



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

010801

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

TECTA Combined *E. coli* and Total Coliform Test

manufactured by

Pathogen Detection Systems, Inc. (TECTA-PDS)

382 King Street East

Kingston, ON Canada K7K 2Y2

This method has been evaluated in the AOAC[®] *Performance Tested Methods*SM Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (December 01, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 01, 2019

Date

METHOD AUTHORS

Stephen Brown, C. E. Dunkinson, E. J. P. Marcotte, A. Luke, S. Ronan, and W. P. Aston

SUBMITTING COMPANY

Pathogen Detection Systems, Inc.
Suite 1619, Biosciences Complex
116 Barrie Street
Kingston, ON, Canada K7L 3N6

CURRENT COMPANY ADDRESS

Pathogen Detection Systems, Inc.
(TECTA-PDS)
382 King Street East
Kingston, Ontario
Canada K7K 2Y2

KIT NAME(S)

TECTA Combined *E. coli* and Total Coliform Test

CATALOG NUMBERS

TECTA-CCA

INDEPENDENT LABORATORY

Laboratory Services Division
University of Guelph
95 Stone Road, West
Guelph, ON Canada N1H 8J7

AOAC EXPERTS AND PEER REVIEWERS

Thomas Hammack¹, Edward Richter², Denise Kaji³
¹ US Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA
² Richter International, Columbus, OH, USA
³ Pacific Coast Analytical Services, Sylmar, CA, USA

APPLICABILITY OF METHOD

Target organism – Total Coliform and *Escherichia coli*

Matrices – Tap water, well water, lake water, vegetable wash water, bottled water, sugar-free lemon iced tea

Performance claims - The PDS method was shown to be equivalent to the reference methods.

REFERENCE METHODS

Anonymous (2002) *Test Method 1604: Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)*. United States Environmental Protection Agency, USEPA, Office of Water, Washington DC EPA 821-R-02-024. (29)
U.S. Food and Drug Administration, Center for Food safety and Applied Nutrition (2002) *Bacteriological Analytical Manual Online, Chapter 4, Section III: Examination of Bottled Water*. <http://www.cfsan.fda.gov/~ebam/bam-4.html>. (30)

ORIGINAL CERTIFICATION DATE

January 07, 2008

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2020

METHOD MODIFICATION RECORD

1. February 2018 Level 1
2. December 2018 Level 1
3. December 2019 Level 1

SUMMARY OF MODIFICATION

1. Name change to Pathogens Detection Systems, Inc. (TECTA-PDS)
2. Editorial changes to update inserts
3. Editorial changes to update inserts

Under this AOAC® *Performance Tested*SM License Number, 010801 this method is distributed by:

NONE

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NONE

PRINCIPLE OF THE METHOD (1)**Overview of *E. coli* and Total Coliform detection**

There are a wide variety of methods available for detection of EC and TC organisms as indicators of water quality [4-10]. In recent years there has been a shift away from traditional cultural techniques using biochemical indicator reactions to detecting EC and TC based on detection of enzyme activity in a culture designed to promote growth of the target organisms. The enzymatic reactions are very specific, rapid and sensitive. It has been shown that about 97% of EC strains produce β -glucuronidase (GLU) while almost all other Enterobacteriaceae lack this enzyme. Therefore detection of GLU activity can be used to detect the presence of EC [9]. β -galactosidase (GAL) is the accepted indicator for the detection of TC, because non-coliforms usually do not produce this enzyme [10]. This approach is not necessarily absolute, as there are potential sources of error in the suitability of broth and incubation conditions for all target coliform types. As well there are non-target organisms which may also possess the specific enzymes. Nonetheless, the reliability of these established methods is high enough that there is broad regulatory acceptance of this approach for assessment of drinking water [11,12].

Enzyme activity detection

The enzymes are detected when they convert a chromogenic or fluorogenic substrate compound to an easily detected product [13,14]. Typically, a glucuronide or galactoside conjugate of a dye compound is added to the sample broth as a substrate, and if the target enzymes are present, then the conjugate is converted to a free dye molecule. This is detected by a change in colour or fluorescence of the free dye molecule compared to the conjugate. Similar substrate schemes are used both for tests performed in solution and on membrane filters or gel plates [15]. Some formats use multiple dye substrates which produce a variety of colours depending on which enzymes are present.

