Enhancing Water Quality Surveillance: A Comparative Analysis of Water Testing Portable Microbiological Lab and the Colilert Quanti-Tray 2000

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Abstract

BACKGROUND

Operational water quality surveillance dominates urban piped systems. Lack of it poses a serious risk to public health as the population is exposed to disease-causing microorganisms; responsible for between four and six million cases of diarrhoea and more than 1,300 fatalities each day. Thus a need to determine the accuracy and reliability of the Portable Microbiology Lab (PML) for point sources of water, both protected and unprotected.

METHODOLOGY

The study evaluated the field test method, PML Kit under different water source conditions by comparing it to a laboratory standard method Quanti-Tray. This was executed by analyzing 27 water samples.

RESULTS

PML and Quanti-Tray 2000 yielded matching risk-level results for 26 samples. For the qualitative test of the 10mL and 100mL Colilert; 4 of the 27 samples' presence/absence tests were not congruent with each other. Thus error for a test with 10mL Colilert of PML resulted in a percentage variation of 14.81%, a sensitivity of 82.6% and a specificity of 100%. The addition of Petrifilm to identify risk levels, the proportional reduction in error relative to water source designation, for improved water source; for moderate levels at 30.78%, low risk 30.78%, high/very high risk was at 7.69% with a statistically significant differenceχ^2 (2, n =13) = 30.78, d.f. =2, (p <0.0001).

CONCLUSION

The Portable Microbiology Laboratory offers accurate and reliable water quality assessment in line with the WHO disease-risk levels and serves as a basis for informed management and public health interventions.

Keywords: *Water Quality, Risk Levels, Portable Microbiology Lab, Quanti-Tray*

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Introduction

In Sub-Saharan Africa's dry and semi-arid regions; industrial, residential, and agricultural sectors need to compete for the few water resources, hence water shortage is a reality. Sub-Saharan Africa has significant difficulty in accessing safe drinking water. Today, one in six people lacks proper access to water, and more than double that number lacks even the most basic sanitation, which requires water. In certain nations, 50% of the population lacks access to clean drinking water, which hurts their health [1]. The Sustainable Development Goals established new goals on access to clean water, sanitation, and hygiene for all (WASH) by 2030 [2].

Waterborne diseases are responsible for between four and six million cases of diarrhoea and more than 1,300 fatalities each day; a number that would drastically decrease if there was enough water for sanitation [3]. It has been acknowledged that it would be impracticable to test for every known waterborne pathogen. *Escherichia coli* was eventually selected as the best microbiological indicator after efforts to find a universal microbial indicator of faecal contamination failed in the year 1900 [3]. *Escherichia coli*, an indicator bacterium, can utilize nutrients that other bacteria are unable to consume. Interestingly, 100 million to one billion *E. coli* cells per gram of human faeces are present in the faeces of healthy and sick people alike. Moreover, it does not multiply when it leaves the body and enters the water. Additionally, it slowly degrades when shed in faeces and survives in water for at least as long as bacteria that cause cholera, typhoid fever, and dysentery. These characteristics make it relatively simple to detect, thus, *Escherichia coli* was chosen as the best microbiological indicator of recent faecal contamination [3]. Based on this, Colilert and Petrifilm, a new generation of *E. coli* tests, was released.

The examination of drinking water for the presence of total coliforms and *E. coli* is most frequently performed using methodologies that detect the presence of the enzymes β-D-galactosidase and β-Dglucuronidaseas markers of these organisms. Coliform is a term used to denote a group of gram-negative bacteria that can ferment lactose with a production of gas within 48 hours at either 35ºC or 44/44.5ºC. These characteristics allow for easy isolation, detection, and enumeration in the lab and are the gold standard for microbial water testing. They are always present when enteric pathogens or viruses are detected in water testing [3].

IDEXX's patented Defined Substrate Technology (DST) nutritional indicator, is used in the Enterolert Test to identify enterococci. This nutritional indicator turns fluorescent when enterococci break it down. DST approaches provide the benefits of simplicity, speed, and accuracy. However, the high expenses of a comprehensive water monitoring program and the related lab expenditures for *E. coli* enumeration continue to be a problem [4]. The price per sample for counting *E. coli* using procedures recognized by government laboratories (membrane filtration and specified substrate technology - Quanti-tray 2000) can range from $30 to $50. Costs can potentially surpass available budgets since multiple-day monitoring throughout a 14-week quarterly reporting cycle might result in more than 100 water samples per sub-county needing analysis for *E. coli* contamination. With the help of Friends of The Old (FOTO), the residents of Nyakach sub-county in Kisumu County, Kenya, have turned to a less expensive method for *E. coli* analysis called PML; as developed by Prof. Bob Metcalf [5]. The 3M EC 10mL Colilert technique and 1mL Petrifilm method is the option for counting *E. coli* (Portable microbiology lab [PML]). These assays are not authorized for use in mass water testing despite being reasonably priced ($2 to $3 per sample) and requiring little equipment [6].

