Detection of Foodborne Pathogens

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Canada's food-safety system is controlled by provincial/territorial and municipal governments, food-producing companies, the agricultural sector, consumer organizations and four federal departments that share responsibility for food safety, *i.e.* Health Canada (HC), the Public Health Agency of Canada (PHAC), the Canadian Food Inspection Agency (CFIA), and Agriculture and Agri-Food Canada (AAFC) (Figure 1).

Part of the responsibility of the AAFC is to develop food-safety programs that are applicable to on-farm production. PHAC maintains surveillance systems that track foodborne illnesses, diet-related chronic diseases and works in the coordinated management of food-related emergencies. The CFIA is responsible for the design and delivery of federal food-inspection programs while monitoring industry's legal compliance. The CFIA enforces policies, regulations and standards set by HC. If a food-safety emergency occurs, CFIA, in partnership with HC, Provincial Agencies and the food industry, operates an emergency response system. And HC establishes food-safety policy and develops methods and standards for the food industry. HC also conducts health-risk assessment, sometimes at the behest of CFIA, and provides information to the public on potential health hazards.
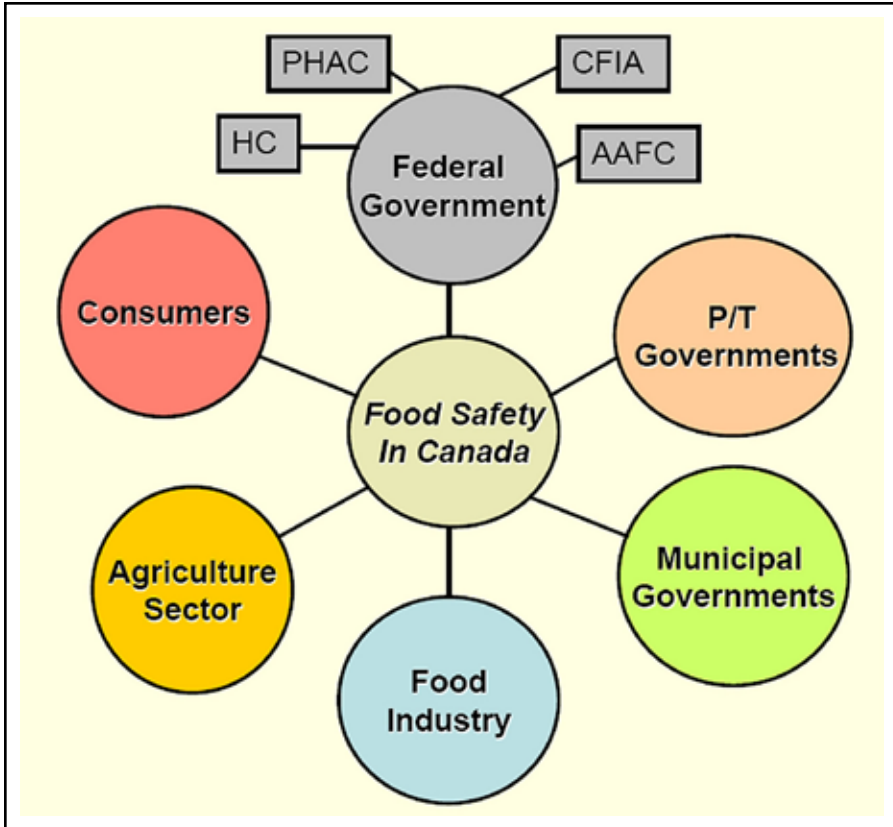
Figure 1. Canada's food-safety system.

Foodborne-Pathogen Detection

Detection of a foodborne pathogen—and validation of a suspected health risk—comprises several steps:

- Sampling
- Sample preparation
- Enrichment
- Pathogen detection
- Pathogen isolation
- Pathogen identification
- Pathogen typing

To protect today's complex food system against intentional or unintentional contamination requires addressing various needs relative to detection.

What?

First of all, what is to be detected? Pathogens and toxins are of many types, and the correct target must be selected for assay development. Also, the target may change during the investigation as the microorganism evolves, and the matrix in/on which the microorganism exists may be air, liquid or solid, or a surface. The end product or the food-processing environment may provide the best source location, or, alternatively, it may be the environment in general.

Where?

In this complex system, a risk-based approach is required to keep the samples to a reasonable number; it is impossible to test everything in a food chain. A trend is emerging of lab-testing being superseded by field-testing as new time-saving methods are developed.

When?

Timing of sampling can be critical to the detection of a pathogen.

Why?

Detection may result from routine surveillance and monitoring, checking for foodborne source attribution, or during regulatory-compliance activities. Alternatively, detection may result from investigation of an outbreak of foodborne disease.

How?

A wide range of technologies are available for investigating the possible presence of pathogens and/or toxins in food. An important factor is the choice of technology appropriate for detection of the micro-organism and/or toxic compound in question.

