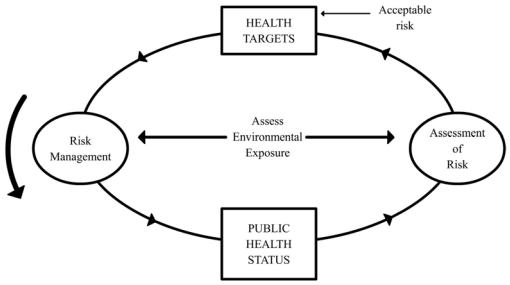

13

Indicators of microbial water quality

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Current guidelines in the three water-related areas (drinking water, wastewater and recreational water) assess quality, in microbiological terms, by measuring indicator organisms. This chapter looks at the history and examines some of the methods used to assess the microbiological quality of water, highlighting the current limitations and also possible future developments.

13.1 INTRODUCTION

Traditionally, indicator micro-organisms have been used to suggest the presence of pathogens (Berg 1978). Today, however, we understand a myriad of possible reasons for indicator presence and pathogen absence, or vice versa. In short, there is no direct correlation between numbers of any indicator and enteric pathogens (Grabow 1996). To eliminate the ambiguity in the term 'microbial indicator', the following three groups (outlined in Table 13.1) are now recognised:

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- General (process) microbial indicators,
- Faecal indicators (such as *E. coli*)
- Index organisms and model organisms.

Table 13.1. Definitions for indicator and index micro-organisms of public health concern (see Box 13.1 for definitions of microbial groups)

Group	Definition
Process indicator	A group of organisms that demonstrates the efficacy of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection.
Faecal indicator	A group of organisms that indicates the presence of faecal contamination, such as the bacterial groups thermotolerant coliforms or *E. coli*. Hence, they only **infer** that pathogens may be present.
Index and model organisms	A group/or species indicative of pathogen presence and behaviour respectively, such as *E. coli* as an index for *Salmonella* and F-RNA coliphages as models of human enteric viruses.

A direct epidemiological approach could be used as an alternative or adjunct to the use of index micro-organisms. Yet epidemiologic methods are generally too insensitive, miss the majority of waterborne disease transmissions (Frost *et al.* 1996) and are clearly not preventative. Nonetheless, the ideal is to validate appropriate index organisms by way of epidemiological studies. A good example is the emerging use of an enterococci guideline for recreational water quality (WHO 1998; Chapter 2 of this volume). Often epidemiologic studies fail to show any relationship to microbial indicators, due to poor design (Fleisher 1990, 1991) and/or due to the widely fluctuating ratio of pathogen(s) to faecal indicators and the varying virulence of the pathogens.

The validity of any indicator system is also affected by the relative rates of removal and destruction of the indicator versus the target hazard. So differences due to environmental resistance or even ability to multiply in the environment all influence their usefulness. Hence, viral, bacterial, parasitic protozoan and helminth pathogens are unlikely to all behave in the same way as a single indicator group, and certainly not in all situations. Furthermore, viruses and other pathogens are not part of the normal faecal microbiota, but are only excreted by infected individuals. Therefore, the higher the number of people contributing to sewage or faecal contamination, the more likely the presence of a range of pathogens. The occurrence of specific pathogens varies further according to their seasonal occurrence (Berg and Metcalf 1978).

In summary, there is no universal indicator, but a number, each with certain characteristics. Therefore, this chapter focuses on elucidating the appropriate

uses for indicator micro-organisms with a view to their role in the management of waterborne microbial risks. To understand the current use of indicators, however, it is necessary to first understand their historical development.

13.2 DEVELOPMENT OF INDICATORS

13.2.1 The coliforms

The use of bacteria as indicators of the sanitary quality of water probably dates back to 1880 when Von Fritsch described *Klebsiella pneumoniae* and *K. rhinoscleromatis* as micro-organisms characteristically found in human faeces (Geldreich 1978). In 1885, Percy and Grace Frankland started the first routine bacteriological examination of water in London, using Robert Koch's solid gelatine media to count bacteria (Hutchinson and Ridgway 1977). Also in 1885, Escherich described *Bacillus coli* (Escherich 1885) (renamed *Escherichia coli* by Castellani and Chalmers (1919)) from the faeces of breast-fed infants.

