water e-journal
Online Journal of the Australian Water Association
ISSN 2206-1991
Volume 1 No 4 2016
http://dx.doi.org/10.21139/wej.2016.041

DETECTION METHODS FOR FAECAL CONTAMINATION EVENTS: THE GAP FOR AUSTRALIA

TRENDS IN PATHOGEN DETECTION AND RECENT DEVELOPMENTS IN THE FIELD OF PATHOGENIC BACTERIA DETECTION

A Anastasi, A Power, S Chandra, L Voss and J Chapman

ABSTRACT

Testing for the detection of human faecal indicator bacteria upon beaches and other bathing waters occurs routinely across Europe and the United States.

Australia does not, as yet, carry out this sampling protocol. With the prospect of inevitable population growth and influx of tourists to recreational water bodies, testing could become a requirement to prevent the outbreak of respiratory and/or potentially fatal gastrointestinal illnesses. Current Escherichia coli detection methods are typically laborious, laboratory-based methods requiring up to 48 hours before the results are obtained.

This is clearly insufficient, and researchers have recently geared efforts towards the development of rapid methods. The advent of new technologies, in the form of sensors, has brought about promising approaches. This review not only offers an overview of the trends in pathogen detection but also describes the current main techniques and traditional methods along with recent developments in the field of pathogenic bacteria detection.

INTRODUCTION

The detection of faecal contamination in natural waters is essential to the users of these water bodies. Faecal contamination occurs from wildlife, domestic animals, stock and human sources which can lead to the outbreak of waterborne diseases such as gastroenteritis or respiratory infections caused by pathogenic microorganisms.[1]

The population groups at greatest risk from serious health complications from these waterborne diseases are the very young, the elderly and the immunocompromised.[1-3] The range of faecal matter-derived microorganisms found in water bodies is diverse and includes both pathogenic and non-pathogenic organisms. Waterborne indicator organisms and their significance to human health are listed by the National Health and Medical Research Council (NHMRC) Table 1.[4]

Generally, it is the bacterial species (Table 1) that are used as water quality indicators, and according to Till[6], there are three reasons to index bacterial indicators to health risks: (1) bacterial indicators are present most of the time in many water bodies, (2) enumeration of indicator organisms is much cheaper than pathogenic enumeration, and (3) a relationship between the health risk and the particular indicator concentration has been established.

While numerous studies are conducted using viral detection methods, bacterial indicators are frequently examined using methods including: culturing techniques, genomics and proteomics.[7, 8] Of the numerous bacterial coliforms, E. coli is described as a specific indicator for faecal contamination as some coliforms are not faecal in origin. Improved methods for the detection of E. coli also allow for increased specificity.9 Where sanitary risk is of concern, E. coli is an appropriate indicator for contaminated waters because it is the most abundant of the coliform group in mammalian faeces.[10-12] Janezic et al.[13] and Garcia-Armisen et al.[14] state that E. coli is the preferred faecal indicator organism as it is always linked to faecal contamination from homoeothermic animals.

Table 1. Table 1. Waterborne microorganisms of faecal origin and their significance to human health, where organisms, health significance and relative infectivity have been listed.

Indicator Organisms	Health Significance	Relative Infectivity
Campylobacter sp.	High	Moderate
Clostridium perfringens spores	Nil – indicator only	Low
Escherichia coli	High[5], mostly indicators	High (for non-indicator strains)
Intestinal enterococci	Nil – indicator only	Low
Salmonella spp.	High	Low
Shigella spp.	High	Moderate
Adenoviruses	High	High
Coxsakie	High	High
Hepatitis A	High	High
Hepatitis B	High	High
Noroviruses	High	High
Rotaviruses	High	High
Cryptosporidium parvum oocysts	High	High
Cryptosporidium hominis oocysts	High	High
Entamoeba histolytica	High	High
Giardia lamblia cysts	High	High

Conventional detection methods are most commonly used despite the long turnaround times, owing to their high selectivity and high sensitivity responses. Biosensors have the potential to shorten these turnaround times between the sample uptake and results. The future development of biosensors for faecal contamination will be in reaching sensitivity and selectivity comparable to conventional methods at a fraction of the cost.

