



Full Length Research Article

COMPARATIVE ASSESSMENT OF FIELD METHODS FOR MICROBIOLOGICAL WATER QUALITY TESTING IN EMERGENCIES

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ARTICLE INFO

Article History:

Received 28th April, 2015
Received in revised form
13th May, 2015
Accepted 29th June, 2015
Published online 30th July, 2015

Key words:

E-coli; Ease of use;
Emergences water supply;
Field Methods; Incubation; Water Quality.

ABSTRACT

The combination of Comparative Analysis Performance and Decision Rank Matrix results were used to assess; accuracy, experimental process, cost per 1000 samples, portability and durability of Delagua, Colilert (P/A), Colilert (MPN) and Petrifilm methods for examination of microbiology water quality during emergencies. The assessment further compared human body incubation techniques with conventional power supplied incubation Colilert MPN is ranked (1st) followed by Petrifilm (2nd) and lastly the Delagua (3rd) method. The ranking indicates that during an emergency, Colilert MPN should be considered first next Petrifilm and last the Delagua method. The equal positive and negative at all levels of dilution of Colilert (P/A) results show that the Human body incubation technique performs well in comparison with the power supplied incubator. Based on simple statistics analysis on the results of detected E-coli, human body incubation techniques has standard error of 0.02, more accurate as compared with the powered incubator with standard error of 0.03.

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INTRODUCTION

Note: The term 'water sample' referred to prepared distilled water diluted with live E-coli and Methods referred to the kits, consumables and accessories needed for testing water samples, involved an experimental procedure that detected/enumerated, counted and confirmed the E-coli diluted in the water sample. The research work was carried out as individual research work for my MSc Research project at Water, Engineering and Development Centre, Loughborough University, Leicestershire UK and the information and costs of the kits used then must have changed

In major disaster and epidemics; which call for emergencies situation, the microbiology quality of water supplied should be considerably ok, in order not to further escalate problems associated with such emergencies by increasing the health impacts on the victims due to contamination of water sources or supplies. According to WHO report, the provision of drinking water and sanitation services in health facilities is a top priority during emergencies. Safe drinking water, basic sanitation facilities and safe disposal of infectious wastes will prevent the spread of disease and improve health conditions.

The microbiology examination should be a focus of all the water quality parameters, which indicates the level of human and animal waste contamination in the drinking water. Human and animal waste contains the harmful pathogens, if properly not disposed, found their ways to the water bodies and affect the human health after consumption. In emergencies, E-coli better indicators of the health risk from water contact and species of faecal coliform bacteria that is specific to faecal material from humans and other warm-blooded animals. There is a need for rapid and quick detection of these "indicator bacteria", using a relatively affordable, portable, efficient, and user-friendly water quality testing method. In recent years, there have been many developments in the rapid testing of water, and in the development of more selective methods that detect strictly E. coli and/or total coliforms: these methods include Colilert and Petrifilm. These tests are performed at 35.5°C, rather than 43.5-44.5° C, as in case of the Delagua method, and thermotolerant coliforms, which it is possible to grow on your own body, can be used: these need neither a bulky incubator, nor lengthy and complicated preparation procedures.

However, as one of the focus of this study, human body incubation as new techniques of incubating bacteria can replace the Delagua method during emergencies where there is no power supply or to take the samples to far laboratory for

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testing and to reduce the long experimental procedure peculiar to the Delagua method and with combination of relative ease of use of other methods- Colilert and Petrifilm and their portability, the victims of the disasters, can even participate in examination of drinking water sources by volunteering to carry out the tests and incubate the water samples on their bodies. Such methods have significant advantages, especially in emergencies. However, how do they compare with the Delagua method, a popular water testing method that is relatively expensive, requires a high incubation temperature, and involves too many procedures that require extensive training?

Aim and objectives

The aim of the project was to compare different methods (simple and rapid) of testing microbiological water quality in the field, during emergencies and to determine their difference, in terms of operating time, costs, handling and portability as well as any limitations. The objectives used to achieve the above aim are evaluation of the following; accuracy of experimental results obtained the ease of use and portability of the methods, cost effectiveness of using the methods and varying the incubation techniques, by using the human body and the incubator in the laboratory, and comparing the results.

