Use of Microbiotest Assay and Membrane Filtration Plate Culture Methods in Screening of Microbiological Well Water Quality, Uasin Gishu County- Kenya

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Abstract

Residents of many urban centers including Eldoret are increasingly dependent on groundwater for drinking purposes. This study examined the microbiological suitability of well water available for drinking purposes among residents within Eldoret town. Fifteen sampling stations were established in three residential estates and sampling was done during the wet and dry seasons of the year. The samples were analyzed for the presence of adenosine triphosphate (ATP), using the ATP Microbiotest ® method and confirmed with membrane filtration plate culture method. Results showed that there was a significant difference in the number of bacterial colonies in the sampled sites using the two analytical methods ($\chi 2$=0.867, p=<0.001, $\chi 2$=3.200, p<0.001) in both the dry and wet seasons respectively. This paper reports high levels of bacterial count during the wet season than in dry season and no significant correlation between relative light units(RLU), *Escherichia coliform (E. coli)* and distance of the pit latrine from the wells in all three sampled residential estates (p>0.05). The ATP Microbiotest ® method produced relative light unit (RLU) values which correlated positively with colony forming units from plate culture method (r=0.64, p=<0.001). The study also showed that wells in the high density residential areas had the greatest number of bacterial contaminants expressed as ATP than the wells in the low density residential areas. Thus the ATP Microbiotest ® method should adequately be applied in the household rapid screening of microbial well water contaminants.

Key words: Microbiotest, RLU, ATP, *E. coli*, culture

Introduction

Natural waters contain living organisms, which like all living creatures on earth will at one moment of time die and be decomposed by bacteria. The amount of microbes in water is therefore a signal for the quantitative importance of bacterial decomposition of biological residues (Nuzzo, 2006) proximity to pit latrines and this may be associated with high rates of waterborne infections (Bianci and Lopardo, 2003). The increasing population of cities and towns by natural growth and by migration from rural areas has made the urban population to be split according to their economic and social well being, and this has eventually lead to high, medium and low residential areas within the towns and cities. However in search of the very basic human needs various anthropogenic activities have been initiated within this residential areas leading to the differences in the groundwater quality (WHO/UNICEF, 2004). All people, whatever their stage of development and their social and economic conditions, have the right to have access to drinking water in quantities and of a quality equal to their basic needs. However in the densely populated and low income areas people have always encountered a lot of challenges in pursuit of the fulfillment of this right. The microbial evaluation of the drinking water has been a major challenge in these highly populated areas. Microbiotest offers a convenient option for measurement of bacteriological quality of water (Chen *et al.*, 2006). The energy source of all living organisms, including bacterial is ATP and the total ATP content of a water sample is composed of both “intracellular” ATP from living biota and “extra cellular” ATP which is the ATP released from dead organisms (Masuda *et al.*, 2000). ATP Microbiotest is a rapid assay that has become a widely accepted method to monitor drinking water sources and supplies. In the presence of firefly enzyme system (luciferin and luciferase system) from *Vibrio fischeri* bacteria, ATP facilitates the reaction to generate light. The light can be measured by a luminometer and used to estimate the biomass of microbial cells contaminating a water sample (Chen and Sandria, 2006). However an effective surveillance programs on the bio indicators of water quality depends on the existence of national regulatory standards of water quality and codes of practice. This, in turn depends on appropriate national legislation and the establishment of a component surveillance unit or agency within government like National Environmental Management Authority (NEMA) in Kenyan government (WHO/UNICEF, 2004).

