NRC Publications Archive Archives des publications du CNRC

Evaluation of media for simultaneous enumeration of total coliform and E.coli in drinking water supplies by membrane filtration techniques

Wang, D. L.; Fiessel, W.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:

[https://doi.org/10.1016/S1001-0742\(08\)60043-1](https://doi.org/10.1016/S1001-0742(08)60043-1)

Journal of Environmental Sciences, 20, 3, pp. 273-277, 2008-03-16

NRC Publications Record / Notice d'Archives des publications de CNRC:

<https://nrc-publications.canada.ca/eng/view/object/?id=a343987a-0cba-43b5-a9d2-610a0a36c86c>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=a343987a-0cba-43b5-a9d2-610a0a36c86c>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

<http://irc.nrc-cnrc.gc.ca>

Evaluation of media for simultaneous enumeration of total coliform and E.coli in drinking water supplies by membrane filtration techniques

NRCC-50037

Wang, D.L., Fiessel, W.

2008-03-16

A version of this document is published in / Une version de ce document se trouve dans:
Journal of Environmental Sciences, 20, (3), pp. 273-277, March 16, 2008

The material in this document is covered by the provisions of the Copyright Act, by Canadian laws, policies, regulations and international agreements. Such provisions serve to identify the information source and, in specific instances, to prohibit reproduction of materials without written permission. For more information visit <http://laws.justice.gc.ca/en/showtdm/cs/C-42>

Les renseignements dans ce document sont protégés par la Loi sur le droit d'auteur, par les lois, les politiques et les règlements du Canada et des accords internationaux. Ces dispositions permettent d'identifier la source de l'information et, dans certains cas, d'interdire la copie de documents sans permission écrite. Pour obtenir de plus amples renseignements : <http://lois.justice.gc.ca/fr/showtdm/cs/C-42>

National Research
Council Canada

Conseil national
de recherches Canada

Canada

Evaluation of media for simultaneous enumeration of total coliform and *E. coli* in drinking water supplies by membrane filtration techniques

WANG Dunling^{1*}, FIESSEL Wanda²

1. Centre for Sustainable Infrastructure Research, Institute for Research in Construction, National Research Council, Suite 301, 6 Research Drive, Regina, S4S 7J7 Saskatchewan, Canada. E-mail: Dunling.Wang@nrc-cnrc.gc.ca

2. Environmental Services, Saskatchewan Disease Control Laboratory, Saskatchewan Health, 3211 Albert Street, Regina, S4S 5W6 Saskatchewan, Canada

Received 16 June 2007; revised 22 August 2007; accepted 23 August 2007

Abstract: This study evaluated three different dehydrated media for simultaneous detection and enumeration of total coliform (TC) and *E. coli* in drinking water samples with a standard membrane filtration procedure. The experiment indicated that the differential coliform agar (DCA) medium was the most effective among the tested media in enumerating TC and *E.coli*, without the need for extensive accompanying confirmation tests. The results for DCA medium were highly reproducible for both TC and *E.coli* with standard deviation of 6.0% and 6.1%, respectively. A high agreement (82%) was found between DCA and m-Endo media on 152 drinking water samples in terms of TC positive. The DCA medium also reduced concealment of background bacteria.

Keywords: drinking water; differential coliform agar (DCA); total coliform (TC); *E. coli*; membrane filtration (MF)

Introduction

Drinking water microbiological quality is primarily determined by using “indicator organisms”, whose presence indicates faecal contamination. The presence of the indicators is often a key in assessing potential public health risks due to pathogens and is used in drinking water quality regulations and guidelines in many countries. In Canada, test results are normally tied to the water supply operation license in the form of an agreement between the operator and

*Corresponding author. Dunling.Wang@nrc-cnrc.gc.ca.

the regulator at the provincial level (Health Canada, 1996). Fast and reliable test results will help operators to take quick action when water quality degrades and poses health risks for the public. To obtain fast and reliable results, water testing laboratories tend to: (1) test indicator organisms that directly confirm faecal contamination; and (2) simplify testing procedures and reduce turnaround time. It was recognized that *E. coli* was a reliable indicator for faecal contamination rather than total coliform (TC) (Stevens et al., 2003). However, the presence of TC in finished drinking water provides an indication of either a failure in the disinfection process, or a strong growth of bio-film in the distribution system, or that the water is prone to surface water infiltration.

