The implications of switching from total coliform to *Enterobacteriaceae* as an indicator organism in a food manufacturing facility: a literature review

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Abstract

Coliform is the traditional indicator organism of choice for testing of the environment and finished food products in the United States, as current reflected in some federal rules, such as the Pasteurized Milk Ordinances. A positive test result can indicate the presence of potential pathogenic activity, which at its worst can cause adverse reactions to human health but can also have no ill effects. Further research, however, has proven that the scope of this test is limited in what it can detect. An alternative indicator test, *Enterobacteriaceae*, can pick up not only the bacteria falling under the standard definition of coliform, but also other gram-negative bacteria and potential pathogens, making this test more robust and thorough. A third option – for gram-negative bacteria, generally – would offer the most comprehensive indicator organism testing program, when combined with *Enterobacteriaceae*. This paper reviews relevant research studies regarding these first two indicator organism tests, concluding that *Enterobacteriaceae* is the superior test for the food manufacturing environment, with a focus on finished ready-to-eat food products.

Testing for these pathogenic indicators has evolved from agar testing, which could take days, to 3M's Petrifilm, which can take several hours to incubate and reveal possible contamination. The Petrifilm test can also help food safety practitioners differentiate between contaminants from the environment versus bacteria that are naturally present in certain foods, such as cheese. In fact, some members of the coliform family can be beneficial to cheese curing and ripening. In switching from coliform to *Enterobacteriaceae*, any company would be catching up to the European Union, which has used this test since 2005 as its pathogenic indicator test for ready-to-eat dairy products. Although the literature reviewed in this paper does point to

Enterobacteriaceae as the superior test, some may be required to continue coliform testing in finished products to meet customer requirements and certain federal rules.

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Introduction

There has been an increasing shift away from the use of coliform as an indicator organism in food processing and food manufacturing facilities, transitioning to *Enterobacteriaceae* ("EB"). Even the European Union uses EB, not coliform, as a food safety requirement, in contrast to the U.S. FDA Pasteurized Milk Ordinance ("PMO"). This movement applies not only to testing of finished products, but also to environmental swabs, and represents a movement over 100 years in the making. Even though the science behind these bacterial indicators is not new, the use of EB to detect potential pathogens in the food manufacturing environment represents a recent shift. All coliforms are members of the *Enterobacteriaceae* family, but not all *Enterobacteriaceae* are coliforms, and thus, a more comprehensive or inclusive indicator organism should lead to a safer food production environment. But is that always the case? What are the drawbacks, if any, to using EB rather than coliform? And if EB is superior to coliform, why would a food manufacturer continue to employ an outdated or limited test?

Through a comprehensive literature review, this paper will dive into this recent food safety trend to provide clarity to companies implementing this sweeping change, with a specific focus on finished food products such as cheese. Tracing the history of coliform and EB as indicator organisms, and their use in the food manufacturing environment, this document will paint a clear picture of how these are used to ensure safe food products for the public. As companies make the change to EB, what are the implications for both the finished product and environmental testing programs when there is a positive or out of specification limit test? Do any suspect test results de facto indicate an unsafe environment or tainted product, or are there aspects to these tests that simply draw from the environment or food itself? What further types of testing, if any, should be

done? What are "safe" limits of EB? As testing of the environment and finished products has progressed throughout the years, what have researchers found to be good practices in this area?

