Efficacy of fast methods for *Escherichia coli* counts in a depressed area water supply

S. Canobbio*, M. Antonelli, V. Mezzanotte***

*** DISAT, Università degli Studi di Milano-Bicocca, Piazza della Scienza 1, 20126 Milano, Italy**
(contact e-mail: sergio.canobbio@unimib.it)
**** DIIAR, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy**

Introduction

The majority of drinking water sources in depressed areas of developing countries are the traditional dams, wells, streams and ponds which can easily be concerned by faecal contamination, as well as by other waterborne and vector borne diseases.
One of the most used indicators for drinking water contamination is *E. coli*, usually counted in plates where colony growth is assured by a culture medium, according to various standard methods. The evaluation of *E. coli* contamination can ensure updates about water quality and help the decision-making processes involving disease control and health care. However, conventional methods for *E. coli* detection can be applied with difficulty in depressed areas, given the lack of logistics and skilled personnel. Thus, fast and sample-ready plate methods could play a key role and grant useful information about contamination, provided that they can be efficiently applied in the given working conditions.
To determine the effectiveness of Fast Count Methods (FCMs) we studied the drinking water level of contamination in 20 wells of the city of Mambasa, Ituri district, Democratic Republic of the Congo (DRC), as well as the operational conditions that could be expected in its laboratories. The city has been involved in a cooperation plan with the Italian branch of *Engineers without Borders* (EWB/ISF), and one of the goals of the project has been to identify reliable and reproducible methods to assess water contamination in the given circumstances. Hence, subsequently experiments were conducted to evaluate the FCMs as possible alternatives to traditional counting methods.

Results & Discussion

Preliminary assessment of drinking water contamination in Mambasa was conducted analyzing total coliforms, the only microbiological parameter that could be determined on site, using a Millipore coliform kit. Meanwhile, the operational conditions available locally were described using a preconceived questionnaire.
In laboratory, a comparison was made between the results of the conventional *E. coli* count plate method and three FCMs: 3M Petrifilm plates (PETRI), R-Biopharm Rida Count (RIDA), and PBI Chromogenic Compact Dry (CCD). The methods were applied to test waters with various levels of contamination, and in two differing operational conditions: (A) laboratory standard; (B) based on the Mambasa questionnaire data. Protocol B included: (1) the use of glass pipettes instead of disposable tips; (2) the use of boiled water instead of demineralised water; (3) incubation in an oven instead of a laboratory incubator. A total of 15 water samples were analyzed, and for each one 5 replicates per each method per each protocol were assessed.

Mambasa Drinking Water

The level of contamination of Mambasa drinking water was assessed evaluating the total coliforms in 20 wells and springs. Results are presented in table 1. Only 2 protected springs showed non contamination, while the mean number of total coliform of the other drinking water sources was comprised between 50 and 2250 CFU/100 ml.

Table 1: Level of drinking water contamination (CFU of total coliforms/100 ml) in Mambasa, DRC.

	n	Mean	SD	Min. value	Max. value
Shallow water wells	10	474	506	50	1350
Deep water wells	2	275	106	200	350
Springs	8	403	771	0	2250

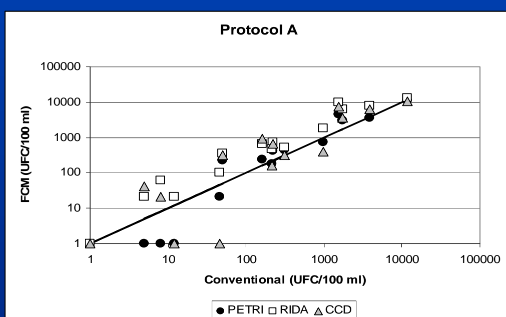

Figure 1: Ratio of the counts obtained by the FCMs to the conventional plate method with protocol (A).

