Discrepancies in the Microbiological Analysis of Foods: Causes and Resolutions

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SUMMARY

This article discusses the types of problematic results that can arise during microbiological testing of food and food processing environments, which potentially undermine the effectiveness of food safety programs. It outlines steps for their resolution and offers readers a structured approach for evaluating the multiple contributing biological, technological, and human factors. To assist in troubleshooting and identifying the root causes of these problems it gives examples of 15 common root causes. It also presents measures to prevent the recurrence of discrepant and ambiguous results. These measures are rooted in a robust laboratory quality system incorporating validated methods, standard operating procedures, calibrated equipment, maintenance of meticulous records, proficiency testing programs, and continuing education opportunities for laboratory personnel. In conclusion, the article advocates for comprehensive risk assessment of problematic results and offers actions to strengthen the application of microbiological methods within food safety programs, in line with the U.S. Food and Drug Administration's objective of preventing adulterated food products from entering commerce.

OVERVIEW

Microbiological analyses of foods are performed for several reasons, including the following:

- Verification of hazard analysis critical control points or food safety plan preventive controls through testing of plant environmental samples, in-process materials, product contact surfaces, and finished products
- Detection of patterns and trends to illustrate process control (20)
- For release of the finished product
- Demonstration of regulatory compliance or conformity with customer specifications
- Troubleshooting a microbiological issue in the product, process, or facility
- In response to a consumer or customer complaint
- During research associated with product or process development

When performed and interpreted appropriately, microbiological analyses generally deliver results upon which we can act. However, the process involved is complex. Representative samples must be prepared and analyzed by an appropriate method that allows the target organism or organisms to grow to detectable levels in suitable media. The detection or enumeration technology must give a reliable signal. Finally, any calculation based on that signal must deliver an accurate result. Given this complexity, sometimes even validated methods can yield results that may be inconsistent with prior knowledge and results from other tests. In addition, results can be reported as indeterminate or inconclusive by rapid methods. Nonconclusive results make decisive actions difficult to determine. In the case of discrepant results, further analysis may be required.

Estimating the risk of such discrepancies is difficult. One approach may be to look at data from proficiency schemes collected over several years, because samples from these schemes are inoculated, standardized, and presented to multiple laboratories and analysts using various methods and equipment. These data suggest a false-positive rate of around 4% and a false-negative rate that varies by pathogen, with atypical strains and low concentrations of bacteria most likely to be missed (1, 10).

This article presents a discussion of the types of discrepancies that commonly occur in food and environmental microbiological testing. Various root causes may exist for any discrepant testing observation. Therefore, the potential scenarios, causes, and resolution strategies are intended not to be fully comprehensive but to provide a structured approach to problem-solving. *Figure 1* summarizes initial approaches to the resolution of problematic results.

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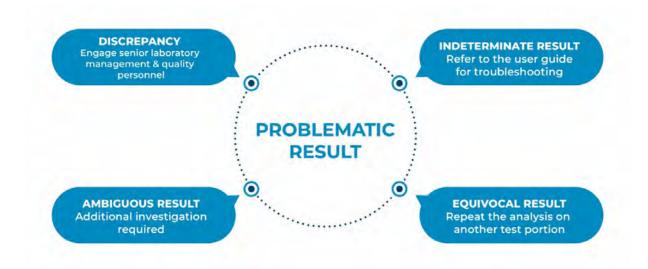
FIGURE 1. Types of problematic results and potential actions to achieve resolution.

DEFINITIONS

Discrepancies

According to the *Merriam-Webster Dictionary Online* *(18)*, a discrepancy is "an instance of disagreeing or being at variance." In our context, a discrepancy may be one or more test results that are inconsistent with what is expected or known about the sample tested or the normal performance of the analytical method or methods. Such results may be inconclusive and prevent decisive action, such as the ability to make a time-critical decision on product release or product disposition. Examples include the following:

- A negative result for an inoculated sample. Because we know the sample is contaminated, we expect a presumptive positive detection or isolation-positive result (assuming the inoculation level is above the limit of detection [LOD] of the method being used).
- A presumptive positive screening result from a rapid pathogen detection method that is not confirmed by cultural isolation. It becomes a question of which result is accurate: the rapid test or the traditional cultural method.
- A coliform count higher than the aerobic plate count (APC) for the same sample. Because coliforms are only a subset of all microbes able to grow on an aerobic plate, the APC should at least be as high as the coliform count.