MATERIALS AND METHODS

Sampling

A stock of 450ml of Ringers solution was prepared by diluting 10 μ l of live E-coli in distilled water. After several trial of detections and enumeration from the prepared stock (by combination of usage of Delagua and Petrifilm) in order to obtain, the three level of dilutions for the study, 10cfu/100ml for low level (3rd), 100cfu/100ml medium level and 135cfu/100ml high level dilutions. From these dilutions, the three methods- Delagua, Colilert and Petrifilm were used with their standard procedures to detect and enumerate the diluted live E-coli in the water samples. For each dilution 20 experiments were performed on each method.

Laboratory procedures

All the experiments were carried out under field testing and procedures. The procedures for enumeration of the E-coli were MF and MPN performed by Delagua, Colilert MPN and Petrifilm. Except Colilert (P/A) that only detect the presence and absence.

Microbiology examination Methods

Delagua method: the standard procedures can be found in the instructional CD-ROM of Delagua water testing kits and it can be found on the website of Robens Centre for Public and Environmental Health.

Colilert (MPN) and Colilert (P/A) method

The standard procedures used for both methods are available on the IDEXX website.

Petrifilm method

The standard procedures for the methods are available on the 3M website.

Procedure of Human Body Incubation

The customized Vest is provided to hold the samples close to the body. At the start of the experiment, the customized vest was laid flat and opens on a clean surface. For each experiment following the standard procedures above, 5 Petrifilm were prepared as above and place inside the vest. Then 5 samples to be tested Presence / Absence (P/A) by Colilert-18. And 50 samples of Colilert MPN techniques (10 dilutions is equivalent to a test) With 60 total samples all together, the vest were put on by the author for 24hours. Every following morning, the vest was removed, the samples were taken out in order they were tucked in; Petrifilm, Colilert (Presence/ Absence) and Colilert MPN. And the readings were taken one after the others.



Fig.1. The customized vest for holding of waste samples and wear on the human body for incubation

Accuracy assessments

Simple statistics were employed to assess which method is more accurate and reliable than other by using the distribution (frequency distribution, histogram and bar chart), the results were graphically displayed which gave visual impacts, the central tendency (mean, median and mode) these measured how the detections of E-coli from water samples were specific and accurate and the dispersion (standard deviation and variance) were used to calculate the standard error from the results obtained.

Experimental procedure assessments

These assessments were carried out to determine the ease of use of each method, by listing and counting the number of steps of the following activities and tasks carried out during laboratory experiments. The activities were experimental procedures (Ease of use), preparation of reagents (solution), sample preparation, media preparation, incubation, timing and temperature, counting and recording of the detected E-coli.

Before the start of each experiment for a method, charts were made, listing all the activities and task (listed above). And the activities were recorded from start till the end represented by number for comparison analysis.

Cost assessments

Capital and consumables costs were collected for each method from suppliers. Based on these figures, the costs of 1000samples for each method were used for the comparative assessment.

Portability

This is measures how is it easy to transport the methods, and to carry the methods about in the field during emergencies. The portability of the methods was assessed by measuring the weights and dimensions of each and compared them. Then, the portability was ranked on the scale of 1-10 based on heavier the methods are.

Durability

The durability, were quantified based on the shelf life of each method, which is measured in years. The durability is ranked between the methods from 1-10 at discretion, of the author, with 1 the most less durable method and 10 for most durable method.

Comparative Performance Analysis (CPA) Method

This was a method used by Harvey and Drouin, 2006 to compare performance of two pumps; the conventional handpump and the rope-pump for water supplies in rural sub-Saharan Africa. They made used of the CPA to determine whether the rope-pump can be an alternative to the convectional handpump using some variable factors. These variables were ranked by NGOs and communities in terms of their relative importance and with average results of the ranking, the different factors were weighted. The use of CPA method is adapted to compare the performances of the methods using the following variables: accuracy of the methods, experimental procedure, cost per 1000 samples, portability and the durability of methods.

