ENHANCING WATER QUALITY SURVEILLANCE: A COMPARATIVE ANALYSIS OF PORTABLE MICROBIOLOGICAL LABORATORY AND THE COLILERT QUANTI - TRAY 2000, IN NORTH NYAKACH WARD, KISUMU COUNTY

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DECLARATION

I declare that this is a thesis that has not been presented for examination at any other University.

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DEDICATION

I dedicate this thesis to all public health practitioners working towards the realization of the fundamental human right to clean and safe water in adequate quantities and the implementation of universal health coverage. Keep up the good work.

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ABSTRACT

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 the need to determine the accuracy and reliability of the Portable Microbiology Lab (PML) for point sources of water, both protected and unprotected. 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. PML and Quanti-Tray 2000 yielded matching risk-level results for 26 samples. For 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%, 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 30.78%, low risk 30.78%, high/very high risk was at 7.69% with a statistically significant difference χ^2 (2, n =13) = 30.78, d.f. =2, ($p < 0.0001$). The Portable Microbiology Laboratory offers accurate and reliable water quality assessment in line with the WHO disease-risk levels and serve as a basis for informed management and public health interventions. This will facilitate consistent and widespread monitoring of water quality, ultimately leading to improved public health outcomes.

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ACRONYMS AND ABBREVIATIONS

3M	Minnesota Mining and Manufacturing Company
BCI	Bromo – Chloro – Indolyl
CFU:	Colony Forming Unit
DSF:	Define Substrate Technology
E. coli:	*Escherichia coli*
FOTO:	Friends of the Old
MPN:	Most Probable Number
MSF:	Médec in Sans Frontières
MUG:	4-methyl-umbelliferyl-β-D-glucuronide
NGO:	Non-Governmental Organization
ONPG:	ortho-nitrophenyl--D-galactopyranoside
P/A:	Presence/Absence
PML:	Portable Microbiology Laboratory
SWAP:	Safe Water and AIDS project
UNICEF:	United Nations Children`s Fund
WHO:	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

In Sub-Saharan Africa's dry and semi-arid regions; industrial, residential, and agricultural sectors need compete for the few water resources, hence water shortage is a reality. Sub-Saharan Africa has significant difficulty when it comes to access to safe drinking water. Today, one in six people lacks proper access to water, and more than double that amount lacks even the most basic sanitation, which requires water. In certain nations, 50% of the population lacks access to clean drinking water, which has a negative impact on their health (*WHO/UNICEF. (2019).*, n.d.). The SDGs establish challenging new goals on access to clean water, sanitation, and hygiene to all (WASH) by 2030.

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 (Dufour et al., 2003). 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 (Dufour et al., 2003). *Escherichia coli*, an indicator bacterium, was shown to be able to utilize nutrients that other bacteria are unable to consume, one hundred million to one billion *E. coli* cells per gram of human faeces are present in the faeces of healthy and sick people alike; it doesn't multiply when it leaves the body and enters the water; it slowly degrades when shed in faeces; it survives in water at least as long as bacteria that cause cholera, typhoid fever, and dysentery; and it is relatively simple to detect based on these characteristics, *Escherichia coli* was chosen as the best microbiological indicator of recent faecal contamination (Dufour et al., 2003). On the basis of 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 laboratory and are the gold standard for microbial water testing. They are always present when enteric pathogens or viruses are detected in water testing (Dufour et al., 2003).

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 laboratory expenditures for *E. coli* enumeration continue to be problems (Parker, 2011). 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 (*Watermicrohistory*, n.d.). The 3M EC 10mL Colilert technique and 1mL Petrifilm method is the option for counting *E. coli* (Portable microbiology laboratory [PML]). These assays are not authorized for use in water testing despite being reasonably priced ($2 to $3 per sample) and requiring little equipment (*3M Petrifilm Plate Certificates*, n.d.).

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, (Morgan, R et al., 2003), compared *E. coli* concentrations in freshwater using membrane filtration, defined substrate, and Petrifilm methods and came to the conclusion that results from all 3 methods were significantly and positively correlated (*Official Methods of Analysis, 22nd Edition (2023)*, n.d.). 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 little amount of water (1 mL/sample) required, which limited the lower limit of detection to 100 colony forming units (CFU)/100 mL (Morgan, R et al., 2003).

According to (Bain et al., 2012), most water quality solutions are advertised for low resource environments, but there is no comparative data on how well these products function in typical low-resource environments. To aid household, 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 since household safe water storage and protection are questionable without microbiological safety verification (Allen et al., 2010).

1.2 Statement of the Problem

While surveillance monitoring of non-piped sources is sparse, operational monitoring of upgraded sources and urban piped systems dominates water quality testing yet 32% of sub-Saharan Africans drink from unimproved sources, and an additional 52% drink from sources that are not piped water to their premises, raising serious public health concerns given that surveillance testing is the only form of oversight used to the informal water supplies and point sources that serve the vast majority of low- and middle-income countries (*WHO/UNICEF. (2019).*, n.d.).

Interventions to reduce the number of waterborne pathogens in drinking water have been restricted to the degree that laboratory space and microbiological skills are available to evaluate the effectiveness of the intervention (Onda et al., 2012). Thus a 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 since household safe water storage and protection are questionable without microbiological safety verification (Allen et al., 2010).

1.3 Objectives

1.3.1 Broad Objective

To assess the effectiveness and reliability of the PML Kit as a field method for water quality testing; By comparing its results with those obtained from the IDEXX Quanti-Tray 2000.

1.3.2 Specific Objectives

i. To compare the determination of Presence/ Absence and risk levels for drinking water sources according to *Escherichia coli tests* in Lower Nyakach, North Nyakach ward for different locations and source types.

ii. To assessing Performance of PML in reducing error relative to improved/unimproved water source designation

iii. To determine PML reliability for point source surveillance testing

1.4 Research Questions

i. What are the risk levels for drinking water sources according to *Escherichia coli* tests in Lower Nyakach, North Nyakach ward?

ii. What is the error of the test for an improved water source?

iii. Are there significant differences in the concentration of *E. coli* in the two test systems (PML and Quanti-Tray 2000)?

1.5 Justification

Access to safe and clean drinking water is recognized as a fundamental human right by the United Nations General Assembly and is enshrined in the Constitution of Kenya. It plays a crucial role in poverty reduction, as it contributes to improved health, enhanced educational opportunities, and increased productivity. Despite significant progress in increasing access to drinking water over the past two decades, the quality of water has become a pressing concern, putting these achievements at risk.

Given the significance of water quality in safeguarding public health, this study aims to contribute to closing the existing data gaps and enhancing understanding of the water quality situation in the study area. By employing E. coli testing and comparing the results to established guidelines, the study will provide valuable information to guide

remediation efforts, and interventions aimed at ensuring access to safe drinking water. as guided by WHO (Table 1).

Table 1: *Correlation of* *E. coli* Levels with WHO Disease Risk and MSF action Categories

Level of *E. coli*	WHO disease risk level a	WHO action priority	MSF* action b
<1 in 100 mL	Very low	None	None
<1 in 10 mL	Low	Low	Consume as is
1-10 in 10 mL	Moderate	Higher	Treat if possible
1-10 in 1 mL	High	Urgent	Must be treated
>10 in 1 mL	Very high	Urgent	Reject or thoroughly treat

b. Médecins Sans Frontières* (1994) Public Health Engineering in Emergency Situations.

1.6 Significance of the Study

The study seeks to introduce a readily available water quality testing and monitoring tool, the Portable Microbiology Laboratory (PML), which offers simplicity and ease of use. By clarifying the most effective procedure for testing water quality in both improved and unimproved water sources, 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 (Parker, 2011).

Successful implementation of the PML as a water testing method could have a profound impact on reducing the incidence of water-related diseases caused by contaminated water in developing countries. By providing a simple and accessible solution for water quality monitoring, this research has the potential to contribute to improving public health and enhancing the effectiveness of development efforts aimed at ensuring access to clean drinking water.