Bioluminescence in bacteria can be used to quantify microbial contaminants in groundwater through regulation phenomenon known as auto induction. Auto induction or quorum sensing was first discovered in *Vibrio fischeri,* which is cell-to-cell communication that ties gene expression to bacterial cell density. Quorum sensing involves the self-production of a diffusible pheromone called an auto inducer (AI), which serves as an extra cellular signal molecule that accumulates in the medium and evokes a characteristic response from cells. Using bioluminescence, once the concentration of the AI reaches a specific threshold (above 10^7 cells mL^{-1}), it triggers the energetically costly synthesis of luciferase and other enzymes involved in luminescence. Thus, by sensing the level of AI, the cells are able to estimate their density and ensure that the luminescent product will be sufficiently high to cause an impact in the environment, making the process cost-effective. The AI for *V. fischeri*, N-acylhomoss serine lactone (AHL), was once thought to be species-specific; however recent studies have established that AHL can serve as a signaling molecule for more than 16 genera of gram-negative bacteria. This suggests that the AI protein can facilitate interspecies communication, allowing quorum-sensing bacteria to monitor the population of other species as well as their own. Quorum sensing is now a widespread regulatory mechanism in bacteria, particularly among a number of pathogens, influencing their ecology and associations with eukaryotic organisms (Masuda *et al*., 2000)

In Eldoret municipality there are increased human activities particularly the indiscriminate location of septic tanks, soak away pits and pit- latrines, disposal of refuse and waste, and other materials that can leach into the groundwater posing a major health concern. The municipality population increase of 3.35 % annually has over- stretched the supply of the essential services such as sewerage system and consistent water supply. As a result many households have resulted to the sinking of shallow wells, pit latrines and septic tanks as a source of water and domestic waste management respectively.This practice is rampant in the peri- urban densely populated areas of Langas, Kimumu, Huruma, Kahoya; Munyaka and Kapyemit residential areas (Eldoret Municipal Council, 2010). The study focused on Langas, Kimumu, and Elgon View estates in Eldoret Municipality with the main objective of determining the effectiveness of rapid Microbiotest assay as a method of assessing and monitoring well water quality in high, medium and low density residential areas . The geology of Eldoret town and its environs is mainly made up of metamorphosed basement system formation, overlain by a sequence of tertiary volcanic strata. The basement system rocks weather to a pink or brown sandy soil which is lighter in texture and less fertile than the phonolite soils (Otieno, 2001). The phonolite soil weathers initially to a red brown murram and eventually to friable silt clay, which is very fertile and drains freely hence suitable for agriculture (Hillel, 2004). Poor permeability, porosity and the storage capacity of the rocks in the Eldoret area has set close limits on groundwater exploitation of shallow wells (such as those found in Langas and Huruma) and produce groundwater contained in the joints of the weathered phonolite.

Methodology

Sampling design and procedure

Purposive sampling method was employed to select residential areas from the municipality to be included in the study. The criterion used in selection was population density and the Municipal service provision like sewer lines and piped water. Thus the Municipality was divided into three strata mainly high population, medium population and low population density residential areas. The selected study areas thus included Elgon View (low density), Kimumu (medium density) and Langas (high density). Stratified random sampling was used to select households based on their water sources and consumption points. All the functional wells were identified from each selected residential area and serial numbers assigned. The wells were then randomly selected based on the serial numbers already given. Locations of selected wells were geo-referenced using Geographical Positioning System (GPS).

Sample Collection

Water samples for bacteriological analysis were collected in 200 ml sterile water sampling bottles which were kept unopened until the time of filling. The cork was removed and bottle held by the other hand around the base of the bottle and it was ensured that the sample bottles were not rinsed with sample during collection and the bottles were not completely filled to allow for shaking prior to analysis. The sampled wells were considered to be representative of the entire study area. For wells equipped with a pump, it was operated for a bout ten minutes to clear any standing water in the water column. The outlet pipe was then sterilized using a flame from a burning cotton swab soaked in Methylated spirit. The pump was operated and allowed to run for 2 minutes and sample collected from the flowing stream of water. Where a bucket was used to draw water a sample from the water collector's bucket was taken, as this was more representative of what was actually being consumed by the household. The sample was poured into the sample bottle directly from the bucket (Yolanda *et al*., 2007). Seven replicate samples were collected both from Langas and from Kimumu and one from Elgon View. The physicochemical analysis of the water samples like pH, temperature and conductivity were all done at the site. The samples were stored in cool boxes containing ice which kept the temperatures between 4^{o}C and 10^{o}C and then taken to the laboratory for analysis. Bacteriological analysis of the samples was done in Eldoret Water and Sanitation Company (ELDOWAS) laboratories in Eldoret and the Moi University Biotechnology Center (MUBC).(Yolanda *et al*., 2007)