Ideal Method

The ultimate method for detecting microbial pathogens in food will be/have:

- Rapid in real-time
- Sensitive
- Specific, with no false positives or false negatives
- Reliable
- Portable and field deployable
- Robust
- Inexpensive in production and operation
- Easy to use
- High throughput
- Customizable (for use with a range of pathogens)
- Usable in multiple food matrices.

However, significant challenges remain in the development of a broadly applicable detection system. A key issue is sample preparation, the importance of which has been underestimated in the past. It has to be unaffected by food type, with no interference from the matrix or background flora. Low numbers of cells of the pathogen in a food are likely to mean non-uniform dispersal and the need for a lengthy enrichment period. There has to be an acceptable balance between the sensitivity of the assay and its specificity. And polymerase chain reaction (PCR)-based detection methods can give false positives resulting from the presence of dead cells. A wide variety of agents—viruses, spores, bacteria, parasites, *etc.*—can cause foodborne diseases, therefore, simultaneous detection of multiple pathogens would be greatly advantageous. Again, because of the range of possible pathogenic agents, coupled with the many food types that may be affected, emerging threats may result from unanticipated food-agent combinations as well as from "traditional" foodborne pathogens. Furthermore, some pathogens require only low numbers of cells to be infectious. Another key issue is cost associated with detection—in time, training, equipment, requirement of consumables, *etc.*—since the numbers of samples tested can be large.

Conventional Culturing

Conventional culture techniques remain the "gold standard" for the isolation, detection and identification of target pathogens, despite the disadvantages that they are applicable only when the microorganism of interest can be enriched and that the enrichment process may be lengthy. Newly developed assays are always compared with the "gold standard" for validation.

Affinity-Based Assays

The specificity of antibodies, including recombinant antibodies, or fragments of antibodies, is being exploited to detect pathogens, *e.g.* via enzyme-linked immonosorbent assays. Phage-display libraries are now being similarly utilized. Disadvantages of methods that use antibodies include their potential lack of stability, specificity and sensitivity. Nucleic acid aptamers that have stable secondary structures that function as ligands are increasingly used, particularly for the detection of non-immunogenic molecules.

Sequence-Based Assays

PCR, real-time PCR, nucleic acid sequence-based amplification, fluorescence *in situ* hybridization, microarrays and nanoarrays are all being used as methods to detect and identify DNA in samples, with the advantage that they are generally more sensitive than affinity-based assays and are highly specific. However, appropriate target selection is critical, and the presence of compounds in foods that inhibit nucleic acid amplification may be problematic. A disadvantage of PCR-based commercial kits is that some enrichment is necessary, requiring up to 18 hours. Where nucleic-acid enrichment is done on a bacterial colony, the PCR portion may be rapid but it can take three days to grow the colony, which, again, is a sample-preparation challenge.

Next-Generation Tools

There is a trend to miniaturize sensors. For example, microbiologists are working with physicists and engineers to develop biosensors, microfluidic systems and magnetic nanoparticles that capture bacteria.

Typing of Bacteria

After a bacterium has been detected and identified, often typing beyond the species or subspecies level is essential in food-safety investigations. Analyses by more than one typing method may be required with some pathogens:

- Serotype
- Phage type
- Antimicrobial resistance profile
- DNA "fingerprinting" by pulsed-field gel electrophoresis (PFGE)
- Multiple-loci variable number tandem repeat analysis (MLVA)
- Whole-genome sequence
    - Pan-genome analysis of core genes or accessory genes.

Opportunities for designing next-generation genotyping targets are constantly being sought.

Currently, PFGE is considered the "gold standard" for most foodborne bacterial pathogens, and is used by PulseNet[1] scientists. However, MLVA is being employed increasingly to type bacteria, as is whole-genome sequencing, as DNA-sequence analysis becomes less expensive, permitting pan-genome analysis of core and accessory genes for strain comparison.

Bureau of Microbial Hazards

At Health Canada's Bureau of Microbial Hazards (BMH), stakeholder needs are addressed by scientists who contribute to policy development, formulation of guidelines and industry standards, provision of advice to consumers and industry, and the maintenance of the *Compendium of Analytical Methods* which provides validated methods, standards and guidelines relative to microbiology and extraneous material for the food industry.

The BMH is organized into two divisions: the Microbiology Research Division and the Microbiology Evaluation Division. In the Research Division, scientists are conducting research and method development for high-risk foodborne pathogens. The Division also houses reference services that investigate botulism and listeriosis in Canada. Scientists in the Evaluation Division are responsible for policy development and conducting health-risk assessments.

Listeriosis Outbreak

In 2008, an outbreak of listeriosis in Canada—the largest on record—resulted in fifty-seven

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[1]http://www.cdc.gov/pulsenet/. Page 174.

confirmed cases in seven provinces, with twenty-two deaths. Subsequently the federal government commissioned an enquiry by independent investigator Sheila Weatherill, to elucidate the causes of the outbreak and propose preventative measures. The report provided fifty-seven recommendations to improve the food-safety system and enhance the responsiveness of laboratories to national foodborne emergencies.