1.7 Limitations of the Study

The WHO recommends using a risk assessment strategy for analyzing water quality. *E. coli* count data and sanitary inspection findings are combined in risk assessment analysis (Davison et al., 2005). However, the study focused on the results of the *E. coli* counts, thus the results will not fully inform the quality status of the sources and whether they

truly represent the standards for improved water sources. Acknowledging this limitation is important to ensure that the study's findings are interpreted within the context of the specific focus on E. coli counts and that further research is needed to encompass a broader range of risk assessment factors for a more comprehensive evaluation of water quality in relation to improved water sources.

CHAPTER TWO
LITERATURE REVIEW

2.0 Introduction

Having access to clean water is crucial for reducing poverty as it gives individuals more energy for work and education and better health. The progress accomplished in the previous 20 years to increase access to drinking water is now in jeopardy due to deteriorating water quality. The World Health Organization (WHO) and United Nations Children's Fund (UNICEF), through the Joint Monitoring Program (JMP) for Water Supply, Sanitation, and Hygiene, are mandated to monitor the SDG global indicators for WASH and released baseline estimates in July 2017 and updated estimates in 2019 (*WHO/UNICEF. (2019).*, n.d.) and 2021 (*WHO/UNICEF 2021*, n.d.). The JMP data support prior research that suggested drinking water pollution was common in low- and middle-income countries (LMICs) and that almost 2 billion individuals drank water from sources that included faecal indicator bacteria (Bain et al., 2012) (Onda et al., 2012). The 2019 JMP report indicated that only 98 of the 193 UN Member States have enough data at the national level to report on water quality for Target 6.1, despite the fact that policymakers are increasingly acknowledging the significance of water quality (*WHO/UNICEF. (2019).*, n.d.), the JMP require a 100 mL which essentially prevent countries from sustainable water testing. To support national efforts to deliver securely managed drinking water services by 2030, it is necessary to enhance the monitoring of drinking water quality. In a global overview of national regulations and standards for drinking water quality second edition 2022, "all 103 countries specified a value of zero per 100 Ml except one country, which specified 20 per 100mL.

Water testing is crucial for ensuring that water sources are functioning properly, ensuring the safety of drinking water, looking into disease outbreaks, and validating procedures and preventative measures (Bain et al., 2012). The use of other tools and resources, such as sanitary surveys and monitoring, to help ensure water quality is necessary because countries cannot rely solely on water quality testing to protect public health. After all, it is neither physically possible nor economically feasible to test all drinking water (Kumpel et al., 2016).

2.1 History and Advancement of Water Testing

It has long been known how important testing is to ensure excellent water quality in general. The discovery of the diarrheal disease in the 1880s as a result of bacteria contaminating the water via faecal pollution led to a broad understanding of the significance of water testing for public health. Since it is not practicable to test for all pathogens individually, *E. coli* has been recognized as the most reliable microbiological indication of faecal contamination (Standridge, 2008).

The fact that *E. coli* is always present in large numbers in the faeces of people and warm-blooded animals as well as, the discovery that *E. coli,* but not other coliforms, contained the constitutive enzyme l-glucuronidase led to the recognition of *E. coli* as the best indicator of recent fecal contamination. The ability of this innovation to directly screen for *E. coli* in water eliminates the need for the inexact coliform and thermotolerant coliform tests (Leclerc et al., 2001). The TtC test was not a reliable substitute for the *E. coli* test because some environmental coliform bacteria can produce false-positive results (Allen et al., 2010) (Water & Team, 2006). In addition, unlike waterborne infections, it does not grow after leaving the body and contaminating the water; it endures there; and it is reasonably simple to identify (Dufour et al., 2003).

2.2.0 *E. coli* Analysis as an Indicator of Water Quality

Faecal pollution, arsenic, and fluoride are the three health criteria with the greatest global priority. Although the lack of *E. coli* is thought to suggest minimal risk but is not a guarantee of safety, it is seen as a credible signal of faecal contamination since short incidents may go undetected even with routine testing (Howard et al., 2010). "Free from contamination" for SDG monitoring indicates that drinking water is devoid of *E. coli* or thermotolerant coliforms (in a 100-mL sample and a 1mL sample).

The new assays, such as Colilert and Petrifilm, readily, quickly, affordably, and without the aid of a fully-equipped laboratory, identify and count *E. coli* (Allen et al., 2010). Due to these advancements, the traditional testing targets of coliforms and thermotolerant coliforms are no longer regarded as appropriate alternative indicators for *E. coli* (Leclerc et al., 2001).

2.2.1 Measurement of Indicator Bacteria, *E. coli*

The bacteriological examination of water quality may be divided into two categories: quantitative and qualitative. By counting the number of colonies that develop on a membrane filter or in a Petri plate, the colony-forming units (cfu) per volume of water used in the quantitative technique are used to express the number of bacteria; additional this is listed as a Most Probable Number (MPN) on Quanti-Tray 2000. Reporting presence or absence (P/A) in a predetermined volume of water, usually 10 mL or 100 mL, is the qualitative alternative.

2.2.2 P/A Testing for *E. coli* with Colilert

The Colilert test was introduced in 1987 by IDEXX Laboratories, Inc. (Westbrook, Maine). It uses MUG (4-methyl-umbelliferyl-D-glucuronide) as a substrate for glucuronidase, which hydrolyzes to produce fluorescent 4-methyl-umbelliferone (MU) and glucuronide, which *E. coli* uses as a source of energy for growth (Edberg et al., 2000). Onitrophenyl-Dgalactopyranoside, a substrate for the -galactosidase enzyme found in all coliform bacteria, is also included in the Colilert test. To liberate the yellow o-nitrophenyl, this is hydrolyzed (ONP). Colilert tests for environmental coliforms only exhibit a yellow coloration, but *E. coli* additionally exhibits a blue fluorescence when exposed to long-wavelength ultraviolet (UV) light. 10 mL of water was initially added to a tiny test tube before using the Colilert procedure.

According to the EPA guidelines in effect in 1988, no coliform was allowed in a sample of 50 mL of water, and the degree of coliform contamination in a sample of 50 mL could be determined by inoculating five 10 mL P/A tubes. The usage of the Colilert changed from a 10 mL P/A to a 100 mL P/A in 1989 when the EPA standard changed to no coliform in 100 mL. In the U.S., Canada, and Japan drinking water markets, the Colilert test is now utilized in more than 90% of state laboratories after receiving approval from the U.S. Environmental Protection Agency (EPA) and international organizations (IDEXX, 2011). Since American drinking water standards are based on the absence of any coliform bacteria in 100 mL of water, a 100 mL Colilert P/A test is typically conducted on treated drinking water in the country (Parker, 2011). Thankfully, IDEXX continues to provide the 10 mL P/A test.

2.2.3 Interpretation of *E. coli* with Petrifilm.

A violet-red bile medium containing the glucuronidase substrate BCIG (5-Bromo-4chloro-3-indolyl-D Glucuronide) contains the *E. coli* Count Petrifilm, developed by the 3M Company (St. Paul, Minnesota), and approved by the Food and Drug Administration (FDA), as the most widely used test to detect *E. coli* in foods. A Petrifilm is filled with one mL of a food dilution, and it is incubated there for up to 24 hours at 35°C. A blue colony will grow if *E. coli* bacteria are present because BCIG is digested to liberate the insoluble blue BCI. Due to the lactose in the medium being fermented into acid and gas, gas bubbles also develop surrounding the *E. coli* and other coliform colonies (Schraft & Watterworth, 2005). The *E. coli* Count Petrifilm has been successful in identifying *E. coli* in raw water sources where *E. coli* concentrations are >1/mL, despite the fact that it was not designed to be used for drinking water in the United States and only samples 1 mL instead of 100 mL (Metcalf, 2006)

2.3 WHO Categories for Risk of Disease from Water Using the Colilert and Petrifilm Tests.

According to the WHO, each country's acceptable risk limits should be determined based on objectives for progressively improving its water supply (World Health Organization, 2017). Four WHO disease-risk categories may be identified by combining the findings of the 10 mL Colilert P/A test with the results of the 1 mL Petrifilm test based on variations in *E. coli* concentrations (Table 2). The Colilert P/A test's minimum sample size of 10 mL is crucial because it separates water with a low disease risk from water with high and extremely high disease risk. The Petrifilm test sample of 1 mL is crucial for identifying water sources with a high or extremely high risk of illness. A four-log variation in *E. coli* counts can be covered by the Colilert and Petrifilm tests when used together. These range from very low, >10 cfu in 1mL, to high, 1-10 cfu in 1 mL, and low, 1 cfu in 100 mL.