In 1891, the Franklands came up with the concept that organisms characteristic of sewage must be identified to provide evidence of potentially dangerous pollution (Hutchinson and Ridgway 1977). By 1893, the 'Wurtz method' of enumerating *B. coli* by direct plating of water samples on litmus lactose agar was being used by sanitary bacteriologists, using the concept of acid from lactose as a diagnostic feature. This was followed by gas production, with the introduction of the Durham tube (Durham 1893). The concept of 'coliform' bacteria, those bacteria resembling *B. coli*, was in use in Britain in 1901 (Horrocks 1901). The colony count for bacteria in water, however, was not formally introduced until the first Report 71 (HMSO 1934).

Therefore, the sanitary significance of finding various coliforms along with streptococci and *C. perfringens* (see Box 13.1) was recognised by bacteriologists by the start of the twentieth century (Hutchinson and Ridgway 1977). It was not until 1905, however, that MacConkey (1905) described his now famous MacConkey's broth, which was diagnostic for lactose-fermenting bacteria tolerant of bile salts. Nonetheless, *coli-forms* were still considered to be a heterogeneous group of organisms, many of which were not of faecal origin. The origins of the critical observation that *B. coli* was largely faecal in origin while other coliforms were not, could be claimed by Winslow and Walker (1907).

13.2.1.1 Coliform identification schemes

Various classification schemes for coliforms have emerged. The earliest were those of MacConkey (1909) which recognised 128 different coliform types, while Bergey and Deehan (1908) identified 256. By the early 1920s,

differentiation of coliforms had come to a series of correlations that suggested indole production, gelatin liquefaction, sucrose fermentation and the Voges–Proskauer reaction were among the more important tests for determining faecal contamination (Hendricks 1978). These developments culminated in the IMViC (Indole, Methyl red, Voges–Proskauer and Citrate) tests for the differentiation of so-called faecal coliforms, soil coliforms and intermediates (Parr 1938); these tests are still in use today.

Water sanitary engineers, however, require simple and rapid methods for the detection of faecal indicator bacteria. Hence, the simpler to identify coliform group, despite being less faecal-specific and broader (for which *Escherichia, Klebsiella, Enterobacter* and *Citrobacter* were considered the most common genera) was targeted. One of the first generally accepted methods for coliforms was called the Multiple-Tube Fermentation Test.

Box 13.1. Definitions of key faecal indicator micro-organisms.

Coliforms: Gram-negative, non spore-forming, oxidase-negative, rod-shaped facultative anaerobic bacteria that ferment lactose (with β-galactosidase) to acid and gas within 24–48h at 36±2°C. **Not** specific indicators of faecal pollution.

Thermotolerant coliforms: Coliforms that produce acid and gas from lactose at 44.5± 0.2°C within 24±2h, also known as faecal coliforms due to their role as faecal indicators.

Escherichia coli (E. coli): Thermophilic coliforms that produce indole from tryptophan, but also defined now as coliforms able to produce β-glucuronidase (although taxonomically up to 10% of environmental *E. coli* may not). Most appropriate group of coliforms to indicate faecal pollution from warm-blooded animals.

Faecal streptococci (FS): Gram-positive, catalase-negative cocci from selective media (e.g. azide dextrose broth or m Enterococcus agar) that grow on bile aesculin agar and at 45°C, belonging to the genera *Enterococcus* and *Streptococcus* possessing the Lancefield group D antigen.

Enterococci: All faecal streptococci that grow at pH 9.6, 10° and 45°C and in 6.5% NaCl. Nearly all are members of the genus *Enterococcus*, and also fulfil the following criteria: resistance to 60°C for 30 min and ability to reduce 0.1% methylene blue. The enterococci are a subset of faecal streptococci that grow under the conditions outlined above. Alternatively, enterococci can be directly identified as micro-organisms capable of aerobic growth at 44±0.5°C and of hydrolysing 4-methlumbelliferyl-β-D-glucoside (MUD, detecting β-glucosidase activity by blue florescence at 366nm), in the presence of thallium acetate, nalidixic acid and 2,3,5-triphenyltetrazolium chloride (TTC, which is reduced to the red formazan) in the specified medium (ISO/FDIS 7899-1 1998).