Coliform

Definition of coliform

The definition of coliform was set relatively long ago as this: "Coliform bacteria are members of the *Enterobacteriaceae* that are capable of fermenting lactose with the production of acid and gas within 48 hours at 35°C (89.6°F)" (National Research Council, 1985). The term itself, however, was first used over a century ago to describe bacteria in water that was known to have contamination from human feces (Tillgren, 2019). To bring slightly more clarity to this category, they can be "aerobic or facultatively anaerobic, gram-negative, non-spore-forming rods" (Masiello, et al., 2016). There are as many as 20 different types of this bacteria, including *Escherichia, Klebsiella*, *Enterobacter*, *Hafnia*, *Serratia*, *Raoultella* and *Citrobacter*. Although these names may not be familiar, these pathogens, and coliforms, are generally undesirable in finished food products (Martin, et al., 2016). *E. coli* bacteria, the type found in feces, is also a part of this group. These bacteria cannot make it past the legal pasteurization step found in dairy plants, and thus can serve as good indicators of any contamination that occurs in a manufacturing environment after the milk is pasteurized (Hervert, et al., 2016). Pasteurization through a High Temperature Short Time ("HTST") system is defined by the PMO as heating raw milk to at least 161°F for at least 15 seconds, a process that will kill most pathogens. Even though coliforms represent a small portion of potential spoilage bacteria, their presence can still alert a food manufacturer to post-pasteurization and sanitation defects, as well as potential illness causing bacteria in food.

Coliform testing is used widely today as an "indicator organism," which is defined as "bacteria that are used to provide evidence of poor hygiene, inadequate processing or post-process contamination of foods" (Metz, et al., 2020). An indicator organism may or may not indicate the presence of harmful pathogenic activity, but is different from an index organism, which typically does lead to the discovery of specific pathogens present in the food product or environment. An indicator test has the ability to show food manufacturers areas of concern, by presenting the manufacturer with evidence of a failure in sanitation, employee hygiene, or process safeguards, such as pasteurization. Today's literature supports the use of coliform as such an indicator, as at least one study noted that much of the current published research relating to indicator organisms in the dairy industry focuses on coliforms as the test of choice for dairy products (Hervert, et al., 2017). The test itself, however, is sometimes considered "unpractical and not ideal to use as an indicator organism," but is used due to the "lack of other indicator organisms" (Tillgren, 2019). It is also still widely implemented as an indicator organism check because Federal or state rules often require coliform testing, leaving the food manufacturing company no choice. (Baylis, et al., 2011). Although the definition of coliform has not changed over the years, it has become increasingly clear to researchers that too many potential pathogens exist outside the scope of this test.

Initially, coliform was identified as a good test for water purity, as its presence correlated with the existence of fecal particles. It is the epitome of an "indicator" organism, which is "an organism whose presence indicates the condition of a substance or environments…[including] the potential presence of pathogens" (Tillgren, 2019). Because the test proved reliable at pointing out problem areas, the dairy industry "quickly adopted" it and indeed, has kept using it in many places (Trmcic, et al., 2016). This test has proved effective in assisting food manufacturers to keep a safe environment, since fecal coliforms are only a small percentage of coliforms, and many commonly

found in milk and cheese – such as *Enterobacter spp.* – are also detected by this test. (Trmcic, et al., 2016). Take note, however, that coliform bacteria may not always be negative, as coliforms present in some cheese actually contribute to the flavor and ripening process (Morales, et al., 2004). Further reducing support for coliform as an indicator, because the definition of coliforms has focused on the type of test used (detection of lactose fermentation), and not taxonomy, this test has left out many potential pathogenic indicators, and can lead to false positives, such as *Aeromonas spp.* (Baylis, et al., 2011). *Aeromonas* is a diverse genus of bacteria that can pose threats to human health (the mesophilic group) but can also be relatively benign to people (psychrotrophic strains) (Janda, et al., 2010).