Table 2: 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

2.4 Water Testing Challenge in Low-Income Countries

Since the majority of the population has access to unimproved water sources, water testing is extremely important for low-income countries. However, because of the lack of resources and properly equipped laboratories, performing standard water quality tests is difficult, as a result, low-income countries' water testing standards should include the following: With regard to the faecal indicator *E. coli,* equipment to use, simply add water, simple to execute and requires little instruction straightforward to understand and link with WHO illness risk levels tested techniques that are affordable and readily available in the market (Metcalf, 2006) Near body temperature, which is 35–37°C, *E. coli* multiplies most quickly. Colilert tubes can be placed in a small bag or sock, and Petrifilm can be sandwiched between thin pieces of cardboard to incubate both tests close to the body in the absence of a suitable incubator if ambient temperatures are below 30°C and delay the appearance of positive tests. Results from both tests should be available in 12–24 hours.

These tests provide the option of body incubation, which is a significant benefit. The necessity to get samples to an incubator in a central laboratory, where bottleneck traffic might make this a time-consuming difficulty, is avoided for service providers testing in the distribution chain (Metcalf, 2006). Colilert and Petrifilm tests may be transported to communities in remote locations so that they may be infected and incubated there. This immediately informs the populace about the illness danger posed by drinking water sources.

2.5 The Portable Microbiology Laboratory

*A Practical Method for Rapid Assessment of the Bacterial Quality of Water, a field-based guide created by the UN Human Settlements Programme for UN-Habita*t, may be used without power, incubators, or laboratory equipment in the field (Metcalf Robert H. & Lars Onsager Stordal, 2010). Sterile plastic pipettes, Petrifilm, 10mL Colilert tubes, collecting bags, and a portable, long-wave UV light are all utilized to construct a "Portable Microbiology Laboratory." The materials for 25 of each test may fit within a one-gallon plastic bag since the tests can be stored at room temperature without the requirement for special storage conditions.

The Portable Microbiology Laboratory, created by Prof. Metcalf; enables water testing at the community level in poor nations to identify and quantify both *E. coli* and coliforms in ranges of concentrations similar to those have been discovered in prior investigations in Lower Nyando. The Colilert and Petrifilm tests are useful for usage in the field since they are ready to use with just the addition of water. A single *E. coli* cell in both tests would have to multiply 20–24 times, respectively, to attain the required *E. coli* numbers, which are 106 for a visible blue colony on Petrifilm and 107/mL for a MUG positive test in Colilert. If results are needed in 12 to 18 hours and ambient temperatures are below 30°C, one can incubate Petrifilm and/or Colilert tubes on their bodies because *E. coli* grows most quickly at a body temperature of close to 35°C (Metcalf Robert H. & Lars Onsager Stordal, 2010).

Additionally, because these tests are straightforward to do and interpret, it is possible to include community people in evaluating local water sources (Parker, 2011). Because the tests include the substrate for the beta-glucuronidase enzyme generated by *E. coli* but not by environmental coliform bacteria, they are the most often employed tests for the target indicator organism, *E. coli*, in the water and food sectors. The PML enables efficient fieldwork using the quick and simple *E. coli* test without the need for autoclaves, incubators, power, or any laboratory scientific knowledge.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Study Area

3.1.1 Location of the study area

This evaluation was conducted in Lower Nyakach, North Nyakach Ward near Lake Victoria in western Kenya, which has a population of 69,000 with over 60% living in extreme poverty. Lower Nyakach contains 180 small villages divided into 12 locations and two wards (Solar Cookers information, 2008). The Luo tribe represents the dominant ethnic population The Luo of Nyakach depends majorly on subsistence agriculture and fishing with some of them engaging in sand harvesting.

3.1.2 Main Water Sources

Lower Nyakach is one of the locations with insufficient sanitation, poor water resource management, and limited water coverage. The water sources were clustered as per WHO water source classification as per table 3

Table 3: World health organization water source classification

Improved Sources of Drinking Water	Unimproved Sources of Drinking Water
Piped water into dwelling, yard, or plot	Unprotected dug well
Public tap/standpipe	Unprotected spring
Tube well/borehole	Vendor-provided water
Protected dug well	Tanker truck water
Protected spring	Surface water (river, stream, dam, lake, pond, irrigation channel)
Rainwater collection	Bottled water*

3.2 Study Design

The study design was a comparative research design that aimed to evaluate and compare the Portable Microbiology Laboratory (PML) with the gold standard water testing method, Quanti-Tray 2000. The study employed both qualitative and quantitative approaches to analyze the similarities and differences between the two methods.comparative study design was adopted which entailed; comparing the PML to the gold standard water testing method Quanti-Tray 2000, drawing conclusions about them by identifying and analysing the similarities and differences in water testing both qualitatively and quantitatively.

Before choosing a location, a reconnaissance survey was carried out to familiarize with the research region, the nearby settlements, and the mapping of the community water sources from which samples would later be collected. The village elders provided the total number of villages and households in each community.

3.3 Sampling

The sampling zones and sample size were calculated using a ratio of one sampling zone for every 2,000 people, dispersed per the proportion of water sources that the locals of that place had access to (e.g. a location with a population of 10,000 would have 5 sampling zones selected).

3.3.1 Sampling Inclusion

The following water sources were chosen based on their accessibility, the fact that at least 10 local families used them, and their WHO classification:

- One of the unimproved sources in each zone that was chosen was randomly chosen and evaluated.
- One water source was randomly chosen for testing for each town that was chosen at random for enhanced level 1-stand-alone point source supply.
- One reservoir was chosen at random from each inlet that was chosen at random for improved level 2 communal point supply, and one of its outlets was examined. The reservoir outlet were the water sources used for the tests.

- One home that obtains water from these sources was randomly selected for testing in each zone for each village that was chosen at random for the improved level-3 private supply testing. In the zone, every tenth home had its water sources analyzed.

3.3.2 Sampling Exclusion

Chlorinated water source supplies were excluded.

Chlorine inactivates most pathogens that cause diarrheal disease in humans.

"A" (Absent, zero tolerance) in 100 mL of water is the WHO threshold for a very low risk of sickness using a P/A test of *E. coli* in water appropriate for human consumption. Additionally, industrialized nations with chlorinated water systems adhere to this drinking water standard (Parker, 2011).