Sulphite-reducing clostridia (SRC): Gram-positive, spore-forming, non-motile, strictly anaerobic rods that reduce sulphite to H_2S.

Clostridium perfringens: As for SRC, but also ferment lactose, sucrose and inositol with the production of gas, produce a stormy clot fermentation with milk, reduce nitrate, hydrolyse gelatin and produce lecithinase and acid phosphatase. Bonde (1963) suggested that not all SRC in receiving waters are indicators of faecal pollution, hence *C. perfringens* is the appropriate indicator.

Bifidobacteria: Obligately anaerobic, non-acid-fast, non-spore-forming, non-motile, Gram-positive bacilli which are highly pleomorphic and may exhibit branching bulbs (bifids), clubs, coccoid, coryneform, Y and V forms. They are all catalase-negative and ferment lactose (except the three insect species; *B. asteroides, B. indicum* and *B. coryneforme*) and one of the most numerous groups of bacteria in the faeces of warm-blooded animals.
Bacteriophages (phages): These are bacterial viruses and are ubiquitous in the environment. For water quality testing and to model human enteric viruses, most interest in somatic coliphages, male-specific RNA coliphages (F-RNA coliphages) and phages infecting *Bacteroides fragilis*.
Coliphages: Somatic coliphages attack *E. coli* strains via the cell wall and include spherical phages of the family *Microviridae* and various tailed phages in 3 families. The F-RNA coliphages attack *E. coli* strains via the sex pili (F factor) and are single-stranded RNA non-tailed phages in four groups (Table 13.3).
***Bacteroides fragilis* bacteriophages**: These infect one of the most abundant bacteria in the gut, belong to the family *Siphoviridae* with flexible tail (dsDNA, long non-contractile tails, capsids up to 60 nm). Phages to the host strain, *B. fragilis* HSP40 are considered to be human-specific, but phages to *B. fragilis* RYC2056 are more numerous and not human-specific (Puig *et al.* 1999).

13.2.1.2 Most probable number method

In 1914, the first US Public Health Service Drinking Water Standard adopted a bacteriological standard that was applicable to any water supply provided by an interstate common carrier (Wolf 1972). It specified that not more than one out of five 10 ml portions of any sample examined should show the presence of the *B. coli* group by the specified Multiple-Tube Fermentation procedure (now referred to as the Most Probable Number or MPN procedure).

Although this test is simple to perform, it is time-consuming, requiring 48 hours for the presumptive results. There are a number of isolation media each with its bias and the bacteria enriched are not a strict taxonomic group. Hence, the total coliforms can best be described as a range of bacteria in the family *Enterobacteriaceae* varying with the changing composition of the media.

Following presumptive isolation of coliforms, further testing is required for confirmation of the coliform type. During the late 1940s there was a divergence between the UK and US approaches to identifying the thermotolerant or so-called 'faecal' coliforms. In the UK, Mackenzie *et al*. (1948) had shown that atypical fermentors of lactose at 44°C were indole-negative, whereas *E. coli* was indole-positive. Confirmation of *E. coli* with the indole test was undertaken in the UK, but lactose fermentation at 44°C alone was used in the US (Geldreich 1966). Thus over a period of some 50 years, water bacteriologists developed the concept of *B. coli* (later *E. coli*) as the indicator of faecal pollution, but continued to attach significance to the total lactose fermenters,

known variously as 'coli-aerogenes' group, *Escherichia-Aerobacter* group, colon group or generally referred to as the 'total coliforms' group.

The range of non-faecal bacteria represented in the coliform group and the environmental growth of thermophilic (faecal) coliforms *Klebsiella* spp. and *E. coli* (Ashbolt *et al.* 1997; Camper *et al.* 1991) have concerned bacteriologists and sanitary engineers since the 1930s (Committee on Water Supply 1930). At the other extreme, recent outbreaks of cryptosporidiosis in the absence of coliforms (per 100 ml) are well known (Smith and Rose 1998), and many earlier classic failures of coliforms to identify waterborne pathogens have also been reported.

