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CALCULATING *E. COLI* CONTAMINATION

Multiplexing and bioinformatics deliver a rapid test that meets the USDA's new standards BY MIKE MAY

The 2011 *Escherichia coli* outbreak in Germany, caused by the Shiga-toxin producing *E. coli* (STEC) with the O104:H4 serotype, claimed 50 lives. The outbreak was a sobering reminder of the dangers of STEC infection, which in humans generates symptoms ranging from mild intestinal complications to more serious kidney problems that, as in this tragic case, can even be fatal. Although the German infections derived from seeds, undercooked ground beef is another source of the bacteria. The U.S. government is now requiring more thorough screening of beef for *E. coli,* and in order to conduct such testing, food suppliers must turn to new analytical technology.

The U.S. Centers for Disease Control and Prevention (CDC) states that as many as 10 percent of STEC infections lead to hemolytic uremic syndrome, which may result in a life-threatening kidney failure. The U.S. Department of Agriculture (USDA) already requires testing of beef for the O157:H7 serotype, but the STECs include more than 100 other strains, generally referred to as non-O157 strains. The USDA's new requirements now mandate testing for six of those—known as "The Big Six"—as well as for O157.

Ground beef appeared to cause several cases of the O26-STEC infections in Maine and New York in August 2010, and other examples have linked beef with non-STEC infections—contaminations that were not being tested for. "We were learning that the states that were looking for it, the states that were doing special research projects, were finding non-O157 STECs in really significant numbers," Elisabeth Hagen, the USDA's Under Secretary for Food Safety, told *Food Safety News*. "And in some cases much higher numbers than O157."

Nonetheless, existing methods did not screen for non-O157 STECs in beef. In fact, the "DRAFT Risk Profile for Pathogenic Non-O157 Shiga Toxin-Producing *Escherichia coli* (non-O157 STEC)," which was published in August 2011 by the USDA's Food Safety and Inspection Service (FSIS), states: "[T]he commercially available diagnostic methods for the isolation of non-O157 STEC are currently quite crude, with around a 10% (or less) recovery rate from PCR-positive samples." So to test for the non-O157 STECs, the USDA first needed a test.

ACCELERATING THE ANALYSIS

In November 2011, the FSIS published "Detection and Isolation of non-O157 Shiga-toxin Producing *Escherichia coli*." This process includes an incubation step alone that can take 22 hours. It scans for the Big Six serotypes—O26,

O45, O103, O111, O121 and O145—which cause about 70 percent of the non-O157 STEC infections. According to statistics from the CDC, those non-O157 STECs could cause more than 180,000 infections a year in the United States alone.

Notably, the equipment and reagents listed in the protocol from FSIS include many items produced by Life Technologies in Carlsbad, California. For example, this protocol—and others for food testing—uses Dynabeads, magnetic beads from Life Technologies that isolate pathogens from

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—Peyman Fatemi

a solution to make an enriched sample. However, the FSIS protocol takes two days for sample screening and six days to complete the entire infection-confirmation workflow.

"We're speeding up that process," says Peyman Fatemi, senior food safety consultant at Life Technologies. The company's test runs in just 8–9 hours, and it combines advances in sample preparation, a sensitive polymerase chain reaction (PCR) step and sophisticated data analysis.

Starting with 375 grams of ground beef, the Life Technologies assay gains speed and accuracy from optimizing the sample before testing it. Keep in mind that just one gram of beef could include 100,000—maybe even a million—bacterial cells that are just background. "So you need to grow up your bacteria while trying to control for background flora so they don't overwhelm the sample," Fatemi says. "Then, you separate out the bacteria you want, extract its DNA and move to the assay."

Using Dynabeads, the Life Technologies procedure enriches the sample for O157:H7 plus the Big Six. The Dynabeads bind to these specific strains of *E. coli*, which can be separated from other cells just by using a magnetic particle processor that pulls out the O157 and Big Six cells.

Much of the speed behind the Life Technologies approach comes from using a two-stage test. As long as a sample turns out negative for O157 and the Big Six, the process takes roughly 8 hours. A PCR confirmation step—in the event of a positive result from the first stage—requires another hour. The company can then make a decision based on that result. If they want to culture confirm and isolate the specific bacteria, then the process goes essentially to the FSIS protocol to identify the specific contaminant.

SOFTWARE & SIMULTANEOUS SOLUTIONS

The efficiency of the first stage in the Life Technologies platform arises from its simultaneous scan for all seven bacterial strains. Although this approach speeds up the analysis, it complicates the process of developing the test. "The targets get diluted when you start mixing them," says Eric Liu, product manager of food and environmental safety testing at Life Technologies.

Even then, it takes time to build the best assay. "You design a number of candidate assays based on the target genes, which identify the strains," says Fatemi. "Then, you test them and pick the one that works the best."

But users of the assay will also need ways to explore the data it generates. Life Technologies's RapidFinder Express software provides different options for this analysis. The data can be viewed in a simple positive or negative format, which clearly indicates if samples turned out infected by one or more of the seven strains of bacteria. Further, users can analyze the raw data if they wish.

Most important to food suppliers and consumers is ensuring the safety of beef. The more forms of *E. coli* that producers can quickly and accurately analyze, the more likely that beef can be safely served. In all food safety, however, the producers and the consumers should both do their parts. For the latter, cooking meat fully and washing hands after touching raw beef goes a long way toward avoiding any infections. In short, as long as beef is properly tested and thoroughly cooked, everyone will stay safe.