DETECTION OF BACTERIA

Pathogenic detection is an area of growing importance, primarily for health and safety reasons. A web of science search consisting of over 25,000 papers indicates the following areas to be relevant to pathogenic organism detection (Figure 1).

The main areas of pathogenic detection research have been categorised in the following areas: defence and security (1%), food safety (11%), clinical (8%), water and environmental (59%), and new and emerging other (21%). Bacterial indicators of water quality have routinely been used since the late 1800s when water contamination was linked to illness and high infant mortality rates, particularly in low socio-economic areas.[9, 15]

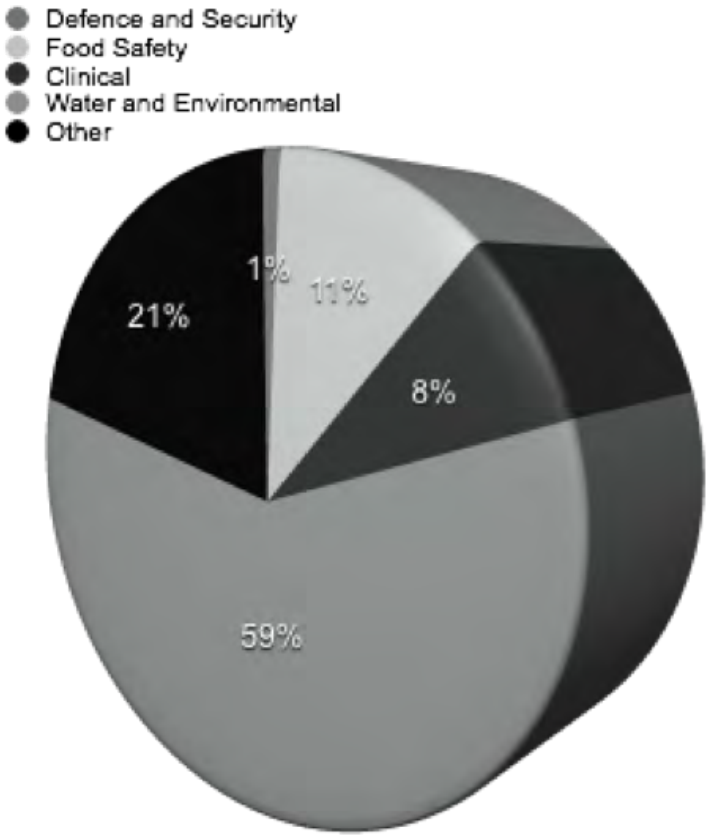

Figure 1. Pathogenic detection search results from 2010 using ScienceDirect distribution by industry and application.

Current methodologies for bacterial detection vary greatly from growth on media to molecular techniques. Gene sequencing through amplification using polymerase chain reactions (PCR) and the use of many chemical properties such as chromogenic, fluorogenic or enzymatic reactions supplement current methodologies.

Media-Based Growth Methods

The earliest forms of bacterial detection were performed using solid gelatin media to establish visible colonies.[9] Litmus lactose agar was alternatively used by sanitary bacteriologists.

In the litmus lactose agar detection method, the acid produced in the digestion of lactose changes the pH and therefore the colour of the agar, and is used as a diagnostic test for enumeration of *E. Coli.* This process is known as the Wurtz method.[9]

Culturing on media is time consuming; sample collection, laboratory-based serial dilutions and an additional 24-hour time frame for growth in no way provides the rapid determination of the presence of faecal coliform contamination required for the notification of the public for use of natural waters.[16]

Multiple Tube Fermenting and Membrane Filtration

Multiple Tube Fermenting (MTF) techniques have been used for over 90 years for the detection of bacteria. On its own, MTF is a slow process, taking 48 hours for a presumptive reaction relying on gas or acid production or growth, plus a possible further 48 hours if subculturing is required.[17]