Following the methodology used (Harvey and Drouin, 2006), the scores for the criteria is between 1-10,the least important parameter received a score of 1, then each factor was attributed a score corresponding to its relative importance ratio to the least important parameter. Therefore, the weight of each factor was then computed as follows:

$$W_i = \text{score for } i^{\text{th}} \text{ factor} / \text{sum of all factor scores, where:}$$

$$W_i = \text{importance weight for the } i^{\text{th}} \text{ factor}$$

To measure the location of each method for each factor, different scales were used with minimum value being score for the worst plausible value and the maximum, the best plausible value. There was normalization of the variables for each method, using the relation below

Where:

- S_{ij} = normalization (scaled position of the jth entity on ith factor.)
- Best_i = best plausible value of ith factor
- Worst_i = worst plausible value of factor
- Value_{ij} = value of jth entity on ith factor

Finally, the overall score of each method was then computed as follows:

Where:

$$S_j = \sum_{i=1}^n (W_i)(S_{ij})$$

Where:

$$j = \sum_{i=1}^n (W_i)(S_{ij})$$

- S_j = overall evaluation score for the jth alternative method
- J = number of alternatives (3)
- W_i = importance weight for ith factors
- N = number of factors of value (?)
- S_{ij} = scaled position of the jth entity on ith factor

Then, the overall total scores were ranked and compare. A method with highest value was being ranked first and least value, last.

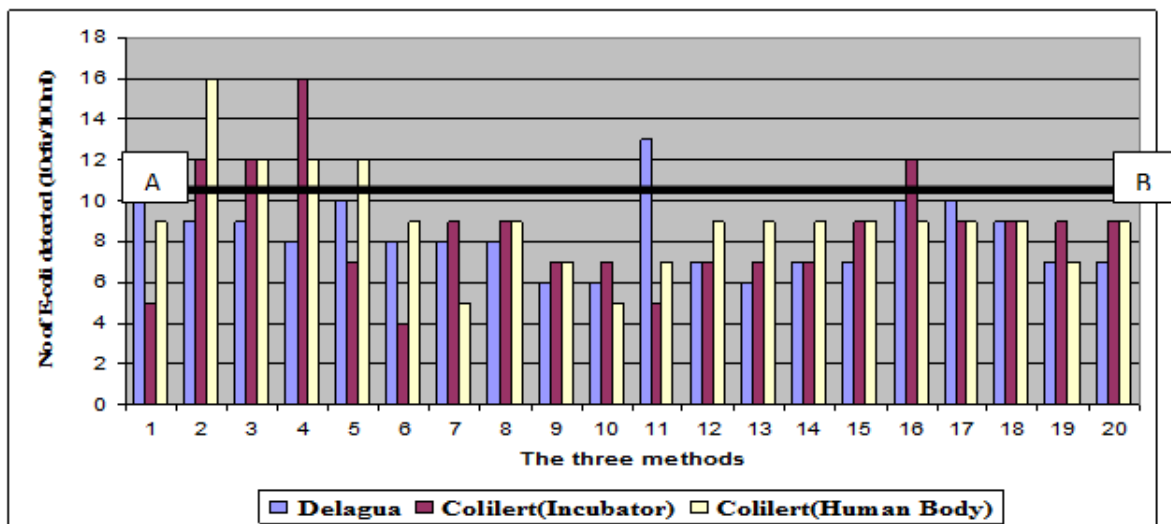


Fig.2. Graphical comparison of results from the three methods at low level (3rd) dilutions

RESULTS AND DISCUSSION

Accuracy of the methods

Delagua, Colilert (MPN) and Petrifilm

Due to ambiguous results from the Colilert MPN method for medium level (2nd) dilution and high level (1st) dilution samples, only results of the low level (3rd) dilution were used to compare the accuracy of the three methods. A summary of all the results of low level (3rd) dilution for all the methods: Delagua, Colilert and Petrifilm are presented in the Figure 2. This also includes the two techniques of incubation: the incubator in the laboratory and on the human body. To help appreciate the significance of the large amount of data, a line AB drawn on the mark 10 represents the low level dilution of E-coli (10cfu/100ml). It can be observed that the majority of the detection by the methods is below 10cfu/100ml, except for a few, which may be due to some abnormality or error. Petrifilm is not included in this comparison, because of the lack of results at low level (3rd dilution).