Microbiotest technique

A sterile syringe was used to draw 10 ml of the water samples from the sample bottles. A Filtravette™ which is a combination of a filter and a cuvette with a pore size of 0.45µm was placed into a 13-mm Swinex® filter holder. The filter holder was screwed onto the syringe and the water sample was pushed through the filter (Dostalek and Branyik, 2003).The Filtravette was removed from the filter holder after filtration of the water sample and was placed onto a sterile blotting paper. A somatic cell releasing agent (NHD) was used to lyse all non bacterial cells and to release the ATP. With a specially converted 10 ml syringe, air pressure was applied to remove the nonbacterial ATP through the filter. At this stage, the Filtravette retained bacteria on top of the membrane filter, and the bacterial ATP remained within the bacterial cell membranes throughout this step of the procedure. The Filtravette was inserted into the microluminometer and the bacterial cell releasing agent was then added to lyse the bacterial cells retained on the surface of the filter. The released bacterial ATP was mixed with 50 µl of luciferin–luciferase obtained from *Vibrio fischeri.* The light emission was recorded after a 10-sec integration of the light impulses; the unit was called a relative light unit (RLU). The results were expressed as RLU/ml by dividing the RLU values by the filtered water volume. The detection limit and sensitivity of the luminometer was tested with a serially diluted standard ATP solution, diluted five times, which gave a directly proportional linear relationship between RLU and ATP. Distilled water was used for the dilution. The activity of the luciferin–luciferase was checked by using an ATP standard. The assay was based on the reaction between the luciferase, luciferine and ATP. Light emitted during the reaction was measured quantitatively using the luminometer indicated in Fig 3.1 and correlated with the ATP quantity extracted from bacteria in the water sample. The RLUs are proportional to the amount of ATP, and the amount of ATP is proportional to the number of bacteria within the water sample analyzed (Dostalek and Branyik, 2003).

Membrane Filtration plate culture Technique

The membrane filtration procedure involved the use of membrane filters of 0.45 microns pore size and sterile Petri dishes for holding the sterilized selective media.

The Erlenmeyer flask was connected to the vacuum source and the porous support placed in position with a second flask placed in between the vacuum pump and the Erlenmeyer flask to act as a water trap and protect the electric pump. The filtration unit was assembled by placing a sterile membrane filter on the porous support, using forceps sterilized by flaming and then the upper container was placed in position and secured with special clamps. One hundred ml well water sample was poured into the upper container of the filtration unit then when all the sample had passed through the filter the vacuum pump was disconnected. The filtration unit were taken apart and using the forceps the membrane filter was removed and placed in the Petri dish containing the Endo agar medium with the grid side up without introducing air bubbles. The Petri dishes were inverted and incubated at 37^{o}C for 24 hr (Rivera *et al.,* 2007).

Colonies of Coliform bacteria were medium red or dark red in colour, with a greenish gold or metallic surface sheen. In most cases the sheen covered the entire colony while in others it appeared at the center of the colony. The colonies were counted with the aid of a glass lens. The number of total Coliform per 100 ml was calculated as a percentage number of Coliform colonies counted over 100 ml of the well water sample filtered (Rivera *et al.,* 2007).

Data analysis

The tabulated data was coded in data forms and entered into a computer database. Data was analyzed using SPSS16 computer package and presented in frequency tables and graphics. Median rank test was used to compare differences in the median bacterial count between wells in high, low and medium density areas. Statistical significance for each variable was also calculated in order to draw appropriate conclusions and test the stated hypotheses. All tests were considered significant at 5% alpha level.