Reference Center

In response to Weatherill's report, a Reference Center for Rapid Diagnostics, Regulatory Science and Food Safety was established as a joint initiative of HC/BMH and the Industrial Material Institute of the National Research Council of Canada (NRC), co-chaired and led by Nathalie Corneau (HC) and Teodor Veres (NRC). The objective of the Reference Center personnel is to design, fabricate and implement next-generation technologies for rapid diagnostic tests for foodborne pathogens and to facilitate deployment of these technologies throughout the food chain. The NRC's Industrial Material Institute (IMI) has a unique infrastructure in Canada, suited to polymer-based micro- and nano-fabrication, which is less expensive than silicon- or glass-based approaches. The objective is to develop portable lab-on-a-chip platforms capable of simultaneously detecting and isolating bacteria—even viable but non-culturable bacteria—viruses and parasites from various food matrices. A sample-preparation method has been designed to accommodate a wide variety of foods and environments, with detection possible without enrichment resulting from high sensitivity and specificity. The portable technology—not yet finalized—is designed so that it can be multiplexed to detect multiple pathogens. The integration of sample preparation, detection and typing will be achieved using microfluidic and micro-array systems (Figure 2).

The system is modular and uses various chips for flexibility. Food samples of 25 g—ground beef, brie cheese and deli meat (*i.e.* of varied fat content) have been tested—are
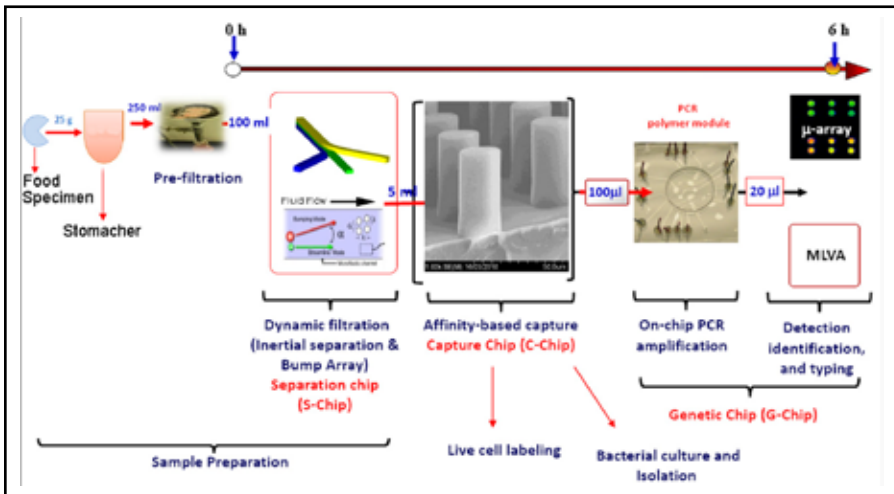

Figure 2. HC-NRC microfluidic detection approach

homogenized with a stomacher, and a pre-filtration step removes large food debris (>100 μm) without significant loss of bacteria. Pathogens are separated from food particles (>8 μm) by inertial focusing, then "bump" arrays are employed as a means of fine filtration and to concentrate the target cells (≤3 μm). Inertial focusing involves microfluidic continuous filtration in which randomly distributed particles are focused near the channel walls, resulting from an inertial lift effect. In rectangular channels, particles are focused at about 20% of the channel height from each wall surface. As the aspect ratio of the channel increases, the majority of the particles are focused near the larger walls, and the central part of the channel containing the smaller particles, *i.e.* the purified sample, can be collected. Bump arrays continue particle separation and can be used also to concentrate particles. Post-separation distance is critical. For example, an inter-post distance of 5 μm will "bump," or tend to exclude, particles larger than 2 μm, and thus separate them from the main stream. At the next stage, the capture chip again has a "forest" of posts (640,000, 25 μm in diameter), and antibodies or surface chemistry can be used to capture cells of interest. Partners at the NRC (John Pezacki and David Kennedy) are working on a click chemistry technique to improve capture of specific live cells and facilitate their release for the last stage, the genetic chip. This on-chip detection and identification approach involves cell lysis, DNA/RNA extraction, multiplex PCR amplification and microarray identification.

Next Steps

We will continue to optimize each microfluidic module, investigating new capture molecules in various food matrices, with particular emphasis on development of more-rapid detection methods and further miniaturization.

Martin Duplessis is the manager of the Food Emergency Preparedness and Response Unit (FEPRU) for the Food Directorate in the Health Products and Food Branch of Health Canada. He is responsible for policy areas on a wide range of issues related to food safety, food defense, and chemical, biological, radiological and nuclear (CBRN) emergency preparedness and response. Additionally, he is a scientific advisor on several research projects involving CBRN-agent detection.

Prior to assuming his current position, he was a scientific advisor responsible for research-development strategies and intellectual property management in universities for the government of Quebec.

He holds a PhD in food microbiology from the University of Laval, Quebec City.