Membrane filtration (MF) is the process of passing a water sample through an ultra-fine filter (0.45 µm) to trap bacterial cells. The filter is then placed in growth/detection media, agar or broth, and relies on either visible colonies or the detection of fluorogenic or chromogenic active enzyme markers.[18] Studies describe this technique as time consuming, producing results that are difficult to interpret[19] with a long time required for incubation. The detection of both slow growing and viable but non-culturable organisms (VBNC) is also limited.[17]

MTF and MF based on the detection of B-GUD activity has been approved by the United States Environmental Protection Agency (US EPA)[14] and is widely used in the analysis of samples for water quality testing in both North America and Europe.[10]

Proteomics and Genomics

One of the most rapidly advancing areas of coliform detection lies in molecular detection. Molecular detection techniques are based on 1) proteomics - the study of proteins, 2) genomics - the study of genetic material contained in deoxyribonucleic acid (DNA), and 3) transcriptomics - the study of the complete set of ribonucleic acids (RNAs).[20]

Falling into this category is PCR, a method of amplifying or copying a particular region of DNA.[21] This is an established area of microbe detection that has been extensively studied (Table 2), however the best type of genetic material to use, DNA or RNA, is debatable, as is the target gene. A number of studies use different genes for detection, with some of the common sequences used displayed in Table 2.

PCR is a high-cost *in vitro* laboratory technique which requires a skilled technician to operate the specialist equipment. The PCR method lacks the ability to differentiate between viable and non-viable cells.[19, 20] Brescia *et al.*[26] indicate that treating samples with photoactive vital dyes (propidium monoazide (PMA) or ethidium monoazide (EMA)) that penetrate non-viable cells will prevent amplification of non-viable cell DNA, thus circumventing this differentiation issue.[26]

Fluorescence In-Situ Hybridisation

Another recent advancement in molecular detection was from the 1980s called fluorescence in-situ hybridisation (FISH). FISH is a non-PCR molecular method to identify microorganisms by either DNA or RNA using specific probes built from and complementing the target nucleic acid sequences.[27] Lopez-Roldan *et al.*[25]

Table 2. Some common genes found in E. coli targeted in PCR

Gene	Coding for	Reference
uidA	B-glucuronidase	Kong, Mak[22] Horakova, Mlejnkova[23] Min and Baeumner[19]
gadA/B	Glutamate decarboxylase	Min and Baeumner[19]
eaeA	Virulence	Janezic, Ferry[13]
rfbE	Lipopolysaccharide	Jothikumar, Narayanan[3]
sfmD	Putative outer membrane export protein	McLain, Rock[24]
lacZ	B-galactosidase	Rompré, Servais[17] Horakova, Mlejnkova[23]
lacY	Lactose permease	Horakova, Mlejnkova[23]
cyd	Cytochrome bd	Horakova, Mlejnkova[23]
stx^1 & stx^2	Shigatoxin	Janezic, Ferry[13]
eaeA	Initimin	Janezic, Ferry[13]
hlyA	Enterohemolysin	Janezic, Ferry[13]

Table 3. Some of the biochemical properties and identification tests for *Escherichia coli*				
Test	E. coli reaction	ETEC/ EPEC	EIEC	% isolates with same reaction as E. coli
Gram staining	-	-	-	99
EMB	BCMS	n.d.	n.d.	99
Lactose fermentation	+	+	V	90
Motility	+	n.d.	n.d.	n.d.
Indole	+	+	V	75
Methyl red	+	+	+	75
Voges-Proskauer	-	-	-	60
Citrate	-	-	-	80
B-Glucuronidase	+	+	+	n.d.

state that FISH with rRNA (ribosomal ribonucleic acid) targeted probes is the most common, non-PCR molecular technique currently used. Potential drawbacks with the FISH method are the complex sample preparation, multiple sample processing stages and limitations when used in the detection of nutrient starved cells.[25, 17]