Results

Determination of microbial count within wells in high, medium and low residential

Results from the three studied sites were analyzed both in the dry and wet seasons and presented in table 1 showing the degree of contamination.

Table 1. Median IQR of Microbial count using the ATP Microbiotest ® method

Location	Dry season		Wet season	
	Median microbial count		Median microbial count	
	Direct	Filtration	Direct	Filtration
Langas	1437(1368,1674) (+++)	47900 (45600-55800) (++++)	1790 (1650-192) (+++)	50000 (36000-61500) (++++)
Kimumu	683(570,1020) (++)	22750 (19000-34000) (++++)	900 (788-1200) (++)	30000 (26250-40000) (++++)
Elgonview	444 (++)	14800 (++++)	825 (++)	27500 ++++

Key: Very high ++++ High +++ Significant ++ Relatively low + Very low _

Direct ATP Microbiotest® and the filtration ATP Microbiotest® methods were used concurrently to determine the level of bacterial count in the wells studied. The filtration ATP Microbiotest ® method reported a high number of bacteria with median of 47900(45600,55800) in Langas, 22750 (19000, 34000) in Kimumu and 14800 in Elgon View for the dry season and 50000 (36000,615000) counts in Langas, 30000(26250, 40000) in Kimumu and 27500 in Elgon View for the wet season as compared to the direct ATP Microbiotest® with median of 683(570, 1020) and 900 (788, 1200) in the dry and wet seasons respectively as shown in table 1.

(A) Direct test screening values

RLU VALUE	DEGREE OF CONTAMINATION	NOTATION
< 50	Very low	-
< 200	Low	+
200-1000	Significant	++
> 1000	Very high	+++

(B) Membrane filter test screening values

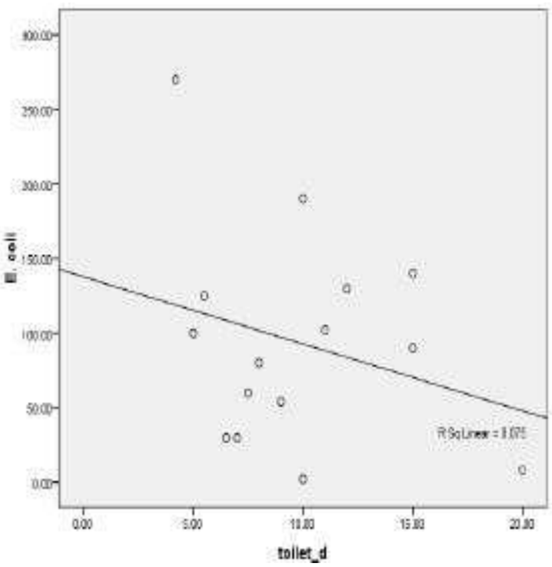
RLU VALUE	DEGREE OF CONTAMINATION	NOTATION
< 200	Very low	-
< 1000	Relatively low	+
> 1000	Significant	++
> 5000	High	+++
> 10000	Very high	++++

Tables A and B above show the standard ATP measurements in form of relative light units (RLU) with the equivalent notation howing the degree of contamination.

Source: Microbiotest® www.microbiotests.be

ATP detection rate and sensitivity

The filtration ATP Microbiotest® method was able to concentrate the bacterial ATP thus increasing the detection and sensitivity rate by an average of 31.5 times than that of Direct ATP Microbiotest ® method. The filtration method was found to be more sensitive in both dry and wet seasons with minimum detection limit (MDL) being <50 and <200 RLU for direct ATP microbiotest and filtration ATP microbiotest methods respectively. The analytical sensitivity of the assay is usually between 0.2 to 20,000pg as established by Chen *et al.*, 2006 using serial dilutions of ATP calibration standards.



Toilet_d= Distance of the pit latrine from the well

r=-0.273, p=0.325

There was a negative correlation between the number of *E .coli* and the distance of the well from the pit latrine (r=-0.273, p=0.325) as indicated in figure 1, though it was not statistically significant (p> 0.001) as in the ATP Microbiotest ® method. The outliers recorded were due to the short distance of the well and the gradient of the well from the pit latrine.