The membrane filtration (MF) procedure has been used for drinking water bacteriological testing for many decades and showed a great advantage in testing large numbers of water samples. Many public and private laboratories across Canada are currently using the MF procedures with m-Endo agar for drinking water bacteriological testing. It has proven to be cost-effective. However, using m-Endo agar, only TC can be detected and enumerated. Frequently background bacterial growth may conceal test results, or cause false positive (APHA, 2005). When the TC result is positive, an accompanying test (usually MUG procedure) is required for confirmation of *E. coli* and a second sample is often needed. This process is time-consuming and may take more than a week to complete. Therefore, this delay often causes potential health hazards when the water supply is found to be contaminated a week later.

The primary objective of this experiment was to find an alternative medium that can be used for simultaneous detection and enumeration of TC and *E. coli* in drinking water samples. To replace m-Endo agar, this alternative medium must provide reliable enumerated results for both TC and *E. coli*, without a need for confirmation and must be less interfered with by background bacterial growth.

1 Materials and methods

1.1 Cultural media

Three dehydrated media were selected for this experimentation, m-Colibblue 24, Chromocult Coliform Agar (CCA) and Differential Coliform Agar (DCA). The m-Colibblue24 broth ampoules were obtained from the Millipore (Catalogue No. M00PMCB24, Billerica, MA USA). The m-Colibblue24 broth contains triphenyltetrazolium chloride (TTC) and 5-bromo-4-

chloro-3-indolyl- β -D-glucuronide. TC bacteria use TTC during their growth by forming red-colored colonies; while *E. coli* metabolizes the latter compound to produce complexes that display blue color. No confirmation test is required for *E. coli*. The CCA was obtained from the MERICK (Catalogue No. 1.10426-0100, Darmstadt, Germany). The CCA contains β -D-galactosidase and X-glucuronide. TC bacteria use β -D-galactosidase during their growth to form pink to red color complexes; *E. coli* colonies display dark blue to violet color, resulting from β -D-galactosidase and X-glucuronide cleavage by β -D-galactosidase. However, confirmation of *E. coli* requires an additional test. The DCA was obtained from the Oxoid Limited (Catalogue No. CM-1038B, Cambridge, UK). The DCA is supplemented with lactose, 5-bromo-4-chloro-3-indolyl- β -D-galactosidase and cefsulodin. The medium was capable to simultaneously detect and enumerate TC and *E. coli*. TC colonies display as light purple to whitish color with a distinct pink rim; while *E. coli* shows as a blue-centred red colony.

1.2 Testing procedures

Two 100-ml aliquots of water sample were filtered through a 0.45- μ m pore-sized membrane. The bacteria in the sample were retained on the surface of the membrane. One membrane was transferred to a previously prepared testing medium plate. The other was transferred to the m-Endo plate as a control. The plates were cultured at a temperature of $35 \pm 0.5^\circ\text{C}$ in an incubator for 24 ± 2 h. The bacterial colonies grown on the plate were inspected. Identified TC and *E. coli* colonies were counted and reported as colony formation unit (cfu).

1.3 Testing organisms

Seven organism cultures from American Type Culture Collection were chosen to evaluate the performance of the selected test media and are listed in Table 1. Table 1 also lists the characteristics of bacterial colonies grown on the test media. *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Escherichia coli* were selected bacterial cultures to represent TC and *E. coli*, respectively. *Pseudomonas aeruginosa* are environmental bacteria; commonly present in surface water sources and some strains do pose health risks. But they do not belong to coliform group, their presence should not interfere TC and *E. coli* enumeration. *Proteus mirabilis* and *Staphylococcus aureus* are bacteria that thrive in the environment and were selected to represent background bacteria. *Aeromonas hydrophila* are also common environmental bacteria in water.

