Detecting Pathogens in Dairy

Pathogens in these products can be difficult to detect due to their complex matrices

BY **ANDREA TOLU**

Food contamination generally depends on two major variables: how easy it is for a particular product to become contaminated and how difficult it is to discover the contamination through the testing methods in use. With milk and dairy products, the combination of these two factors make the probability of food safety incidents much higher than with other foods.

The risk starts at the very source. "Milking happens in a non-sterile environment that can harbor different pathogens," says Dino Demirovic Holmquist, vice president of business development at Eurofins. "For bacteria to grow, you need humidity, the right temperature, and food. Milk has the perfect combination: It's liquid, nutritious, and is drawn from the cow at a temperature between 32º and 34ºC."

The other variable is not favorable either: Pathogens in dairy products can be difficult to detect because of their complex matrices and the interaction among different microorganisms. One of the effects of this interaction is a phenomenon called metabiosis, which happens when a microorganism creates the right conditions for the growth of another one. A typical example, says Holmquist, is a pathogen that lowers the pH in milk, creating a perfect environment for another pathogen that was already there, but in very small quantities. As this second pathogen grows, it produces a substance or other favorable conditions in which a third one can flourish and make a product unsuitable for consumption, he adds.

In fermented products, these interactions may have the opposite effect of keeping bacteria undetected when using standard plating techniques. "Fermentation often uses lactic acid bacteria," says Luke Thevenet, a pathogen technical sales specialist at 3M. "These can produce antimicrobial compounds that compete for resources with the pathogen that you're trying to detect, preventing it from growing."

The same phenomenon occurs in dairy powders: "Powdered dairy is probably one of the most difficult matrices to recover pathogens from and prevent interference if using an unvalidated detection method. Their low-water-activity environment is not conducive for low numbers of pathogens to survive and grow rapidly, which affects the detection and recovery rate of molecular platforms," says Celina To, regional technical sales manager at Hygiena.

Whether it is metabiosis or competition between microorganisms, the result is that the pathogen is there, but invisible to standard plating methods. "You can have the best technology, but if the pathogen hasn't grown to levels above the limit of detection, it is not going to provide valuable information," says Thevenet.

To complicate this situation even further, dairy is one of the most dynamic segments in the food industry, with new products and formulations launched every week: "If you're introducing new ingredients all the time, you might not have data on their pathogenic risk, their interaction with the rest of the formulation, or whether the tests you've been running are still valid for that new matrix," says Thevenet.

Using an aggressive heat treatment such as ultra-high temperature (UHT) to sterilize milk in all products would not be a viable solution, says Holmquist: "Ultra-high temperatures oxidize lipids and caramelize sugars [and] will change the taste, which is the main reason we buy milk and dairy products these days. What's more, the dairy industry has always claimed to interfere very little with milk and keep it very close to its natural state. With the clean label trend, this has become even more important."

The Need for Speed

To be sure, plated methods are not any less valid because of these challenges. With the right strategy, the right enrichment process can always be found. For example, says Thevenet, "You might have to adjust the pH or select antibiotics to target the competing microorganisms, while promoting a positive growth environment for the pathogen."

"Any ingredient could be problematic without any preliminary testing to validate the method for that dairy facility" says To. "Also, it's not the type of dairy processing that creates challenges; rather, these depend on whether the dairy facility has a robust and easy-to-use environmental sampling plan, where technicians are trained to look for areas that are difficult to clean and swab. This is one of the major hurdles with environmental detection."

The real problem is time: "Heat-resistant and spore-forming bacteria like *Clostridium* can survive in plant-based dairy formulations, while *Geobacillus stearothermophilus* has been detected in extended shelf life and aseptic dairies before. Even with the right enrichment conditions, these can take up to 10 to 14 days to be detectable on plates. But many facilities can't wait that long to hold and release products," says To.

"Speed is crucial," says Thevenet. "Processing environments are dynamic, and if you're waiting several days for a result, a lot can happen: Microorganisms can be spread around processing plants by forklifts, carts, or employees."

For dairy processors, a successful food safety program is a matter of preparation,

says Thevenet: "A lot of money is invested in a product and people's lives could potentially be at risk, so picking the right pathogen test is extremely important. You need to consider the matrix and size of the sample you're testing, the manufacturing and lab environments, the available technical resources, and the expertise levels of your technicians. You also need the data to prove that a method is appropriate for your samples."

A Holistic Approach

Because speed is crucial, detection solution providers are striving to make tests faster, either with improved enrichment media or with alternative methods. 3M has developed methods based on loop-mediated isothermal amplification (LAMP), while Hygiena's methods focus on ATP and DNA-based PCR technology.

Making test execution faster will also become more important, says To: "Lab automation and optimized, reliable, and validated methods will help reduce staff turnover and allow technicians to allocate their time to other tasks, making results more reliable and repeatable. Also, with cloud-based software, a lab can quickly identify process challenges onsite and make data-driven decisions, from environmental monitoring to pathogen identification."

Improved speed and accuracy, however, are just part of the solution. One of the most recent advancements in pathogen detection is using next generation sequencing (NGS) to look more deeply into complex food matrices: "With standard plating techniques, you have to know what to look for, while NGS can be used both for identification and characterization," says Holmquist. "The first approach is called shotgun metagenomic sequencing, where you sequence the DNA of all microorganisms in a sample and see in what proportion they are present. The other is called targeted metagenomics, or barcoding, where you identify family genus, species, serotype, type, and strain of a known microorganism."

Targeted metagenomics is proving very useful for tracing outbreaks of foodborne illness and can be applied successfully inside the processing plant, too. "When you find a pathogen in your plant, the challenge is to determine whether it's a transient microorganism that comes from the outside or a persistent one," says Holmquist. "Some of the recent recalls were based on the same pathogen that had caused a recall from the same facility several years before."

Shifting the mindset from pathogen detection to strain tracking, says Holmquist, makes it possible to know what is really going on in a facility, instead of just sampling random points throughout the processing chain.

An important piece to this holistic approach, says Thevenet, is to integrate existing data points and technologies other than pathogen testing, such as data from raw ingredients and seasonality, to build predictive models. "This way, you would know with what product, or pathogen, or at what time of the year you're more susceptible to having a contamination," he adds. "For scientific vendors, the next step will be to create software that is able to track and integrate data from different platforms and make these types of predictions." ■

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