Immunological

Immunological detection methods for *E.coli* use specific antibody/antigen recognition with either polyclonal or monoclonal antibodies depending upon the specificity of the target organism.[17, 28]

An advantage of this method is that VBNC organisms are still detected, these are important because these non-culturable bacteria do not grow colonies, but may still be pathogenic.[29]

Immunological antigen-antibody techniques have also been used with enzyme-linked immunosorbent based assays (ELISA), however, results have shown this method to lack sensitivity, and a pre-cultivation of the sample is required to boost the cell count, which takes 24 hours.[17] ELISA is a useful technique, though due to the low sensitivity without a culture stage, use for rapid *in situ* detection of waterborne coliforms is limited.

Biochemical Properties Detection

The most rapid detection of specific bacteria species comes from the biochemical properties of the organism itself. β-D-glucuronidase, along with the β-D-galactosidase (BGAL), enzymes involved in the breakdown of carbohydrates, are frequently used in conjunction with other techniques for *E. coli* detection.

During the development of methods of detection for waterborne microorganisms, many biochemical properties are utilised in detection and identification of bacterial species. Table 3 shows common tests using biochemical properties of *E. coli.*

The tests shown in Table 3 include stains that react with particular bacterial cell walls, pH indicator dyes, enzymatic reactions and measures of motility.

Another biochemical test involves analysing for adenosine triphosphate (ATP), a molecule found in the cells of all living organisms, used in cellular metabolism. To measure microbial content of a water sample, the ATP is released into the sample by lysing the cells in the solution. ATP reacts with the catalyst luciferase, breaking down the ATP molecule to release a photon of light.[25] This process is only an indicator of bacterial load, and gives no information of the particular species present, so could be useful in preliminary water quality testing.

Light production produced by cleaving of a high energy compound by a specific enzyme is known as chemiluminescence.[4] 1,2-dioxetane compounds produce chemiluminescence by reaction with BGAL and BGUD, and are useful for *E.coli* detection. This reaction provides analytical results in less than an hour and results in a detection limit of between 100 and 1000 *E.coli* cells per 100 mL. A sensitivity detection limit in this range does not provide suitable sensitivity for water for human consumption, nor for recreational waters.[4]

Enzyme Detection

Chromogenic enzyme substrates are a culture media containing enzyme substrates associated with a chromogen, a colour changing reaction.25 A common enzyme substrate used with E.coli is ortho-nitrophenyl-βD-galactopyranoside (ONPG). As the E.coli colonies grow, the use of their enzyme B-GAL in the metabolism of ONPG, changes the colour of the substrate colourless to yellow.25 Chlorophenol red-β-galactopyranoside (CPRG) is also a B-GAL chromogenic enzyme substrate producing a yellow to red-magenta indicator result.30, 31

Chromogenic substrates detecting B-GUD include: p-nitrophenyl-β-D-glucuronide (PNPG) which produces a yellow indicator and 5-bromo-4-chloro-3-indoyl-β-D-glucuronide (XGLU) producing a blue indicator.[30]

One issue with the use of chromogenic enzyme substrates as indicated in literature is the effect phenolic compounds have on enzyme based substrates.

The above listed chromogenic enzyme substrates (ONPG, CPRG, PNPG and XGLU) are all phenolic compounds.[30]

Phenols can appear in natural waterways from sources including pesticides, wood preservatives, dyes and from industrial processes such as petroleum refining, pulp processing and leather tanning.[30]

Fluorogenic enzyme substrates are non-fluorescent substrates which when reacted with certain enzymes produce fluorescent products. These in turn can identify organisms containing the specific enzyme present.[10] The most frequently used substrate in the detection of BGUD is 4-methyl-umbelliferone-β-D-glucuronide (MUG).[17, 19, 32, 33]

Unfortunately, for non-fluorescent detection methods *E.coli* is not the only microorganism to produce BGUD. Others include: some *Shigella* and *Salmonella* strains, *Yersinia*, *Flavobacterium* species, *Bacteriodes* species, *Staphylococcus* species, *Streptococcus* species and *Clostridium* species.[32, 34-36]

