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Utility of EC 3M™ petrifilms™ for fecal assessment of surface drinking water sources in Southwestern Uganda

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Abstract

This study examined bacterial contamination levels from 47 drinking water sources (92% were surface sources) from two rural Ugandan villages using EC 3M™ petrifilms™ incubated for 24 h at 37ºC. *E. coli* counts in 1ml water samples ranged from 0-120 cfu/ml. Confirmation of presumptive *E. coli* was performed by transferring colonies onto Blood agar followed by characterization by subculture on selective media and standard biochemical analysis. Selected isolates were verified by sequencing of their 16S rRNA genes. A total of 184 colonies were isolated from petrifilms representing 70% of the water sources and 92% of the isolates were identified to genus level (n = 169). Only 24% of presumptive *E. coli*, isolates were confirmed as *E. coli* (n = 40). Additionally 61% of isolates were found to be lactose negative and were confirmed as *Salmonella*, *Yersinia* and *Proteus* (n = 103). Purified bacterial isolates from the Ugandan water samples and laboratory strains were re-plated on new petrifilms at various dilutions to determine the potential for misclassification of colony color or gas production. Upon inoculation of new petrifilms, misclassification of coliforms was observed. While the level of bacterial contamination in the rural Ugandan drinking water sources is not unexpected, isolation of lactose negative bacteria from presumptive *E. coli* colonies and misclassification of coliforms by the petrifilms was unexpected.

Introduction

- Many people in developing countries rely on contaminated, untreated surface water as their drinking water source
- Basic monitoring of the bacteriological quality of source waters would facilitate the ability to select the best quality water source available
- Standard methods for monitoring accepted fecal indicators are frequently impractical in developing countries.
- Petrifilms may offer a less expensive, low technology for assessing fecal contamination in source water in developing countries.
- This study examined bacterial contamination of drinking water sources using petrifilms in rural Uganda.

Figure 1: Drinking water source

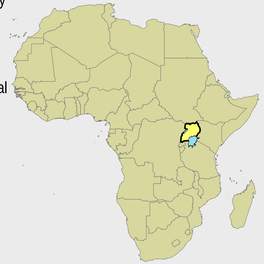


Figure 2: Study site: Uganda

Materials and Methods

Water Sources

- 47 water sources serving 2 villages in Nyabushozi County, Mbarara District; 90% were surface water sources
- Duplicate 100 ml water samples from each source concurrently

Bacterial Analysis of Water Samples

- 1ml of each water sample onto an EC 3M™ petrifilm™ (< 6 h of collection) and incubated at 37ºC for 24 h
- Fecal coliforms colonies red with gas bubbles; *E. coli* blue with gas bubbles (Fig. 3)
- Counts exceeding 150 (1.5 X 10^3) were too numerous to count (TNTC)

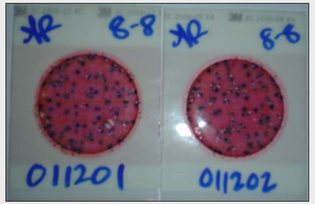


Figure 3: Presumptive *E. coli* on petrifilm

Identification of isolated colonies

- Blue colonies subcultured on Blood agar plates (Difco Laboratories), incubated at 36.5ºC for 24 h
- Isolates identified using selective agars (e.g. MacConkey, CHROMAgar™, Brilliant Green), API 20, and classical biochemical tests
- Selected isolates verified by sequencing 16S rRNA genes

Figure 4: CHROMAgar plate

Figure 5: Brilliant Green Agar plate

Laboratory testing of petrifilms

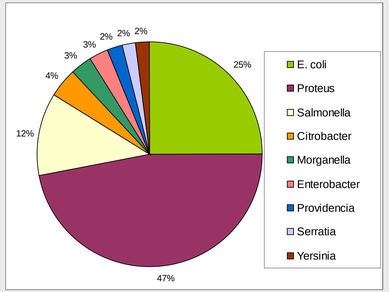
- Sterile saline dilutions of identified isolates and laboratory strains (10-100 CFU) used to inoculate new EC petrifilms™
- Samples incubated at 36.5º C for 24 h and examined for color and gas

Results

Over-prediction of E. coli

- Bacteria isolated and identified from 79% of water sources
- Of 160 presumptive *E. coli* colonies: 40 (25%) were confirmed *E. coli;* 103 (64%) were other coliforms or lactose negative species(Fig. 6)
- Presumptive *E. coli* counts ranged 0-120 (mean = 20, median = 7), other coliforms equal or higher

Figure 6. Isolation and identification of bacteria



Laboratory testing

- Distinction between lactose positive and negative bacteria (Fig. 7)
- *E. coli* in laboratory gave blue colonies with gas bubbles (Fig. 3)

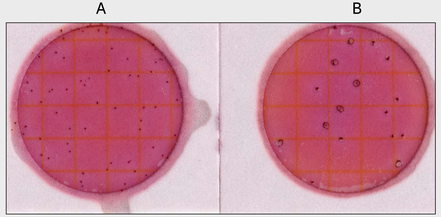


Figure 7. Lactose negative bacteria (A) *Providencia,* (B) *Serratia*

Discussion & Conclusions

- False positive detection of *E. coli* on petrifilms may be related to:
  - High concentrations of bacteria
  - Potential masking due to proximity of colonies and/or prevalence of other coliforms
  - Bias from time from petrifilm inoculation to isolation of colonies
- Misclassification of coliforms may be related to having 'atypical', tropical environmental strains (Schraft et al. 2005)
- Overprediction of *E. coli* suggests that petrifilms may have utility in tropical regions for assessing levels of bacterial contamination of water
- Petrifilms could be an effective tool to facilitate transport of environmental isolates to the laboratory

Acknowledgements

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References

SCHRAFT H and WATTERWORTH LA (2005) Enumeration of heterotrophs, fecal coliforms and Escherichia coli in water: comparison of 3M Petrifilm plates with standard plating procedures. *J Micro Methods* 60 335– 342.