The usefulness of *E. coli* detection by Petrifilm in water monitoring has been examined in a few prior studies, however, the interpretation of these data is difficult because of faulty screening methods and/or a disregard for the manufacturer's advised protocols. For instance, when blue colonies surrounded by gas bubbles were recognized as *E. coli* on Petrifilm tests, [7] compared *E. coli* concentrations in freshwater using membrane filtration, defined substrate, and Petrifilm methods; the results from all 3 methods were significantly and positively correlated [8]. But when blue colonies without gas were discovered to be *E. coli*, this was not the case; one drawback of the Petrifilm test was the small amount of water (1 mL/sample) required, which limited the

lower limit of detection to 100 colony forming units (CFU)/100 mL [7].

According to Bain R. [9], many water quality solutions are advertised for low- or medium-resource environments, but there is no comparative data on how well these products function in typical low-resource environments. To aid households and development practitioners, policymakers, and researchers in choosing suitable products for low or medium-resource settings; rapid, simple-to-learn, and simple-to-use field testing for the detection of *E. coli* in drinking water is required in low-income countries. Household-safe water storage and protection are questionable without microbiological safety verification [10]. The study seeks to identify economically feasible water testing methods without compromising efficacy as the existing standardized tests require specific tools and training, making them difficult to implement in the field.

Methodology

Study design

A comparative study design was adopted which entailed comparing the PML to the gold standard water testing method Quanti-Tray 2000.

Study area

This evaluation was conducted in Lower Nyakach, North Nyakach Ward near Lake Victoria in western Kenya. Lower Nyakach comprises 180 small villages divided into 12 locations and two wards.

Sampling

The sampling zones and sample size were determined using a ratio of one sampling zone for every 2,000 people, dispersed per the proportion of accessible water sources. classification for improved water source levels was as follows: enhanced level 1-stand-alone point source supply, reservoir - level 2 communal point supply and level-3 private supply as in appendix 1.

The water sampling adhered to WHO's classifications as:

- **Improved sources of drinking water**: Piped water into dwelling, yard, or plot, public tap/standpipe, tube well/borehole, protected dug well, protected spring and rainwater collection.
- **Unimproved sources of drinking water:** Unprotected dug well, unprotected spring, vendor-provided water, tanker truck water, surface water (river, stream, dam, lake, pond, and irrigation channel).

Water sampling procedures and transportation

The FOTO and SWAP staff were trained on sampling and sample transfer during pre–testing. Water samples were collected into a 100 mL sterile, Whirl pack. Samples were transported to the lab in cold boxes and analyzed within 4 hours of collection. Every sample label contained information about the sample location (e.g., household, source), sample description (e.g., inlet water, storage bucket water), ID number, date and time of sampling, initials of the person collecting the sample, And other relevant information such as the date the sample was received in the laboratory. The samples were field-lab-tracked using the sample tracking form.

Sample processing

PML method: A volume of 10 mL water was aseptically transferred using a sterile pipette from the well-mixed samples into the Colilert tubes.

Using an aseptic pipette, 1 mL of water from the Colilert tube was transferred to a Petrifilm plate for examination. Triplicate Petrifilm plates were prepared and then incubated at body temperatures.

Following successful incubation, the Petrifilm plates were counted following the manufacturer's instructions, and information was recorded [6]. Blue colonies with a gas bubble around them were counted as *E. coli*, but blue colonies without one were not.

Although estimating the amount of *E. coli* present can be done by counting the colonies on one square of the Petrifilm and multiplying the

result by 20, the suggested counting limit for each Petrifilm is 150 colonies (150 CFU/mL).

IDEXX Quanti-Tray 2000®: To leave 100 mL in the sample vial, the water from the samples used for the PML was decanted.

Following the manufacturer's instructions, Colilert with Quanti-Tray 2000 (IDEXX Corp., Portland, ME) was used to count the amount of *E. coli* present [11], [12].