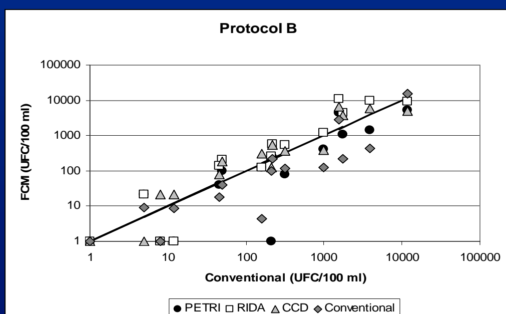

Figure 2: Ratio of the counts obtained by the conventional plate method and the FCMs with protocol (B) to the conventional plate method (Protocol A).

Fast Count Methods Test

Starting from the collected data, an experimental design involving three classes of *E. coli* water contamination was determined to assess the efficacy of the FCM methods in laboratory: from 0 to 100, from 101 to 1000 and above 1000 CFU/100 ml. The first of the assessed classes, especially, could prove tricky because the FCMs are all based on a water sample of 1 ml, which can lead to false negative results in determining the number of CFU in 100 ml.

All the results obtained with FCMs and conventional count plate methods in conditions (A) are reported in table 2, while in conditions (B) are reported in table 3. The FCMs, in laboratory conditions, are apt to overestimate the number of CFU, if compared with conventional plate counts (see figure 1). This is a desirable condition, because leads to a precautionary approach. On the other hand, with low contamination (class 0-100 CFU/100 ml), some false negatives were found. This is to be expected, because the FCMs are based on the analysis of 1 ml of water and, thus, a low *E. coli* load (<1 CFU/ml) may require more replicates to be detected.

Table 2: Results of the analyses made on 15 samples of contaminated water with protocol (A).

Class		Protocol "A" Conventional	PETRI	RIDA	CCD
0-100 CFU/100ml (n=6)	*CFU*	19 ± 22	40 ± 88	93 ± 135	63 ± 127
	ratio of false negatives	n/a	0.50	0.00	0.33
101-1000 CFU/100ml (n=5)	*CFU*	379 ± 346	404 ± 218	830 ± 540	484 ± 290
	ratio of false negatives	n/a	0.00	0.00	0.00
1001+ CFU/100ml (n=4)	*CFU*	4726 ± 4800	5436 ± 3641	9257 ± 2853	6839 ± 2778
	ratio of false negatives	n/a	0.00	0.00	0.00

Table 3: Results of the analyses made on the same 15 samples of contaminated water with protocol (B).

Class		Protocol "B" Conventional	PETRI	RIDA	CCD
0-100 CFU/100ml (n=6)	*CFU*	12 ± 15	27 ± 39	60 ± 88	50 ± 70
	ratio of false negatives	0.17	0.33	0.33	0.17
101-1000 CFU/100ml (n=5)	*CFU*	111 ± 76	210 ± 143	544 ± 424	340 ± 152
	ratio of false negatives	0.00	0.00	0.00	0.00
1001+ CFU/100ml (n=4)	*CFU*	4731 ± 7273	3042 ± 2114	8564 ± 2836	5282 ± 1233
	ratio of false negatives	0.00	0.00	0.00	0.00

Operational conditions, tested with protocol (B), brought to partially different results. Conventional counts and PETRI FCM showed an underestimation of bacterial load if compared with conventional counts made in laboratory conditions. On the other hand, RIDA and CCD FCMs were still overestimated (figure 2). The comparison of the results obtained by the same method within the two protocols showed that all four the tested methods underestimate the *E. coli* load in operational (B) conditions.

The results were tested with a two-way ANOVA model (categorical variables: 1–method; 2–protocol), and the model was not significant (p=0.868 for the methods, p=0.522 for the protocols). A Tukey's HSD test performed on the various method*protocol differences showed no significant differences as well. Thus, the methods can be considered, overall, interchangeable. It should be noticed that the rough cost for each analyzed sample is similar among the methods, too, including traditional plate counts (between 1.00 and 1.90 € for each count, based on a provision of 100 plates).

Conclusions

The FCMs can be considered as efficient as conventional count methods, while they are far simpler to use. The tendency to overestimate the counts (although not significant) can be considered as an advantageous precautionary approach. The methods applied using the worse operational conditions showed no significant drift and can be applied to real-case scenarios. However, the FCMs showed a tendency to produce false negatives with low levels of contaminations (up to 100 CFU/100 ml). This should be taken in account and a higher number of replicates for negative results should be made.