Ambiguous results

An ambiguous result could be "doubtful or uncertain, or capable of being understood in two or more different ways" *(18)*. An example is the interpretation of a colony type. For example, *Salmonella* is described as yellow with a black center on a xylose lysine desoxycholate (XLD) selective and differential plating medium, but if the colony is yellow with a charcoal gray center, more confirmation

is required, such as replating onto alternative *Salmonella* differential or chromogenic media. Another example is colony counts in the presence of matrix particles that could be mistaken for colonies.

Indeterminate results

An indeterminate, or questionable, result usually arises when a rapid screening method encounters an internal technical issue. For example, many rapid polymerase chain reaction (PCR)-based methods contain an internal positive control that is a nontarget DNA sequence coamplified in the same reaction tube as the target pathogen *(7)*. If the internal control fails to amplify, then the instrument software will report an indeterminate result, from which no decisive action can be taken. Faced with an indeterminate result, the analysis should be continued by a cultural method until complete. We do not consider indeterminate results further because they are usually flagged by the method platform, allowing action to be taken to troubleshoot the issue and deliver a reliable result.

Out-of-specification results

An out-of-specification result exceeds a value established by the end user of the result to define acceptability. Typically, any pathogen detection would be "out of specification," as would indicator or spoilage organism counts above the user-defined limit. These results do not necessarily suggest a problem with the method, but an out-of-specification result usually causes the end user to question laboratory performance.

Equivocal results

An equivocal result comes from following a process to investigate an out-of-specification, discrepant, or ambiguous result when it has been determined that an identifiable error

DISCREPANCY OR AMBIGUOUS RESULTS

1 METHODS NOT COMPARING LIKE WITH LIKE
Carefully compare results obtained by 2 different methods (similar LOD)

2 DIFFERENT TEST PORTIONS
Take into account heterogenous distribution of target organisms

3 NON-CONFIRMING PRESUMPTIVE POSITIVES
Use other confirmation technologies (NGS Metagenomics)

4 MATRIX INTERFERENCE
Matrix verification before implementing a new method

5 MEDIA INTERFERENCE
Use media validated within the chosen method

6 PRE-ENRICHMENT & SELECTIVE ENRICHMENT MEDIA BIAS
Use validated media and several of them as needed to decrease enrichment bias

7 EXOGENEOUS (DEAD) DNA
Depending on the DNA concentration, consider the significance of this presence of DNA & if needed, use enzymatic solutions to get rid of it

8 CROSS-REACTING ORGANISMS
Consider the exclusivity study of the method and use another validated method if needed

9 COLONIES WITH SIMILAR MORPHOLOGY
Confirm with a method based on a different technology (PCR)

10 ATYPICAL STRAIN
Confirm with a molecular method (PCR/NGS)

11 BACKGROUND FLORA
Consider using more specific media or adding additional separation technology (IMS)

12 VIABLE BUT NOT CULTURABLE (VBNC) CELLS
Consider potential microscopic and molecular techniques

13 RELATIVE SENSITIVITIES OF RAPID AND CULTURAL METHODS
The LOD of PCR can be lower than culture

14 CROSS-CONTAMINATION
Confirm any presumptive positive result with a different technology

15 OTHER OPERATIONAL ISSUES
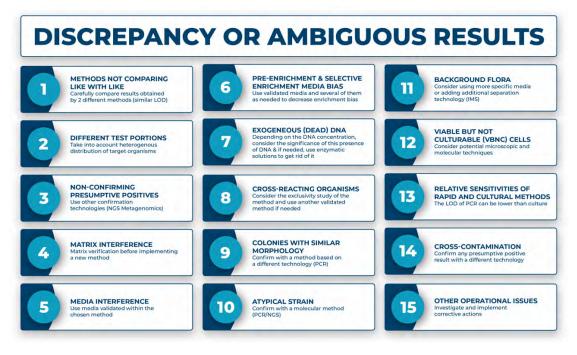Investigate and implement corrective actions

FIGURE 2. Approaches to investigating ambiguous and discrepant results. See the text of the Causes section for a detailed discussion. IMS, immunomagnetic separation; NGS, next-generation sequencing.

during the analysis has rendered the result untrustworthy. In such a situation, the only way to repair the error is to repeat the analysis, but the decision to do so should involve senior laboratory management and quality personnel and be clearly documented. It is critical to understand that repeated analysis is never conducted with the purpose of obtaining a different result but only when there is no other way to provide reliable data to the decision-maker. We do not discuss equivocal results further.