Several fresh and marine water algal species also express BGUD activity, which may provide false-positive results particularly if algal blooms are present.[32] To reduce the number of false-positive indications from non-target bacteria, the media will contain inhibitors. These inhibitors will hinder the growth of gram-positive bacteria and may include the BGAL enzyme substrate to further eliminate specific BGUD positive species such as *Shigella* and *Salmonella*.[37]

CONCLUSION

Extensive research into microbial detection as a water quality indicator is evident. It is also clear that the time required for traditional culture methods, at 24 to 48 hours, is not acceptable for rapid assessment of faecal contamination in recreational waters for human health and safety.

Using genomic and biochemical characteristics of *E. coli* to provide a rapid detection method is an important research topic requiring further investigation. Additionally, it is important to have a detection system which is both portable and affordable. In comparison to North America and Europe, Australia generally has very good water quality with routine testing not performed as extensively.

However, with the expected expansion of population growth and increases in volume and relative ease of tourism, natural water bodies are becoming more popular with bathers.

Routine testing for faecal contamination will become necessary throughout Australia. Current genomic approaches involve numerous detection methods, all requiring extended amplification times and costly laboratory equipment, as well as skilled personnel to operate them. Exploring the opportunities enzyme assays present, in order to provide cheap rapid results, would be opportune.

THE AUTHORS

Dr Amie Anastasi is a lecturer of permaculture at CQUniversity. She obtained her PhD in 2015 and has since worked as a research fellow and now lecturer in the School of Medical and Applied Sciences.

Dr Aoife Power is a lecturer of chemistry at CQUniversity and works in the school of medical and applied sciences. She has been at the university for 2 years since moving from Ireland. Aoife's research interests are in analytical and materials chemistry.

Dr Shaneel Chandra is a lecturer of chemistry at CQUniversity. He joined the university in November 2015 from University South Pacific. His research interests are in electrochemistry and sensors.

Leanne Voss is a lecturer of chemistry at CQUniversity. Leanne has worked in the school of medical and applied sciences for over 10 years and has a passion for first year student learning in chemistry in the school of medical and applied sciences.

Piumie Rajapaksha is a research assistant and group member of Dr James Chapman's innovative biomaterials group at CQUniversity.

Salina Cosford is a research assistant and group member of Dr James Chapman's innovative biomaterials group at CQUniversity. She is currently reading a Bachelor of Science degree at the university and conducts research in the school of medical and applied sciences.

James Chapman is currently the group leader of the innovative biomaterials group at CQUniversity (www.brainybiomaterials.com). He is also the discipline leader for Chemistry and Senior Lecturer in the School of Health, Medical and Applied Sciences. His research portfolio centres on nanotechnology in water research, antifouling materials and design of sensors for applied monitoring parameters. He is also a proponent of STEM excellence through engaged research activities with teachers, educators and students in Australia and internationally.

REFERENCES

1. NHMRC, Guidelines for Managing Risks in Recreational Water, N.H.a.M.R. Council, Editor, Australian Government: Canberra ACT, 2008

2. H Stender, K Oliveira, S Ribgy, F Bargoot, and J Coull, 'Rapid detection, identification, and enumeration of Escherichia coli by fluorescence in situ hybridization using an array scanner', J Microbiol Methods, Vol 45, 2001, p. 31-9.

3. P Jothikumar, J Narayanan, and V.R. Hill, 'Visual endpoint detection of Escherichia coli O157:H7 using isothermal Genome Exponential Amplification Reacti,on (GEAR) assay and malachite green'. Journal of Microbiological Methods, 2014. Vol 98, p. 122-127.

4. AS Bukh, and P Roslev, 'Characterization and validation of a chemiluminescent assay based on 1,2-dioxetanes for rapid detection of viable Escherichia coli', Appl Microbiol Biotechnol, 86, 2010, p. 1947-57.