This group of bacteria is chosen because they have been found to create false positive TC counts on m-Endo agar (APHA, 2005).

Table 1 The growth characteristics of bacterial colonies on CCA, DCA and m-Endo media

ATCC organisms	CCA	DCA	m-Endo
<i>Enterobacter cloacae</i>	Light purple spreaded colonies	Light purple colonies with pink rim	Red colonies with metal sheen
<i>Klebsiella pneumoniae</i>	Light purple rounded colonies	Whitish colonies with pink rim	Red colonies with metal sheen
<i>Escherichia coli</i>	Blue centered and purlish colonies	Blue colonies with pink rim	Red colonies with metal sheen
<i>Pseudomonas aeruginosa</i>	Light pink-clear colonies	No growth	Clear colonies
<i>Proteus mirabilis</i>	Light-pink clear colonies	Clear colonies	Clear colonies
<i>Staphylococcus aureus</i>	No growth	No growth	No growth
<i>Aeromonas hydrophila</i>	No growth	No growth	Red colonies with metal sheen

ATCC: American Type Culture Collection; CCA: Chromocult Coliform Agar; DCA: Differential Coliform Agar.

1.4 Preliminary evaluation of ColiBlue 24 broth

The ColiBlue 24 broth ampoules do not require previously prepared plates, as the ampoules were added to the absorbent pad before the filter was transferred to the petro-dish plate. Both TC and *E. coli* colonies that developed on the medium were visually identifiable. However, as preparation of the plate on site was labour intensive and time-consuming. Occasionally, it created a mess on filter bench where the medium spilled. It was recognized that this medium was not suitable for large volume laboratories. Therefore, no further investigation was performed on this medium.

1.5 Water samples

A total of 174 drinking water samples from both public and private supplies were tested with the selected test media, in comparison with m-Endo agar as a control during a period of 3 month. Of the 174 drinking water samples, 22 were used for testing CCA medium and 152 were

used for testing DCA medium. All water samples were collected in sterilized 220 ml plastic bottle containing a pullet of 10 mg sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) to remove free chlorine residual. After collection, the samples were immediately shipped to the Saskatchewan Provincial Laboratory in a refrigerated container. Samples were analyzed upon receipt. If a sample could not be analyzed immediately, it was stored in walk-in cooler. Analysis started within 48 h of collection.

2 Results and discussion

2.1 CCA agar

Experimental results indicated that both *E. coli* and TC could be dark red in color, which is atypical for either group. Some violet colonies (potential *E. coli*) showed a slightly pinkish color, which was closer to the color of TC colonies. The test result of drinking water samples with CCA was given in the Table 2, with the m-Endo as controls. “Overgrown background” implied that greater than 200 non-coliform bacterial colonies were observed on each plate. On these overgrown plates, when TC colonies could be identified, the TC colonies were reported in cfu, in addition to being reported as “overgrown”. When TC colonies could not be identified, they were reported only as “overgrown”, meaning that the test results were non-ascertainable. On the CCA medium, 12 of the 22 samples were observed with an overgrown background; 11 were negative with no TC colony developed; 9 were positive with different TC cfu. Three of the 9 TC positive results on the CCA coincided with positives on m-Endo, which gave an agreement rate of 33% between CCA and the control medium (Table 2).

Table 2 Comparison of TC results between CCA and m-Endo medium with drinking water samples

	CCA		m-Endo		Percentage of CCA in agreement with m-Endo (%)*
	Total number	Agreed number	Total number	Agreed number	
TC positive	9	3	5	3	33
TC negative	11	9	15	9	81
“Overgrown”	12	10	12	10	83

Total number of samples: 22. *Calculated by dividing the total number (from CCA or m-Endo whichever is higher) by the agreed number. CCA: Chromocult Coliform Agar; TC: total coliform; “Overgrown”: greater than 200 non-coliform bacterial colonies grown on each plate.

There were 9 samples that showed opposite results for the two media: 6 samples were negative on CCA, but positive on m-Endo; 3 samples were positive on CCA, but negative on m-Endo. Although CCA was supplemented with cefsulodin, suppression of background bacteria did not show an apparent improvement. There were still 12 samples with overgrown background, which was the same as the m-Endo medium.