In order, to compare the accuracy (reliability) of each method, standard error is used to make the analysis simple and easy to follow. The mean score with the smaller standard error provides a more reliable estimate of the true value than does the mean score with a higher standard error. As you can see from the Table 1 below, the Delagua method is more reliable than other methods with a standard error of 0.396; followed by the Colilert MPN method, which uses the human body to incubate the E-coli, having a standard error of 0.561. Colilert MPN method which uses an incubator should be considered next, having a standard error of 0.6343. The Petrifilm method has a standard error of 9.883 showing that it is very unreliable at low level (3rd) dilution. The increasing order of standard errors from 0.396, 0.561, 0.6343 and 9.883 indicates that the methods' accuracy or reliability decreases. So Delagua is the most reliable followed by Colilert MPN which uses the human body, then Colilert MPN with the incubator and finally the Petrifilm method appears to be the least reliable.

The Delagua method is most accurate, relatively with a value of 9 followed by the results from Colilert MPN using the human body with a value of 7, followed by Colilert MPN with an incubator with 6. The Petrifilm using the incubator scored 2 and on the human body, 0, and then only at low level (3rd dilution). *Colilert (P/A)*: To be able to compare and analyse the results from Colilert (P/A) method, 0 and 1 were used to represent the data: 0 indicates a negative result (absence of E-coli) while 1 indicates a positive result (presence of E-coli). The results of presence/absence were summed up and represented by bar charts below. It can be seen from Figure 3 above that the results from the incubator and the human body are identical; there is no difference in results between using a laboratory incubator or the human body to incubate the samples. For the high level (1st) dilution the 20 samples were all positive, also 20 samples were positive using the medium level (2nd) dilution and similar results occurred with low level (3rd) dilution, but 15 were positive and 5 negative. Generally, the results from both the laboratory incubator and the human body were consistent.

Experimental procedure assessments

It was used to evaluate the ease of use of each method by considering all the experimental procedure for each method particularly: (1) number of steps for the experimental procedure, (2) equipment preparation and (3) the number of steps involved in the media preparation. If we look at the three items listed above in relation to the ease use of a method, there is an inverse relationship between ease of use and experimental procedure. Thus a complex experimental procedure can be considered to have a 'low' ease of use and *vice versa* The relationship between Ease of use and Experimental procedure can be expressed mathematically as:

Ease of use=1/Experimental procedure. (No of steps of procedure)

As can be seen from Table 4, 0.33 (1/3) is the highest values for both Petrifilm and Colilert P/A, followed by Colilert MPN

Table 1. Standard errors of the results from all the methods

	Delagua	Colilert MPN		Petrifilm	
		Incubator	Human Body	Incubator	Human Body
Mean	8.25	8.55	9	25	0
Standard Deviation	1.773	2.837	2.511	44.2	0
Standard Error	0.396	0.6343	0.561	9.883	-

