An update on rapid detection methods for common foodborne bacteria

New assay methods shorten the time food is held up in quarantine waiting for an OK to ship, while automation is taking the human error out of test results.

Wayne Labs, *Senior Technical Editor*

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Source: bioMérieux.

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FE: **Time is money, and holding food in quarantine while waiting for lab results eats up valuable time when food could be in the supply chain. What can be done to speed up the results of lab assays?**

Bailey: Ideally, we would love to be able to look at a product and discover if it had a pathogen on it, or test it and have true real-time results—a couple of minutes or even an hour. Unfortunately the technologies that would detect pathogens from food products within a matter of an hour or so will not work on real world foods. Detecting bacterial pathogens from foods is like finding a needle in a haystack.

Generally speaking, most of the pathogens we deal with are not present in high numbers in a typical situation. They're present in very small numbers, and they're homogeneously distributed. So we have to be able to know how to sample, and then, be able to grow any organisms in the sample up to a high-enough number that our technology will be able to detect them.

Many of the molecular techniques have the ability to detect one or two cells if they're present, but in practical terms, detection at this level is not yet possible with standard assay technologies.

We now have a number of technologies within the industry that can give us next-day detection, and in some cases, same-day detection. Usually the same-day detection is more limited in scope for food products. In other words, in ground beef, there are several technologies that will give us about 8 hours to grow the bacteria and another 1-3 hours to run the tests—depending on what test we're running.

The industry has greatly improved assay time. In a broader scale, when looking for other pathogens like Salmonella across a wide variety of foods, there are now technologies that are pretty reliable as what we'll call next-day technologies—somewhere between 18-24 hours. That's a dramatic improvement in time-to-result. Before rapid methods became available, it took about 4-7 days for traditional cultured methods. We're talking 25-30 years ago. Then when we started moving into rapid screening methods, whether ELISA

(enzyme-linked immunosorbent assay), or early PCRs (polymerase chain reaction), we could realize a 48-hr screening procedure. Now we're moving into generations of next-day (24-hr) tests, with a few selected cases, primarily for E. coli O157:H7 and maybe some Salmonella in raw meat products in an 8-16 hour range.

From an engineering standpoint, automation in testing is really important. This is another dramatic area where we've seen improvements in reducing the number of steps and simplifying the handling of samples in testing. Every time a person touches an assay, there's an opportunity for an error—not that there will be an error, but there's an opportunity for an error. So everything we can do to lessen the number of times a person touches an assay reduces the possibility or probability of an error.

That's one of the things we've worked on at bioMérieux through the years, and I think bioMérieux has been known for its automation—whether it's in the pathogen testing or quality testing (indicators). We've taken routine tasks and automated them.

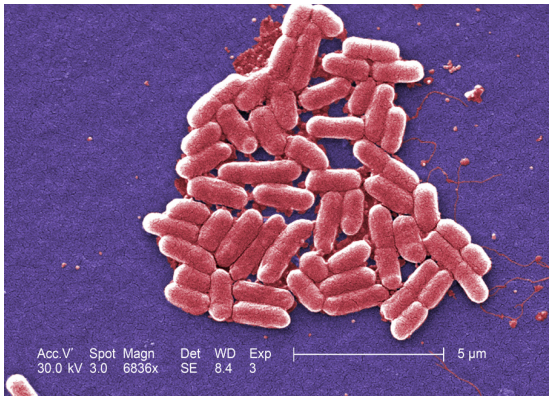
FE: **So you take them out of peoples' hands when you can?**

Bailey: Basically we've automated the actual pathogen testing, but it's difficult to eliminate the manual preparation steps. Someone still has to take the sample, put it in some broth, place it in an incubator and grow the bacteria. Someone still has to make a physical transfer into a test strip, and that requires picking it up and placing it in a machine and pushing a button. Everything else in the detection procedure is done automatically by the machine—all the transfers, broths, wash steps, and all that.

***FE*: Can you build up data to get an SPC curve on it?**

Bailey: For quality indicators, total bacteria count or coliform count, we have an instrument called a TEMPO, which uses an automated, most-probable-number (MPN) technique. MPN was the original way of counting bacteria—even before we used plates—and that's where you take advantage of serial dilutions.

What we did was automate the process. As with the automated pathogen testing, the analyst only has to prepare the sample and place it into a vial. The automated TEMPO system then uses a vacuum to pull the sample into a small card about the size of a credit card and disperse the sample into the appropriated wells to give a sixteen tube MPN. After approximately 24 hours of incubation the instrument reads the cards and automatically calculates the number of bacteria in the sample.



This colorized scanning electron micrograph depicts a number of Escherichia coli bacteria of the strain O157:H7. An estimated 73,000 cases of infection, and 61 deaths occur in the United States each year. Source: Janice Haney Carr, Centers for Disease Control (CDC).