Many different substrates have been reported and used in commercial detection applications. For detection in solution cultures, fluorogenic dyes tend to be preferred. Conjugates of umbelliferone and umbelliferone derivatives (e.g. 4-methylumbelliferone, trifluoromethylumbelliferone) [16] are favoured both because of good contrast in fluorescence between the product and the substrate, as well as rapid kinetics for reaction of conjugates with either the GLU or GAL enzymes. Chromogenic substrates such as nitrophenyl conjugates are commonly used in solution cultures as the product is a soluble compound. Substrates which produce insoluble products, including indolyl [17] and indoxyl conjugates [18], tend to be favoured for use in membrane or gel supported cell detection. Methods using all of these substrates have been accepted as "equivalent" for EC and TC detection, indicating that a variety of molecules can be conjugated onto glucuronic acid or galactoside without affecting the reliability of the resulting substrate for detection of the target enzyme [10].

All commonly used commercial tests use visual detection of the coloured or fluorescent product by a human observer. A small number of tests with instrumental detection of the product have been reported [19-21], and have been demonstrated for various applications [22-25]. None of these has been widely adopted for routine monitoring, however. It has been reported that these tests are not sufficiently reliable for some samples owing to optical interference from the sample matrix [26].

PRINCIPLE OF METHOD CONTINUED (1)**Outline of Pathogen Detection Systems technology**

The Pathogen Detection Systems (PDS) test utilizes standard solution culture broth medium (LB Lennox) composed of ingredients selected from methods already in use in laboratories accredited for testing drinking water. Therefore, the PDS test is really an adaptation of current tests, and it is expected to perform the same in terms of detecting particular organisms, excluding “non-target” organisms, and recovering stressed organisms.

The most significant difference between the PDS medium and the conventional tests is the specific substrates used as EC and TC indicators. Current tests use a variety of molecules as specific substrates. The common feature for all EC indicator substrates is a glucuronic acid molecule which is conjugated onto an aromatic molecule. The product of the enzyme reaction is a free aromatic molecule with colour or fluorescence properties which must be different from the substrate in order for the product to be visually differentiated. The EC indicator substrate in the PDS test is also a glucuronic acid molecule conjugated onto an aromatic molecule, however both the product and substrate are fluorescent. The difference is that the free aromatic molecule from the PDS substrate is extracted into a small localized clear polymer element within the test cartridge while the substrate is not. A schematic of this process is given in the PDS literature [25]. Similarly, current TC tests and the PDS TC test use aromatic molecules conjugated onto a galactose molecule. Both indicators are fluorescent, however the EC and TC indicators emit two different wavelengths. Using a single excitation light source for both the EC and TC products, a CCD spectrometer is used to simultaneously resolve the fluorescence of both products within the polymer. Isolating optical detection to the polymer makes the test resistant to optical interference from the sample matrix.

DISCUSSION OF THE VALIDATION STUDY (1)

The PDS method was generally successful at recovering and detecting both EC and TC organisms in a variety of samples. The inclusivity and exclusivity tests demonstrated detection of the expected organisms for a large group of reference strains and natural isolates. A few non-coliform organisms were detected as apparent coliforms, indicating some discrepancy between microbiological classification and enzyme profile. This was consistent with literature reports discussing the reliability of enzyme activity indicators, and it's also notable that the US-EPA reference method gave the same result.

Lot-to-lot testing showed no significant changes in performance between lots of PDS test cartridges. This includes comparisons between cartridges which were new, 6 months old and more than 12 months old. Similarly, ruggedness testing demonstrated that the PDS method is effective even with variations in volume and temperature above and below normal operating values.

Method comparison testing demonstrated equivalent performance of the PDS method compared with either the US-EPA or FDA-BAM reference methods using fractionally positive samples. Chi-squared calculations showed no statistically significant difference between the PDS method and the reference methods for EC and TC presence/absence results for all matrices. Two presumptive positive TC results were not confirmed for the Lake Water samples, where there were natural NECC organisms present. This affected both the PDS method and the reference method, and most likely reflects a small discrepancy between the organism classes and the enzyme activity profiles, as expected for all directed substrate-type tests.