The CCA medium has been reported to be an efficient medium for detection of *E. coli* in polluted surface water, food samples and fecal materials (Alonso et al., 1998; Frampton et al., 1988; Finney et al., 2003). In particular, it showed a high level of discrimination efficiency for differing fecal contamination levels of surface water polluted by faecal materials (Byamukama et al., 2000; Farnleiter et al., 2000). To date, there is no report of using CCA agar for drinking water bacteriological testing. From this experiment, it was noted that at low TC counts in drinking water supplies, the results could be different with the m-Endo medium. The major disagreement showed as negative on CCA and positive on m-Endo. This might be partially caused by interference of *Aeromonas* bacteria in the water sample. Historically, *Aeromonas* were considered nuisance organisms because they interfered with TC testing and created false positive on m-Endo agar (APHA, 2005). In addition, all potential *E. coli* colonies must be confirmed by the indole procedure, which required adding Kovacs reagent to each colony and takes about 1 min to determine if a pink color develops (*E. coli*). After testing with 22 water samples, it was clearly shown that the medium would not be suitable for drinking water testing. No further investigation was performed using the CCA medium.

2.2 DCA agar

On the DCA medium, *E. coli* displayed as a blue-centered colony with pink rim, which could be easily differentiated from the whitish to pink colonies with a pink rim for TC. The background bacteria were identified as clear or transparent colonies. The TC, *E. coli* and background bacteria were usually differentiable to the naked eye (Fig.1).

Fig. 1 Growth feature of TC, *E. coli*, TC and *E. coli* and “overgrown” with *E. coli* on DCA medium.

Agreement rate between the DCA and m-Endo agar on TC positive was 82%. The major differences were observed on the number of TC negative and “overgrown” (Table 3). It was expected that more samples would be TC positives on the m-Endo than on the DCA medium, based on the fact that many of the water samples were from private wells, which lacked a treatment facility and most likely contained *Aeromonas* bacteria. There was a low agreement (19%) between DCA and m-Endo (Table 3). This could be attributed to the large number of overgrown background on the m-Endo.

Table 3 Comparison of TC results between DCA and m-Endo medium with drinking water samples

	DCA		m-Endo		Percentage of DCA in agreement with m-Endo (%)**
	Total number	Agreed number	Total number	Agreed number	
TC positive*	77	63	95	63	82

TC negative*	75	14	17	14	19
“Overgrown”	27	21	60	21	78

Total number of samples: 152. * include the plates with “overgrown background” when it is identifiable.

**Calculated by dividing the total number (from DCA or m-Endo whichever is higher) by the agreed number.

DCA: Differential Coliform Agar; TC: total coliform; “Overgrown” is the same as in Table 2.

As expected, the DCA medium had a greater suppression of background bacteria; there were only 27 overgrown plates (Table 4). Even with an overgrown background, TC and *E. coli* colonies could be clearly identified and counted as shown in Fig.1. The TC (and *E.coli* as well) can be detected and enumerated on all 27 overgrown DCA agar plates, giving an identification rate of 100%. In contrast, TC colonies could only be identified on 10 of the 60 overgrown plates with m-Endo medium, which gave an identification rate less than 17%. That left more than 83% of the overgrown plates without ascertainable results and required a separate procedure to confirm *E. coli* presence. A second water sample was always required.

Table 4 TC identifiable rate on overgrown plates with the DCA and m-Endo media

	DCA	m-Endo
Number of overgrown	27	60
TC identifiable (<i>E. coli</i> on DCA)	27	10
TC unidentifiable	0	48
Identifiable rate (%)	100	17

DCA: Differential Coliform Agar; TC: total coliform.

It may be noted that occasionally, a dark blue colony grew on the DCA medium. A confirmation test with the MUG (4-methylumbelliferyl- β -D-glucuronide) procedure found that the dark blue colony was not *E. coli* colony. The other concern was that the prepared DCA plate is pinkish in color, and this might require initial training for staff to differentiate TC’s pink rim and the background bacteria pink edge from the medium.

