Short communication

Utility of EC 3M™ Petrifilm™ and sanitary surveys for source water assessment in Nyabushozi County, south-western Uganda

Amber L Pearson[1], Marilyn C Roberts[2], Olusegun O Soge[2], Iana Ivanova[2], Jonathan D Mayer[3] and John S Meschke[4]*

[1] *Departments of Geography and Global Health, University of Washington, Box 353550, Seattle, WA 98195, USA*
[2] *Departments of Pathobiology and Environmental and Occupational Health Sciences, University of Washington, Box 357234, Seattle, WA 98195*
[3] *Departments of Geography and Epidemiology, University of Washington, Box 353550, Seattle, WA 98195, USA*
[4] *Department of Environmental and Occupational Health Sciences, University of Washington, Box 354695, Seattle, WA 98195*

Abstract

The majority of people in developing nations rely on untreated or minimally treated surface and shallow groundwater sources which are prone to faecal contamination. This study evaluated the utility of EC 3M™ Petrifilm™ and sanitary inspection forms (SIFs) as tools to assess 47 water sources and identify hazards of contamination in two rural Ugandan villages (90% were surface sources). Water samples were cultured on EC 3M™ Petrifilm™, which are intended for the enumeration of *E. coli* and total coliforms following 24 h incubation at 37ºC. Isolated bacteria were cultured on MacConkey agar and identified using standard biochemical tests, while selected isolates were verified by sequencing 16S rRNA genes. From 105 Petrifilms, 110 presumptive *E. coli* were isolated and identified to genus level. However, only 33 presumptive *E. coli* isolates from 14 water sources (representing 27 distinct strains as determined by PFGE) were confirmed *E. coli*. The other presumptive *E. coli* isolates were identified as *Citrobacter*, *Enterobacter*, *Proteus, Salmonella* and *Yersinia* species. SIFs used an adapted survey designed for urban water sources of Uganda. The form yielded an SIF score based on binary data and characterized potential sources of contamination. SIF scores alone offered little information to distinguish between contamination levels of surface water sources, but the information collected in the surveys could be used to identify ways to improve sources. The results of this study suggest that the use of sanitary surveys may assist in identifying potential pollution sources that may be targeted to protect water sources. Bacterial monitoring using EC 3M™ Petrifilms™ may be effective for the screening of relative levels of contamination of source waters, including surface sources.

Keywords*:* drinking water, developing countries, sanitary survey, EC 3M™ Petrifilm™

Introduction

The quality of drinking water is dependent on the initial quality of the source water used and the level of treatment provided. In much of the developing world, people rely on untreated or minimally (e.g. point of use) treated water. Contaminated water is a major source of infectious diseases and highlights the importance of selecting the best possible quality of source water to protect public health (WHO, 2002). Historically, indicator organisms (such as total coliforms or *E. coli*) have been used to indicate the sanitary quality of water due to their normal presence in high numbers in the faeces of humans and warm-blooded animals, and the impracticality of direct pathogen detection (Grabow, 1996; LeClerc et al., 2001).

No *E. coli* in a 100 mℓ sample is the standard of The World Health Organization and of Uganda for drinking water (WHO, 1993; Howard et al., 2003); however, the absence of *E. coli* does not ensure that water is safe for consumption (WHO, 2006). Therefore, a multifaceted approach where bacterial analyses are complimented by sanitary inspection forms (SIFs) to assess water quality is preferred (Smith and Husary, 2000). SIFs are standardized, low cost surveys which have been used to quantify risks of contamination, identify hazards, and offer possible explanations of water quality analyses (Smith and Husary, 2000; WHO, 2000). SIFs have been successfully used for the assessment of groundwater sources in urban areas of Uganda (Pedley and Howard, 1997; Howard et al., 2003). However, in rural areas of Uganda, the majority of drinking water sources are surface water sources and SIFs for surface water sources have not been developed. The application of SIFs and bacterial monitoring to assess sources in tandem may aid in drinking water source selection and in development of protection strategies for those sources.