The protocol developed for this testing was effective. Inoculating a large sample volume and then testing over two days produced similar fractional positive rates on both days. The distilled water matrix showed some evidence of “die-off” between the two days, and possible evidence of stressed organisms leading to slower growth. The number of samples possibly affected was not large enough to affect the statistical comparison of the methods, however it might be recommended for future studies of this matrix to test all samples on one day. Inoculating with a mixture of EC and NECC organisms was effective for matrices where the US-EPA method was the reference method. It was less effective for the FDA-BAM reference method studies, however, since it was not always possible to distinguish the typical EC and typical NECC colonies on the mEndo plates. This made confirming the PDS method samples difficult since a large number of colonies was recovered and all could not be easily confirmed unless it was possible to visually distinguish the EC and NECC colonies by appearance. In future studies, it would be recommended to inoculate EC and NECC organisms separately when using the FDA-BAM membrane filter method, or to use a different reference method such as the MI medium.

Table 1: Results of various Bacteria strains used in the Inclusivity study (1)

EC Organisms				
ATCC Strains	Source/Location	Identification	PDS EC Result	PDS TC Result
<i>E. coli</i> 11229		N/A	Positive	Positive
<i>E. coli</i> 25922	Clinical	N/A	Positive	Positive
<i>E. coli</i> 10536		N/A	Positive	Positive
<i>E. coli</i> 35218	Canine	N/A	Positive	Positive
<i>E. coli</i> 11775	Urine	N/A	Positive	Positive
<i>E. coli</i> 13706	in ATCC water list	N/A	Positive	Positive
<i>E. coli</i> 23848		N/A	Positive	Positive
<i>E. coli</i> 35421		N/A	Positive	Positive
<i>E. coli</i> 51813	Food	N/A	Positive	Positive
<i>E. coli</i> 9637		N/A	Positive	Positive
<i>E. coli</i> 33605		N/A	Positive	Positive
<i>E. coli</i> 51446	Clinical	N/A	Positive	Positive
<i>E. coli</i> B-type ¹		N/A	Positive	Positive
Isolates				
<i>E. coli</i> KS1	Kingston Sewage sample	<i>E. coli</i>	Positive	Positive
<i>E. coli</i> KS2	Kingston Sewage sample	<i>E. coli</i> or <i>E. coli</i> -inactive	Positive	Positive
PWW1	Paterson Well Water	<i>E. coli</i>	Positive	Positive

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LOWS1	Lake Ontario - St. Lawrence Ave.	<i>E. coli</i>	Positive	Positive
LOWS-B6	Lake Ontario - St. Lawrence Ave.	<i>E. coli, prev - Levinea amalonaticus</i>	Positive	Positive
CCL1	Commodore's Cove Lake Water	<i>E. coli</i>	Positive	Positive
EAP1	Elliot Ave - rain runoff	<i>E. coli</i>	Positive	Positive
CMP-B4B	Cataraqui Mall – small pond	<i>E. coli</i>	Positive	Positive
LOWS6	Lake Ontario - St. Lawrence Ave.	<i>E. coli</i>	Positive	Positive
LOWM22	Lake Ontario - Murney Tower	<i>E. coli</i>	Positive	Positive
CMP-B1A	Cataraqui Mall – small pond	<i>E. coli</i>	Positive	Positive
CMP-B11A	Cataraqui Mall – small pond	<i>E. coli</i>	Positive	Positive
NECC Organisms				
ATCC Strains	Source/Location	Identification	PDS EC Result	PDS TC Result
<i>K. pneumoniae</i> 13883	periodontal pocket (dental)	N/A	Negative	Positive
<i>K. pneumoniae</i> 13882	Water	N/A	Negative	Positive
<i>C. freundii</i> 8090		N/A	Negative	Positive
<i>E. aerogenes</i> 35029		N/A	Negative	Positive
<i>E. cloacae</i> 13047	spinal fluid	N/A	Negative	Positive
<i>K. pneumoniae</i> 31488	Soil	N/A	Negative	Positive
<i>K. oxytoca</i> 43086		N/A	Negative	Positive
<i>C. braakii</i> 43162	Clinical	N/A	Negative	Positive
<i>K. pneumoniae</i> 13882	Water	N/A	Negative	Positive
<i>K. pneumoniae</i> C6 ¹		N/A	Negative	Positive
Isolates				
CMP1	Cataraqui Mall – small pond	<i>K. oxytoca</i>	Negative	Positive
CMP6	Cataraqui Mall – small pond	<i>E. agglomerans</i>	Negative	Positive
BU1-3	Basta Well Water	<i>E. agglomerans</i>	Negative	Positive
BU1-4	Basta Well Water	<i>E. agglomerans</i>	Negative	Positive
BU1-5	Basta Well Water	<i>E. agglomerans</i>	Negative	Positive
BU1-6	Basta Well Water	<i>E. agglomerans</i>	Negative	Positive
LOWS-B12	Lake Ontario - St. Lawrence Ave.	<i>K. oxytoca</i>	Negative	Positive
BSP-B3A	Groundwater - non-potable building sump	<i>E. agglomerans</i>	Negative	Positive
BSP-B11	Groundwater - non-potable building sump	<i>Citrobacter sp 10</i>	Negative	Positive
BSP-B13	Groundwater - non-potable building sump	<i>Serratia fonticola</i>	Negative	Positive
CMP-B13	Cataraqui Mall – small pond	<i>Serratia rubidaea</i>	Negative	Positive
CMP-B14	Cataraqui Mall – small pond	<i>K. pneumoniae</i>	Negative	Positive
CCL17	Commodore's Cove Lake Water	<i>K. pneumoniae</i>	Negative	Positive
CCM3	Collin's Creek Marsh	<i>Serratia rubidaea</i>	Negative	Positive
DPF1-2B	Days Road Farm rain runoff	<i>E. agglomerans</i>	Negative	Positive