Table 3.2: 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	Sampling Zones (SZ)	#Villages	#SZ	#Villages	#SZ	#Villages	#SZ	#Villages	#SZ	Total
Rangul	28	Y	N	Y	N	2056	7370	4	3	3	0	0	1	1	0	0	4
North Nyakach	22	Y	N	N	N	1775	7930	4	4	4	0	0	0	0	0	0	4
East Nyakach	14	Y	N	N	N	1003	3000	2	1	1	0	0	1	1	0	0	2
North East	25	Y	N	Y	N	2444	6370	3	2	2	0	0	1	1	0	0	3
Jimo East	18	Y	N	Y	N	3255	8010	4	3	3	0	0	1	1	0	0	4
Asao	22	Y	Y	Y	N	2209	5392	3	0	0	1	1	1	1	1	1	3
Agoro West	37	Y	Y	Y	Y	2551	15000	7	1	1	3	3	1	1	2	2	7
Total	166	6	5	6	2	15293	53072	27	23	14	4	4	6	6	3	3	27

Y – Yes N – No HH – Household SZ – Sampling Zone

3.4 Sampling procedures and transportation

The sampling procedure was conducted as follows:

i. Collection Method: Water samples were collected using 100 mL sterile Whirl packs. This collection method ensured a standardized and hygienic approach to sample collection.

ii. Transportation and Analysis: After collection, the samples were immediately transported to the laboratory in cold boxes to maintain the integrity of the samples. To minimize degradation or changes in water quality, the samples were analyzed within 4 hours of collection.

iii. Sample Labeling: Each sample was carefully labeled to include essential information, such as the location of sampling (e.g., household or water source), a description of the sample (e.g., inlet water or storage bucket water), an identification (ID) number, the date and time of sampling, the initials of the person collecting the sample, and any other relevant information. This comprehensive labeling ensured proper identification and documentation of each sample.

iv. Sample Tracking: The samples were tracked throughout the field-to-laboratory process using a sample tracking form. This form documented the relevant details of each sample, including its unique ID, collection location, date of receipt in the laboratory, and other pertinent information. The sample tracking system facilitated efficient organization, monitoring, and traceability of the samples during analysis.

3.5 Sample processing

3.5.1 PML method

i. Aseptic transfer: Using a sterile pipette, a volume of 10 mL of water was aseptically transferred from the well-mixed samples into the Colilert tubes.

ii. Petrifilm preparation: 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.

iii. Colony counting: After incubation, the Petrifilm plates were counted following the manufacturer's instructions provided in the 3M Petrifilm Plate Certificates. Blue colonies with a gas bubble around them were counted as E. coli, while blue colonies without a gas bubble were not considered as E. coli. The count of E. coli colonies on

one square of the Petrifilm plate was multiplied by 20 to estimate the amount of E. coli present. However, it is important to note that each Petrifilm plate has a suggested counting limit of 150 colonies (150 CFU/mL).

3.5.2 IDEXX Quanti-Tray 2000

i. Decant 100 mL of water in the Quanti Tray from the samples used for the PML method
ii. Seal the tray in Quanti-Tray Sealer PLUS.
iii. Incubate the tray and count positive wells, with reference to appropriate MPN table.

Quality controls: Controls for positive, negative, and proficiency tests were created in accordance with the laboratory's quality assurance strategy. Distilled water served as the negative control, while E. coli was used as the positive control using IDEXX Corp.'s Quanti-Cult.

Results reporting: All results were reported as the most probable number (MPN) of E. coli per 100 mL of water, as per the APHA guidelines. The MPN method is statistically equivalent to the CFU/100 mL designation.

3.6 Data Analysis

The Quanti-Tray 2000 and PML test results were tabulated to determine the disease-risk level for each source. A count of the point sources associated with each disease-risk level was produced for distinct source categories.

Frequency distribution tables were used for proportionate reduction in error calculation to determine the error for the PML test kit.

Given the classifications of unimproved and improved water sources, two statistical analyses were done to evaluate the precision of the PML as a tool for monitoring water quality. We used the formula below to determine error and reduction in error:

$$\lambda = \frac{\text{(error from knowing water source type)} - \text{(error from knowing water source type and additional water quality test)}}{\text{(Error from knowing the water source type)}}$$

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.

The t test was then used to determine whether the comparable PF Av x 100 were statistically significant to the MPN value. Using a p-value, the degree of statistical significance was assessed.

3.7 Data Management

To ensure data integrity, all electronic data was protected on password sites and computers, and physical data on lock and key cabinets.

3.8 Data Dissemination

The PML water quality test results were shared with the community members through the Village Access Facilitators. Copies of the thesis availed at the university library.

3.9 Ethical Consideration

This study adhered to all ethical considerations involved in the use of human participants, the PML, and Quanti – tray users. 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 Referral Hospital Ethics and Review Committee, and NACOSTI. The water sampling at sampling zones approval was obtained through FOTO by the local administration and the community.

CHAPTER FOUR

RESULTS

4.1 Determining risk levels for drinking water sources

Qualitative Analysis: In Table 4.1, 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). 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).

Table 4.1: 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**

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 4.2 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; with1 sample yielding low for PML and Moderate for Quanti – Tray.

Table 4. 2: *Escherichia coli* **counts and risk levels in lower NyakachTable**

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**

4.2 Performance of PML in reducing error relative to improved/unimproved water source designationon

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 4.3. 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 4.3.

Table 4. 3: Calculations for Error for PML – ColilertTable

		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**

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 4.4: 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 differenceχ^2 (2, n =13) = 30.78, d.f. =2, p <0.0001) as in Table 4.4.

Table 4. 4: Calculations for Error for PML – Improved water sourceTable

		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

4.3 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. 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 4.5).

Table 4. 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

To determine a comparable value for the Portable Microbiology Laboratory (PML), the average count of the Petrifilm test was multiplied by 100 (PF-MPN/100 = Av x 100). This adjustment was made because the Petrifilm test enumerates bacteria for a 1mL volume of water, while the Quanti-Tray 2000 measures for a 100mL volume. This conversion allows for a standardized comparison of results between the two methods and a t- test was done.

Table 4.6 presents the results of the sample t-test and p-test series for various locations and water source types. The table includes information on MUG (+ or -), OPG (+ or -), PF MPN/100 (Petrifilm Count and Most Probable Number per 100 mL), t-test, and p-value. For Agoro West, Kobita well. The t-test value is -71.0141, and the p-value <0.001. The negative t-test value suggests a significant difference between the Petrifilm and Quanti-Tray 2000 results. The (p <0.49) indicates that this difference is statistically significant at a high level of confidence this is consistent with the water sources whose PML petrifilm multiple count resulted in a number beyond the maximum MPN value and the

A high (P >0.5 <1.0), in the 5 water sources that the difference was not statistically significant has the PML petrifilm multiple count were close to the MPN count of Quanti Tray

Table 4. 6: Sample t-test and p-test series

Location	Source/types		MUG, + or - OPG + or -	PF MPN/100 Av x 100	Q-*2000E.coli MPN*/100	t-test	p-value
Agoro west	**Kobita well**	**I**	**OPG +**	**0.00**	**4.10**	**-71.0141**	**<0.001**
	Kanyarera dam	I	+	33.33	90.90	-1.72674	0.159
	Jeniffer tap	**I**	**OPG+**	**0.00**	**5.10**	**-88.3346**	**<0.001**
	Kanyalwal borehole	I	+	233.33	372.40	-1.57683	0.190
	Kolwal borehole	I	+	66.67	98.80	-0.96388	0.39
	Pawtenge borehole	I	-	0.00	0.00	-	-
	Kopige pond	**U**	**+**	**5866.67**	**2419.70**	**9.90479**	**<0.001**
Asao	**Samwel tap**	**I**	**OPG+**	**0.00**	**4.10**	**-71.0141**	**<0.001**
	Lisana borehole	I	-	0.00	0.00	-	-
	Koyuga pond	**I**	**OPG+**	**0.00**	**15.80**	**-24.8785**	**<0.001**
East nyakach	Komuono spring	I	+	66.67	16.90	1.49278	0.21
	River Sibion	U	+	833.33	920.80	-0.99175	0.38
Jimo east	Kajatap borehole	I	-	0.00	0.00	-	-
	Kamula dam	U	+	333.33	267.83	1.96488	0.121
	Ko-okoto dam	U	+	200.00	120.50	0.794992	0.47
	River Asao	U	+	1100.00	1119.90	-0.13028	0.90
N. nyakach	Oremo pond	U	+	500.00	456.90	0.373252	0.72
	River Awach	U		1333.33	1266.37	0.449494	0.68
	River Ataro	U	+	1800.00	1421.27	1.81813	0.143
	St. Alloys pond	U	+	1100.00	1413.60	-0.77596	0.48
North east	Katuk dam	I	+	166.67	93.50	2.19479	0.093
	River Sare	U	+	1500.00	1046.20	1.48541	0.21
	River Awach	**U**	**+**	**8300.00**	**2419.70**	**20.37**	**<0.001**
Rangul	Kochuka water tank	I	-	0.00	0.00	-	-
	Ka-Elias pond	U	+	933.33	1046.20	-0.46955	0.66
	Kajole pond	U	+	2600.00	2419.60	1.181	0.30
	River Nyando	**U**	**+**	**5666.67**	**2419.70**	**7.36343**	**<0.001**

CHAPTER FIVE

DISCUSSION

5.1 Determining P/A and risk levels for drinking water sources

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 North Nyakach. Improved and unimproved water sources were sampled in different locations and tested using PML and Quanti-Tray 2000 and risk levels were classified according to WHO.