Samples were processed in the Kisumu-based SWAP lab. Controls for positive, negative, and proficiency tests are created per the quality assurance strategy for the laboratory. Distilled water was utilized as a negative control, while *E. coli* was employed as a positive control (IDEXX Corp., Quanti-Cult, and Portland, ME).

All results were reported as the most probable number (MPN) of *E. coli per* 100 mL of water The MPN method is statistically equivalent to CFU/100 mL designations [11] and risk levels determined as guided by WHO in Table 1

In each day cluster, blank tests were conducted to provide confidence in the results. Water for the blank test was obtained from a reliable brand of mineral water or distilled/deionized water.

Accuracy of test

Water sources were classified as unimproved and improved water sources, therefore, two statistical analyses were done to evaluate the precision of the PML as a tool for monitoring water quality. The formulae below were used to determine error and reduction in error:

$$\lambda = \frac{(E \text{ knowing source type}) - (E \text{ knowing source type and additional test})}{(E \text{ from knowing source type})}$$

The λ value, defined as "proportional reduction in error," is a measure of how good a test kit becomes at making predictions for both improved and unimproved water sources.

Data analysis

Frequency distribution tables were used for proportionate reduction in error calculation to determine the error for the PML test kit. The Chi-Square was then used to determine whether the comparable PF Av x 100 were statistically significant to the MPN value.

Ethical consideration

This study was approved by the Board of Postgraduate Studies of Jaramogi Oginga Odinga University of Science and Technology, ethical approval was obtained from Jaramogi Oginga Odinga Teaching and Referral Hospital Ethics Review Committee and a research license was obtained from NACOSTI.

Results

Risk levels for drinking water sources

Qualitative Analysis: In Table 2, it was observed that 4 out of 27 samples showed non-congruent results between the two tests. Specifically, the 10mL Colilert test indicated the Absence of *E. coli* (MUG -ve and OPG -ve), while the 100mL Colilert test indicated the Presence of *E. coli* (MUG -ve and OPG +ve).

Table 1:
Determination of WHO disease-risk categories for drinking water and correlation of Colilert and E. coli Count Petrifilm result with risk categories

Disease-risk Level	Colilert MUG +	*E. coli in* sample CFU per mL (PML)	Colilert MUG +	*E. coli in* sample (CFU/100 mL) (MPN)
Very low	-	0	-	< 1
Low	-	0	-	1-10
Moderate	+	1-10	+	10-100
High	+	> 10	+	100-1000
Very High	+	TNC	+	> 1000

Table 2:
Frequency Distribution Table of 10mL Colilert- PML and 100mL Colilert- Quanti-Tray 2000 for E. coli Contamination for Lower Nyakach

		100mL Colilert		
		Presence MUG +ve and OPG +ve	Absence MUG -ve and OPG -ve	Total
10mL Colilert	Presence MUG +ve and OPG +ve	19	0	19
	Absence MUG -ve and OPG -ve	4	4	8
	Total	23	4	27

Table 3:
Escherichia coli counts and risk levels in lower Nyakach

Location	**Source/types**		**MUG, + or - OPG + or -**	**PF MPN/100 Av x 100**	**Q-2000 *E. coli* MPN/100**	**Risk levels**
Agoro West	Kobita well	I	- OPG +	0.00	4.10	Low
	Kanyarera dam	I	+	33.33	90.90	Moderate
	Jeniffer tap	I	- OPG+	0.00	5.10	Low
	Kanyalwal borehole	I	+	233.33	372.40	Very high
	Kolwal borehole	I	+	66.67	98.80	Moderate
	Pawtenge borehole	I	-	0.00	0.00	Low
	Kopige pond	U	+	5866.67	2419.70	Very high
Asao	Samwel tap	I	- OPG+	0.00	4.10	Low
	Lisana borehole	I	-	0.00	0.00	Low
	Koyuga pond	I	- OPG+	0.00	15.80	Low/Moderate
East Nyakach	Komuono spring	I	+	66.67	16.90	Moderate
	River Sibion	U	+	833.33	920.80	High
Jimo East	Kajatap borehole	I	-	0.00	0.00	Low
	Kamula dam	U	+	333.33	267.83	High
	Ko-okoto dam	U	+	200.00	120.50	High
	River Asao	U	+	1100.00	1119.90	Very high
N. Nyakach	Oremo pond	U	+	500.00	456.90	High
	River Awach	U		1333.33	1266.37	Very high
	River Ataro	U	+	1800.00	1421.27	Very high
	St. Alloys pond	U	+	1100.00	1413.60	Very high
North East	Katuk dam	I	+	166.67	93.50	Moderate
	River Sare	U	+	1500.00	1046.20	Very high
	River Awach	U	+	8300.00	2419.70	Very high
Rangul	Kochuka water tank	I	-	0.00	0.00	Low
	Ka-Elias pond	U	+	933.33	1046.20	High
	Kajole pond	U	+	2600.00	2419.60	Very high
	River Nyando	U	+	5666.67	2419.70	Very high