1. These strains are not from ATCC but are reference strains used previously in our laboratory.

Table 2: Results of various Bacteria strains used in the Exclusivity study (1)

Non-Coliforms				
ATCC Strains	Source/Location	Identification	PDS EC Result	PDS TC Result
<i>P. rettgeri</i> 9250	human dysentery	N/A	Negative	Negative
<i>P. alcalifaciens</i> 51902		N/A	Negative	Negative
<i>P. stuartii</i> 33672		N/A	Negative	Negative
<i>P. aeruginosa</i> 27853	blood culture	N/A	Negative	Negative
<i>A. hydrophila</i> 7966	tin of milk with a fishy odor	N/A	Negative	Negative
<i>A. caviae</i> 15468	epizootic of young guinea pigs	N/A	Negative	Positive
<i>P. fluorescens</i> 13525	pre-filter tanks	N/A	Negative	Negative
<i>B. diminuta</i> 19146	found as a contaminant in a culture of <i>B. cereus</i>	N/A	Negative	Negative
<i>P. hauseri</i> 13315		N/A	Negative	Negative
<i>Enterococcus hirae</i> (Gram+) 8043		N/A	Negative	Positive
<i>P. aeruginosa</i> 10145		N/A	Negative	Negative
<i>S. aureus</i> 13565	ham involved in food poisoning	N/A	Negative	Negative
<i>B. cereus</i> 4342	Milk	N/A	Negative	Negative
Isolates				
LOP2	Lake Ontario Park	<i>Moraxella prev. CDC group lif</i>	Negative	Negative
BU1-9	Basta Well Water	<i>W. virosa formerly CDC group lif</i>	Negative	Negative
MATW2 (fluorescent)	Marcotte Stored Tap Water	<i>P. fluorescens-35</i>	Negative	Negative
MATW5	Marcotte Stored Tap Water	<i>Moraxella prev. CDC group lif</i>	Negative	Negative
HWW4	Hampel Well Water	<i>Moraxella spp.</i>	Negative	Negative
HWW1	Hampel Well Water	<i>Moraxella spp.</i>	Negative	Negative
CWW1	Casselman Well Water	<i>P. aeruginosa</i>	Negative	Negative
PLTW1	PDS Lab Tap Water	<i>Pasturella haemolytica</i>	Negative	Negative
MGW3	Mike - Ground Water	<i>Flavobacterium meningo-septicum</i>	Negative	Negative
GWW7	Gallant Well Water	<i>Burkholderia cepacia</i>	Negative	Negative
LOWM9	Lake Ontario - Murney Tower	<i>W. virosa formerly CDC group lif</i>	Negative	Negative
PWW3	Paterson Well Water	<i>P. fluorescens</i>	Negative	Negative
LOWSS	Lake Ontario - St. Lawrence Ave.	<i>Stenotrophomonas maltophilia</i>	Negative	Negative
DFP1-1B	Days Road Farm rain run-off	<i>Achromobacter. xylosoxidans ss.xylos</i>	Negative	Negative
IL-1	Queen's Research Lab	<i>Pseudomonas putida</i>	Negative	Negative
PRB3	Paterson - Rain Barrel	<i>Moraxella sp.</i>	Negative	Negative
CCL16	Comodore's Cove Lake Water	<i>Pseudomonas stutzeri</i>	Negative	Negative

