for both identificatio ns and strain comparisons Can be used to analyze genes of interest to predict behavior (e.g., virulence, resistance to compounds)	May not detect organisms present at very low levels May not detect target organisms if high levels of other DNA (e.g., cow DNA in milk) are present	$$$	Long	High

Note: There are also several advanced methods that are related to specific testing platforms. Some of these advanced methods can only be utilized if that specific testing platform is used for the initial detection analysis, while others can be used independent of the testing platform, as long as an isolated colony is available. Some examples of these methods include:

a. GENE-UP® TYPER: used in conjunction with the GENE-UP® Real-time PCR platform from bioMérieux
b. Clear Safety®: targeted next generation sequencing used in conjunction with the Clear Labs testing platform

Table Key:

Cost per Test ($, $$ or $$$): The test cost for 1 isolate at a contracted laboratory is variable and can range from <$10 to >$500. Cost can also be impacted by the requested time to result. The cost will be different if the testing is done at an internal laboratory, which typically includes higher startup costs, followed by lower individual testing costs.

Turnaround Time (Fast, Medium or Long): The test turnaround time will depend on the specific method duration, as well as any data analysis that is needed. This timing can also be impacted by laboratory batching needs. For some methods, it is more cost effective to test several samples together because of high consumable costs, which can increase turnaround time. Sometimes, a faster turnaround time is available for an increased cost. For this document, the turnaround time is defined as the time to receive results after the isolate is available at the testing laboratory. We developed the following definitions as guidance: Fast (<24 hours), Medium (24 hours-5 days), Long (>5 days).

Complexity (Low, Medium or High): Defined as the level of expertise needed to properly understand and apply the results. If the testing is performed at a contracted laboratory, the result interpretation would typically be completed by that laboratory before results are shared.

METHOD USE CASE STUDIES

Powder Example #1

- Background
  - The plant produces non-fat dry milk (NFDM).
  - During baseline testing, *Salmonella* Typhimurium was detected in finished product.
  - After the detection of *Salmonella* Typhimurium in finished product, a CAPA analysis was initiated.
- Information Collected During the CAPA Process
  - A review of historical results showed that the plant has detected *Salmonella* species as part of their environmental monitoring program, but never in finished product.

- Recently, the packaging room, which has exposed product, had a water intrusion event caused by a leak in the room directly above. Employees observed water dripping down a wall and pooling on the floor.
- At the time of the water intrusion event, additional environmental monitoring samples were collected from the packaging room.
- One of the additional environmental monitoring samples had tested positive for *Salmonella* Typhimurium.

- <u>If Advanced testing methods were NOT Utilized as part of the CAPA Process</u>
  - The team concluded that the water intrusion event in the packaging room was the plausible root cause of the product contamination because the environmental sample and product sample both yielded a result of *Salmonella* Typhimurium.

- <u>If Advanced testing methods were Utilized as part of the CAPA Process</u>
  - The team tested the finished product isolate and the recent packaging room environmental isolate by an advanced testing method with high discrimination ability.
  - The packaging room isolate was not a match to the finished product isolate, and therefore the water intrusion event may not have been the root cause of *Salmonella* spp. contamination in finished product. Therefore, the investigation continued.
  - As the team reviewed historical results, they realized there were 3 other environmental detections of *Salmonella* Typhimurium on record. The team compared those isolates to the recent finished product isolate using the advanced method database.
  - One of the historical isolates was a match to the finished product isolate. This isolate was detected a few weeks prior to the finished product positive test and was from a zone 2 location near the fluid bed.
  - Upon further investigation, they also realized there is a planned system breach into the fluid bed every day to check on processing conditions. They also realized the breach procedure was not very robust from a GMP perspective.
  - The team concluded that the plausible root cause of the product contamination was an uncontrolled breach into the fluid bed from an area that had an increased risk based on recent environmental activity. The other issue also needs attention (water intrusion event in the packaging room contaminating the environment) but likely did not contribute to the finished product contamination.

- Conclusion:
    - There were 2 separate issues, and both required a CAPA analysis. Without the use of an advanced test, the most plausible root cause of the product contamination would have been missed, and there would have been an ongoing product contamination risk.

Natural Cheese Example #2

- Background
    - The plant manufactures RTE natural cheese.
    - The main pathogen of concern is *Listeria monocytogenes*.
    - When *L. monocytogenes* is detected in the environment, the follow-up procedure includes vector sampling, cleaning, and completing a minimal investigation.
    - There was a roof leak near the brine tank room, which led to the initiation of a CAPA analysis.

- Information Collected During the CAPA Process
    - The plant conducted extensive environmental testing near the brine tank room, resulting in 4 new detections of *L. monocytogenes.*
    - Shortly after the roof leak the plant also detected *L. monocytogenes* in 3 other locations in a different area of the plant as part of their baseline testing program. These locations were near the draining, matting, and cutting area (DMC) and the whey storage area.

- If Advanced testing methods were NOT Utilized as part of the CAPA Process
    - The team concluded that the roof leak was the plausible root cause of the initial environmental contamination near the brine tank room, and then traffic patterns spread the contamination to the DMC and the whey storage areas since the timing of all detections were close.

- If Advanced testing methods were Utilized as part of the CAPA Process
    - Using advanced testing methods, the team showed that the *L. monocytogenes* isolates within each cluster matched but there was a difference between the groups overall (Cluster #1: Near the brine tank room, Cluster #2: DMC and whey storage areas). Therefore, there appears to be more than one issue and root cause.

- One of the clusters (DMC and whey storage areas) also matched a few historical findings that were tested by the same advanced method. These isolates were from situations where no root cause was determined.
- The team decided they needed more information to determine the root causes and conducted a large-scale swabbing exercise throughout the plant.
- This large swabbing event yielded a *L. monocytogenes* detection on a maintenance tool, and the advanced testing showed a match of this isolate to the cluster locations near the DMC and whey storage areas, as well as the historical findings.
- The team concluded that there were 2 plausible root causes:
  - The roof leak near the brine tank room resulted in a transient pathogen issue.
  - The maintenance tool that contaminated several areas of the plant for a longer period of time, resulting in a resident pathogen issue.

- Conclusion:
  - There were 2 separate issues, and both required a CAPA analysis. Without the use of an advanced testing tool, the resident pathogen issue would have been missed, and there would have been an ongoing product contamination risk.

FINAL CONCLUSIONS

There have been significant advances in microbiological testing technology in recent years. Advanced testing methods, as an addition to a thorough baseline testing program, can be powerful tools to address pathogen risks. These methods can link isolates with great precision, differentiate transient contamination from resident, and lead to faster, more confident food safety-based decisions. It is important to understand the potential benefits of these methods and to consider the value they can offer toward achieving specific goals, while considering any regulatory implications. Embracing these methods as best practice maximizes food safety programs and ultimately helps to maintain consumer trust in the safety of food products by protecting public health. For additional guidance and training on baseline testing programs, hygienic separation and sanitary design best practices, visit www.usdairy.com/foodsafety.