High-tech tests—simpler, cheaper, faster.

New Detection Methods Improve Food Safety

By developing new detection techniques and processes that incorporate principles from many different scientific disciplines, Agricultural Research Service scientists in Wyndmoor, Pennsylvania, are improving food safety.

Regulatory agencies and the food industry need fast, automated, cost-effective analytical methods that are accurate, reliable, and safe and that minimize waste. Several of the research unit's chemists are using advanced technologies to develop methods to screen, detect, and confirm multiple chemical residues—such as veterinary drugs and pesticides—and pathogenic bacteria and their toxins in food products.

Shu-I Tu, research leader of the Microbial Biophysics and Residue Chemistry Research Unit at the Eastern Regional Research Center, oversees development of such techniques. He says the laboratory works with state-of-the-art scientific instrumentation and biosensor-based methods that can detect chemical signals and provide information about specific biological activities.

"Many procedures we use have been modified from diagnostic systems used for medical analysis," Tu says. "We are taking several different approaches to prevent distribution of contaminated foods."

One method they use, fluorescence spectroscopy, involves recording optical spectra from molecules absorbing and emitting light. There are several ways to attach highly fluorescent probes to make biological targets—such as pathogenic bacteria—glow. Fluorescence spectroscopic methods, which rely on monitoring changes in the wavelengths and intensity of the signal, have been used for decades. Researchers use changes in the emission spectrum to achieve valuable insight into the concentration and behavior of the emitting molecules.

Detecting low levels of protein or DNA targets in or on a sample is sometimes difficult and prone to errors because specific fluorescence signals can be low or masked by interferences. One approach to improve detection and isolate the desired spectroscopic signal is to use what's known as time-resolved fluorescence (TRF) and luminescence (TRL) reagents. Time-resolved spectroscopic techniques help reduce background noise and increase sensitivity.

Scientists Monitor Food for Residues

Some veterinary drugs are not approved for use in food-producing animals. Others, such as beta-lactam antibiotics, have tolerance levels—maximum amounts of a given chemical or its breakdown products allowed to remain in or on food commodities—established by the U.S. Food and Drug Administration (FDA). Time-consuming and laborious methods are currently used for regulatory purposes to ensure that these drugs are not present in samples.

Chemist Guoying Chen has developed a prototype of a portable, suitcase-sized device to detect contaminants, such as tetracycline antibiotics, in meat, milk, and fish. The 25-pound prototype was designed for regulatory-agency investigators to take directly to a site for field analysis. Its user-friendly custom software is already completed and will run in a Microsoft Windows environment.

The filter-based fluorometer uses TRL to detect trace amounts of target chemicals by removing interference from fluorescent background signals given off by other organic substances present in a meat sample. The system requires 1-5 g of meat from which to extract the antibiotics present and concentrate them into liquid solution. Testing can be conducted on site and results provided on the spot. A quick change of filters

STEPHEN AUSMUS (K11684-1)

Chemist Steve Lehotay prepares extracts of fruits and vegetables for analysis of pesticide residues.

allows the user to move from one targeted drug to another. The device is capable of analyzing key antibiotics in chicken and beef at slaughterhouses, and it could be used to check for contaminants in liquids, such as milk, water, or urine.

Marilyn Schneider, another chemist in the research unit, is interested in analyzing animal-derived foods for veterinary drug residues. She is developing assays using natural fluorescence, as well as TRF, to screen for the presence of fluorescent antibiotics. Schneider takes advantage of the fluorescence of a fluoroquinolone antibiotic called enrofloxacin to first screen muscle extracts for its presence. After two reagents are added to this solution, the same sample can be screened for tetracycline antibiotics, eliminating the need for a separate extraction.

QuEChERS stands for "quick, easy, cheap, effective, rugged, and safe."

In another approach, Schneider and Chen use a buffer to extract samples from chicken, clean the samples by solid phase extraction, add TRL reagents, and then measure the samples for tetracyclines using TRL.