Table 7: Summary of methods comparison results for all test matrices. (1)

Matrix	cfu EC/ 100 mL	cfu NECC/ 100 mL	Test portions	PDS EC Pos ²	PDS EC ³ Confirmed	False pos/ False neg rates (%)	Ref EC Confirmed	Chi- Square ⁴	PDS TC Pos ²	PDS TC ³ Confirmed	False pos/ False neg rates (%)	Ref TC Confirmed	Chi- Square ⁴
(1) Tap Water	1.1	1.2	20	15	15	0% / 0%	14	0.12	17	17	0% / 0%	17	0.00
	uninoc	uninoc	5	0			0		0			0	
(2) Well Water	1.5	1.9	20	18	18	0% / 0%	16	0.76	18	18	0% / 0%	19	0.35
	uninoc	uninoc	5	0			0		0			0	
(3) Distilled Water	1.2	1.7	20	12	15	0% / 20%	15	0.00	16	16	0% / 0%	18	0.76
	uninoc	uninoc	5	0			0		0			0	
(4) Lake Water - Lot 1	5.3	47.6	20	20	20	0% / 0%	20	---	20	20	0% / 0%	20	---
	uninoc	uninoc	N/A										
(4) Lake Water - Lot 2	1.0	2.0	20	9	9	0% / 0%	13	1.58	15	13	13% / 0%	16	0.31
	uninoc	uninoc	N/A										
(5) Vegetable Wash Water	0.8	1.5	20	10	10	0% / 0%	11	0.10	18	18	0% / 0%	17	0.22
	uninoc	uninoc	5	0			0		0			0	
(6) Bottled Water	0.5	1.4	20	8	8	0% / 0%	10	0.39	16	16	0% / 0%	14	0.52
	uninoc	uninoc	5	0			0		0			0	
(7) Iced Tea	0.9	1.8	20	15	15	0% / 0%	12	1.00	16	16	0% / 0%	18	0.76
	uninoc	uninoc	5	0			0		0			0	
(6) Bottled Water ¹	0.9	1.2	20	16	16	0% / 0%	15	0.14	19	19	0% / 0%	18	0.35
	uninoc	uninoc	5	0			0		0			0	
(8) Remineralized Bottled Water ¹	0.8	0.8	20	12	12	0% / 0%	11	0.10	19	19	0% / 0%	20	1.00
	uninoc	uninoc	5	0			0		0			0	

1. These results are from tests performed in the Independent Laboratory
2. This is the total number of samples which were presumptive positive by the PDS test
3. This is the total number of sample broths, in the PDS test cartridges, which were culturally confirmed as either EC or TC positive
4. Chi-Square parameter is calculated using the PDS Confirmed and Reference test Confirmed numbers