In terms of an engineering aspect, the automation is key. An assay can be very complex—whether we're looking for pathogens or counting the number of bacteria that are present. Automation takes those arduous and sometimes time-consuming tasks and simplifies the overall procedure as much as possible, removing human error from the equation.

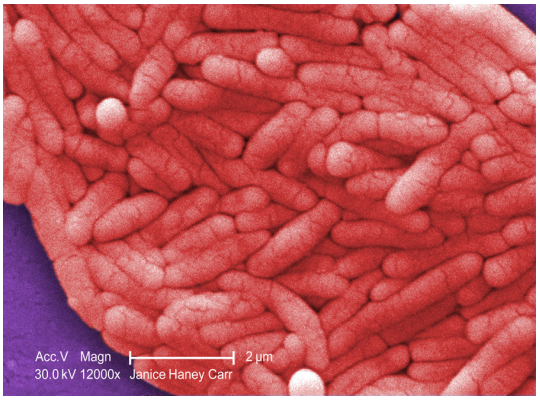
On the horizon, there are other devices that are being developed, but there will still be some time before they're practical or cost-effective. For example, biosensors will be placed immediately in the broth, and they will provide data without human hands coming in contact with the medium. The biosensor can sense bacteria coming in contact with it. I think it will be at least two or three years, however, before a sensor that is robust enough to handle complex food samples will be available. It will be more expensive—like any new technology—and it won't be suited to every application.

***FE*: USDA and FDA recalls seemed to be down in 2010 for serious problems, or should I say, really large recalls? Are we getting better at detecting contamination and preventing problems?**

Bailey: Most people would probably say that the analysis you were just talking about, that in fact it looks like we're having more foodborne outbreaks every year, but in reality we are detecting more foodborne outbreaks.

***FE*: Smaller quantities of contaminated food, then?**

Bailey: The reason we're detecting more foodborne outbreaks every year is because we have better tools. In particular, the one thing that's led to more detection of foodborne outbreaks is PulseNet, which is the program CDC put in place a few years ago, and pretty much all the state health departments participate now. So that every time a doctor has a patient who presents with a reportable bacterial disease, medical personnel run tests with very specified procedures. For example, if you run tests in



This colorized scanning electron micrograph depicts a colony of Salmonella typhimurium bacteria. Source: Janice Haney Carr, Centers for Disease Control and Prevention (CDC).

Ohio and I run them in Georgia, we should be able to compare the results.

Those results are uploaded into a central computer that's housed at CDC, and that computer is constantly [searching] to find like patterns. This has enabled us to detect outbreaks we would have never ever known were an outbreak before. Does that mean there are more outbreaks than there were before PulseNet existed? Nobody can prove that, but I would say, based on all of my years of experience, the outbreaks were always there; we just didn't detect them.

Does that mean the food is not as safe because we're detecting more outbreaks? Actually, I think it's totally opposite. It means the food is safer because we're detecting more outbreaks, and by detecting them, we can trace them more quickly. Think back to the Peanut Corporation of America outbreak a couple of years ago. Without PulseNet technology, tracking down affected products could still be going on years later; instead all related recalls, numbering more than 2,000, were finished in a few months.

By having the tools to detect outbreaks quickly, we can now pull all of those products out of commerce. One could make the argument that because we have better tools, even though we see more outbreaks, in fact our food is safer. It's a little counter-intuitive to people, but in fact, I believe that absolutely to be true.

***FE:* What seems to have improved is that most of the E. coli recalls were not 200,000, 500,000 or a million pounds of beef. It was more like this recall was a thousand pounds, this one was 500 lbs.**

Bailey: That's telling me what great ability we have to detect. There are two ways we get recalls. One is we detect them because many people got sick. The second is through routine testing, where pathogens are picked up before distribution. Therefore, we recall the product, and we don't have any sicknesses. The latter certainly happens frequently, too.

That's where test-and-hold is important. If you are holding contaminated product after the tests are pulled, and haven't shipped it, then technically you don't have to report it. Rather you just destroy it, cook it, or do something else with it. That's what most people are doing with test-and-hold. You get results before you ship the product.

***FE:* In some of your testing methods you make use of bacteriophages (or phages). Phages have been used as a method to kill specific bacteria. Can you explain how you use them for detection?**

Bailey: There are two different ways that phages can be used. You alluded to one way. Bacteriophages are viruses that are specific to a very-well defined group of bacteria. That's one way of using phages—to kill bacteria. I did some of that work in my research. The negative side of using bacteriophages to kill bacteria is that the bacteria can mutate enough such that the bacteriophage will kill most of the, say Salmonella, but the remaining Salmonella will begin to change or evolve over time, and the phage will no longer kill the mutated Salmonella.

Killing bacteria isn't the reason we're using bacteriophages. It's somewhat related. For the phage to kill Salmonella or an E. coli, the phage, which looks like a little spaceship with legs, uses its legs to recognize targets on the bacteria, and the legs attach and hold on, and this allows the phage to inject its genetic material into the bacteria, and it [phage] replicates and causes bacteria to lyse. Then millions of little viruses go out and find some other bacteria and the cycle repeats again and again. That's an oversimplification, but it's exactly right.