"These screening assays are alternatives to biological assays used by regulatory agencies," Schneider says. "The fluorescence assay is very rapid and detects the drugs when they are at or above the tolerance level. TRL methods require more cleanup, but have greater sensitivity."

Chemist Steven Lehotay, along with a previously visiting scientist, Michelangelo Anastassiades, developed the QuEChERS method, which stands for "quick, easy, cheap, effective, rugged, and safe" and is pronounced "catchers." The streamlined approach makes it easier and less expensive for analytical chemists to examine fruits and vegetables for pesticide residues. Lehotay says the method reduces procedural steps—and that lessens the chance for a mistake. A single, easy-to-clean Teflon tube is the only item to be washed and reused, eliminating all the glassware used in conventional methods. Furthermore, less than 10 mL of solvent waste is generated—much less than the 75-450 mL generated by other methods.

"Several monitoring laboratories, including a few in FDA, are evaluating QuEChERS for use in routine monitoring and other approaches designed to safeguard the food supply," Lehotay says. He and colleagues are now working to adapt the concept to analyze meats for veterinary drugs.

Chemist Keith Fagerquist, along with Lehotay and chemist Alan Lightfield, developed a method to measure and confirm beta-lactam antibiotics in pork and cattle kidney tissue. The method—which uses liquid chromatography/tandem mass spectrometry—is fast and looks for multiple residues in tissue samples, where antibiotics tend to concentrate the most.

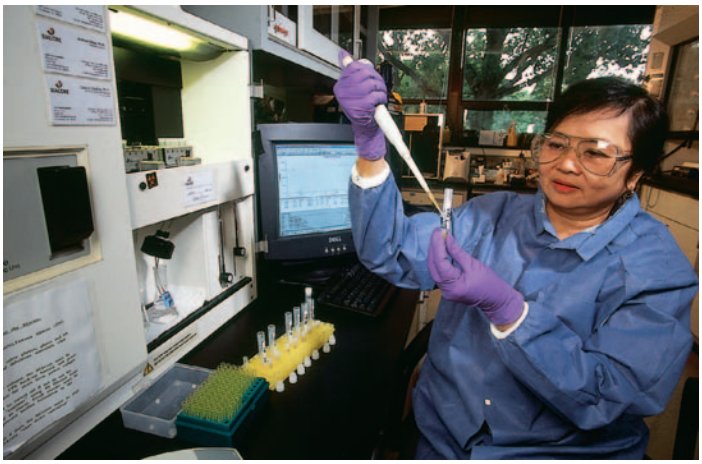
Methods Detect Pathogens and Toxins in Food

Antibodies are protein molecules that bind to antigens—such as bacteria—and remove them from the body. Researchers can use antibodies to isolate pathogens or chemicals in food products as well.

STEPHEN AUSMUS (D009-1)

To detect *E. coli* O157:H7 in foods, chemist Shu-I Tu and microbiologist Marsha Golden use immunomagnetic capture and time-resolved fluorescence.

STEPHEN AUSMUS (K11685-1)



Chemist Marjorie Medina prepares egg samples for analysis of *Staphylococcus aureus* enterotoxins by surface plasmon resonance.

Andrew Gehring, another chemist in the unit, is working with Tu on a procedure that combines immunomagnetic capture with TRF to simultaneously detect *Escherichia coli* and *Salmonella* in ground beef, ground turkey, alfalfa sprouts, and seeds. The procedure uses magnetic beads that are coated with pathogen-specific antibodies. The antibodies bind to the bacteria, and the magnetism pulls them out of complex mixtures of food. Once extracted, the bacteria can be more easily detected.

Gehring is also developing a luminescence-based method coupled with an ELISA (enzyme-linked immunosorbent assay) to detect and confirm *E. coli* O157:H7. An ELISA is a sensitive laboratory test that uses antibodies and enzymes to detect and measure specific antigens in samples. Gehring's test can be completed in 8 hours and can detect 1-10 bacteria per gram of ground meat. USDA's Food Safety Inspection Service (FSIS) would ultimately like to be able to detect 1 bacterium in 25 grams of meat.