CONSEQUENCES

Within verification activities for a food safety program, specific methods are selected to evaluate the effectiveness of the program and the safety of products for distribution to consumers. Examples include detecting the presence of a target organism or pathogen, quantifying the levels of microorganisms in a product, or screening for contaminants, toxins, or other detrimental metabolic products. Discrepant or ambiguous test results can give a false impression of the effectiveness of the food safety program and should be examined for root cause and resolution, often requiring significant time and expense.

CAUSES

Many biological, technological, and human factors can contribute to discrepant results in analytical testing, including matrix interference; the presence of atypical strains or cross-reacting organisms; the detection of exogenous DNA

by molecular methods such as PCR; high populations of background organisms overwhelming detection methods, including the ability to find typical colonies on selective and differential plates; equipment failures; cross-contamination in the laboratory; and technician error. Discrepant results create doubt and necessitate root cause analysis for immediate resolution, corrective actions, and future mitigation. What potential root causes does experience tell us are likely? What approaches can we take to investigate these potential causes? Some common causes are described later, and paths to investigate them are detailed in *Figure 2*.

Not comparing like with like

Confounding test results can seem to occur when the results of two disparate methods are compared. This is often because of a misunderstanding of the type of information each method can provide and the limits of detection afforded by each method.

When present, pathogens generally occur at low concentrations and are nonuniformly distributed in the product, environment, or sample. Hence, many food producers verify process controls using indicators such as APC, Enterobacteriaceae, total coliforms, or *Escherichia coli*. These methods quantify the level of microorganisms in a sample. If there is no growth of the target organism or organisms, the result is reported as "less than" the LOD, typically <10 colony-forming unit (CFU)/g or another unit of measure. A result of <10 CFU/g is an unquantified result

and not the same as a negative or not-detected result from a qualitative method using a larger test portion.

Qualitative methods are used to screen for the presence of specified microorganisms, such as *Salmonella* or *Listeria*. These methods generally use enrichment, capture, or other amplification approaches to detect as few as one cell per sample test portion. Because it is common for test portions to be 25–375 g, the qualitative method is nominally sensitive to between 1 CFU per 25 g and 1 CFU per 375 g of the target pathogen. Compared with the 10 CFU/g sensitivity of the quantitative indicator method, this represents a 250- to 3,750-fold increase in sensitivity. Hence, a sample with an unquantified indicator organism count that is below the LOD could test positive for a pathogen with no errors in either method.

Comparing results from these two classes of methods may indicate a misunderstanding of their capabilities and purpose. The indicator test is intended to provide insight as to the likelihood of a pathogen being present, but only the qualitative pathogen test is designed to specifically detect it.

Different test portions

Even with sensitive and specific methods, pathogens can be hard to detect. The low prevalence, low concentration, or nonuniform distribution of pathogens creates reduced odds of detection within the environment or foodstuff lot. Increasing the number of test portions increases the likelihood that the target organism will be detected if present. However, people generally assume uniform distribution of microorganisms across a decision unit (i.e., the product lot from which sample portions are taken), whereas organisms are often randomly distributed and may be found in hot spots within a product. Therefore, retesting a product lot following a positive pathogen test result is never permissible if the intention is to obtain a different result and any decision from such testing will be used to release products into commerce. The 2008–2009 *Salmonella* outbreak associated with Peanut Corporation of America products *(28)* and the egregious decisions of executives associated with analytical testing results *(4)* illustrate the potential dire consequences of such decisions.