Methods for testing for coliform

Testing can be difficult, as multiple different methods – be they Violet Red Bile Glucose Agar, 3M Petrifilm, culture-based tests, or Polymerase Chain Reaction ("PCR") assays – yield different levels of effectiveness. The Violet Red Bile Glucose Agar has been a reliable method, showing coliform colonies with a red halo that is easily identifiable. 3M built on this foundation, creating Petrifilm, a sample-ready gel that contains the Agar, but also a "tetrazolium indicator that facilitates colony enumeration" (3M Coliform Plate Instructions). At least two genera of coliform that can be detected by Petrifilm – *Leclercia* and *Kluyvera* – are known to cause infections in humans, showing that the simplified method offered by 3M does have good use (Masiello, et al., 2016). One study found that culture-based methods only found coliform in 79% of contaminated water samples, while PCR testing detected coliform in anywhere from 67% to 80% of such samples (Maheux, et al., 2014). Another study added to the potential drawbacks of these methods, pointing out that *E. coli* O157:H7, which is a pathogen originating in human feces, and one that causes health issues if ingested, did not show up on some culture-based coliform test methods (Zhang, et

al., 2015). Furthermore, reliable testing can depend not just on the effectiveness of the test method selected and verified, but also on the level of training each lab technician undergoes, how easy the test is to conduct, time and equipment needed for each test, and the quality controls in the lab (incubation temperature, e.g.) (Maheux, et al., 2014). There are pros and cons to each, as "culture-based methods simply rely on the activity of one single gene," and can therefore "give false positives or negative results when detecting coliforms" (Tillgren, 2019). Even the most reliable methods to test for coliforms, as stated succinctly by a thorough research study, can produce inaccurate results (Tillgren, 2019).

Implications of coliform test results

Detecting pathogens in dairy products, potable water, and the environment is a challenge of chief concern for all food processors, and especially those in the dairy industry. In fact, coliform is one common bacterium found in pasteurized fluid milk (Masiello, et al., 2016). Its presence can indicate contamination after the pasteurization process, or an issue with the pasteurizer itself. More specifically, a positive coliform test result can point to potential biofilm development. These films are a network of microorganisms and extracellular polymeric substances that fester and grow in hard-to-reach places such as cracks or pipe junctions. These films are tough to remove, and permit "colonization of populations of microorganisms" that can break free from the film and end up in finished food products (Martin, et al., 2016). Interestingly, of the coliforms detected by researchers in pasteurized cheese products, the bacteria were those most commonly found in the environment (*Hafnia* and *Raoultella*), not coliforms one would expect to find in raw milk (*Escherichia* and *Enterobacter*). *Hafnia alvei*, for instance, is common in some cheese, and is not known to cause food safety issues (Trmcic, et al., 2016). *Raoultella*, however, can cause illness in humans if digested (Tominaga, T., 2018). This strengthens the argument that a coliform test is useful as an

indicator of environmental cleanliness and can differentiate between contamination from a faulty pasteurizer (if subtyping of the coliform genus is done), thus helping the food safety practitioner root out dangerous growths such as biofilms (Masiello, et al., 2016).

Coliform has been an effective test to use because "detection is frequently used as a hygienic indicator for dairy products" (Masiello, et al., 2016). This has been the case since at least 1924, when the U.S. Public Health Service published its first edition of the Grade "A" Pasteurized Milk Ordinances, or "PMO"s (Martin et al., 2016). Many of the coliforms commonly found in fluid milk contamination after pasteurization can increase in their destructive activity over time, even under refrigerated conditions (Masiello, et al., 2016). This can cause complete loss of any finished food products due to spoilage, as well as quality issues, such as splits or cracks in cheese. In one study, 43% of coliforms found in pasteurized milk increased a process known as "lipolysis", which is "the release of fatty acids from triglycerides in the milk," and can leave milk tasting "rancid" (Masiello, et al., 2016). Lipolysis also affects the texture and flavor of cheese. That same study found that 10% of coliforms detected contributed to increased proteolysis, which is the breakdown of proteins in the milk, and makes it taste bitter (Masiello, et al., 2016).