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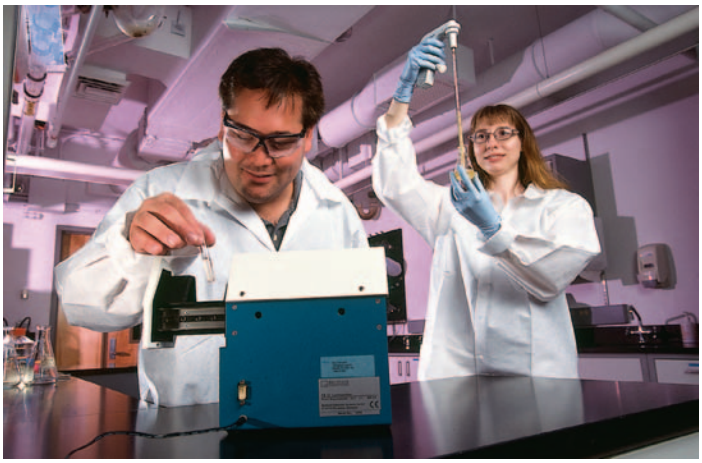
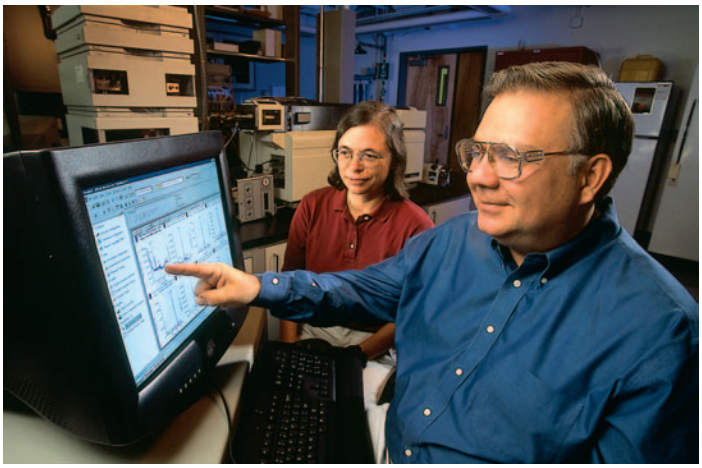


Chemist Andy Gehring inserts a meat sample into a luminometer to check for the presence of bacteria as technician Sue Reed prepares other samples for analysis.

STEPHEN AUSMUS (K11688-1)

The tube held by technician Sheri Mazenko contains concentrated immunomagnetic beads coated with antibodies that bind to specific bacteria, making it possible to detect contaminants in food.

STEPHEN AUSMUS (K11686-1)



Chemists Marilyn Schneider and Alan Lightfield analyze veterinary drug data from the liquid chromatograph/tandem mass spectrometer.

STEPHEN AUSMUS (K11689-1)

Chemist Guoying Chen tests a portable fluorometer he designed and built to screen for drug residues in food extracts.

Chemist Marjorie Medina developed a biosensor immunoassay using surface plasmon resonance (SPR) to detect *Staphylococcus aureus* enterotoxin A (SEA) and B (SEB)—toxins that cause gastroenteritis—in foods such as ham, milk, and eggs. Conventional heating and processing kills the bacterium but not its toxins. Bacteria produce toxins under stressful conditions, such as when they are too crowded or denied food or when they're fighting back against antibiotics.

"SPR uses light reflected off thin metal films," Medina explains. "Toxin molecules in the sample bind to the sensor surface, and the refractive index at the surface changes. The time it takes for a response from the interaction provides a measure of how much toxin, if any, is actually present in the food sample."

Medina says that FSIS is interested in an alternative to the conventional method to detect enterotoxins in whole eggs. Her semi-automated method has several advantages over other methods and may detect multiple bacterial toxins in a single food sample.

Medina also developed a latex particle agglutination assay for detection of SEA and SEB that causes the toxins to clump together. The method takes advantage of an antibody's ability to bind to a unique antigen in pathogen cells. The assay is simpler to use than other methods and can detect as little as 10 parts per billion of toxin per gram of sample.—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

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