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 (*WHO/UNICEF. (2019).*, n.d.). The sampled water sources had 70% recording high and very high-risk levels. This is concurrent with a baseline study in Lower Nyakach, which reported 75% of sampled sources being contaminated; the quality of the "raw" water from the 2017 study in the database, which has a 76 % chance of very high risk for disease (Blodgett, 2018). 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. It also shows the 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.

Testing for *E. coli* has been a crucial component of risk analysis studies. The findings of this study show that the Portable Microbiology Laboratory tests can fill this gap and help community units better detect and manage health risks associated with drinking and household use water since regulatory authorities' data on water quality in many LMICs are scarce, especially for rural areas and non-piped supplies (Kumpel et al., 2016) as even small improvements in water quality can have a significant positive impact on health, the Portable Microbiology Laboratory is especially well suited to identify these high and very high disease-risk sources. It can also help improve the early detection and management of health risks associated with contaminated drinking water (*Photo 25 of 127, The Goal Is ZERO*, n.d.)

As the responsibility of the service provider typically ends at the household connection or public tap, regulators rarely collect samples from inside the home; therefore, the 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 (Kumpel et al., 2016).

The idea of incorporating communities in water testing operations may alter how a community treats its drinking water (Metcalf Robert H. & Lars Onsager Stordal, 2010). Community-led monitoring has the benefit of fostering chances for behavior change communication, which is integrated into, among other behavior change initiatives, community-led water safety planning. The FOTO project's approach involves incorporating local communities in evidence-based microbiological testing of water supplies. Through this participatory method, the local community may identify and reduce the risks of pollution to their drinking water sources. Particularly when it offers a visual indication of polluted water, water quality testing can be crucial in raising community awareness. The prevalence of waterborne illness in Lower Nyakach has decreased as a result of FOTO's provision of affordable treatment options to low-income families. Since the intervention was first made available to 4,800 families in February 2012, FOTO has noticed a 73% decrease in the prevalence of diarrhea (Blodgett, 2018); according to survey findings, 95% of people treat their drinking water, with 65.7% of those people treating it after being introduced to FOTO's evidence-based microbiological behavioral change communication program.

5.2 Performance of PML in reducing error relative to improved/unimproved water source designation

Various E. coli tests may be included in point source monitoring (Bain et al., 2012); one of them uses an adapted membrane filtering method that produces accurate quantitative data (Brown et al., 2020). Field and test kit disposal costs are also very low, although it is anticipated that they will go down with time.

The sensitivity of 10 mL at 82.6% and a specificity of 100%; it is 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 (Saboor et al., 2021) with all the household reporting the test as an incentive enough 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 (*WHO/UNICEF. (2019).*, n.d.); 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.

Piped water, public standpipes and boreholes, secure drilled wells or springs, and even rainwater collecting are examples of improved drinking water technologies that are more likely to deliver safe drinking water than those that are considered to be unimproved (*WHO/UNICEF. (2019).*, n.d.). However, a lot more people than initially thought to; drink dangerous water from sources that have been upgraded since it is difficult to verify that it is safe to drink at the home level (Bain et al., 2012). Consequently, a sustainable water surveillance approach is required; PML is an effective test kit for predicting the risk levels of both improved and unimproved water sources. The Portable Microbiology Laboratory tests may be integrated into ongoing work with communities receiving universal health care and give prompt (within 24 hours), clear feedback to assist the communities in meeting the minimal WHO monitoring standards (Standridge, 2008). The utilization of PML tests within community health programs 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.

5.3 Determining PML reliability for point source surveillance testing

The results implies that both the PML field and Quanti-Tray methods provide similar outcomes when testing unimproved water sources. Some samples had Petrifilm *E. coli* levels that exceeded the maximum Quanti-Tray 2000 MPN count as a consequence of

multiplying averages of PF MPN/100 Av by 100; nevertheless, these counts were also statistically significant. The initial objective is often to increase access and the amount of water available such that all communities reach intermediate access service levels in order to satisfy health-based objectives in point source supply zones (Howard et al., 2010). 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 if the Portable Microbiology Laboratory was used in combination with a routine sanitary inspection. Once the community had access to evidence-based microbiology, it could independently identify sources of contamination and implement remedial measures, much as the FOTO and SWAP initiatives had done.

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 outline a disease-risk management framework (World Health Organization, 2017), this can be accomplished by the use of PML. Based on these findings, it can be concluded that the PML kit is a reliable field test method for the determination of contamination levels in water sources, both for improved and unimproved sources. The absence of significant differences between the PML field and Quanti-Tray methods suggests that the PML kit can be a suitable alternative to the Quanti-Tray 2000, providing accurate and comparable results in the field. To implement the recommendations within a national context, each country is also advised to develop a water safety plan (Water & Team, 2006). To effectively implement these recommendations at a national level, it is crucial for Kenya to develop a comprehensive water safety plan. The development of a water safety plan will require collaboration among various stakeholders, including national and county government agencies, water utilities, public health departments, and community representatives. This participatory approach will help ensure that the plan addresses the diverse water quality issues present and incorporates local knowledge and expertise.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the utilization of the Portable Microbiology Laboratory (PML) tests can effectively bridge the gap in water quality monitoring and enable communities to better identify and manage health risks associated with drinking water, particularly in high disease-risk areas. The study's findings support this conclusion by demonstrating that the risk levels determined by the PML and Quanti-Tray 2000 were consistent.

The PML's efficacy in differentiating between the risk categories acknowledged by the World Health Organization (WHO) validates its usefulness. The ability of the PML to provide a comprehensive range of risk assessments contributes significantly to establishing a foundation for the development of water policies and strategies.

The study reveals that while the Colilert test alone can provide reasonably accurate predictions, the addition of the Petrifilm test further enhances the accuracy. The PML, with its combined tests, proves particularly effective in identifying high and very high disease-risk sources, as evidenced by the calculations of error and proportional reduction in error for both unimproved and improved water sources.

In summary, the Portable Microbiology Laboratory offers a valuable tool for accurate and reliable water quality assessment. Its implementation can contribute to mitigating the risks associated with contaminated water and serve as a basis for informed decision-making in water management and public health policies.

6.2 Recommendations

Based on the findings of this study, the following recommendations are suggested:

i. The PML demonstrated its effectiveness in water quality assessment. Therefore, it is recommended that the PML be integrated into existing water monitoring programs and surveillance efforts. This will enable efficient and reliable identification of health risks associated with drinking water and prompt action taken.
ii. The findings of this study support the use of the PML as a practical and affordable method for water quality testing for both improved and unimproved water sources.

Thus, PML will facilitate consistent and widespread monitoring of water quality, ultimately leading to improved public health outcomes.

iii. Given the simplicity and ease of use of the PML, it is recommended for water quality monitoring at the community level.