It is important to balance this assertion, however, with the question of whether coliforms "indicate" the presence of other pathogens at all, since coliforms themselves are found in many environments and food products (Trmcic, et al., 2016). One study did not find pathogenic *Salmonella spp.*, *Staphylococcus Aureus*, or Shiga toxin-producing *E. coli* in any coliform positive samples but did find *Listeria monocytogenes* ("*L. mono.*") in a small portion of them (Trmcic, et al., 2016). *L. mono.* causes death in hundreds of people each year, and is especially dangerous to pregnant women, newborn babies, and some with weakened immune systems (cdc.gov). The researchers, however, could not state with certainty that a coliform test correlates or even suggests

the presence of *L. mono.* Another researcher flatly stated that a prior study found no "significant increases in coliform levels in pathogen-positive samples" representing *Bacillus cereus, E. coli O157:H7, L. mono.* and *Salmonella spp.* (Martin, et al., 2016). One researcher went further, stating that "it is clear that coliforms are not appropriate index organisms for the presence of public health hazards in dairy products," as at least one study has clearly shown that a higher coliform count does not correlate to the presence of dangerous pathogens and indeed, can be part of the cheese curing process (Martin, et al., 2016). In fact, coliforms are common in raw milk prior to pasteurization, including such types as *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*, meaning that a coliform test higher than zero should not immediately cause immediate concern. Lower levels in milk do not trigger alarm, but "colony forming units," or cfu/ml, above 1,000 can mean environmental contamination or be a sign of cattle disease, even in raw milk. (*Ibid*.). Even though this test is still useful in highlighting potential issues in the product and/or the environment around the product, coliform can also have a positive impact on the ripening and flavor processes of some cheeses, leaving its presence ambiguous without further testing to confirm which pathogens, if any, are present (Martin, et al., 2016).

There are also rules, regulations, and industry standards for acceptable levels of coliforms in food products and the environment, such as the PMO's, which require no more than 10 colony forming units per milliliter of pasteurized, grade "A" milk and milk products for consumption; if the milk is in a bulk tanker, limits can reach 100 (2017 PMO). Many organizations purchasing and distributing cheese products today still require a coliform count of less than 10 in order to safely process such foods, however some will enforce a limit of less than 100, so long as other pathogenic tests are within specification limits (*E. coli*, *salmonella spp.*, *listeria spp.*, etc.). These regulations and practices are in contrast to a bold finding from one study that flatly stated "generic

coliform testing cannot be used to assess the safety of natural cheese," leaving one to wonder if *Enterobacteriaceae* is the right path (Trmcic, et al., 2016). Furthermore, since the European Union (EU) has adopted EB as its indicator organism of choice for milk and milk products, with limits of less than 10, it is worth questioning whether the American PMO's need updating (EU Commission Regulation 2073/2005).

Enterobacteriaceae Family

Definition of *Enterobacteriaceae*

These are "gram-negative, heat-labile, glucose fermenters [that] represent a broad range of dairy-related genera with the potential to indicate post-pasteurization contamination" (Hervert, et al., 2016). EB is a vast group of microorganisms containing many, but not all, coliforms (*Aeromonas* being an exception) (Martin, et al., 2016). It also does not include other potential contaminants, such as *Pseudomonas*, but does include *Salmonella spp.*, *Shigella*, *Escherichia coli*, *Citrobacter freundii*, and *Edwardsiella tarda*. (Silbernagel, et al., 2002). The greatest benefit over coliform testing is that EB tests (and tests for total gram-negative bacteria) can detect coliforms that have been part of the traditional definition, as well as all other non-coliform, common contaminants known to occur after pasteurization (Hervert, et al., 2017). More importantly, EB testing is superior to coliform testing because it can pick up a broader array of organisms, such as *Salmonella spp.* and *Yersinia spp.* (Boor, et al., 2017). It has grown in popularity and importance as an indicator test in recent years since members of the *Enterobacteriaceae* family naturally occur in the intestinal tract of animals and are thus a good indicator of contamination (Mladenovic, et al., 2021).

