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**CRITICAL REVIEW**

# Foodborne Pathogenic Bacteria Detection

AN EVALUATION OF CURRENT AND DEVELOPING METHODS

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### ABSTRACT

Epidemics arising from foodborne pathogenic bacteria are a major public health concern. There is a critical need for the development and integration of sensitive and efficient methods for foodborne pathogen detection. Beyond this, detection should ideally be rapid, inexpensive, and easy to operate without extensive training or expertise. Although conventional techniques, involving plating followed by various biochemical tests can reliably detect bacteria at low concentrations, the time required to obtain a result is often impractical. Techniques used in conjunction with conventional methods include immunological tests and nucleic acid-based tests; these have been adapted for simultaneous screening of multiple bacterial strains. Flow cytometry has recently been applied to bacterial detection with considerable success. Biosensors, devices that convert biological activity into a measurable electrical signal, have recently gained attention as a potential method for rapid sample screening. This review aims to summarize and evaluate current methods for foodborne pathogenic bacteria detection.

### INTRODUCTION

Foodborne pathogens are a growing public health concern. Estimates for the annual number of foodborne disease episodes in Canada range from 4 million<sup>1</sup> to 11 million,<sup>2</sup> depending on the methods of approximation used. Of these cases, only 40% are attributed to 30 known and accepted foodborne pathogens, suggesting the existence of additional pathogens that are not yet identified.<sup>1</sup> Canadian cases of food-related acute gastrointestinal illness are believed to represent an annual per capita cost of \$115 CAD.<sup>2</sup> The global nature of food trade further increases the spread of foodborne pathogens.<sup>3,4</sup>

Common foodborne pathogenic bacteria causing human disease include *Staphylococcus aureus*, *Campylobacter*, *Salmonella* serotypes, *Listeria monocytogenes*, *Shigella*, and enterohemorrhagic and enteropathogenic strains of *Escherichia coli*.<sup>4-6</sup> Food poisoning is often associated with raw or undercooked meats and eggs; however, many different strains of pathogenic bacteria have also been reported in prepared foods, dairy products, leafy vegetables, and shellfish.<sup>4,7</sup>

Effective bacterial detection methods are essential for controlling the spread of foodborne pathogens, as they permit the withholding of food products suspected to be contaminated.<sup>5</sup> These methods must be rapid, as susceptible foods often have a limited shelf life. They must also be sensitive enough to detect trace amounts of bacteria (the infectious dosage of *E. coli* O157:H7 can be as low as 10 cells), and to distinguish pathogenic bacteria from the many

non-pathogenic strains that may also be present in foods.<sup>8</sup>

Culture methods are conventional detection methods that begin with pre-enrichment of food samples to increase pathogenic bacterial concentrations to detectable levels, and to resuscitate bacteria injured but not killed by the treatments used in food processing.<sup>7-9</sup> These samples are then transferred to selective media, which only allows specific bacterial strains to grow, and the selectively enriched samples are subjected to various biochemical assays to test for the presence of the bacteria of interest.<sup>5</sup> Although this process is highly effective, it requires complex, labour-intensive tests.<sup>8</sup> Furthermore,

these tests typically require approximately three to seven days to obtain a result, which is often impractical in situations involving screening of foods with short shelf lives.<sup>5,10</sup>

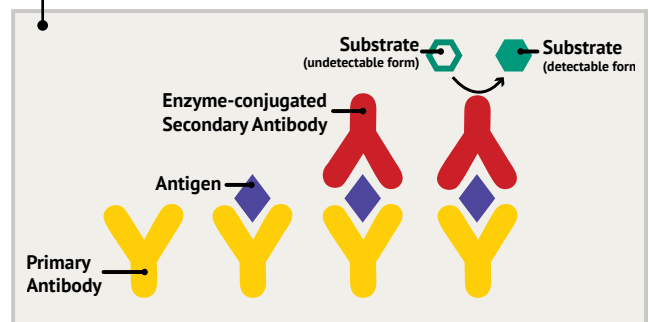
In recent years, the focus has been on the development of more rapid and sensitive procedures for detecting and enumerating bacteria from food samples.<sup>9</sup> Some of these methods include antibody-based tests, nucleic acid-based tests, flow cytometry, and biosensors.

### IMMUNOLOGICAL TESTS

Immunological assays are based on antibody-antigen interactions to detect bacteria. Antibodies specific to an antigen, whether it be the bacteria itself or a compound secreted by the bacteria, are raised by exposing animals to those antigens.<sup>11</sup> One of the most common immunological techniques is the enzyme-linked immunosorbent assay (ELISA), which involves forming a two-antibody complex with the target antigen between them, and allows for quantification of the bound antibody through an enzyme-mediated colour change reaction (see figure 1).<sup>11-13</sup>

Specific antibodies are raised to a bacterial strain and as a result, only one type of bacteria can be screened for in each assay. However, various

**FIGURE 1:** Sandwich Elisa Protocol.  
1) Primary antibody is fixed onto the surface of reaction. 2) The food sample is added, and target antigens (either bacterial cells or specific toxins secreted by bacteria) bind to the primary antibody. Any unbound substances are then washed off. 3) A secondary, enzyme-conjugated antibody is added. This antibody will only bind if the antigen is present. Unbound antibody is then washed off. 4) A colourless substrate is added. The enzyme, if present, converts the substrate to a coloured, detectable form.



