

COMPARISON OF BACTERIOLOGICAL METHODS FOR DETECTING TOTAL COLIFORMS AND *ESCHERICHIA COLI* IN WATER

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Bacteriological analysis of water, using indicator bacteria is a routine practice around the world to assure the microbiological quality of water. The current study investigated the performance of four alternative methods compared with the reference Sri Lanka Standard-Multiple Tube Fermentation (SLS-MTF) method, for the detection and enumeration of total coliforms and *Escherichia coli* in different water sources (tap water, bottled water, well water, surface water and effluent water-to cover a wide range of contamination levels), collected from different geographical areas. The four alternative methods were American Public Health Association (APHA)-MTF method, Colilert-MTF method, Sri Lanka Standard-Membrane Filtration (SLS-MF) method and m-ColiBlue24-MF method. Colilert and m-ColiBlue24, which are enzymatic methods, detect total coliforms and *E. coli* simultaneously, by the activity of enzymes β -D galactosidase (in total coliforms) and β -D glucuronidase (in *E. coli*), while the other methods are based on lactose fermentation.

Variance analysis results revealed that Colilert, m-ColiBlue24 and SLS-MF methods detected significantly higher ($p \leq 0.05$) total coliform counts, compared to the SLS-MTF method. In *E. coli* detection, Colilert ($p \leq 0.05$) and m-ColiBlue24 ($p \leq 0.1$) methods detected significantly higher counts compared to SLS-MTF method. Simple Linear Model showed, the three alternative methods detected several folds higher total coliforms counts than SLS-MTF method (3.8, 1.75 and 1.55 times higher counts were detected by Colilert, m-ColiBlue24 and SLS-MF methods respectively). For *E. coli*, 2.93, 1.83 and 1.35 times higher counts (than SLS-MTF) were detected by m-ColiBlue24, Colilert and SLS-MF methods respectively. ISO performance criteria (sensitivity, specificity, efficiency, false positive and false negative ratios), showed the two enzymatic and SLS-MF methods were superior to the conventional SLS-MTF method. Method performances by paired count evaluations showed inconclusive results due to inadequate valid data resulted by contaminations and missing data during subculturing.

Confirmational rates obtained for both bacterial types were higher in three alternative methods, than SLS-MTF method. Values being: for coliforms, Colilert (78.2 %), SLS-MF (75.2 %), m-ColiBlue24 (72.1 %) and SLS-MTF (71 %) and for *E. coli*, Colilert (66.6 %), m-ColiBlue24 (50 %), SLS-MF (50 %) and SLS-MTF (37.5 %).

Cost comparison showed that MTF methods (SLS, APHA and Colilert) were more expensive compared to MF, for drinking water analysis. The most economical method for drinking water analysis was SLS-MF method (6.7 times cheaper than SLS-MTF); followed by m-ColiBlue24 (2.1 times cheaper). For surface water analysis SLS-MF method was the cheapest (4.3 times cheaper), followed by the Colilert method (2.1 times cheaper) than the SLS-MTF method.

Conventional MTF methods (SLS and APHA) showed several drawbacks such as, need for longer incubational periods, confirmational tests, more labour and subjective nature of results interpretation. In comparison, Colilert was very efficient in all aspects. Among MF methods, m-ColiBlue24 was more efficient than the conventional SLS-MF method with the advantages of simultaneous detection of both types of bacteria, absence of heavy background growth or atypical colony formation, easy preparations and easy result interpretations, less time and labour requirement, etc.

Bacteriological identifications revealed 60 % identifications of non-coliforms by the SLS-MTF method. In contrast, Colilert, m-ColiBlue24 and SLS-MF methods identified more than 60 % of coliform bacteria. Identifications showed that, even the drinking water sources tested were contaminated with fecal (pathogenic) organisms, suggesting threats on drinking water quality in Sri Lanka.

In conclusion, results of the current study revealed that the conventional SLS-MTF method is less efficient, compared to the Colilert, m-ColiBlue24 and SLS-MF methods. SLS-MF method was the most economical method for analyzing both drinking and surface water samples. m-ColiBlue24 and Colilert methods, with their superior performance could be recommended as alternative methods for analyzing drinking water and surface water samples respectively, when cost is not the limiting factor.