REFERENCES CITED

1. Brown, S. Dunkinson, C. E., Marcotte, E. J. P., Luke, A., Ronan, S., and Aston, W. P., Validation of New Technology for Automated Detection of Coliform Bacteria and Specifically *Escherichia coli* in Water, AOAC® Performance TestedSM certification number 010801.
2. AOAC Research Institute Validation Outline for New Technology for Automated Detection of Coliform Bacteria and Specifically *Escherichia coli*, Approved – January 2008.
3. Mantel, N., & Haenszel, W. (1959), *Journal of the National Cancer Institute*, **22**, 719–748.
4. Health Canada (1981) *Compendium of Analytical Methods: Vols. 1-3*. Polyscience Publications Inc., Montreal, QC.
5. Rompré, A., Servais, P., Baudart, J., de-Roubin, M.-R., & Laurent, P. (2002) *J. Microbiol. Meth.* **49**, 31–54.
6. Deisingh, A.K., & Thompson, M. (2002) *Analyst*, **127**, 567–581.
7. Leclerc, H., Mossel, D.A., Edberg, S.C., & Struijk, C.B. (2001) *Ann. Rev. Microbiol.* **55**, 201–234.
8. Tortorello, M.L. (2003) *J AOAC Int.* **86**, 1208-1217.
9. Frampton, E.W., & Restaino, L. (1993) *J. Appl. Bacteriol.* **74**, 223-233.
10. Greenberg, A.E., Clesceri, L.S., & Eaton, A.D., Eds. (1995) *Standard Methods for the Examination of Water and Wastewater*; 19th ed., American Public Health Association, Washington, D.C.
11. Olstadt, J., Schauer, J.J., Standridge, J., & Kluender, S. (2007) *J. Water Health* **5**, 267-282.
12. *Guidelines for Drinking-water Quality*, 3rd ed. (2004) World Health Organization, Geneva, Switzerland.
13. Manafi, M., Kneifel, W., & Bascomb, S. (1991) *Microbiol. Rev.* **55**, 335-348.
14. Geissler, K., Manafi, M., Amoros, I., & Alonso, J.L. (2000) *J. Appl. Microbiol.* **88**, 280-285.
15. Yassien, N., Ibrahim, A., & Elmossalami, M.K. (1998) *Fleischwirtschaft* **78**, 735-736.
16. Nelis, H., & Van-Poucke, S. (2000) *Wat. Air Soil Poll.* **123**, 43-52.
17. Flowers, D.G., & Sternfeld, M. (1994) *Chromogenic compounds and methods of using same*. United States Patent 5,364,767.
18. Ley, A.N., Rickey, N.E., & Taylor, M.J. (1990) *Escherichia coli (E. coli) test method*. United States Patent 4,923,804.
19. Van Poucke, S.O., & Nelis, H.J. (1995) *Appl. Environ. Microbiol.*, **61**, 4505–4509.
20. Apte, S.C., Davies, C.M., & Peterson, S.M. (1995) *Water Res.*, **29**, 1803–1806.
21. Berg, J.D., & Fiksdal, L. (1988) *Appl. Environ. Microbiol.*, **54**, 2118–2122.
22. Anglés D'Auriac, M.B., Roberts, H., Shaw, T., Sirevåg, R., Hermansen, L.F., & Berg, J.D. (2000) *Appl. Environ. Microbiol.*, **66**, 4401–4407.
23. Tryland, I., Samset, I.D., Hermansen, L., Berg, J.D., & Rydberg, H. (2001) *Wat. Sci. Tech.*, **43**, 217–220.
24. Eckner, K.F., Jullien, S., Samset, I.D., & Berg, J.D. (1999) In *Rapid microbiological monitoring methods, Proceedings, IWSA/AISE specialised conference 23–24 February*, Warrington, United Kingdom.
25. Tryland, I., Surman, S., & Berg, J.D. (2002) *Wat. Sci. Tech.*, **46**, 25–31.
26. Davies, C.M., & Apte, S.C. (2000) *Let. Appl. Microbiol.*, **30**, 99–104.
27. Pathogen Detection Systems (2007) Document #L-PDS-003-02 “Theory of Operation”, Pathogen Detection Systems, Inc., Kingston, ON. <http://www.pathogendetec.com>
28. The Walkerton Inquire Reports, Part 1 and Part 2, are available on the Government of Ontario Website at: <http://www.attorneygeneral.jus.gov.on.ca/english/about/pubs/walkerton/part1/>; <http://www.attorneygeneral.jus.gov.on.ca/english/about/pubs/walkerton/part2/> or by writing to: Publications Ontario, 880 Bay Street, Toronto, Ontario, M7A 1N8.
29. Anonymous (2002) *Test Method 1604: Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)*. United States Environmental Protection Agency, USEPA, Office of Water, Washington DC EPA 821-R-02-024.
30. U.S. Food and Drug Administration, Center for Food safety and Applied Nutrition (2002) *Bacteriological Analytical Manual Online, Chapter 4, Section III: Examination of Bottled Water*. <http://www.cfsan.fda.gov/~ebam/bam-4.html>.
31. Pathogen Detection Systems (2007) Document # L-DTU-003-02 DTU “Operating Instructions”, Pathogen Detection Systems, Inc., Kingston, ON. <http://www.pathogendetec.com>