Fig .1: Relationship between *E .coli* and toilet (pit latrine) distance

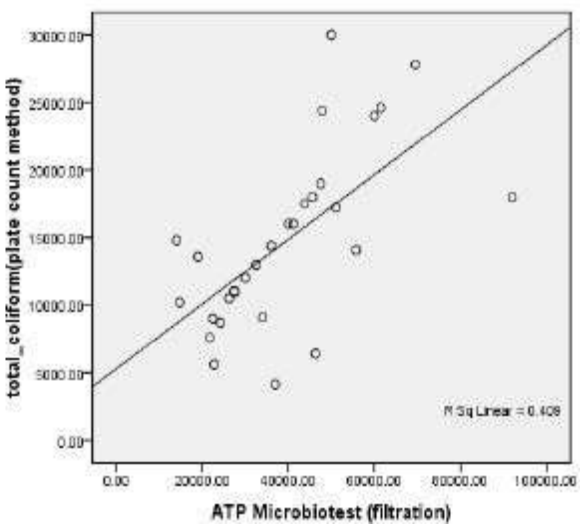
ATP Microbiotest ® method versus conventional membrane filtration plate culture technique

Using the ATP Microbiotest ® method, the median bacterial count for direct ATP microbiotest® and filtration ATP microbiotest® were 1110(653, 1437) and 37000(21750, 47900) per 100mls of water for dry season, and 1300(825, 1790) and 36000(27500, 50000) per 100mls of water for wet season. Using the conventional membrane filtration plate culture method the median bacterial count was 13600(7600, 17200) Cfu and 14400(11000, 24000) Cfu for dry and wet seasons, respectively. Further there was significant difference in the number of total bacterial count per 100ml using the conventional membrane filtration plate culture method and ATP Microbiotest ® method (χ^2=0.867,p=<0.001, $\chi2$=3.200,p<0.001) for dry and wet seasons respectively.

Table 2: Relationship between ATP Microbiotest® rapid assay method and the conventional membrane filtration plate culture methods

Sampling site	MFT (Conventional Method)	Microbiotest	
	Median Total coliform count	Median Direct Count	Median Indirect count (Filtration)
Langas	13,600 (7600-17200) (++++)	1,790 (1650-1920) (++++)	50,000 (36000-61500) (++++)
Kimumu	14,400(11000-24000) (++++)	900 (788-1200) (++)	30,000 (26250-40000) (++++)
Elgon View	12,350 (++++)	825 (++)	27,500 (++++)

The Microbiotest® method showed high values of bacterial count as compared to the membrane filtration technique as shown in table 2. This in turn increased the sensitivity of microbial detection using the Microbiotest filtration technique (indirect) by an average value of 2.7 times in comparison to conventional method. The median direct Microbiotest® count method sensitivity was found to increase by 31.50 times to that of the indirect Microbiotest technique (filtration).



r=0.64, p=0.002

As indicated by the scatter plot in fig 2, there was a significant positive correlation between Cfu and RLU(r=0.640, p=0.002) which showed that, as the ATP numbers (RLU) increases, the amount of bacterial total coliform units (Cfu) also increased. There was a significant difference in the number of bacterial counts between wells with top cover and those without using the ATP Microbiotest ® method which measured the amount of bacteria in RLU (χ^2=0.067, p= 0.037 and χ^2=3.200, p= 0.021and) respectively. Those without top cover had the highest bacterial count as compared to those with top cover.

Fig 2: Relationship between ATP Microbiotest ® method and Convectional membrane filtration plate count method

DISCUSSION

From the study it was found that most of the wells were poorly constructed in almost all the sampled locations, and the ones which were properly constructed with a top then a pit latrine was found located just a few meters from the well, therefore increasing the amount of microbial contaminants. In Elgon View a low population density area of the Eldoret Municipality there was an extensive use of the sewerage system and the low contamination reported was thought to be from the leaking sewer pipes since the existence of a public sewer system does not assure that other sources of wastewater don't contaminate the aquifers (Hirata *et al.,* 2002).