Nonconfirming presumptive positives

For historical reasons, pathogen detections from rapid screening platforms are formally called presumptive positive results, or presumptives for short, because they are not based upon the isolation of the target as a colony on a plate. These presumptive results are often subsequently confirmed using a cultural isolation method, such as those detailed in the *Microbiology Laboratory Guidebook* from the U.S. Department of Agriculture's Food Safety Inspection Service *(30)* or the U.S. Food and Drug Administration's *Bacteriological Analytical Manual (33)*. Sometimes, the cultural method fails to produce an isolated, morphologically typical colony of the target pathogen that can be confirmed by biochemical or molecular means. This cultural "not detected" result gives

a "nonconfirming presumptive result" by the screening method, often considered a "false positive." This discrepancy can occur for numerous reasons, which are detailed in subsequent sections, but both results could be correct and nonconfirming presumptives are not always false positives. Rapid screening tests usually use a cellular component (a DNA sequence or a cell-surface antigen) as the test target, serving as a proxy for the viable, infectious pathogen that is the primary concern. When that test target is present but associated with dead cells (e.g., after pasteurization), the test may correctly detect its target, but cultural confirmation will not yield an isolate. It is also possible that the LOD of a PCR test is lower than the LOD of the cultural method, so analysts should take care if calling a PCR result a false-positive result when the cultural result is negative.

Ultimately, many strategies can be employed to investigate the sample; however, all must be interpreted within the context of what decision or decisions will be made. One investigational approach for resolving such discrepancies is to use a molecular method to "confirm" an isolate, even if it is atypical in appearance. This involves testing an isolated colony with a molecular method and is a validated procedure for multiple molecular platforms. Ideally, the confirmation platform would not be the one that gave the initial presumptive result so that each platform would detect a different DNA sequence associated with the target organism. Another approach is to use an alternative method that employs a detection chemistry different from that of the initial screening method. For example, a result from a nucleic acid–based method "confirmed" by an immunoassay could be considered the strongest indication of a true positive without a cultural confirmation, because in general, an immunoassay would be less sensitive, giving a greater likelihood that it detected an organism that grew during the enrichment culture. A not-detected result using any rapid confirmation method cannot be used to overturn the first presumptive result. This is further discussed in *Case Study 1* and stands in contrast to cultural results, which can be used to overturn initial rapid screening result presumptives. The inability to overturn initial presumptive results may greatly limit the appeal of molecular confirmation for many pathogen test users. Finally, in cases with a presumptive positive from a rapid screen but no growth on culture plates, we can consider a metagenomic analysis of the enrichment to determine whether the sample contains DNA from the target organism. If this is a rare occurrence, cost would be outweighed by ensuring the safety of the product before it is released into commerce. Confirmation is not necessary to take corrective action but can support effective corrective actions and, if necessary, modifications to the food safety plan.

Matrix interference

As discussed in previous articles *(5, 17)*, third-party method validation studies, such as from AOAC International

CASE STUDY 1

Samples may be submitted for rapid PCR testing to detect enterohemorrhagic *E. coli* (EHEC) or STEC. For many rapid PCR methods, the test may use multiplexing to target DNA specific to the detection of Shiga toxin genes (stx_1/stx_2) and either intimin *(eae)* or hemolysis *(hlyA)* virulence genes characteristic of EHEC or STEC. These primary screens may include or may be reflexed to a specific O antigen serogroup gene or a gene panel associated with multiple serogroups of regulatory focus and most commonly associated with human foodborne illness (O157 and non-O157 EHEC or STEC). Discrepancies may manifest, for example, when a positive EHEC or STEC screen fails to culturally confirm an isolate or a specific serogroup, yielding a presumptively false-positive screening result and likely yielding a costly decision to divert or destroy the product represented by the sample.

Although there can be issues with the cultural isolation method or methods, a predominant root cause underlying problematic EHEC or STEC PCR screening is the detection of multiple targets that do not reside within the same bacterial cell. In other words, the sample may contain various *E. coli* that have an *stx* gene and others that harbor the *eae* gene or a serogroup gene, but there may be no evidence, established by cultural isolate testing, that the sample has *E. coli* cells harboring all gene targets together, which would be considered the isolate or isolates capable of causing serious foodborne illness. As an example of the natural complexity and diversity of this issue, researchers collected 13,650 samples across 33 farms in a major leafy greens production region in the United States. Of 1,912 samples testing positive for non-O157 STEC, 3,256 unique isolates were selected for further characterization. Of these isolates, stx_1, stx_2, and both stx_1 and stx_2 were detected in 63%, 74%, and 35%, respectively, whereas the virulence genes *eae* and *hly* were observed in 25% and 79%, respectively. Only 23% of STEC isolates belonged to the top 6 non-O157 serogroups *(8)*.