Conversely, congruent results were observed in 19 of the 27 samples, with both the 10mL and 100mL Colilert tests indicating the Presence of *E. coli* (MUG +ve and OPG +ve).

Quantitative Analysis: Risk levels were categorized according to the World Health Organization (1997) guidelines, and the risk levels were classified as either low, moderate, high or very high determined using the results from the petrifilm count and Quanti – Tray MPN count as outlined. Table 3 presents the risk level classifications for all 27 samples, showing that both the PML and Quanti-Tray 2000 yielded the same risk level results for 26 of the samples; with 1 sample yielding low for PML and Moderate for Quanti–Tray.

The sensitivity and specificity of PML in reducing error relative

The 4 out of 27 samples showed non-congruent results between the 10 mL Colilert and 100 mL Colilert tests hence, calculation for error in the 10 mL and 100 mL comparison was performed by determining the percentage variation as presented in Table 4. Specifically, the error associated with the water quality test using 10mL Colilert of the PML method resulted in a percentage variation of 14.8%. The sensitivity of 10 mL Colilert is 82.6% and a specificity of 100% as calculated based on Table 2.

The addition of Petrifilm to the Portable Microbiology Laboratory (PML) method for identifying risk levels resulted in a proportional reduction in error relative to water source designation. The analysis revealed the following findings, as presented in Table 4: the highest percentage was noted for moderate levels at 30.78% for improved water source followed by low risk (30.78%), while high/very high risk was at 7.69% with a statistically significant difference χ^2 (2, n =13) = 30.78, d.f. =2, (p <0.0001) as in Table 4.

Determining PML reliability for point source surveillance testing

To ascertain whether a PML kit is a reliable field test method for local application beyond this study for determination of contamination levels of water sources, Quanti-Tray 2000, PML lab, and PML field data were compared having standardised PML Petrifilm count value by multiplying by 100.

Table 4:
Calculations for Error for PML – Improved water source

		Quanti-Tray 2000		
		Low	Moderate	High/very high
PML	Low	4	4	0
	Moderate	0	4	0
	High/ very high	0	0	1
	Total	4	8	1

Table 5:
Comparison of Quanti-Tray 2000, PML lab, and PML field

		Improved source of water		Unimproved source of water
	Count	Average±sd	Count	Average±sd
PML field	13	1152.94±2360.91	14	3211.11±2755.20
PML lab	13	470.59±681.69	14	2655.56±3041.84
Quanti-Tray 2000	13	420.84±685.27	14	1452.79±825.67
f-test	1.31			1.24
p-value	0.2789			0.306

For improved water sources, the highest averages were recorded in the PML field (1152.94±23.60.91sd), while the lowest was recorded for Quanti-try (420.84±683.26sd) with no significant difference ($F_{0.05\ (2,\ 48)} = 1.31$, p=0.2789). For the unimproved water sources, the PML field portrayed the highest averages (3211±2755.20sd) while Quanti-try recorded the lowest (1452.79±825.67sd) with no significant difference ($F_{0.05\ (2,\ 24)} = 1.24$, p=0.3060) as illustrated in Table 5.

Discussion

The findings of this investigation supported the use of a novel method PML to find *E. coli*, indicator bacteria for faecal contamination, in multiple point sources of drinking water in the North Nyakach region of Kenya. The tests presented the same risk level for 26 samples of the 27 samples; just as field tests conducted in Bangladesh had 85% of samples record the same risk level [1]. The sampled water sources had 70% recording high and very high-risk levels, this was concurrent with a baseline study in Lower Nyakach, Kenya, which reported that 75% of sampled sources were contaminated[12]. This shows that a significant portion of the population is exposed to very high *E. coli* levels, and the deterioration in quality suggests that interim targets and approaches are needed to reduce risks and strengthen water quality surveillance.

