Indicator organisms and faecal contamination

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Micro-organisms that can cause disease are called pathogens. Pathogens that can be spread through drinking water and cause water-borne disease include bacteria, viruses, and protozoa. Faecal organisms can also directly contaminate foodstuffs or indirectly cross contaminate foods via water supplies.

The number of different types of pathogens that can be present in water as a result of pollution with human or animal faeces is very large and it is neither practical nor economic to test for each specific pathogen. Accordingly, simple, easy, rapid and reliable test(s) are required to convey the potential associated risk and permit early investigative and remedial action.

Marker organisms

The concept of 'marker organisms', which includes 'indicator organisms' to evaluate hygienic food preparation practices and 'index organisms' to evaluate the potential risk of enteric pathogens in the food processing area has been proposed.

Coliforms and Enterobacteriaceae are used as indicator organisms, and Escherichia coli is used as index organism. In drinking water, coliforms and E. coli are used as indicator organisms to evaluate water quality and the potential presence of enteric pathogens in water environments.

'Coliform' was the term first used in the 1880s to describe rod-shaped bacteria isolated from human faeces.

Total coliform bacteria are a functional group consisting of four genera of the family Enterobacteriaceae that could all ferment lactose. These genera were Escherichia, Klebsiella, Enterobacter and Citrobacter.

Of the total coliforms present in the human gut, Escherichia coli (E. coli) represents the majority of the population (>90%). Total coliforms represent only about 1% of the total population of bacteria in human faeces in concentrations of about 1000 million bacteria per gram.

Test method	Average log count	Lower range	Upper range
Expected ACC 30C	5.45	5.20	6.20
Measured ACC 30C	5.70	5.40	5.90
Expected ACC 22C	5.45	5.15	6.15
Measured ACC 22C	5.65	4.45	5.85
Expected coliform	3.25	3.05	4.05
Measured coliform	3.55 (3500)	1.95 (90)	5.65 (440,000)
Expected enterobacteriaceae	4.80	4.45	5.45
Measured enterobacteriaceae	4.95 (90,000)	2.55 (350)	5.75 (560,000)

*Data from 60 European accredited laboratories

Table 1. Data from formal proficiency testing scheme (food microbiology).

Coliforms are the most commonly used indicator organisms and coliform testing is a useful means of assessing the adequacy of food processing and post-processing contamination in heat-sterilised foods.

However, some problems have been encountered. First, slow lactose fermenting or lactose-negative Enterobacteriaceae, including enteric pathogens such as salmonella and shigella, would be underestimated or undetected by coliform testing and this can lead to dangerous false conclusions.

Accordingly, specific methods are required to detect specific pathogens.

The Enterobacteriaceae is a diverse family group of bacteria where only 12% of genera are coliforms and where 88% are harmless genera associated with plants.

Enterobacteriaceae are thought to be use-

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Fig. 1. Enumeration of E. coli in cod in six hours.

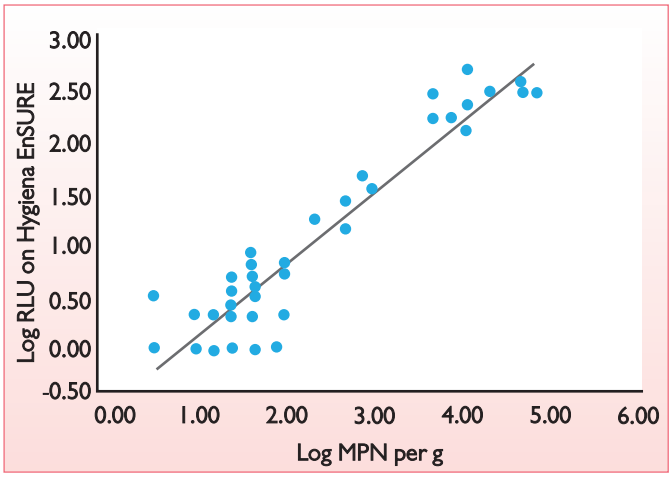
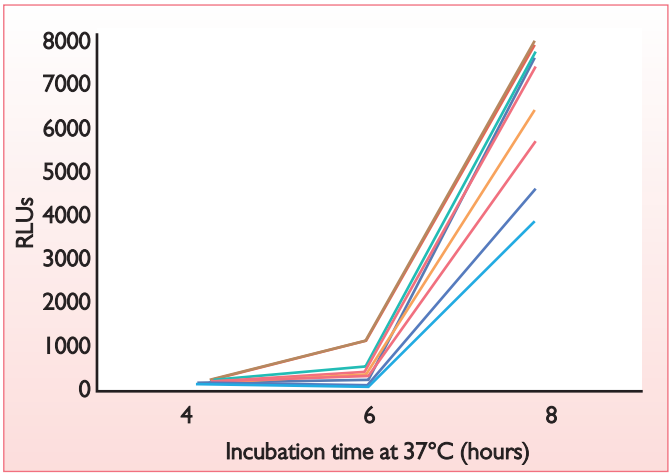


Fig. 2. Detection of coliform and E. coli in water and beverages.



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ful indicators of hygiene and of post-processing contamination of heat processed foods. Their presence in high numbers ($>10^4$ per gram) in ready-to-eat foods indicates that an unacceptable level of contamination has occurred or there has been under processing (for example inadequate cooking).

However regulatory agencies recommend that testing for Enterobacteriaceae is not applicable to fresh fruits and vegetables or foods containing these.