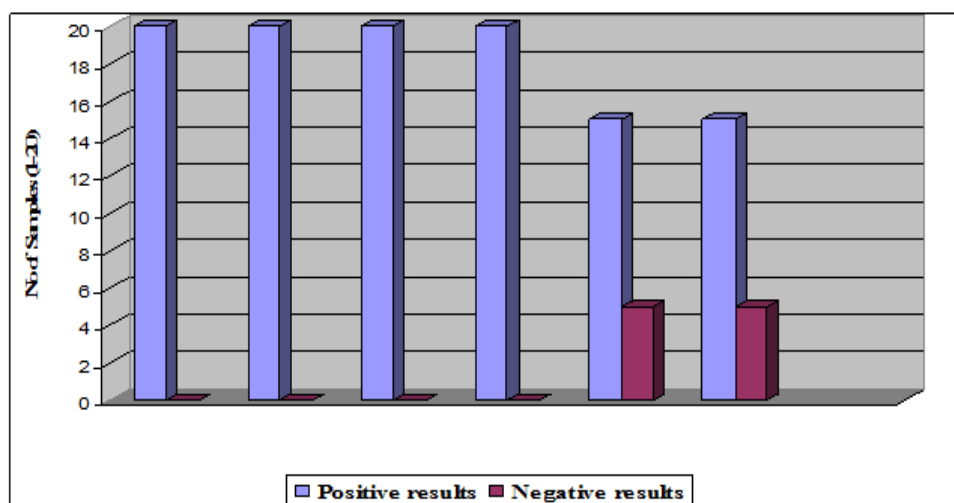


Fig.1. Summary of Colilert Presence /Absence results at all the level of dilutions

with a value of 0.20 (1/5) and lastly the Delagua method with a value of 0.04 (1/25). If we rank these methods; on the scale of Ease of use, Petrifilm and Colilert P/A will be at the top, followed by Colilert MPN and lastly the Delagua at the bottom of the ranking. That means that the Petrifilm and Colilert PA methods are easy to use, followed by Colilert MPN with the Delagua method being not easy to use. This clearly shows that the Delagua method might not be desirable for use in field testing during emergencies. The need for quick results, the long procedure and high level of skills needed to test for water quality are peculiar to the Delagua method and make it impracticable as a rapid field test.

consumables like disinfectants, cleaning materials. The Petrifilm cost is lowest at £1,159. It should be noted that the overall cost can be reduced for both Colilert and Petrifilm methods in the following way: The conventional incubator can be replaced for the Colilert and Petrifilm methods, by using a customized vest or putting the samples in the pouch. The vest used for the research cost a sum of £39.80, which is considerably less costly than the conventional incubator.

Portability

On a scale from 1-10, 1 being the least portable and 10 the most portable, 8 is assigned to Petrifilm, 6 to Colilert P/A, 5 to

Fig.2. Ranking of the Ease of use

	Delagua Method	Colilert Method		Petrifilm Method
		P/A	MPN	
No of steps for experimental procedure / media preparation equipment preparation.	25	3	5	3
Ease of use (ranking).	0.04*(1)	0.33*(8)	0.20*(5)	0.33*(8)

* these values are multiplied by a factor of 100 and divided by smallest products 4

Fig.3. Analysis of experimental procedure

Criteria	Delagua Method	Colilert Method		Petrifilm Method
		P/A	MPN	
Ease of use	0.04	0.33	0.20	0.33
Time to complete a procedure	10min.	2min	12min	2min
Time to get the results	24hours	18hours	18hours	24hours
Incubation Temperature	44.5 ₀ C	35 ₀ C	35 ₀ C	35 ₀ C

To further analyzed the experimental procedure, using the Ease of use, and other three criteria as shown in the Table below. As can be seen from the above table, the Delagua Method takes 10min to complete an experimental procedure, although the Colilert MPN method takes 12min, this is because it involves drawing 10ml from the 100ml Colilert snap pack, diluting the sample and transferring into 10 test tubes. Thus, although the method is more time consuming than the Delagua method, the processes involved are not as difficult making it very easy to use compared to Delagua. The Colilert P/A and Petrifilm methods each take a period of 2min. The Delagua method requires a high level of training and experience, which make it difficult for the layman to use because, although the whole procedure takes 10 minutes with an experienced and skilful user, it takes 25 steps to carry out each analysis. The Colilert MPN method followed the Delagua taking 5 steps to complete an analysis, albeit in 12minutes. Although, the Colilert MPN takes a longer time than the Delagua, the number of steps for the procedure and the equipment preparation are less, making it ease to use. Therefore it requires no high level of skills and training to handle. The Petrifilm and Colilert (P/A) methods required less time to complete an analysis with a three-step experimental procedure. As a result of this, training and experience to use these methods and have the results interpreted is minimal.