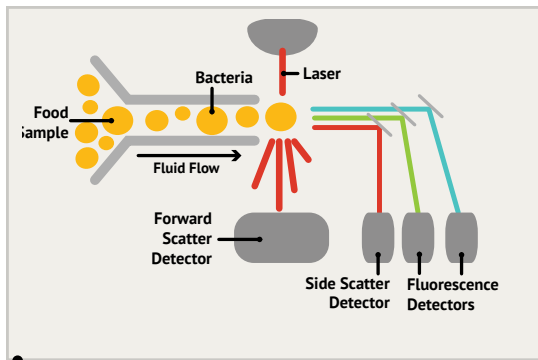


FIGURE 2: Schematic of a typical flow cytometry system setup. Bacteria passing the laser beam cause light diffraction, which is measured as a combination of forward scatter and side scatter. Photoreceptors are also present to capture light emitted by the fluorescent labels. Lenses filter out wavelengths of light so that each photoreceptor only detects a specific wavelength.

detect multiple strains of bacteria. Multiplexed immunoassays have been demonstrated to be effective in detecting a combination of *Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Salmonella typhimurium*, and *Listeria monocytogenes*.<sup>14,15</sup> Immunochromatography strip tests have primary and secondary antibodies fixed on different areas of a strip; the presence of the target antigen will allow the primary antibody to be displaced and migrate to interact with the secondary antibody, which produces a qualitative colour change. This can be used as a rapid and inexpensive initial screening for a specific foodborne pathogen.<sup>16</sup>

Although immunological detection methods are generally reliable, the specificity of the test is limited by the quality of the antibody used.<sup>5,11</sup> Inadequate sensitivity often necessitates an enrichment step to increase bacteria count in the food sample, thus increasing the time required to deliver the result.<sup>5</sup>

### NUCLEIC ACID-BASED TESTS

Nucleic acid-based tests allow for recognition of target pathogens by probing for specific DNA or RNA sequences. Traditional DNA probes consisted of a 15-30 nucleotide base sequence labelled radioactively or fluorescently for detection.<sup>11</sup> However, modern DNA-based methods usually employ polymerase chain reaction (PCR). In this procedure, primers with nucleotide sequences complementary to a sequence of the target bacteria's genome are added *in vitro* to a genomic DNA sample, along with the DNA polymerase enzyme. If the target DNA sequence is present, the primers will anneal and allow this sequence to be amplified and subsequently detected through gel electrophoresis. Because PCR involves the amplification of genetic information from the original sample, it has higher sensitivity than most other tests.<sup>17,18</sup>

Although PCR traditionally involves the use of a single pair of primers to detect the presence of a single DNA sequence, multiplex PCR systems have been designed to allow for the use of multiple primer pairs to simultaneously screen for several

forms of ELISA have been developed to combat this issue, as well as to increase assay sensitivity and decrease assay time. In multiplexed immunoassays, each ELISA plate well has multiple internal sub wells. This allows for the simultaneous use of multiple antibodies (one in each sub well) in order to

bacterial strains.<sup>19</sup> Other modifications of PCR detection include real-time PCR, which detects the amplified DNA in real time and allows DNA to be quantified.<sup>20,21</sup> This also eliminates the need to perform gel electrophoresis after PCR in order to visualize amplified DNA.<sup>21</sup>

A primary drawback of PCR foodborne pathogen detection is the requirement for an extremely clean DNA template: various contaminants derived from food samples can interfere with DNA polymerase activity to produce false-negative results.<sup>4,11</sup> Additionally, because PCR only detects the presence of target genomic DNA, it cannot provide information on whether toxins secreted by bacteria are present, or whether the bacteria are alive.<sup>22,23</sup>

### FLOW CYTOMETRY

Flow cytometry differs from immunological and nucleic acid-based techniques because it detects bacteria by measuring physical parameters, rather than through chemical interaction.<sup>8,24</sup> Cells in a test sample, which may be fluorescently stained by a specific antibody or dye, are passed single-file through a very narrow channel across a beam of light, usually a laser beam. As the bacteria flow past the laser beam, lenses and photocells detect light scatter and fluorescence (See Figure 2). These light scatter patterns provide information about the size and shape of the bacteria, as well as particle density within the cell.<sup>11,24</sup> However, the physical data provided by flow cytometry is not specific enough to distinguish between similar strains of bacteria. To overcome this, fluorescent labels specific to strains of interest are used.<sup>24</sup> Modern instruments can simultaneously measure fluorescence at more than ten different wavelengths, allowing for the detection of many bacterial strains at once.<sup>25</sup> Fluorescent stain systems have also been adapted for use in distinguishing between live and dead cells: these employ a fluorescent stain that can only enter the cell and bind to its nucleic acid target if the cell membrane is compromised.<sup>26</sup>