Method developers and researchers continue to innovate new assays, targets, and workflows to minimize risk through increased specificity.

CASE STUDY 2

While examining *Listeria* isolates in agricultural soil, researchers at Cornell University identified five previously unknown and novel *Listeria* spp. *(6)*. This research increases the number of known *Listeria* spp. that could potentially populate a food production environment. Nonpathogenic *Listeria* spp. are often found coexisting in environments that support the growth of *L. monocytogenes*. Food manufacturers will often analyze environmental swabs for all *Listeria* spp. to verify the effectiveness of their sanitation program. If a presumptive positive result occurs, the isolate can be speciated to determine whether pathogenic *L. monocytogenes* is present and corrective actions are required.

Some unique characteristics of *Listeria* spp. that are closely related to *L. monocytogenes* were described by the researchers. As an example, one of the novel species, *Listeria immobilis*, lacks motility. Motility is a common characteristic among *Listeria* closely related to *L. monocytogenes* and is a pivotal assay in *Listeria* detection methods. This lack of motility could contribute to discrepant results in analytical testing for environmental pathogens, because traditional cultural methods used in confirmation may not propagate the newly recognized species, despite being detected by rapid screens. In addition, because the newly identified *Listeria* spp. are closely related to *L. monocytogenes*, existing methods of detection may misidentify them as L. monocytogenes. Some standard identification protocols may need to be reevaluated.

or the French Standardization Association, do not address all sample matrices that may be tested. Some products may have unique intrinsic attributes that can produce a false signal on a rapid screening test or may inhibit the test's chemistry. For example, dark or colored matrices may interfere with the interpretation of a lateral flow test strip or with fluorescent detection in a rapid screening platform. It is always best practice to validate and verify a matrix on the rapid screening method before using the method for the first time. This ensures that the matrix is free from inhibitory substances that may interfere with the method. Detailed protocols for first-use method and matrix verification are described in International Organization for Standardization (ISO) 16140-3:2021 *(15)*.

Media interference

Enrichment media are used to propagate microorganisms to detectable concentrations and to isolate them from complex samples. Some components of enrichment media can interfere with detection technologies, such as PCR, by inhibiting the amplification reaction or recognition of the target nucleic acid sequence *(27)*. Other components–such as acriflavine, a selective agent in certain media–may fluoresce and interfere with signals in real time PCR *(26)*. To mitigate potential media interference, it is important to use media validated for the target pathogen and detection method. Quality control measures, such as including positive and negative media controls in the detection method, can also help to identify and correct for media interference.

Preenrichment and selective enrichment media bias

In recent years, researchers have described the effect of media bias. In simple terms, a combination of the sample's intrinsic microbial flora and the medium's formulation may result in the biased selection or repression of a given microbial target. Gorski *(13)* showed that both enrichment medium formulation and presence of competing organisms affected the dominant *Salmonella* serovar from a mixed strain inoculum. Ottesen et al. *(23)* used metagenomic methods to show that specific taxa (e.g., *Paenibacillus* spp.) may enrich alongside *Salmonella*, resulting in the inhibition or death of *Salmonella* in the sample. This could lead to a discrepant result involving molecular detection of *Salmonella* but no cultural confirmation, despite the presence of viable *Salmonella* in the original sample. Cox et al. *(9)* sampled commercial broiler carcasses for *Salmonella* using various combinations of enrichment broth and plating media, reporting that different combinations influenced the serotypes that were ultimately recovered. Obe et al. *(21)* confirmed these findings, demonstrating that both the number and the sequence of selective enrichment media before plating affected the detection and recovery of *Salmonella* serovars from poultry flocks.