Cost

Assessment of the cost on testing per 1000 samples, is trying to bring the cost to a certain level of equality. But this might be difficult because, each method does not have the same contents or use the same accessories. However, Colilert method cost more (£3,000) followed by the Delagua method (£1666.42) but its experimental procedure uses more

Colilert MPN and 1 to the Delagua method In conclusion, Petrifilm is most portable and cheapest to transport to emergency sites followed by Colilert P/A and Colilert MPN whilst Delagua is the least portable and costly to transport to emergency sites.

Durability

Delagua has a durability of 2years (when used regularly and constantly), the Colilert method has a durability of 1year and Petrifilm’s is 1year and 6months. Thus, Delagua is the most durable method, very tough and robust, followed by Petrifilm. Colilert is the least durable with a shelf life of only 1year, less than Petrifilm’s 1 year and 6months and Delagua’s 2years.

CPA results

The scores for the criteria were allocated between the values of 1-10, and the Experimental Procedure is allocated a score of 10, which means it is the most important criterion and other criteria were then allocated scores according to their importance relative to others and also to field and emergency situations. For example, durability has little effect during emergencies; it was scored 1.because there is no point in stocking a product (because it will last longer) during emergencies. Experimental procedure, which is related to ease of use of a method, is scored 10, then second most important is the Portability (9) of the method because there is no point in wasting time in emergencies using a method with lengthy procedures with high skill and experience requirements for its use and at the same time one which is very bulky to carry about. The accuracy of the experimental results, is not the most important thing during emergencies in order to tackle any problems of water quality appropriately, that why the MSF the

Fig. 4. Importance of weighting

Criteria	Scores	Importance Weight
Experimental Procedure	10	31.3
Portability	9	28.1
Accuracy of Experimental results	7	21.8
Cost per 1000 samples	5	15.6
Durability	1	3.1
Total	32	100

Fig.5. Scaled positions for each factor for the three methods

	Best	Worst	Delagua		Colilert MPN		Petrifilm	
	Plausible value	Plausible value	Value	Score	Value	Score	Value	Score
Experimental Procedure	10	0	3	0.30	4	0.40	6	0.60
Portability	10	0	1	0.10	7	0.70	9	0.90
Accuracy of Experimental results	10	0	9	0.90	7	0.70	2	0.20
Cost per 1000 samples	£1159	£3000	£1666.4	0.72	£3000	0	£1159	1.0
Durability	5yrs	0yr	2yrs	0.4	1yrs	0.10	1.2yrs	0.12

Fig.6. Overall evaluation scores

	Score			Importance Weight	Weighted score		
	D	C	P		D	C	P
Experimental Procedure	0.30	0.40	0.60	31.3	9.39	12.52	18.78
Portability	0.10	0.70	0.90	28.1	2.81	19.67	25.29
Accuracy of Experimental results	0.90	0.70	0.20	21.8	19.62	15.26	4.36
Cost per 1000 samples	0.72	0	1.0	15.6	11.23	0	15.6
Durability	0.4	0.10	0.12	3.1	1.24	0.31	0.372
TOTAL					44.29	47.76	64.40

Fig.7. Decision Rank Matrix

Alternatives	Delagua	Colilert (MPN)	Petrifilm	Row Sum	Rank
Delagua		0	0	0	3 rd
Colilert (MPN)	1		1	2	1 st
Petrifilm	1	0		1	2 nd

research collaborator, uses a range of microbiological pathogen levels during emergencies and sets out what type of treatment should be used. Apart from the values that are obtained from previous assessments and the scores that are calculated from the formulae given in CPA method above, the least and most plausible values for the first three criteria (Table 7) were based on the previous ranking of 0-10. The durability figures are based on the life span of the methods provided by the manufacturers. The final overall scores were 44.29 for Delagua, 47.76 for Colilert (MPN) and 64.40 for Petrifilm (on a scale of 0 to 100). The high value of Petrifilm is due to its ease of use based on the fewer number of steps in its experimental procedure and high portability and low cost compared to other methods. The Delagua method has a low value due to its being difficult to use, because of the large number of steps for its experimental procedures. Also its bulky nature causes it to be ranked low. The Colilert MPN method falls in the middle of the two, but very close to the Delagua method in performance.

