

## Rapid Methods in Water Microbiology

Professor C. William Keevil

Man cannot live without a wholesome supply of water for drinking, washing and preparing food. Despite rapid progress in science, the evolution of the built environment and improvements in sanitary practice, modern society continues to suffer from waterborne outbreaks of disease. Those in Western society should not be complacent because waterborne outbreaks can be larger there than those recorded in the Third World, a notable example being the outbreak of Cryptosporidiosis in Milwaukee, USA in 1993 which affected over 400,000 people. Consequently, the water and wastewater microbiology testing market runs into billions of dollars each year worldwide. Most testing is concerned with detection of faecal indicators of pollution, principally *Eshcherichia coli* and other faecal coliforms, as surrogates for the potential presence of dangerous pathogens such as *Salmonella enterica* or *Vibrio cholerae*. These tests and the use of chlorination over the past 100 years have caused a major improvement in human health and saved millions of lives each year.

The tests originally depended on classical culture techniques, with the stipulation that there should be no coliforms detected in say 100 ml of treated water, but now rapid enzyme-based chromagenic and fluorogenic tests have been approved for use in many countries. These tests have arisen due to the development of new technologies but have been driven by the factors of speed of test, cost of test, simplicity of test to be undertaken by relatively untrained staff, and large throughput of samples in each laboratory. Several of these factors are particularly important in the use of such tests in the Third World. These factors also relate to the drive to automate tests wherever possible, increasing speed and saving on manpower and sometimes reagent costs. Nevertheless, it should not be overlooked that a test must be fit for purpose and have the usual requirements of sensitivity, i.e. a very low limit of detection but sometimes taken to mean that there are no false negatives, and specificity (i.e. no false positives). Every test must go through a vigorous validation procedure and receive regulatory approval from organisations such as ISO and CEN.

The wastewater treatment industry has been relatively slow to adopt the newer technologies developed in the clinical, veterinary, food and potable water industries. However, there has been recent concern about new and re-emerging pathogens being transmitted by faecal contamination and the risk of their presence in wastes when recycled to agricultural land. This has caused a reappraisal of what tests are appropriate for stored and/or treated human and animal wastes. In the USA, the EPA Part 503 Sludge Regulation now stipulates that a sludge is acceptable for use on land if it contains less than 1000 faecal coliforms per g dry weight of sludge and less than 3 salmonellae, 1 virus and 1 viable helminth per 4 g (Federal Register, 1993). These stringent regulations have highlighted the need for better methods of detection for each type of microorganism in sludge. Moreover, there is a growing realisation that stored and treated wastes may contain sub-lethally damaged pathogens with the possibility of them still being able to cause disease. Consequently, newer culture techniques are addressing the requirement of including a resuscitation, pre-enrichment step before enumeration on agar media or detection with molecular and immunological technologies. This extra step inevitably slows down the detection method, making it less than rapid and probably more expensive. Perhaps the future holds a compromise of using simple tests, such as immunological dip sticks, for rapid on-site monitoring for a pathogen's presence, followed by a subsequent full

laboratory resuscitation analysis of the presumptive positives to look for the viable organism. This approach may be criticised however because of the insensitivity of immunological techniques to detect low numbers of the target organism. This is certainly one area where the diagnostics industry should look to increase the sensitivity of detection. Virus detection has become easier and rapid with the development of PCR, RT-PCR and immunological detection technologies. However, it is unlikely that the assessment of viability will become rapid or cheap because of the need for cell culture-based assays with the caveat of their sensitivity to toxic inhibitors in the wastes. As a consequence, rapid methods are unlikely to dominate the water testing market in the near future but useful advances will be made by integrating some of their technology into more tedious assay protocols.