However no significant relationship in electrical conductivity between wet and dry seasons in Langas, Kimumu and Elgon View areas of the municipality. This implies that while seasonal changes caused variations in electrical conductivity levels, rainfall had no significant influence. The findings suggest that direct ingress of bacterial contaminants through the study soils to the well is prevented due to low permeability of the soils. Temporal linked variations could therefore be attributed to the precipitation of ions to the sediment and evaporation or changes in pollution levels, Spatial linked variation could only be as a result of the differences in the soluble mineral content of the geological material (Otieno, 2001). The median pH for the well water obtained in Langas, Kimumu and Elgon View were found to be within the recommended range by WHO and NEMA being 6.5-8.5 and 6.0 and 9.0 respectively. The recommended range of pH for drinking water acceptable by World health organization(WHO) standards is 6.5-8.5 and 6.0 and 9.0 respectively (Harp, 2000). The study further showed that the median ATP microbiotest bacterial counts were low during the dry season and high in the wet season.

These results when compared to plate culture method showed high bacterial counts in both dry and wet seasons respectively and that the two methods showed a positive correlation when compared together. The results were therefore comparable with a previous study by Chen *et al.*, 2006 on comparison of a rapid ATP Microbiotest® assay and standard plate count methods for assessing microbial contamination of water. A strong positive correlation between relative light units (RLU) and colony forming units (Cfu) in drinking water samples was established as shown in fig 2. In his findings Chen *et al.*, 2006 found out that the ATP Microbiotest® method detected more bacterial contaminants for the same amount of water sample measured than the convectional plate culture method. Both the direct and filtration ATP Microbiotest® method proved to be more reliable and sensitive in the screening of the microbial contaminants which were detected high above those of the convectional membrane filtration plate culture method. The findings from this study therefore seemed to compare very well with those of Chen *et al.*, 2006 as stated above and those of Hirata *et al.* ,2002 were he found out that Poorly constructed wells were the cause of elevated pathogenic bacterial contamination in the Patino Aquifer in Asuncion, Paraguay, where 70% of drilled wells are contaminated with feacal coliforms. Hirata *et al.*, 2002 also found out that in the medium density and low density residential areas of Sao Paolo, Brazil, 60% of drilled wells without top covers had high counts of pathogenic microbial contamination. In another study done by Mangore and Taigbenu (2004) in Zimbabwe it was found that 27% of wells in Bulawayo were contaminated with coliform bacteria which were thought to be caused by leaking sewers.

Therefore from the above study ATP Microbiotest® method provided a rapid means of enumerating total numbers of viable bacterial cells than the convectional membrane filtration plate culture method, and that all the wells in the sampled locations of Eldoret Munipality Uasin Gishu County were all found to be feacally contaminated.

However the above study experienced a shortage of ATP Microbiotest® reagents thus limiting the sampling locations(residential estates) to three which represented the low, medium and high density residential areas within the Municipality.

CONCLUSION

The results showed that ATP Microbiotest® method provided a rapid means of enumerating total numbers of microorganisms in well water as compared to the other conventional methods like the membrane filtration plate culture method and the most probable number (MPN) methods. The high density residential area namely Langas had the highest number of bacterial contaminants. The study also showed that there was no significant correlation between groundwater qualities in all the three studied locations because the sources of groundwater contaminations were all different from each location. The construction of pit latrines, leaking sewer pipes, and poor abstraction methods were the main cause of contamination or increased level of bacterial ATP count

RECOMMENDATION

The Municipality should provide an increased coverage of the Sewerage system in all the residential areas especially the high density areas where the most urban poor live. The Municipality must also develop a clear definition of water rights (separate from property rights) by enforcing licencing and fee payment for sustainable groundwater exploitation.

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