Flow cytometry is a rapid technique that can detect up to 100 000 cells/s.<sup>25</sup> Another distinct advantage is its ability to identify subpopulations of bacteria even if nothing is known about them. This is contrasting with immunological and nucleic acid-based methods, which can only detect bacteria for which antibodies have been developed, or genomic data is known.<sup>25</sup> Flow cytometry has been demonstrated to successfully detect strains of *E. coli* in food products such as apple juice, milk, and ground beef. However, a pre-treatment process must be performed to eliminate non-bacterial particles that interfere

with effective bacterial detection because they diffract light to generate background noise.<sup>27,28</sup>

## BIOSENSORS

One of the most rapidly growing areas in foodborne pathogen detection is the development of biosensors. Biosensors are detection systems consisting of a bioreceptor, which recognizes target bacteria, and a transducer, which converts the bacteria-bioreceptor interaction into a measurable electrical signal.<sup>4,29</sup> There are currently multiple types of bioreceptor-transducer systems available: most modern transducers are either optical, mass-sensitive, or electrochemical.<sup>4,12,30</sup>

Optical transducers are based on the recognition of an optical change. In a surface plasmon resonance (SPR) biosensor, a certain wavelength of electromagnetic radiation generates resonance in the electron cloud of a thin metal (usually gold) coat on the transducer surface. Interaction of the antigen of interest with this surface alters its refractive index, which changes the wavelength required for electron resonance.<sup>4,23,30</sup> SPR has been successful in detecting both whole bacteria and secreted toxins.<sup>30</sup> SPR-based biosensors are limited by the high cost and large size of equipment required, but portable systems have been made commercially available, and have been demonstrated to successfully detect *E.coli* O157:H7.<sup>4,12,23</sup>

Mass-based biosensors employ a piezoelectric crystal, which can be induced by an electrical signal to vibrate at a certain frequency.<sup>4</sup> This crystal is coated with antibodies for the antigen of interest. When antigens from the sample bind to the antibodies coated on this crystal, they decrease its vibrational frequency by a magnitude that corresponds directly to the added mass.<sup>4,12,30</sup> A flow-through piezoelectric assay has been shown to detect *E. Coli* with a measuring cycle of under 10 minutes; however, the system was not sensitive enough to reliably detect bacteria at concentrations less than 10<sup>6</sup> colony forming units (CFU)/mL.<sup>31</sup>

Electrochemical transducers are further broken down into amperometric, potentiometric, impedimetric, and conductometric types, which detect

changes in current, voltage, impedance, and conductance respectively, as a result of antigen-bioreceptor interactions.<sup>4,30</sup> These systems are inexpensive, and can even be manufactured as disposable chips.<sup>30</sup> Pal et al.<sup>32</sup> report a conductometric biosensor detecting pure *Bacillus cereus* cultures that were inoculated into food at concentrations as low as 35.3 CFU/mL, and with a detection time of six minutes. However, lack of specificity in electrochemical transducers is a common problem; other electroactive compounds present in the sample can often interfere with detection.<sup>30</sup>

Biosensor systems have the common advantage that the pathogens do not need to be labelled prior to detection, and they are thus capable of producing results very rapidly. These methods generally require little to no sample pre-treatment as compared to conventional methods.<sup>8</sup>

## CONCLUSION AND FUTURE STEPS

Early screening of food products is an important measure to prevent epidemics relating to foodborne pathogens. Current emphasis is on the development of techniques that allow for the rapid, inexpensive characterization of food samples. Conventional, immunological, and nucleic acid-based methods provide reliable results, but are slow and require specialized equipment and personnel. Flow cytometry is an effective detection method with potential for integration into the food inspection system, especially with the recent development of disposable microfluidic chips.<sup>33</sup> Biosensors, notable for their speed, inexpensiveness, and minimal sample pre-treatment, are another emergence in the field of foodborne pathogen detection. However, biosensor performance, particularly in terms of sensitivity and specificity, is still unreliable. Further advancements must be made before biosensors are robust enough to be accepted as a standard in food microbiological testing. ■

## REVIEWED BY DR. MIN LIN

Dr. Min Lin is a microbiologist at the Canadian Food Inspection Agency, and an adjunct professor at the University of Ottawa. Current research being conducted in his lab uses immunological and molecular biology techniques to characterize the cell surfaces of various pathogenic bacterial strains, and to develop bacterial detection methods based on these cell surface features.

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