It is widely accepted that the total coliform group of bacteria is diverse and they can be considered normal inhabitants of many soil and water environments which have not been impacted by faecal pollution. Even though the presence of E. coli is considered an appropriate and specific indicator of faecal pollution, uncertainty surrounds the use of total coliforms as a health indicator.

As microbiological understanding about the nature of disease and the pathogens responsible increases, techniques have been developed to isolate and enumerate pathogenic viruses and protozoa from water.

These techniques, however, are not sensitive, specific, reliable, reproducible or inexpensive enough to replace the use of bacterial indicators.

Detection methods

There are several methods employed routinely for the detection of coliforms and E. coli including traditional based broth and agar methods, molecular methods and defined substrate methods.

Traditional methods

MPN (Most Probable Number) Method is a tube based broth method that employs biochemical characteristics of the thermotolerant coliforms and E. coli to be able to grow at the elevated temperature and produce gas and acid from certain sugars.

However, traditional liquid media such as Lauryl Tryptose Broth (LST) and Brilliant Green Bile Broth are very inhibitory such that some true coliform cannot grow and produce a false negative result. Similarly, some Gram positive, lactose fermenting bacilli will also cause a false positive.

Agar based methods employ solid media to recover the bacteria either as spread plates or pour plates.

These methods can be limited by the microflora present, for example bacteria who are oxidative or thermally stressed will struggle to be recovered from solid media.

The preparation and use of agar based methods require careful attention as well as the interpretation and counting of characteristic colonies. There are numerous sources for error that can give rise to very wide variations in results even when tests are conducted by accredited laboratories taking part in a proficiency testing scheme.

Table 1 shows some typical data from an international proficiency test scheme and also shows that the Enterobacteriaceae count gives very similar information to a total viable count. This is because tests for Enterobacteria offer little more than a gross semi-selective test for bile tolerant bacteria. It gives poor information about faecal contamination or risk of pathogens.

Molecular methods

Polymerase Chain Reaction (PCR) can be rapid and very specific but requires samples to be relatively clean and gives no guarantee of living bacteria due to the nature of the assay measuring nucleic acids, which can occur in dead or non-viable organisms.

Incorporating an incubation period is required in order to detect the viable organisms as well as increasing their cell numbers. Overnight incubation is typically required which negates the benefit of this very sensitive and specific technique.

Defined substrate methods

The use of biochemical markers is used routinely to detect and characterise certain bacteria, for example the majority of E. coli contain the enzyme beta-glucuronidase, and most coliforms possess beta-galactosidase.

These enzymes are well established identifiers of these faecal organisms. The use of defined substrates are used to detect these characteristic bacterial enzymes rapidly. Some chromogenic substrates can work in a time frame of 14-24 hours and produce visible colour reactions either in solid agar or simple liquid broth formats.

The use of fluorogenic defined substrates can produce results faster but the large automated systems have a higher capital cost. Time to result is important to some manufacturers where a rapid pro-active response to potential microbiological issues is required viz. high value, short shelf life, ready-to-eat products.

A new range of bioluminogenic substrates for the common faecal bacteria including thermotolerant coliforms, E. coli and streptococci have been developed giving results in the same working day or shift. These defined substrates can be detected in less than eight hours using a small hand-held luminometer that also reduces costs and enables testing to be conducted at locations outside the laboratory if required.

Bioluminogenesis

A new bioluminogenic test (MicroSnap) has been developed for the rapid qualitative and quantitative estimation of coliforms and E. coli in water and food. This assay uses the ability of the bacteria to grow in a non-specific media and produce diagnostic enzymes that are then used to quantify the specific target bacteria.

The MicroSnap system consists of two phases, first the enrichment phase – the bacteria are grown in broth to increase both the number of bacteria and titre of the diagnostic enzyme. This enrichment phase can be as short as two hours but depends on the starting inocula.

The second phase is the measurement of the specific enzyme; this phase takes 10 minutes and is used to determine the total enzyme produced which is directly proportional to the starting inocula.

Fig. 1. shows the detection and enumeration of E. coli in raw cod after six hours. There is a direct relationship over a 4 log range with an excellent correlation coefficient of 0.8853 i.e. 94% agreement between the rapid test and the reference method. Similar results are obtained with other foodstuffs and for low level contamination to give equivalent presence or absence test results in seven hours.

The test can also be applied to liquid samples such as water or beverages and detect coliform and E. coli down to 1 bacterium in 100ml in seven hours.

Fig. 2 shows a range of beverages, both carbonated and still, inoculated with 70 Escherichia coli cells per 100ml sample.

The results also show that significant levels of Escherichia coli were detected between 5-6 hours.

Conclusions

The use of indicator and index organisms have been used successfully for >60 years to provide simple assessment tools for faecal contamination and associated risks from pathogenic micro-organisms. The definition of inclusive organisms is still debated.

Whereas the coliform group may be a more pragmatic functional representation of faecal contamination, the more phylogenically correct Enterobacteriaceae test is too broad and inclusive of irrelevant microbes.

Accordingly, no single test can provide a definitive answer, and precision of all microbiological methods is lacking, particularly when trying to detect low numbers or provide evidence of absence.

Modern food manufacturing procedures require rapid test results to verify compliance, quality and safety and also to respond promptly to deviations and facilitate immediate corrective action. Simple, rapid, low cost test systems can provide solutions within the same working day/shift. ■

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