6.3 Recommendation for Further Study

While this study provides valuable insights into the efficacy of the PML, further research is recommended to explore its applicability in different geographical regions and diverse water sources.

Ethnographic Research on Communication Strategies: This research will help implement agencies make informed decisions on data sharing and establish the best practices for communicating water quality information in a manner that is easily understood and actionable by the community.

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APPENDICES

Appendix 1: Sample tracking form

JSF SAMPLE TRACKING FORM

Sample particulars

Sample type:

Sample ID:

Date of collection:

Locality/Location:

Sample site/Source:

Sample site classification:

Unimproved.

Improved (Level 1). Improved (Level 2) Improved (Level 3)

Sampling containers used:

IDEXX bottles. Whirl packs. Others (describe)

Samples transported in cold chain to the laboratory: Yes.
No.

Sample triple packaged and received in cold chain into the laboratory:

Yes. No.

Sample test requested:

Microbiological (Bacteriology) Coliforms/E.coli Chemical analysis.

Samples collected by: Signature/Initials:

Samples received by: Signature/Initials:

Appendix II: Test Methods

The specific test products and methods evaluated and compared in this paper are described below:

1. IDEXX Quanti-Tray 2000®: The Quanti-Tray 2000® 2000 is an enzyme-substrate coliform test that utilizes the Most Probable Number (MPN) method for enumeration of contamination. The Quanti-Tray 2000® is both reliable and accurate, producing results from 100 mL samples with 95% confidence limits.

While EPA has approved it for wide use in the U.S., the high cost per test limits its suitability for resource-constrained settings. Moreover, in contrast to the other test to be performed, the Quanti-Tray 2000® 2000 requires a laboratory setting.

It is utilized in this study as the standard against which the performance of the other product is measured.

2. PML: *E. coli* /Coliform Count Plate (Petrifilm) are carded media plates that provide a quantitative count (Colony-Forming Units, or CFU) of total coliform bacteria and *E. coli* from a 1 mL sample.

The media contains differential indicator sugars that allow for differentiation between total coliform colonies (red with gas bubbles) and *E. coli* colonies (blue with gas bubbles).

Appendix III: Map of Lower Nyakach sampling locations

Above: Map of Lower Nyakach Sampling Locations

Appendix IV: List of sample frame

1. **Rang'ul Location**

N0.	Name of Villages	Households	Water sources	Total Population 7370
1.	**Kabuor Upper A**	**80**	✓ Kowano Pond ✓ Polo Tap Water ✓ Erick Borehole ✓ Koluoch Tap Water ✓ Kopuge Tap Water	
2.	**Kabuor upper B**	**50**	Nill	
	Kabuor upper C	**120**	✓ Ephraim Borehole ✓ Tap water Kasimon ✓ Kaperes Tap Water ✓ Tap water Kasalome ✓ Tap water Kamary ✓ Tap water Kowiyo ✓ Therdias Tap water ✓ Kongoya Pond	
3.	**Kabuor Lower**	**70**	✓ Borehole Kashem ✓ Borehole Kajoyce	
4.	**Kowuor Upper**	**91**	✓ Borehole Kaelias ✓ Dam Kasusana	
5.	**Kowuor Lower**	**75**	Nill	
6.	**Koyola**	**22**	Nill	
7.	**Koloo Bwaja**		✓ River Nyando	
8.	**Upper Koloo**	**20**	✓ Kabolo Borehole	
9.	**Jimo Kambero Village**	**45**		
10.	**Kangombe Kamgomre**	**120**	Nill	
11.	**Kangombe Kowiti**	**65**	✓ Hesbon Borehole ✓ Kahesbon Pond	
12.	**Kangombe Kakimiri**	**115**	✓ Kolang Borehole	

13.	**Komen Upper**	**102**	✓ Apida Pond ✓ Kandega Pond
14.	**Komen Lower**	**30**	✓ Kanyalwal Pond
15.	**Kobongo Upper A**	**50**	Nill
16.	**Kobongo Upper B**	**89**	
17.	**Kobongo Upper c**	**86**	
18.	**Kobongo Lower A**	**70**	✓ Omondo River
19.	**Kobongo Lower B**	**96**	nill
20.	**Kobongo Lower**	**60**	nill
21.	**Kogondi Kosenya A**	**60**	nill
22.	**Kogondi Kosenya B**	**57**	✓ Kajole Pond
23.	**Kogondi Kadhetho**	**90**	✓ Ondiek Borehole ✓ Barnaba Borehole
24.	**Kogondi Sirindwa**	**75**	✓ Wasare Primary Borehole ✓ River Ochuoga ✓ River Omondo
25.	**Kogondi Mariwa**	**57**	✓ River Asawo
26.	**Kogondi Lower**	**60**	
27.	**Kogondi Kandaya**	**64**	✓ River Nyando
28.	**Kogondi Kamuga**	**48**	

1. **North Nyakach**

NO.	Name of Villages	Households	Water source	Total Population 7930
1.	**Koloo A**	**140**	✓ River Awach ✓ canals	
2.	**Koloo B**			
3.	**Koloo C**	**163**	✓ Kongaro borehole ✓ Kanyasembo tap water ✓ Kolaka tap water ✓ Kambugu borehole ✓ River Awach	
4.	**Karabok A**	**60**	✓ Canals ✓ River Awach	
5.	**Karabok B**	**65**	✓ River Awach ✓ Kolal borehole ✓ Kaluoch tap water	
6.	**Karabok C**	**63**	✓ St. alloys pond ✓ Kangoya tap ✓ Kasimba tap	
7.	**Karabok D**	**57**	nill	
8.	**Kawakungu A**	**127**	✓ Kondijo tap	

			✓ River Nyando
9.	**Kawakungu B**	**120**	✓ River Nyando ✓ Kodima tap
10.	**Kosano A**	**128**	✓ River Awach ✓ Rae borehole
11.	**Kosano B**	**41**	✓ Karoko tap ✓ Kokombo tap
12.	**Kosano lwala**	**96**	✓ River Awach
13.	**Kasik Awach**	**80**	✓ River Awach ✓ Kojienda tap ✓ Kobwa tap
14.	**Kasik Atoyiengo**	**68**	✓ Kosimbo tap ✓ Oremo borehole
15.	**Kajieyi**	**56**	✓ River Awach ✓ Rae borehole
16.	**Kogelo Orayo**	**56**	✓ River Awach
17.	**Luala**	**96**	✓ River Awach
18.	**Karabok(Kanyo Chieng')**	**65**	✓ River Awach ✓ Kolwal borehole
19.	**Kanyamango**	**105**	✓ River Awach ✓ Kogera tap ✓ Komondi tap
20.	**Kaluth**	**64**	✓ River Awach ✓ Oremo pond ✓ Kacrito tap
21.	**Kanyikwaya**	**58**	✓ River Awach ✓ Ataro river
22.	**Karabok A (Matama)**	**67**	✓ River Awach ✓ Kolwal borehole ✓ Kanyarongo tap water

2. Jimo East

No.	Name of Villages	Households	Water source	Total Population 8010
1.	**Kouko Ola**	**250**	✓ Asawo river ✓ 15 tap water points	
2.	**Kogol**	**100**	✓ Asawo river ✓ Tap water	
3.	**Kasaye West**	**200**	✓ Asawo river ✓ 20 tap water points	
4.	**Kasaye Central**	**180**	✓ Asawo river	
5.	**Kasaye Cherwa**	**100**	✓ Kochura dam	