There is a need for countries to locally adapt the SDG targets to the national context, create plans to gradually improve drinking water quality, and identify and target populations at greatest risk using the PML. PML offers a cheap and simple *E. coli* testing alternative to generate nationally representative data from water points surveys, provide a cost-effective means of filling these data gaps in the short term, and draw attention to inequalities in service levels in the absence of regulation [13].

The idea of incorporating communities in water testing operations may alter how a community treats its drinking water [14]. PML use for Community-led monitoring has the benefit of fostering chances for behaviour change communication, which is integrated into, among other behaviour change initiatives, and community-led water safety planning. The FOTO project's evidence-based microbiological behavioural change communication program in February 2012, FOTO has noticed a 73% decrease in the prevalence of diarrhoea [15]; according to survey findings, 95% of people treat their drinking water, with 65.7% of those people treating it after being introduced to the project.

The sensitivity and specificity of PML in reducing error

The high sensitivity and specificity of PML make it an excellent test kit for both improved and unimproved water sources. A low-cost kit has also been successfully tried in the Afghanistan Living Conditions Survey [16] with all the households reporting the test as an incentive and wanting to get feedback on the quality of their water. Long-term replacement of the culture-based and laboratory methods that now dominate the market for water quality testing is anticipated by the development of new, quick tests [1]; which will allow water testing to be included in nationally representative water point surveys, as shown by the integration of water testing into the Multiple Indicator Cluster Surveys (MICS), and the data gathered can be used to track the SDG on the indicator for safely managed drinking water services.

The Portable Microbiology Laboratory tests can therefore be integrated into ongoing work with communities towards universal health care [17] as it enhances the accessibility and availability of microbiological monitoring. This approach empowers community health providers and community leaders to proactively address potential health risks and implement necessary interventions. By providing clear and actionable feedback, the PML tests enable communities to better understand their health status and take

appropriate measures to safeguard their well-being.

Determining PML reliability for point source surveillance testing

The protection of public health is the main objective of the WHO Guidelines for Drinking Water Quality. The World Health Organization (WHO) has published guidelines for such a plan that outlines a disease-risk management framework [20], this can be accomplished by the use of PML.

The initial objective is often to increase access and the amount of water available such that all communities reach intermediate access service levels to satisfy health-based objectives in point source supply zones [18 19]. The limitations of Quanti-Tray 2000 are caused by the lack of labs that can perform conventional monitoring tests as well as the expense and inconvenience of transferring samples. These significant constraints result in few tests and lengthy gaps between microbiological testing. However, the village access facilitator would be able to monitor the community water sources and communicate the findings of these tests to the community to implement remedial measures, much as the FOTO and SWAP initiatives had done.

Limitations of the study

Limited sample size since North Nyakach ward faces water scarcity and thus limits the sampling zones which then translates to a low sampling size.

Conclusion

In areas where access to well-equipped laboratories may be limited, the PML emerges as a practical and accessible alternative, providing real-time assessments and data for timely decision-making. Moreover, the PML's comparability with the well-established Quanti-Tray method further strengthens its credibility and applicability in various settings and regions. By offering a convenient and efficient means of assessing water quality, the PML empowers decision-makers, public health authorities and communities to take proactive measures in safeguarding communities from waterborne illnesses and related health hazards. With its reliable results, the PML can act as a reliable foundation for developing effective strategies and interventions to address water contamination challenges and track SDG 6.

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Appendix 1

Sampling zones identification

Improved

									Unimproved		Level 1		Level 2		Level 3	
Location	No. Villages	Unimproved sources Y/N	Level 1 Y/N	Level 2 Y/N	Level 3 Y/N	Total HH*	Est. pop	#Villages	#SZ	#Villages	#SZ	#Villages	#SZ	#Villages	#SZ	Total
Rangul	28	Y	N	Y	N	2056	7370	3	3	0	0	1	1	0	0	4
North Nyakach	22	Y	N	N	N	1775	7930	4	4	0	0	0	0	0	0	4
East Nyakach	14	Y	N	N	N	1003	3000	1	1	0	0	1	1	0	0	2
North East	25	Y	N	Y	N	2444	6370	2	2	0	0	1	1	0	0	3
Jimo East	18	Y	N	Y	N	3255	8010	3	3	0	0	1	1	0	0	4
Asao	22	Y	Y	Y	N	2209	5392	0	0	1	1	1	1	1	1	3
Agoro West	37	Y	Y	Y	Y	2551	15000	1	1	3	3	1	1	2	2	7
Total	166	6	5	6	2	15293	53072	23	14	4	4	6	6	3	3	27