Petrifilms were initially developed for the bacterial analysis of food items (3M St. Paul Minnesota, USA). Their ease of use and perceived specificity for target organisms has led to their use for monitoring water (Baumgartner et al., 1993; Vail et al., 2003; Schraft and Watterworth, 2005) in developed countries and has largely focused on testing the sensitivity and specificity of Petrifilms against reference methods (Vail et al., 2003; Schraft and Watterworth, 2005; Hörman and Hänninen, 2006; Wohlsen et al., 2006). Only one study was identified as using Petrifilms for bacteriologic screening of water in sub-Saharan Africa (McCa-

* To whom all correspondence should be addressed.
☎ 206-221-5470; fax: 206-543-8123;
e-mail: jmeschke@u.washington.edu
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rthy et al., 2004). This study screened for total coliforms and *E. coli* in ground- and surface water sources near wastewater effluent. One study compared the accuracy and precision of Petrifilms to seven other culture-based methods with 30 colony-forming units (CFUs) of bacteria and found Petrifilms to be the most accurate and consistent method of enumeration (Wohlsen et al., 2006). Another study evaluated Petrifilms relative to the reference membrane filtration method (ISO 9308-1:2000) using Lactose TTC agar with Tergitol-7 for surface and drinking water and found that Petrifilms had weak positive predictive value and a high negative predictive value for *E. coli* and high specificity for coliforms and *E. coli* based on challenge with a panel of laboratory isolates (Hörman and Hänninen, 2006). However, the study did not account for the quantity of bacteria in samples, by using only presence or absence to determine positive or negative predictive values. Vail et al. (2003) found bacterial enumeration using Petrifilms to be highly correlated with a number of culture-based methods, but the utility of Petrifilms was limited by the 1 mℓ assay volume. Despite this, the authors suggest that Petrifilms were suitable for screening water sources that exceed recreation standards for bacterial contamination.

The purpose of the current study was to evaluate the potential to use a low-tech method for enumerating *E. coli* in potential drinking water sources (EC 3M™ Petrifilm™; hereafter Petrifilms) and to evaluate the appropriateness of sanitary surveys of surface water sources in Uganda.

Materials and methods

Sanitary inspection form*:* Binary data (0, 1) were collected using an SIF, adapted from the form designed for urban water sources in Uganda (Niwagaba et al., 2003). Since the drinking water sources were primarily surface water sources, questions regarding reservoir covers and the quality and potential impacts on tanks were omitted from this adapted SIF. This form helps tabulate an ordered categorical sanitary risk score and includes: protection of source, adequacy of protection, drainage of the area, animal accessibility, elevation of nearby latrines, surface water collection uphill of the source, presence of a diversion ditch and other pollution sources uphill. These factors have been identified as important influences on water quality (Howard et al., 2003). A final risk score was quantified for each water source by calculating the number of positive factors (protective) as a percentage of the total number of factors being assessed (score = 0 to 10). Lower scores indicate lower protection and highest sanitary risk of the water source. In addition, households were asked to estimate the number of jerrycans (about 2 ℓ) used for domestic purposes by all households sharing the water source.

Water samples: Forty-seven water sources that included every water source within 2 villages in Nyabushozi County in the Mbarara District of south-western Uganda were sampled during June to August 2006. These two villages were purposely chosen, because these communities of Bahima pastoralists have undergone dramatic water resource change as a result of land privatization (since 1989). They rely predominantly on small surface drinking water sources. Duplicate 100 mℓ water samples were taken from each source concurrently (90% were surface water). These samples were kept on frozen gel packs in insulated containers and were plated on Petrifilms within 6 h of collection.

Bacterial assessment: One mℓ of each water sample was plated on the Petrifilms according to manufacturer's instructions and incubated at 37°C for 24 h. The EC 3M™ Petrifilm™ growth medium contained lactose and an indicator dye, such that total coliform bacteria appear as red colonies with the presence of gas bubbles. In addition, 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (BCIG) is present in the Petrifilm medium for the detection of the enzyme beta-glucuronidase which cleaves BCIG giving a blue precipitate to beta-glucuronidase positive bacteria such as *E. coli* (3M, 2001). Blue colonies were counted as presumptive *E. coli* and were enumerated as CFUs per mℓ, while red colonies representing total coliforms were not enumerated. *E. coli* counts > 150 were labelled as too numerous to count (TNTC).