			✓ Kobura dam ✓ Tap water
6.	**Kabuor**	**300**	✓ River Asawo ✓ 10 tap water points
7.	**Kabura lower**	**145**	✓ Asawo river ✓ 5 tap water points
8.	**Kabura upper**	**180**	✓ Kokoto dam ✓ Asawo river ✓ 2 tap water points ✓ Koburu borehole
9.	**Kamula Upper**	**170**	✓ Asawo river ✓ Tap water
10.	**Kagure lower**	**150**	✓ Asawo river ✓ 5 tap water points
11.	**Kagure Upper**	**180**	✓ Kobura dam ✓ Tap water
12.	**Kagaya**	**120**	✓ Kamula dam ✓ Tap water
13.	**Kowala**	**160**	✓ Asawo river ✓ Tap water
14.	**Kanjira**	**180**	✓ Asawo river ✓ Tap water
15.	**Kakelo**	**170**	✓ Asawo river ✓ Tap water
16.	**Kochuka**	**270**	✓ Kamula dam ✓ 15 tap water points
17.	**Kamwana 4k**	**200**	✓ Asawo river ✓ Tap water
18.	**Kamwana welfare**	**200**	✓ Asawo river ✓ Tap water

3. North East

N0.	**Name of Villages**	**Households**	**Water sources**	**Total Population 6370**
1.	**Kanyibana A**	**98**	✓ Aluny borehole ✓ Tap water	
2.	**Manyatta**	**120**	✓ Tap water	
3.	**Waingu B**	**109**	✓ Awach river ✓ Tap water	

4.	**Upper Kodiwuor**	**120**	✓ Awach river	
5.	**Obingo central**	**108**	✓ Awach river	
6.	**Miyolo**	**110**	✓ Sare river ✓ Awach river ✓ Tap water	
7.	**Odeyo**	**130**	✓ Tap water	
8.	**Ache go**	**80**	✓ Borehole ✓ Tap water	
9.	**Kasrunda**	**132**	✓ Awach river ✓ Obaje borehole	
10.	**Kamango**	**98**	✓ Tap water	
11.	**Nyibana Obingo**			
12.	**Kamloma A**	**100**	✓ Kibogo borehole	
13.	**Kamloma B**	**96**	✓ Tap water	
14.	**Katuk**	**98**	✓ Katuk dam ✓ Tap water	
15.	**Waingu A**	**95**	✓ Wanga borehole ✓ Tap water	
16.	**Kanyibana B**	**110**	✓ Awach river ✓ Tap water	
17.	**Warieya Kanyagol**	**130**	✓ Ouma borehole	
18.	**Kamadhi**	**48**	✓ Awach river ✓ Tap water	
19.	**Maraba**	**96**	✓ Tap water	
20.	**Obingo Border**	**130**	✓ Sare river ✓ Tap water	
21.	**Cherwa**	**98**	✓ Cherwa borehole	
22.	**Obinju**	**110**	✓ Awach river ✓ 1Tap water	
23.	**Kibuon**	**60**	✓ Obam dam ✓ 1Tap water	
24.	**Siany**	**72**	✓ Awach river ✓ 1Tap water	
25.	**Warieya**	**96**	✓ Awach river	

4. Agoro West location.

No.	Name of Villages	Households	Water source	Total Population 1500
1.	**Kamorogi**	**56**	✓ River Awach ✓ 2Ponds	
2.	**Kanyibana Kowire**	**104**	✓ Borehole ✓ 4Ponds	
3.	**Kanyibana Katito**	**70**	✓ Borehole ✓ Tap Water	

4.	**Kanyagol Kojwach**	**86**	✓ Kopige Pond
5.	**Kanyagol Kopige**	**67**	✓ Kopige Pond
6.	**Kanyagol Olwa**	**42**	✓ Kopige Pond
7.	**Kanyagol Awach**	**40**	✓ River Awach
8.	**Kanyagol Nduga**	**43**	✓ Kogola Pond
9.	**Koyowe upper**	**58**	✓ Kasam Pond
10.	**Kauma**	**52**	✓ Atoyiengo River ✓ Ponds
11.	**Koyowe lower**	**62**	✓ River Awach ✓ Ponds
12.	**Kasrunda upper**	**74**	✓ River Awach
13.	**Warieya Upper**	**53**	✓ River Awach ✓ Pond
14.	**Warieya lower**	**48**	✓ River Awach
15.	**Kabongo A**	**84**	✓ Atoyiengo River ✓ Ponds
16.	**Kanyarera**	**62**	✓ Kanyarera Dam ✓ Kasam Pond
17.	**Kabudho A**	**56**	✓ Pond ✓ Kowire Borehole
18.	**Kabudho B (Kanyadani).**	**32**	✓ Kowire Borehole
19.	**Kabudho B (Koyoyo Area)**	**31**	✓ Kowire Borehole
20.	**Kabongo B**	**96**	✓ Kojwach Pond ✓ Kokungu Pond
21.	**Kamaina Kambuta**	**49**	✓ Pond ✓ Kowire Borehole
22.	**Kamaina Koswe**	**46**	✓ Pond ✓ Kowire Borehole
23.	**Kanyibana Kokungu**	**69**	✓ River Awach ✓ Pond
24.	**Kakeyo**	**54**	✓ Pawtenge Borehole ✓ Pond
25.	**Kanyibana Oseno**	**70**	✓ Borehole ✓ Karao Pond
26.	**Kanyibana Kowire**	**104**	✓ Kowire Borehole ✓ Pond
27.	**Kanyibana Katito**	**70**	✓ Palace Borehole ✓ Pond
28.	**Konuong'a**	**59**	✓ River Awach
29.	**Korinda**	**69**	✓ Awach River

30.	**Pawtenge complex**	**96**	✓ Kanyarera Dam
31.	**Kamron**	**55**	✓ Pawtenge Borehole
32.	**Katito A**	**123**	✓ Borehole ✓ 1 Tap Water Point
33.	**Katito B**	**113**	✓ Borehole ✓ 1 Tap Water Point
34.	**Katito C**	**117**	✓ Borehole ✓ 1 Tap Water Point
35.	**Katito D**	**127**	✓ Borehole ✓ 1 Tap Water Point
36.	**Kasrunda Middle**	**48**	✓ River Awach
37.	**Kasrunda Lower**	**66**	✓ Pond