What we do is we take advantage of the phage's ability to recognize and attach to the bacteria. Those little fibers (legs) that attach use a protein of the phage, which we have identified. We then replicated the protein artificially through a recombinant DNA process to make the protein material. Our first test was for E. coli O157:H7. A new test for Salmonella (VIDAS UP Salmonella) was launched in June of this year. This protein has the advantage of being very specific; it creates very tight attachments with the bacteria.

So in our case, we're running an ELISA-type procedure. When you run a conjugate ELISA with a color-detect probe on it, you can detect pathogens that are present in the food sample. Because we have such specificity of the protein, we've increased significantly the sensitivity of the assay. That's allowed us to reduce the time, so our E.

coli test is 8-10 hrs depending on sample size. For testing Salmonella, [the test is based on] a combination of phage and antibody protein in order to cover the 2500 serotypes of Salmonella. For most products, the [Salmonella] test will require 16-18 hours incubation: One broth, one strip, one hour for the actual run time. The test combines the sensitivity of the phage detection with the automation of the VIDAS screening platform to provide a test that is extremely fast with the least number of handling steps.

The things in an assay you want to work toward are simplifying, automating and shortening the time required to perform the test. That's what we're able to do using the phage technology in combination with all the things we learned through the years on how to grow bacteria.

***FE:* Is the quantitative result you get important as knowing whether…**

Bailey: In this case we're not getting a quantitative result; this is a qualitative result. In the case of Salmonella, at least from a regulatory perspective, there are no quantitative limits. It would nice to quantify, but quantifying is not so straightforward. The cost of a procedure to determine quantity would add time, complexity and cost.

Detection technology is only as good as how we handle the sample up front. You have to properly sample, distribution wise.

***FE:* Are you looking for E. coli O157 serotypes now?**

Bailey: Tests are being developed that would allow testing for other serious E. coli. These will be completely different assays. At the current time, it's a question of a diminishing-returns investment. Typically, 90 percent of E. coli cases are from O157, so that leaves the other 10 percent for non-STEC (Shiga toxin-producing E. coli) bacteria. But everybody is challenged economically—government, suppliers, processors. Everybody has a limited budget to spend on testing, so if the non-O157 STECs maybe make up 5, 10, maybe even 20 percent of the illnesses, should the money be spent for testing serotypes? That's one issue. Second, the serotypes are quite different to detect. We were very fortunate in one way with O157 because it's a very bad actor, especially with kids. It has a unique phenotypic trait that allows us to be able to detect it fairly readily if it's present.

Unfortunately, the non-O157s don't have the same phenotypic trait. They look like every other run-of-the-mill E. coli on earth, which is about the most common bacteria there is. So being able to pull these non-STECs out of the rest of the E. coli population in a sample is much more problematic than finding O157. The non-STECs are only important if they're present and they possess certain pathogenic virulence factors. We're doing a lot of research in this area now and so are a lot of other people, and we've made some strides but right now for raw ground beef, E. coli O157:H7 is an adulterate by legal definitions. If any E. coli O157:H7 is found in meat, it has to be destroyed.

If we were to suggest the same treatment for meat contaminated with non-O157 STECs, most survey results would indicate that somewhere in the neighborhood of between 15 and 30 percent of meat would have to be destroyed. Not that it's going to make you sick, but because it would take so long to ultimately delineate whether or not one of those non-STECs were there. Now we have the ability in 8 to 10 hours to say that a sample is OK or not OK for O157:H7, but it would take a minimum of 48 - 72 hrs for all the gyrations we have to go through to test for the non-STECs, and processors can't hold product that long. They wouldn't necessarily throw it in the trash, but they would cook it, which from their perspective, loses 50-60 percent of the value of the meat.

There is a procedure that FSIS is putting in place, a technique where they're going to baseline work starting this summer for a year or two to try to establish how much non-O157 is really out there. Conducting this project from a research perspective can make sense, but from a practical perspective, it would disrupt completely the supply chain. It's problematic, it's an issue.

About Dr. Bailey

J. Stan Bailey, PhD., director scientific affairs of bioMerieux has authored or coauthored more than 500 scientific publications in the area of food microbiology, concentrating on controlling Salmonella in poultry production and processing, Salmonella methodology, Listeria methodology, and rapid methods to detect and identify bacteria in foods. Dr. Bailey is currently the director of scientific affairs for bioMerieux Industry and was the president of The International Association for Food Protection in 2008-2009. In 2002, he was named Outstanding Senior Research Scientist for the USDA, Agricultural Research Service and is a Fellow of the American Academy of Microbiology and The International Association for Food Protection. Internationally, Dr. Bailey has served as an expert consultant to the Foreign Agriculture. Organization and has presented invited talks in numerous countries around the world.