Decision Rank Matrix

Starting with the Delagua row, its preference was compared with each of the other alternatives. 1 was scored for Delagua, when it was preferred and score 0 entered in the column alternative more preferred. The scoring was continuing for each alternative row. In the "Row Sum" column, the total of score is entered for each row.

The Colilert MPN is ranked 1st followed by Petrifilm and lastly the Delagua method. The ranking indicates that during an emergency, Colilert MPN should be considered because of its importance and the advantages it has over the other two methods and the next consideration should be Petrifilm and last the Delagua method if the first two are not available.

Conclusion

- Based on systematic analysis of Accuracy, Experimental procedure, Cost, Portability and Durability. By combining Comparative performance analysis and Decision Ranking Matrix results, Colilert (MPN) was ranked first, Petrifilm second and lastly Delagua method.
- The human body is as a reliable alternative incubation technique for the microbiological detection of E-coli in the field using Colilert and Petrifilm compared to conventional incubation. It has advantages in terms of being light in weight and easy to wear as a pouch around the waist whilst still providing reliable results. This is a new innovation in the microbiological examination of water away from exclusive reliance on bulky incubators that depend on a power supply. The Delagua method is not very suitable for the microbiological examination of water when field testing during emergencies because it very bulky, difficult and long experimental procedure and requires high skill to operate.

- The Colilert (P/A) method can only be used for detecting the presence or absence of E-Coli in water samples.
- The Colilert (P/A) method can be effectively incubated by the human body to detect the presence or absence of E-coli.
- The Colilert (MPN) method can only be used to enumerate level of contamination between 0-23cfu/100ml of E-coli.
- The Colilert (MPN) method can use the human body as an incubator and can detect all levels of contamination.
- The Petrifilm method can be used to enumerate only high levels of contamination and is not suitable for low and medium levels of contamination.
- The Petrifilm method can use either the human body or a conventional incubator to enumerate high levels of contamination. It is not suitable for low and medium contamination.

Recommendations

As results of research findings, the following procedures are recommended to Médecins Sans Frontières and other agencies working in water supply in emergencies. (The flowchart for the procedures is attached in appendix.

- At least four samples for each water sources should be tested, using Colilert (P/A) to detect the presence or absence of E-coli and these can be incubated on the human body.
- The positive results from above should be tested by Petrifilm (quick procedure and easy to use). This will determine if the contamination is of high level
- If there is no detection of E-coli, that means is it low level of contamination. Then Colilert MPN should be used to enumerate the no of E-coli in the samples.
- Colilert (MPN) incubated on the human body can be used to enumerate low-level contamination within the range (0-23cfu/100ml) and it can be used to complement the Petrifilm method to enumerate medium and high-level contamination.
- One person can incubate 10 test tubes (of 10ml each) on the body at one time for the Colilert (MPN) method for 24hours.
- At least four batches of 20 water samples can be tested using Petrifilm method and incubated on the human body at any one time to enumerate any level of contaminations.
- The use of the human body as an incubator should be promoted among rural dwellers in remote areas where there is no laboratory or skilled and experienced water quality experts for Colilert and Petrifilm for microbiological monitoring of drinking water.

- There is a need to develop guidelines for simple use of descriptive statistics in the field. Because of the reliance on the use of sophisticated software like Excel and SPSS data may not be available or easy to interpret in the field.
- As further research, different sources of water from the field should be used for the Colilert and Petrifilm methods when using the human body as an incubator: to test in a real field situation.

Acknowledgements

I thank Peter Harvey for excellence supervision of this research. Thanks to Jeff of MSF Netherlands for his support and funding from MSF. Finally, thanks to Mrs. J.Bhuptani and Mr. G Russell of Water Engineering Laboratory, Civil Department of Loughborough University for their assistance in the laboratory.

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Appendix