5. Asao Location

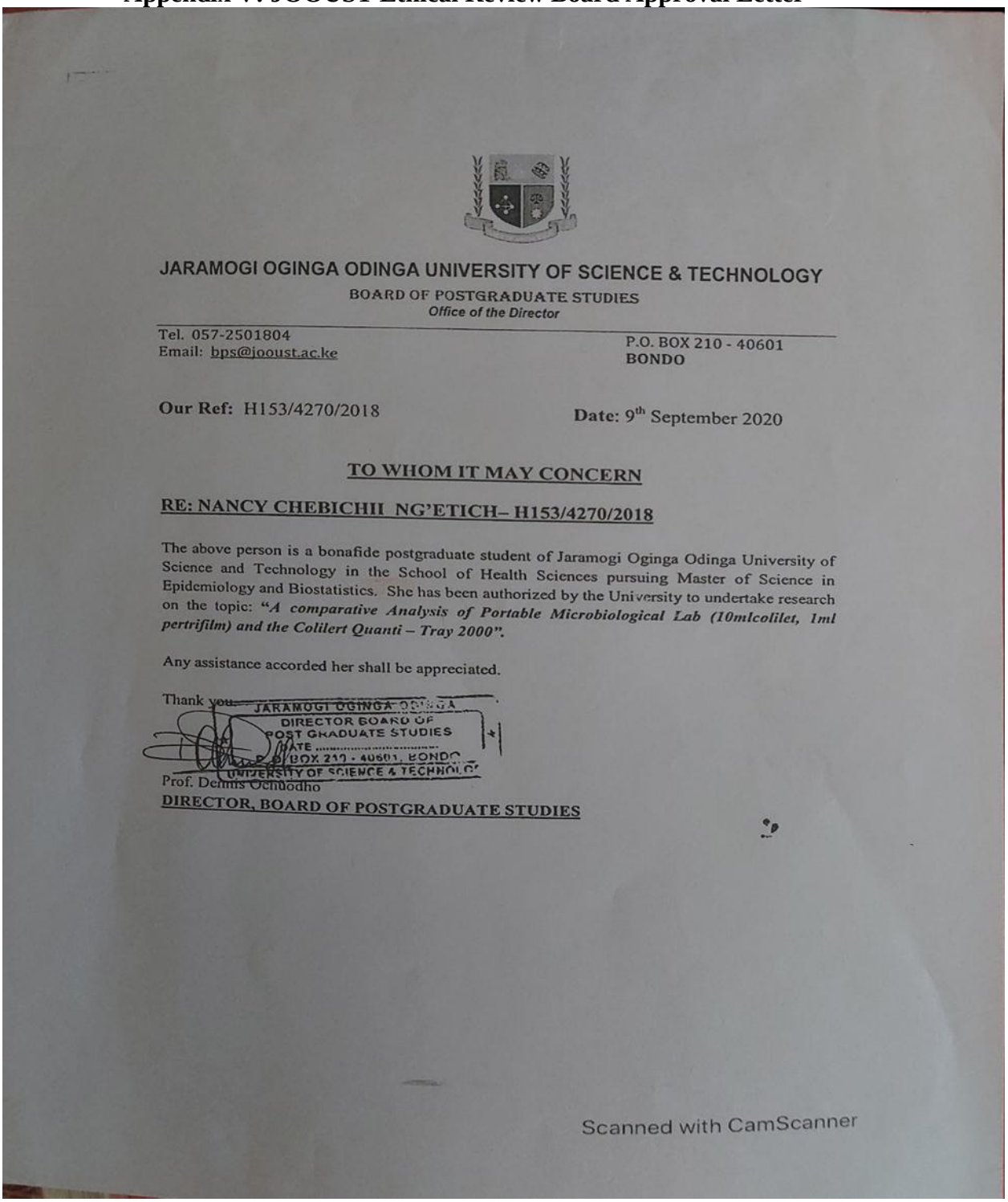
No.	Name of Villages	Households	Water source	Total Population 5392
1	**Kochola west**	**79**	✓ River Asawo ✓ 1 tap water point	
2	**Kochola Central A**	**150**	✓ 2 boreholes ✓ 1 tap water point	
	Kochola central B	**170**	✓ River Asawo	
3	**Kochola East B**	**30**	✓ 1 borehole	
4	**Kochola East A**	**52**	✓ 1 tap water point ✓ borehole	
5	**Kochola East C**	**22**	✓ borehole	
6	**Kolal West**	**130**	✓ 2 ponds ✓ River Asawo	
7	**Kolal Central**	**78**	✓ borehole	
8	**Kolal East**	**140**	✓ 3 ponds ✓ River Asawo	
9	**Karandiga**	**48**	✓ 1 tap water point ✓ Borehole ✓ Dam	
10	**Kanyago West**	**115**	✓ 3 ponds	
11	**Kanyago North**	**72**	✓ Borehole ✓ pond	
12	**Kanyago Central**	**120**	✓ borehole ✓ 1 tap water point	
13	**Kanyago East**	**100**	✓ borehole	
14	**Kowinyo**	**60**	✓ borehole	

	West		✓ pond
15	**Kowinyo East**	**108**	✓ 1 tap water point ✓ River Asawo
16	**Kobunde North**	**173**	✓ Borehole ✓ 3 ponds
	Kobunde South	**85**	✓ 3 ponds
17	**Kotiang**	**100**	✓ Borehole ✓ River Asawo
18	**Kotula West**	**38**	✓ Borehole ✓ River Asawo
19	**Kotula East**	**64**	✓ Borehole
20	**Kawiti West**	**170**	✓ Borehole ✓ 1 tap water point
21	**Kawiti East**	**60**	✓ 1 tap water point
22	**Kanyamlori Border**	**45**	✓ 2 ponds ✓ borehole

6. East Nyakach Location

No.	Name Of Villages	Households	Water source	Total Population 3000
1	**Nduga**	**85**	✓ Sibuon river	
2	**Doll**	**80**	nill	
3	**Thim**	**90**	✓ Kasalak pond	
4	**Ligusa**	**109**	✓ Kawere spring ✓ Komwono spring ✓ Kajabuya pond	
5	**Soko A**	**75**	✓ Ligusa river	
6	**Soko B**	**45**	✓ Kanyaundi spring	
7	**Achia**	**80**	✓ Kanyaoke spring	
8	**Koloo**	**92**	✓ Ligusa river	
9	**Bungu**	**70**	✓ Moro Stream	
10	**Sibuon**	**96**	✓ Kobudo spring	
11	**Lorgot**	**45**	nill	
12	**Withur**	**60**	nill	
13	**Ridri**	**40**	✓ Kaka Chama pond	
14	**Maram**	**36**	✓ Michura pond	

Appendix V: JOOUST Ethical Review Board Approval Letter



JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE & TECHNOLOGY

BOARD OF POSTGRADUATE STUDIES

Office of the Director

Tel. 057-2501804
Email: bps@jooust.ac.ke

P.O. BOX 210 - 40601
BONDO

Our Ref: H153/4270/2018

Date: 9th September 2020

TO WHOM IT MAY CONCERN

RE: NANCY CHEBICHII NG'ETICH– H153/4270/2018

The above person is a bonafide postgraduate student of Jaramogi Oginga Odinga University of Science and Technology in the School of Health Sciences pursuing Master of Science in Epidemiology and Biostatistics. She has been authorized by the University to undertake research on the topic: ***"A comparative Analysis of Portable Microbiological Lab (10mlcolilet, 1ml pertrifilm) and the Colilert Quanti – Tray 2000".***

Any assistance accorded her shall be appreciated.

Thank you.

JARAMOGI OGINGA ODINGA
DIRECTOR BOARD OF
POST GRADUATE STUDIES
DATE .....
P.O. BOX 210 - 40601, BONDO
UNIVERSITY OF SCIENCE & TECHNOLOGY

Prof. Dennis Ochiodho
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Scanned with CamScanner

Appendix VI: JOORH Ethical Review Approval Letter

COUNTY GOVERNMENT OF KISUMU
DEPARTMENT OF HEALTH

Telephone: 057-2020801/2020803/2020321
Fax: 057-2024337
E-mail: ercjoorth@gmail.com

When replying please quote

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Ref: IERC/JOOTRH/396/21

Date: 23rd April, 2021

To: Nancy Chebichii Ngetich

Dear Nancy,

RE: STUDY TITLE:-
A COMPARATIVE ANALYSIS OF PORTABLE MICROBIOLOGICAL LAB (10MLCOLILERT, 1ML PERTRIFILM) AND THE COLILERT QUANTI-TRAY 2000, NORTH NYAKACH WARD, KISUMU COUNTY

This is to inform you that ***JOOTRH IERC*** has reviewed and approved your above research proposal. Your application approval number is ***IERC/JOOTRH/396/21***.The approval period is ***23rd April, 2021 – 23rd April, 2022.***
This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***JOOTRH - IERC***.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***JOOTRH - IERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***JOOTRH - IERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JOOTRH - ***IERC***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

In case the study site is JOOTRH, kindly report to Chief Executive Officer before commencement of data collection.

Yours sincerely,

SECRETARY, IERC

Appendix VII: NACOSTI Ethical Review Approval Letter

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **537582**

Date of Issue: **25/May/2021**

RESEARCH LICENSE

This is to Certify that Miss.. Nancy Chebichii of Jaramogi Oginga Odinga University of Science and Technology, has been licensed to conduct research in Kisumu on the topic: A COMPARATIVE ANALYSIS OF PORTABLE MICROBIOLOGICAL LAB (10mlcolilert, 1ml pertrifilm) AND THE COLILERT QUANTI - TRAY 2000, , NORTH NYAKACH WARD, KISUMU COUNTY for the period ending : 25/May/2022.

License No: **NACOSTI/P/21/10672**

537582

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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