As early as 1985, the National Research Council was debating the issue of whether or not *Enterobacteriaceae* should replace coliform as a microbiological test (National Research Council, 1985). 30 years later, however, coliform was still the predominant test, with more researchers still asking whether there should be a change, and others advocating for the EB test, since it offers the advantage of broader detection of potential pathogens (Trmcic, et al., 2016; Martin, et al., 2016). EB as an indicator is favored by many as a viable alternative indicator test to coliform because EB testing detects more potential pathogens, while still registering a hit for any members of the coliform family (Maheux, et al., 2014). Interestingly, the EB family has greater resistance to environmental conditions than coliform, and thus may be better than coliforms testing when the goal is to detect indicators of the effectiveness of the sanitation process (Maheux, et al., 2014). It should be noted, however, that because *Pseudomonas* species are not detected by coliform test, and are difficult to spot on an EB test, there is a significant gap in pathogenic control for any facility using this test. This bacterium "frequently contaminates dairy products after contamination," but is not routinely sought during testing using only coliform or EB as an indicator (Martin, et al., 2016).

Methods for testing for *Enterobacteriaceae*

Europe is more proactive and ahead of the United States in this area, as EB is "the primary indicator used for pasteurized milk and milk products" (Martin, et al., 2018). This is because coliforms, over the years, were shown to be present in the environment, and not necessarily correlated with the presence of other pathogens that could pose a danger to human health (Metz, et al., 2020). Amazingly, even some types of well-known coliform bacteria do not ferment lactose and would therefore not be called "coliforms" according to the definition thereof (Martin, et al., 2018). Because these varieties of "coliforms" are picked up by an EB test, this is a strong reason

to switch to EB or total gram-negative testing, because it offers a more effective method for detecting potential pathogens (Martin, et al., 2018). The EB test was initially performed on a pour plate using "selective agar," and took 72 hours to produce results (Owen, et al., 2010). Researchers later moved to a Most Probable Number (MPN) test that took four (4) days to incubate, "is subject to variation…expensive, labor-intensive" and requires precise heating and cooling of samples to produce reliable results (Leclercq, et al., 2002). Scientists have now developed a "TEMPO" test that can produce results in just 24 hours (Owen, et al., 2010).

One of the most popular and currently accepted best practices is the use of 3M's "Petrifilms" to test for EB, an easy-to-use pre-made media plate that simply requires a small amount of test material (milk or cheese, e.g.), and an incubation period of just 24 hours. Petrifilm was built upon the standard, reliable Violet Red Bile Glucose (VRBG) method, but added a "tetrazolium indicator that facilitates colony enumeration" (3M Petrifilm Instruction Guide). A detailed study found that the new Petrifilm method was "as sensitive as and more selective than the VRBG method," meaning that this product "performed as well as or better than the standard VRBG method" (Silbernagel, et al., 2002). This test can uncover *Salmonella spp.*, *Shigella spp.* and *Yersinia spp.*, thus offering a broader test platform than traditional coliform methods (3M Petrifilm handbook). Interestingly, in a 2016 study, the researchers found that Petrifilm could accurately identify at least 82% of gram-negative bacteria in contaminated samples. Additionally, the same tests found coliforms in only 62% of those same samples (Hervert, et al., 2016). They were able to determine that Petrifilm was the "most sensitive" of the testing methods they reviewed, which bodes well for today's food safety practitioner, as Petrifilm is readily available, easy to use, and reliable (Hervert, et al., 2016). What's more, the EB Petrifilm identified 100% of

the coliforms found in the contaminated samples, thus proving that it can be a more far-reaching test (Hervert, et al., 2016).