5. AH Farnleitner, N Kreuzinger, GG Kavka, S Grillenberger, J Rath and RL Mach, , 'Simultaneous detection and differentiation of Escherichia coli populations from environmental freshwaters by means of sequence variations in a fragment of the beta-D-glucuronidase gene', Appl Environ Microbiol, Vol 66, 2000, p. 1340-6.

6. D Till, D, G McBride, A Ball, K Taylor and E Pyle, 'Large-scale freshwater microbiological study: rationale, results and risks', J Water Health, Vol 6, 2008, p. 443-60.

7. SM McQuaig, TM Scott, JO Lukasik, JH Paul and V Harwood, 'Quantification of human polyomaviruses JC virus and BK Virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples', Applied and Environmental Microbiology, Vol 75, 2009, p. 3379-3388.

8. EM Symonds, DW Griffin, and M Breitbart,'Eukaryotic viruses in wastewater samples from the United States', Applied and Environmental Microbiology, Vol 75, 2009, p. 1402-1409.

9. ST Odonkor and JK Ampofo, 'Escherichia coli as an indicator of bacteriological quality of water: an overview', Microbiology Research 2013, Vol 4, 2013, p. 5 - 11.

10. J Prats, T Garcia-Armisen, J Larrea and P Servais, 'Comparison of culture-based methods to enumerate Escherichia coli in tropical and temperate freshwaters', Lett Appl Microbiol, Vol 46, 2008, p. 243-8.

11. J Baudart, P Servais, H De Paoli, A Henry and P Lebaron, 'Rapid enumeration of Escherichia coli in marine bathing waters: potential interference of nontarget bacteria', Journal of Applied Microbiology, Vol 107, 2009, p. 2054-2062.

12. NHMRC, Recommendations to change the use of coliforms as microbial indicators of drinking water quality, N.H.a.M.R. Council, Editor, Australian Government: Canberra, 2003

13. KJ Janezic, B Ferry, EW Hendricks, BA Janiga, T Johnson, S Murphy, ME Roberts, SM Scott, A KF Hung and SL Daniels, 'Phenotypic and Genotypic Characterization of Escherichia coli Isolated from Untreated Surface Waters', Open Microbiol J,Vol 7, 2013, p. 9-19.

14. T Garcia-Armisen, J Prats, and P Servais, 'Comparison of culturable fecal coliforms and Escherichia coli enumeration in freshwaters', Can J Microbiol, Vol 53, 2003, p. 798-801.

15. ST Shulman, HC Friedmann, and RH Sims, 'Theodor Escherich: the first pediatric infectious diseases physician?' Clin Infect Dis, Vol 45, 2007, p. 1025-9.

16. TJ Wade, E Sams, KP Brenner, R Haugland, E Chern, M Beach, L Wymer, CC Rankin, D Love, Q Li, R Noble, and AP Dufour, 'Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: a prospective cohort study', Environ Health, Vol 9, 2010, p. 66.

17. A Rompré, P Servais, J Baudart, M-R De-Roubin, and P Laurent, 'Detection and enumeration of coliforms in drinking water: current methods and emerging approaches', Journal of Microbiological Methods, Vol 49, 2002 p. 31-54.

18. H Nelis and S Van Poucke, 'Enzymatic detection of coliforms and Escherichia coli within 4 hours'. Water, Air, and Soil Pollution, Vol 123, 2000, p. 43-52.

19. J Min and AJ Baeumner, 'Highly Sensitive and Specific Detection of Viable Escherichia coli in Drinking Water'. Analytical Biochemistry, Vol 303, 2002 p. 186.

20. K Sen, and N Ashbolt, Environmental Microbiology: Current Technology and Water Applications. 2011, Norfolk, UK: Caister Academic Press.

21. Jackson, R.W. and J.M. Jackson, Forensic Science. 2 ed. 2008, Harlow, Essex: Pearson Education Limited.

22. RYC Kong, MMH Mak, and RSS Wu, 'DNA technologies for monitoring waterborne pathogens: A revolution in water pollution monitoring', Ocean and Coastal Management, Vol 52, 2009 p. 355-358.