Identification of isolated colonies: Randomly selected presumptive *E. coli* colonies were transferred from each Petrifilm to non-selective blood agar plates made with Bacto Brucella Agar supplemented with 5% sheep blood (BA) (Difco Laboratories, Div of Becton Dickson & Co, Sparks, MD USA) and incubated at 36.5°C for 24 to 48 h. Those isolates that grew were then plated on MacConkey agar (Difco Laboratories) and CHROMagar™ Orientation (DRG International, Inc., Mountainside, NJ USA) to give an initial genus identification followed by standardized tube biochemical identification (Remel, Lenexa, KS USA) (Funke, 2003) and/or API 20E strips (Biomerieux, Hazelwood, MO USA). Representative *E. coli* and randomly selected isolates from other genera had the variable region of their 16S rRNA gene sequenced, to verify the species as previously described (Greisen, et al., 1994). Strains confirmed as *E. coli* were analyzed by RFLP/PFGE as described below.

PFGE to confirm *E. coli* strains: All 33 *E. coli* isolates were examined by PFGE analysis using previously described protocols (Verdu, et al., 1996; Xia et al., 1995). Each isolate was grown overnight in LB broth to a bacterial density of 3 McFarland. The pellet was centrifuged at 10 000 r/min and re-suspended in 2 mℓ cold Pett IV buffer (1 M NaCl, 10 mM Tris pH 8.0 plus 10 mM Na_2-EDTA). Then it was centrifuged at 10 000 r/min and washed twice in 500 µℓ cold Pett IV buffer, re-suspended in 500 µℓ Pett IV buffer and added to 500 µℓ of 2% low melting agarose (Bio-Rad Richmond CA, USA) for agarose plugs. The agarose plugs were incubated at 37°C with gentle shaking at 60 r/min for 2 h in 6 mℓ of lysis buffer (1 M NaCl, 10 mM Tris-HCl pH 8.0, 200 mM Na2-EDTA, 0.5% N-Lauroyl Sarcosine, and 0.2% deoxycholic acid sodium salt) and incubated overnight in 6 mℓ of fresh ESP buffer (0.5 M Na_2-EDTA, 1% N-Lauroyl Sarcosine, Proteinase K 20 mg/mℓ stock [AMRESCO Inc., Solon, OH, USA]) at 57°C with gentle shaking at 60 r/min, washed in TE-PMSF (10 mM Tris, 1 mM Na_2-EDTA, and 100 mM PMSF: phenylmethylsulphonyl fluoride) and stored in 1 X TE buffer pH 8.0 at 4°C (Verdu et al., 1996).

The agarose blocks were digested separately with *Xba*I (New England Biolabs, Beverly, MA USA) overnight at 37°C and electrophoresed in a contour-clamped homogeneous electric field (CHEF DR II system; Bio-Rad) for 18 h at 14°C with switch times of 5 s (initial) and 25 s (final) at 6 V/cm. The gels were stained with ethidium bromide, de-stained in distilled water, and photographed under UV trans-illumination. PFGE patterns were evaluated for relatedness from the photographs. If two strains from the same water site had indistinguishable PFGE patterns or ≤ 2 band differences between the two PFGE patterns then isolates were considered to be the same strain, as previously described (Xia et al., 1995).

***In vitro* test of Petrifilms:** Bacteria, originally identified as *E. coli* but confirmed as other genera, were retested on Petri-

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TABLE 1
Amount of water used, households served and mean presumptive *E. coli* by source type and SIF score

Type, Score (n)	Mean ℓ drawn/d (SD)	Mean households served (SD)	Mean presumptive *E. coli* CFUs (SD)
Borehole			
SIF Score 2 (2)	370 (276)	77 (52)	0 (0)
SIF Score 4 (1)	40	20	0
SIF Score 5 (1)	4	2	1
Farm pond			
SIF Score 0 (16)	36 (55)	7 (7)	22 (34)
SIF Score 1 (25)	17 (21)	4 (5)	34 (64)
Valley tank			
SIF Score 0 (1)	175	40	6
SIF Score 1 (1)	120	40	2

films in the laboratory. A 10-fold bacterial suspension was prepared and 10 to 1 000 CFU per mℓ were inoculated onto new Petrifilms, incubated at 36.5°C for 24 h prior to observation.