Bolstering the use of EB testing, an in-depth analysis of biofilms found on stainless steel equipment in dairy plants concluded that many of the gram-negative bacteria found in those biofilms were types identified by an EB Petrifilm test (*E. coli*, *Klebsiella*, and *Proteus*), showing that this test can identify harmful pathogenic growth (Cherif-Antar, et al., 2016). This is important, as "biofilms are the dominant mode of the community of microorganisms in nature," and can be resistant to sanitation chemicals and other cleaning methods (Mladenovic, et al., 2021). Weigh that against an older study that concluded that some EB tests may be inferior to coliform in their efforts to find types of bacteria that contribute to quality and safety defects in finished products, including increased proteolysis (which can result in bitter or off flavors) (Wessels, et al., 1989). That study, however, was later criticized and updated by a 2016 study that found proteolysis could be more common from coliform activity in dairy products than prior studies had let on, meaning that the EB test is also useful for identifying markers that can lead to quality defects (Masiello, et al., 2016). The utility of the Petrifilm test is now beyond doubt, as one research study called it "convenient, space-saving, waste-reducing, sample-ready medium," and one with a long shelf life (Silbernagel, et al., 2002).

Implications of *Enterobactericeae* test results

Put bluntly and succinctly, "the presence of *Enterobacteriaceae* in cheese is of great concern for the dairy industry because of their public health and technological significance" (Morales, et al., 2004). In fact, EU regulations require less than 10 cfu/ml in milk and other dairy products, and a zero count in infant formula (Baylis, et al., 2011). A positive EB count can manifest itself

in quality defects, such as off flavors or smells, or even risks to health, stemming from more dangerous pathogens that may be indicated by the presence of EB, such as *Salmonella spp.*, *Shigella spp.*, and *Yersinia spp*. Making it even more concerning, the "resistance of *Enterobacteriaceae* to various antibiotics is a major problem of current medicine" (Mladenovic, et al., 2021). Foodborne illness stemming from *Listeria spp., Salmonella spp.* and *Yersinia spp.* are known to cause gastroenteritis in some, and an even greater health risk to those who are immunocompromised, pregnant women and infants (Sobeih, et al., 2020; cdc.gov). There is even the possibility of Guillain-Barre syndrome, pneumonia and/or reactive arthritis from infection by these pathogens, as well as "diarrhea, dysentery, septicemia…and meningitis" (Silbernagel, et al., 2002). A 2020 study out of Egypt showed that a decent portion of "random samples of raw milk and some dairy products" collected from stores contained *Klebsiella pneumonia*, which is "responsible for pneumonia and upper respiratory tract infection and may be responsible for meningitis" and other maladies. (Sobeih, et al., 2020). More broadly, an earlier investigation found that "*Enterobacteriaceae* were present on 44% of food handler's hands and on 16% of aprons," meaning that potential pathogenic activity is all around. (Mladenovic, et al., 2021).

Weighing further in favor of EB testing, at least one study has shown that "coliforms represent less than 50% of the bacterial contaminants" that can affect fluid milk after pasteurization (Hervert, et al., 2016). That same team found that 39% of contaminants went undetected by coliform Petrifilm, leaving out a huge portion of potential bacteria that could cause major issues for finished food products, including *Salmonella spp.* and others, all of which can be picked up by an EB test (Hervert, et al., 2016). Contaminants detected by an EB test can lead to both unsanitary food products, as well as spoiling the food prior to sale (Juven, et al., 1981). EB can even influence the pH of your cheese products, leading to off flavors, consistencies, and tastes. Members of the EB

family can also adapt to a broad pH range (3.8 up to 9), temperatures, and differing water activity, making their presence in the food manufacturing environment frightening (Mladenovic, et al., 2021). The enzymes detected by an EB test can destabilize proteins found in milk and cheese, and can "modify or even prevent the coagulation of milk…directly [affecting] the formation of the product" (Amorim, et al., 2017). Due to the numerous pathogens associated with a positive EB count, and their effects on human health, it seems prudent that an EB count greater than 10 is unlikely to be safe in most food products. Even a count of 10 – which can be one colony on a Petrifilm test, depending on the dilution – should prompt the food safety practitioner to confirm that no other common pathogens are present in the environment or food product, such as *Salmonella spp.* and *E. coli O157:H7*.