Despite the obvious failings of the total coliform group to indicate health risk from bacterial pathogens, they provide valuable information on process efficiency which is clearly important in relation to health protection.

13.2.1.3 Membrane filtration method

Until the 1950s practical water bacteriology relied almost exclusively, for indicator purposes, on the enumeration of coliforms and *E. coli* based on the production of gas from lactose in liquid media and estimation of most probable numbers using the statistical approach initially suggested by McCrady (1915). In Russia and Germany, however, workers attempted to culture bacteria on membrane filters, and by 1943 Mueller in Germany was using membrane filters in conjunction with Endo-broth for the analysis of potable waters for coliforms (Waite 1985). By the 1950s membrane filtration was a practical alternative to the MPN approach, although the inability to demonstrate gas production with membranes was considered a major drawback (Waite 1985).

The arbitrary definitions adopted for *E. coli* and the related coliforms were all based upon cultural characteristics, including the ability to produce gas from lactose fermentation (HMSO 1969). Hence, the thermotolerant coliforms include strains of the genera *Klebsiella* and *Escherichia* (Dufour 1977), as well as certain *Enterobacter* and *Citrobacter* strains able to grow under the conditions defined for thermotolerant coliforms (Figureras *et al.* 1994; Gleeson and Gray 1996). This phenotypic approach has also resulted in *E. coli* or a related coliform being ignored simply because they failed to ferment lactose, failed to produce gas from lactose or were indole-negative at 44.5°C. The approach had been repeatedly questioned (Waite 1997), and was only resolved in the UK in the 1990s (HMSO 1994).

It has long been recognised that artificial culture media lead to only a very small fraction (0.01–1%) of the viable bacteria present being detected (Watkins and Xiangrong 1997). Since MacConkey's development of selective media for *E. coli* and coliforms at the beginning of the twentieth century (MacConkey 1908), various workers have shown these selective agents inhibit environmentally or oxidatively stressed coliforms.

13.2.1.4 Defined substrate methods

Media without harsh selective agents but specific enzyme substrates allow significant improvements in recoveries and identification of target bacteria. In the case of coliforms and *E. coli*, such so-called defined substrate methods were introduced by Edberg *et al*. (1988, 1990, 1991). What has evolved into the Colilert® technique has been shown to correlate very well with the traditional membrane filter and MPN methods when used to test both fresh and marine water (Clark *et al*. 1991; Eckner 1998; Fricker *et al*. 1997; Palmer *et al*. 1993). Furthermore, these enzyme-based methods appear to pick up traditionally non-culturable coliforms (George *et al.* 2000).

These developments have led to further changes in definitions of total coliforms and *E. coli*. In the UK, for example, total coliforms are members of genera or species within the family *Enterobacteriaceae*, capable of growth at 37°C, which possess β-galactosidase (HMSO 1989, 1994). In an international calibration of methods, *E. coli* was enzymatically distinguished by the lack of urease and presence of β-glucuronidase (Gauthier *et al.* 1991). Furthermore, the International Standards Organisation has recently published miniaturised MPN-based methods for coliforms/*E. coli* and enterococci based on the defined substrate approach (ISO/FDIS 1998, 1999).

13.2.2 Faecal streptococci and enterococci

Parallel to the work on coliforms, a group of Gram-positive coccoid bacteria known as faecal streptococci (FS) were being investigated as important pollution indicator bacteria (Houston 1900; Winslow and Hunnewell 1902). Problems in differentiating faecal from non-faecal streptococci, however, initially impeded their use (Kenner 1978). Four key points in favour of the faecal streptococci were:

(1) Relatively high numbers in the excreta of humans and other warm-blooded animals.
(2) Presence in wastewaters and known polluted waters.
(3) Absence from pure waters, virgin soils and environments having no contact with human and animal life.
(4) Persistence without multiplication in the environment.

It was not until 1957, however, with the availability of the selective medium of Slanetz and Bartley (1957) that enumeration of FS became popular. Since then, several media have been proposed for FS and/or enterococci to improve on the specificity.