Results and discussion

Previous studies have suggested that sanitary risk scores are a useful indicator of microbial contamination of groundwater in developing countries, but have not been evaluated for inspection of surface water sources and have only rarely been applied in Uganda (Lloyd and Bartram, 1991; Niwagaba et al., 2003). The SIF results of are sanitary risk scores, recommendations for action, and improvement plans. In this study, SIFs were completed and water samples collected from the 47 water sources including 4 boreholes, 41 ponds and 2 valley tanks (Table 1). SIF scores ranged from 2 to 5 for boreholes, and 0 to 1 for ponds and valley tanks. Not surprisingly, the scores indicate that boreholes are more protected than ponds and valley tanks. Ponds and valley tanks received the lowest scores on the ranking scheme consistently. The majority of drinking water, serving ~60% of households, in the region was obtained from ponds. Individual farm ponds tended to serve fewer families, while boreholes and valley tanks served large numbers of families.

The mean presumptive *E. coli* bacterial counts were 26.63 CFUs/1 mℓ for farm ponds, 3.5 CFUs/1 mℓ for valley tanks and 0.13 CFUs/1 mℓ for boreholes, thus international drinking water standards were not met. However, the detection limit of the Petrifilm assay (1 CFU/1 mℓ) which may not be sufficient to ensure that a water source meets the WHO drinking water standard of 0 CFU/100mℓ for *E. coli* due to the differences in sampling volume. This is consistent with other studies that found Petrifilms to be an insensitive measure of CFU to assure safe drinking water, but sensitive enough for screening and monitoring of recreational water (Vail et al., 2003; Beloti et al., 2003). The range of colonies presumptive of *E. coli* was 0 to >150 (TNTC) with a median of 8. Despite the fact that total coliforms were not enumerated on Petrifilms from water samples, numerous total coliform colonies were noted on Petrifilms from most water samples. The distribution of presumptive *E. coli* in pond sources is illustrated in Fig. 1. One pond site was negative, while 27 sites had concentrations > 15 presumptive *E. coli* CFU/mℓ. One borehole was positive (although the colony was not able to be isolated from the Petrifilm), while the other 3 boreholes were negative for presumptive *E. coli*. Sources with SIF scores of 0 to 1 (highest sanitary risk) were the most contaminated. However, the relationship between SIF score and bacterial contamination was not consistent. The *E. coli* positive borehole also had the best SIF score (Table 1).

The 110 presumptive *E. coli* isolates came from 29 (61%) of the water sources. Using biochemical methods and 16S rRNA gene sequencing only 30% of the presumptive *E. coli* isolates (n = 33) were confirmed as *E. coli* from 14 water sources. Using PFGE analysis, the 33 *E. coli* isolates represented 27 distinct PFGE patterns with 1 to 3 unique PFGE *E. coli* isolated from a single Petrifilm. Multiple *E. coli* isolates, from six of the water sites, had different PFGE patterns suggestive of faecal contamination from multiple sources. The implications of these findings are that water quality improvement efforts may need to target a number of potential sources of contamination, such as cattle, goats, chickens, and humans. The remaining 77 isolates were confirmed as *Citrobacter*, *Morganella*, *Proteus*, *Salmonella*, and *Enterobacter* (Fig. 2). Randomly selected isolates from each of the 5 genera were retested onto new Petrifilms. Under laboratory conditions the non-*E. coli* produced red colonies with or without gas as expected of non- *E. coli* members of the *Enterobacteriaceae*. We hypothesize that the poor confirmation rate for the current study may relate, at least in part, to high concentrations of coliform and non-coliform bacteria in the water samples