Exogenous DNA

Exogenous DNA from the target pathogen present in a sample matrix can lead to presumptive results on nucleic acid–based methods. In general, postenrichment concentrations of approximately 10,000 cells/ml are detectable. An equivalent concentration of DNA from "dead" cells of the target pathogen (e.g., following a lethality process) may trigger a presumptive result that cannot be culturally confirmed because the target pathogen is not viable. However, a nonconfirming presumptive screening result associated with "dead" cell detection indicates that the pathogen was present at high levels at or before the point of sampling. We would be wise to understand why the pathogen had been present and how it was rendered nonviable. With the advent of bacteriophage-based interventions, target-organism nucleic acid or other components may be present in the environment or product tested as a result of the phage manufacturing process. Therefore, many nucleic acid-based test method manufacturers have developed optional sample preparation procedures that include enzymatic degradation of exogenous nucleic acid before analysis.

Cross-reacting organisms

Method validation studies generally include as few as 30 exclusivity strains *(2, 35)* and nonselected, similar organisms from industry settings may give false-positive results when using a rapid test method. In molecular methods like PCR, cross-reacting organisms are rarely observed. The major foodborne pathogens and the most relevant nearest neighbors have been well characterized genetically. Test method developers perform extensive in silico testing of proposed primer or probe sequences to assure very low or no potential for cross-reactivity. Immunoassays present a greater risk of cross-reaction with nontarget organisms. For example, in *Salmonella* testing, some rapid methods using either antibodies *(25)* or bacteriophage binding proteins as affinity or capture ligands are known to cross-react with some *Citrobacter, Proteus,* and *Hafnia* spp. If these strains are routinely detected in processing facilities, they can cause significant disruption for food manufacturers. In such circumstances, seeking an alternative but equivalent molecular method may be the most effective solution.

Colonies with similar morphology

During cultural isolation and confirmation of presumptive screening results, colonies may appear morphologically typical of the target organism but not be the target organism, thus extending the confirmation process in a cascade of apparently confirming indications until their identity can be determined. For example, *Citrobacter braakii* can mimic the appearance of *Salmonella* on XLD agar and autoagglutinate with antisera used for serological testing *(24)*. To meet the food manufacturer's turnaround time, a common practice is to use an alternate testing method, such as a genomic PCR test, that would not result in antigen cross-reactivity.

Atypical strain

Some strains of *Listeria* can be hard to distinguish from one another, including atypical hemolytic *Listeria innocua* strains that can appear to be *Listeria monocytogenes* or atypical nonhemolytic *L. monocytogenes* strains that can seem to be *L. innocua* (16, 19). In these instances, FDA recommends simultaneous confirmation of *Listeria* spp. and *L. monocytogenes* using a DNA probe (32) and real-time PCR (31). Confirmation of an atypical colony by PCR is easy and fast. Another option for atypical colonies is sequencing. Although this is more expensive and time consuming, it leaves no doubt about the identification of the colony and provides genus and species information to guide corrective actions. It can further determine whether virulent gene sequences are present that could affect the manufacturer's product disposition decision. Chromogenic media can also help to differentiate among some related strains.

Background flora

Even with selective enrichment media, high levels of background microflora can outcompete the target pathogen for available nutrients. This may leave a final concentration that is sufficiently high to detect using PCR or another sensitive technology yet so low that only a few cells would be transferred to isolation plates by a streaking method. Because the selective principles are often similar in both the enrichment media and the selective plating media, background microflora able to grow in the enrichment broth is likely to grow on the selective plating media, preventing the formation of visible colonies of the few target cells transferred. In *Salmonella* cultural methods, multiple enrichment and plating media are used for this reason but still do not guarantee success. For most other pathogens, the isolation paths are narrower. An extreme example is in confirmation of Shiga-toxigenic *E. coli* (STEC) strains, which are difficult to isolate from other *E. coli*. STEC strains are not always phenotypically different, and cultural methods may involve additional separation technologies, such as immunomagnetic beads. Even so, the STEC confirmation rate is lower than that for other pathogens (1).

Background flora can also be a major confounder of *Listeria* spp. and *L. monocytogenes* confirmation. Today, most rapid screening methods for *Listeria* use single primary enrichment, whereas historically, two-stage enrichment was preferred for *Listeria* recovery and detection. One consequence of this change has been that any given sample sees less variety of selective antibiotics for less time than in the past. This can result in significantly more breakthrough growth of nontarget organisms. *Bacillus* spp. in particular have been problematic for many *Listeria* enrichment and plating media used in rapid methods. They can grow to high numbers in *Listeria* primary enrichment, potentially interfering with growth and detection of the *Listeria* target through competition for nutrients in the enrichment phase,

as described earlier. Moreover, they may confound cultural confirmation and can show heavy growth on standard *Listeria* plating media, making it impossible to identify typical *Listeria* colonies for final confirmation.