Current Best Practices/Conclusions

Methods to employ

In determining which indicator organism to target in a food manufacturing facility, and which test should be used to identify that organism, current research offers some guidance. If a producer chooses to stay with coliform, solely, as an indicator organism in finished products, they "limit their ability to detect and correct instances of post-pasteurization contamination" (Hervert, et al., 2016). This test is still required in finished products by some regulations, including the Pasteurized Milk Ordinances, and therefore cannot be eliminated altogether. The sensitive company should also pay attention to customer requirements, as many could demand coliform testing, even if EB results are available. Although it began as a reliable indicator of contamination, the coliform test is no longer thorough or comprehensive enough to guarantee that the consumer is eating a safe, quality product. By switching to EB testing, the company can still detect all

coliforms, but will now also find other organisms that can contribute to unsafe or unsatisfactory food products. This is especially true for any dairy plants or dairy product manufacturers, since "milk possesses characteristics that may promote biofilm production on surfaces" (Mladenovic, et al., 2021). It would be wise, however, to also test for gram-negative bacteria as well, as this will pick up such contaminants as *Pseudomonas*, which is known to be a significant source of contamination that occurs after milk pasteurization in the United States (Martin, et al., 2016).

Petrifilm has been shown to be the best method, not only for reliability (detecting up to 95% of contaminants), but also for lack of false positive results (16% in that study) (Hervert, et al., 2016). This method, however, takes 24 hours to plate and incubate, leaving the food safety practitioner with a dangerous window in which pathogens could grow and multiply. A newer method of EB detection, "flow cytometry", can be done in 13 hours (Hervert, et al., 2017). Should a company wish to maximize their environmental and finished product testing program, the prudent food safety practitioner could add screening for total gram-negative bacteria, which would detect *Pseudomonas spp*. To this end, an additional testing method, Crystal Violet Tetrazolium Agar (CVTA) "showed the highest detection efficiency for the presence of PPC…compared with" other testing (Martin, et al., 2018). Indeed, other studies endorse CVTA as the standard method for detecting gram-negative bacteria, including *Pseudomonas spp.*, while also preventing false positive test results from gram-positive bacteria (Hervert, et al., 2016). This test, however, may not always add much to the food safety environment, as one study showed only a minimal detection of two additional potential pathogens above the results of an EB test (Hervert, et al., 2016).

Whatever test is employed, it will be necessary to sub-type or further test samples of finished food products above a certain threshold. Each company or food safety practitioner should determine acceptable levels of coliform, EB, and *Pseudomonas spp.* based on governmental rules

and regulations, customer specifications, and internal quality metrics. Any samples testing outside those limits should be further tested. For instance, an EB sample greater than 10 cfu/ml or gram could be further tested for *E. coli O157:H7*, coliform, and *E. coli spp.*, or could be typed for *Klebsiella*, *Shigella spp.*, or *Yersinia spp*. Each company will need to make its own determination on further testing based on such qualifying factors as products produced, geographic locations, and the company's historical pathogenic data, while also consulting with any internal or external microbiology laboratories that the company uses to perform testing.

Although the use of EB as an indicator organism provides a more comprehensive test in the search for pathogens in food products and food manufacturing facilities, a wholesale switch to this indicator may not comply with governmental rules and regulations, or customer specifications. Where possible, companies should make this switch, as EB will help to identify the potential pathogens found by coliform testing, but will also expand this search to include other relevant bacteria, such as *Shigella spp.* and *Salmonella spp*. If a company deems it appropriate or necessary, they could also adopt broad testing for gram-negative bacteria, such as CVTA. As more and more companies adopt this testing method, governmental rules and regulations are sure to follow. Moving from coliform to EB will help to ensure that the broadest net possible is cast in the search for, and removal of, harmful bacteria from finished food products such as cheeses.

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