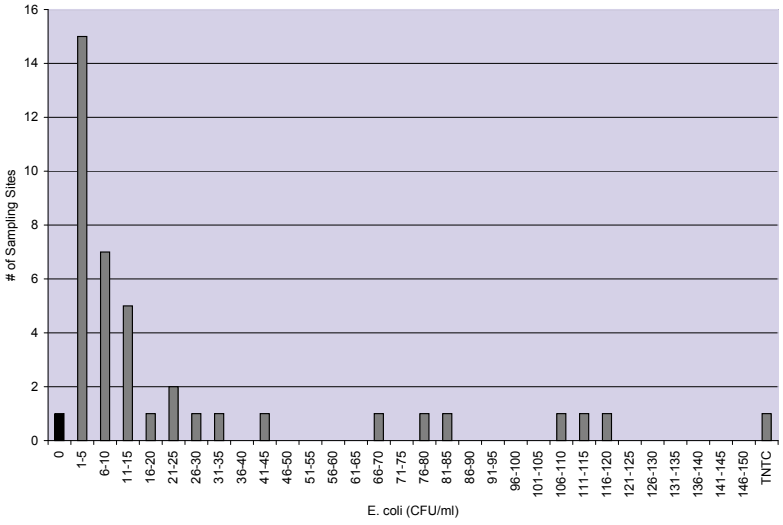

Figure 1
Distribution of presumptive E. coli *levels in farm ponds*

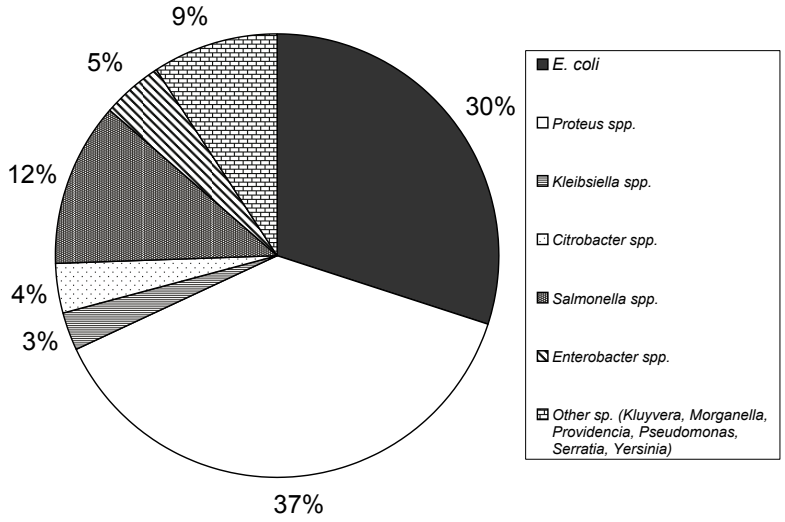

Figure 2
Distribution of confirmed identities for presumptive E. coli *using EC 3M™ Petrifilms™*

resulting in coincidence of *E. coli* and other bacterial colonies. This could have led to mixed populations of bacteria being isolated from an apparently single colony, which were then resolved during confirmation in favour of the non-*E. coli* bacteria.

Assessment of drinking water contamination in tropical areas is complex. In warm climates, total coliform bacteria are not ideal indicators for faecal contamination, as they occur from environmental sources in almost all untreated supplies (Janke et al., 2006). As a result, faeces-specific indicators such as *E. coli* have been recommended for evaluation of water quality in tropical areas, particularly in Uganda (Byamukama et al., 2005). International drinking water standards suggest that drinking water should be free of *E. coli* (0 CFUs/100 mℓ) (WHO, 2006). Complicating assessment of contamination levels further, culture-based methods for detection of *E. coli* in tropical areas may detect naturally occurring environmental strains that cannot be linked to recent faecal contamination (Byappanahalli and Fujioka, 2004). While EC 3M™ Petrifilm™ may not be adequately sensitive to ensure this standard in drinking water (due to the 1 mℓ sampling volume), they do have potential utility in developing/under-resourced regions of the world for screening potential sources of drinking water. In the current study, we found that when *E. coli* is a minor component of the overall bacterial population in the water sample, the ability to distinguish *E. coli* from other bacteria and confirm its identity may be considerably diminished. Still, confirmation of presumptive *E. coli* may not be necessary (or even practical) for screening relative differences in the water quality from several potential drinking water sources.

On their own, sanitary risk scores for surface water sources were not particularly illustrative, as almost all surface sources received the lowest or second lowest SIF value regardless of number of presumptive *E. coli* counts. Still sanitary inspections helped identify potential hazards and could be used to instruct efforts to protect the drinking water sources. Common potential hazards identified include livestock near drinking water sources, lack of latrines and sources of contamination at higher elevations than drinking water sources.

Quantifying the potential sanitary risk for water sources using SIFs paired with low-tech bacterial assessment of contamination using Petrifilms may aid in drinking water source decision-making in tropical, resource-poor settings. By choosing a less contaminated source, treatment of the drinking water would be more effective in reducing pathogen levels (Medema et al., 2003).

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