Viable but not culturable cells

Some bacteria adopt a viable but not culturable (VBNC) survival state in response to adverse environmental conditions. These VBNC cells are unable to multiply in or on a medium normally supporting their growth. However, they do retain reduced metabolic activity, cellular structure, and gene expression, including the expression of virulence factors (12). Resuscitation can occur when VBNC pathogens infect an appropriate host and resume normal metabolic activity, possibly leading to illness. Bacteria capable of entering the VBNC state include *Campylobacter jejuni*, *Enterobacter* spp., *E. coli*, *Shigella*, *L. monocytogenes*, and *Salmonella* Typhimurium. Bacteria in the VBNC state have been studied in water and food products, such as pasteurized milk. Root cause investigations for outbreaks, product contamination, or environmental contamination by pathogens or indicators that rely on cultural methods of detection and confirmation could be compromised when complicit bacteria cannot be detected. Bacteria in the VBNC state can also affect the shelf life and microbial stability of food and beverages while remaining undetectable. If the presence of VBNC cells is suspected, these cells can be detected using various microscopic and molecular techniques (12).

Relative sensitivities of different methods

Molecular platforms may display higher sensitivity than traditional plating. As noted earlier, this can be particularly problematic when high levels of background organisms make cultural confirmation difficult.

Cross-contamination

Cross-contamination may have occurred during preparation so that the sample was not truly positive. In this case, the cultural confirmation is accurate, because the target was never present.

Other operational issues

The many less common laboratory procedural errors and failures of control could include the following:

- Using expired materials
- Failure to correctly maintain and calibrate equipment
- Failure to use proper positive and negative controls to detect internal method errors
- Mislabeling of samples
- Cross-contamination and improper sanitation
- Overzealous bleach applications during lysate preparation

Generally, these challenges are overcome by out-of-specification investigations and implementation of corrective actions.

MITIGATION

Laboratory quality systems are designed to ensure that test results are trustworthy *(29)*. Reputable modern laboratories are usually accredited to ISO 17025:2017 *(14)*, which sets the expectation for elements of a laboratory quality system, including the following:

- Use of appropriate methods and standard operating procedures
- Use of quality control procedures to monitor and assess the accuracy and precision of test results
- Maintenance and calibration of laboratory equipment
- Use of appropriate documentation and recordkeeping procedures
- Training and competence of laboratory staff *(3, 11)*
- Use of proficiency testing programs to assess the laboratory's performance relative to other laboratories *(11, 22)*

Successfully implemented laboratory quality systems ensure consistent and reliable laboratory performance and help to eliminate mistakes and operational errors. They cannot overcome the inherent variability of microorganisms and fundamental limits of detection technologies, but they can, at least, make sure that the limits of performance are understood and documented. Each time an investigation of ambiguous results and discrepancies identifies a root cause, the relevant elements of the quality system should be reviewed and, if necessary, modified to capture the learning and reduce the chance of seeing the same issue again.

CONCLUSIONS

There are many potential causes of discrepancies and ambiguous results. We have attempted to show the most common causes and approaches that can be used to investigate them. Once root causes are identified, procedures can be put in place to prevent or reduce the risk of reoccurrence, and these steps become part of the normal laboratory quality system.

Much of the stress caused by discrepancies and ambiguous results arises from the need to take some action, and the action may be hard to determine if the result is not clear. In this context, the greatest consequences arise with pathogen detections. FDA requires cultural confirmation of its own results for proof of viability and source tracking. Perhaps this is why it is widely believed that results from rapid screening platforms must be confirmed by culture. However, FDA and ISO standards do not preclude industry from accepting an instrumental presumptive result and taking corrective action without cultural confirmation, and many pathogen test users take this approach. FDA's concern is ensuring that adulterated foods do not go into commerce. In a guidance for industry document *(34)*, FDA states, "you should not have to perform another test or obtain a cultural isolate from a presumed positive sample if you choose to not ship the food based on the presumptive positive result." The safest approach is to act on the presumptive result, even if confirmation and identification are done in parallel to gain additional information.

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