23. K Horakova, H Mlejnkova, and P Mlejnek, 'Specific detection of Escherichia coli isolated from water samples using polymerase chain reaction targeting four genes: cytochrome bd complex, lactose permease, beta-D-glucuronidase, and beta-D-galactosidase'. J Appl Microbiol, Vol 105, 2008 p. 970-6.

24. JE McLain, CM Rock, K Lohse and J Walworth, 'False-positive identification of Escherichia coli in treated municipal wastewater and wastewater-irrigated soils', Can J Microbiol, Vol 57, 2011, p. 775-84.

25. R Lopez-Roldan, P Tusell, S Courtois & JL Cortina, 'On-line bacteriological detection in water', TrAC Trends in Analytical Chemistry, Vol 44, 2013, p. 46-57.

26. CC Brescia, SM Griffin, MW Ware, EA Varughese, AI Egorov & EN Villegas, 'Cryptosporidium propidium monoazide-PCR, a molecular biology-based technique for genotyping of viable Cryptosporidium oocysts', Appl Environ Microbiol, Vol 75, 2009, p. 6856-63.

27. B Bottari, D Ercolini, M Gatti and E Neviani, 'Application of FISH technology for microbiological analysis: Current state and prospects', Applied Microbiology and Biotechnology, Vol 73, 2006, p. 485-494.

28. JR Geary, GM Nijak, JR, SL Larson, and JW Talley, J. W, 'Hydrolysis of the soluble fluorescent molecule carboxyumbelliferyl-beta-D-glucuronide by E. coli beta-glucuronidase as applied in a rugged, in situ optical sensor'. Enzyme Microb Technol, Vol 249, 2011, p. 6-10.

29. G Caruso, F De Pasquale, M Mancuso, D Zampino and E Crisafi, 'Fluorescent antibody-viability staining and beta-glucuronidase assay as rapid methods for monitoring Escherichia coli viability in coastal marine waters', J Immunoassay Immunochem, Vol 27, 2006, p. 1-13.

30. S Abboo and BI Pletschke, 'Effect of phenolic compounds on the rapid direct enzymatic detection of βD-galactosidase and βD-glucuronidase',. Water South Africa, Vol 36, 2010, p. 133 - 138.

31. SM Hossain, C Ozimok, C, Sicard, SD Aguirre, MM Ali, Y Li, & JD Brennan, 'Multiplexed paper test strip for quantitative bacterial detection', Anal Bioanal Chem,Vol 403, 2012, p. 1567-1576.

32. I Tryland and L Fiksdal, 'Enzyme characteristics of beta-D-galactosidase- and beta-D-glucuronidase-positive bacteria and their interference in rapid methods for detection of waterborne coliforms and Escherichia coli', Appl Environ Microbiol., Vol 64, 1998, p. 1018-23.

33. D Wildeboer, L Amirat, RG Price and RA Abuknesha, 'Rapid detection of Escherichia coli in water using a hand-held fluorescence detector', Water Research, Vol 44, 2010, p. 2621-2628.

34. Cheung, W.H., et al., 'Methods for enumerating Escherichia coli in subtropical waters', Epidemiol Infect, Vol 106, 1991 p. 345-54.

35. L Fiksdal and I Tryland, 'Application of rapid enzyme assay techniques for monitoring of microbial water quality', Curr Opin Biotechnol., Vol 19, 2008, p. 289-94.

36. CR Fricker, PS Warden, and BJ Eldred, 'Understanding the cause of false negative beta-D-glucuronidase reactions in culture media containing fermentable carbohydrate'. Lett Appl Microbiol, Vol 50, 2010, p. 547-51.

37. CR Fricker, M Desarno, PS Warden, & BJ Eldred, 'False-negative beta-D-glucuronidase reactions in membrane lactose glucuronide agar medium used for the simultaneous detection of coliforms and Escherichia coli from water'. Lett Appl Microbiol, Vol 47, 2008 p. 539-42.