3 Method reproducibility and accuracy

Method reproducibility was measured on a prepared water sample with expected numbers of TC and *E. coli*. A solution with expected value of 80 cfu for TC and 70 cfu for *E. coli* was prepared for method validation. Test results were closely clustered around the expected numbers. The standard deviation was 6.0% and 6.1% for TC and *E. coli*, respectively (Table 5). Method accuracy was calculated on the test result of samples provided by the Clinical Microbiology

Proficiency Testing Program at the University of British Columbia (Table 6). Both TC and *E. coli* numerations were accurate compared to the reference values, which took an average of the reported results from over 200 laboratories across North America. The DCA medium did not produce false positive or false negative results.

Table 5 Method reproducibility test

Replicate	TC count	<i>E. coli</i> count
1	82	69
2	81	65
3	78	70
4	81	75
5	68	62
6	88	78
7	91	76
8	78	72
9	73	66
10	79	81
Mean (x)	79.9	71.4
Standard deviation (s)	6.0	6.1
Variability (1.96 s)	11.8	12.0

TC: total coliform.

Table 6 Method accuracy test (with Clinical Microbiology Proficiency Test-UBC)

	TC			<i>E. coli</i>		
	Reference ^a	Reported ^b	Difference ^c	Reference ^a	Reported ^b	Difference ^c
Sept. 2005	8	9	1	8	9	1
	17	17	0	15	17	2
	0	0	0	0	0	0
	34	29	5	0	0	0
Jan. 2006	33	40	7	0	0	0
	33	31	2	0	0	0
	16	8	8	15	8	7
	6	6	0	8	6	0
Standard deviation			3.3			2.4
Average deviation			2.8			1.6

^aReference is an average cfu number from participating labs across North America (213 labs). ^bReported is the cfu number determined by the DCA. ^cDifference is the difference between the reference and reported. TC: total coliform.

4 Conclusions

The DCA medium was the most efficient medium among the media tested for simultaneous detection and enumeration of TC and *E. coli* bacteria in drinking water samples. Both TC and *E. coli* could be visually identified, even on an overgrown background. The DCA medium showed greater suppression of background bacterial growth. The enumerated results for both TC and *E. coli* were accurate and reproducible. However, the occasional presence of the dark blue colony requires that a follow up confirmation test be carried out.

References

- Alonso J L, Soriano K, Amoros I, Ferrus M A, 1998. Quantitative determination of *E. coli* and fecal coliforms in water using a chromogenic medium. J Environ Sci Health, 33: 1229--1248.
- APHA (American Public Health Association), 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. Washington DC: American Public Health Association.
- Byamukama D, Kansiime F, March R L, Farnleitner A H, 2000. Determination of *Escherichia coli* contamination with Chromocult Coliform Agar showed a high level of discrimination efficiency for differing fecal pollution levels in tropical waters of Kampala, Uganda. Appl Environ Microbiol, 66(2): 864--868.
- Frampton E W, Restaino L, Blaszkowski N, 1988. Evaluation of the β -glucuronidase substrate 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (X-GLUC) in a 24 h direct plating method for *Escherichia coli*. J Food Prot, 51: 402--404.
- Finney M, Smullen J, Foster H A, Brokx S, Storey D M, 2003. Evaluation of Chromocult Coliform Agar for the detection and enumeration of enterobacteriaceae from fecal samples from healthy subjects. J Microbiol Methods, 54: 353--358.
- Farnleitner A H, Kreusainger N, Kavka G G, Grillenberger S, Rath J, Mach R L, 2000. Simultaneous detection and differentiation of *Escherichia coli* populations from environmental freshwaters by means of sequence variations in a fragment of β -D-glucuronidase gene. Appl Environ Microbiol, 66(4): 1340--1346.

Health Canada, 1996. Guidelines for Canadian Drinking Water Quality. 6th ed. Ottawa.

Stevens M, Ashbolt N, Cunliffe D, 2003. Recommendation to change the use of coliform as microbial indicators of drinking water quality. Australia Government National Health and Medical Research Council. ISBN 1864961651.