Advancing ECC Vial Technology: Large-Format Prototype Development

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Abstract

Access to safe drinking water remains a critical public health challenge in low- and middle-income countries. The ECC Vial, a low-cost chromogenic presence/absence test developed by ENPHO and EcoConcern, detects *E. coli* and total coliforms in drinking water, but is currently limited to a 10 mL sample volume, insufficient to meet WHO and UNICEF Target Product Profile requirements. This project develops and characterizes a 100 mL large-format ECC Vial prototype using HiCrome Coliform Agar and a tissue-based media delivery system. We tested six *E. coli* concentrations ranging from 0.1 to 100,000 CFU/mL in triplicate, measuring optical density and visual color change at 0, 1, 2, and 24 hours of incubation. Results characterize the detection sensitivity, time-to-detection, and cost of the scaled prototype relative to WHO and UNICEF benchmarks, laying the groundwork for field validation in Nepal.

Introduction

- Diarrheal disease kills ~1.2 million people annually, with children under five in Nepal disproportionately affected
- WHO guidelines require zero *E. coli* in any 100 mL drinking water sample; UNICEF TPP requires detection of <10 CFU/100 mL
- The **ECC Vial** (ENPHO & EcoConcern, supported by MIT J-WAFS) is a low-cost, field-deployable water quality test using chromogenic enzyme detection
- Current 10 mL format falls short of WHO and UNICEF volume requirements
- The aim of this project is the **development and evaluation of a 100 mL large-format ECC vial prototype**

Specific Objectives

- We aimed to scale the protocol of 10 mL ECC vial and implement it for a 100 mL volume
- We aimed to test bacterial dilution concentrations ranging from 10^7 CFU / 100 mL to 100 CFU / 100 mL (despite being 10-fold higher than the WHO guidelines) to validate (and troubleshoot) sensitivity at higher concentrations as positive controls
- We aimed to analyze cost and propose a cheap container for use in Nepal

Materials

- *E. coli* MG1655 (non-pathogenic K-12 strain)
- HiCrome Coliform Agar w/ SLS (Himedia M1300) as detection medium
- 250 mL wide-mouth glass Erlenmeyer flasks
- Folded tissue paper (media carrier, ~3 mL absorption)
- Sterile PBS
- Parafilm
- 37°C incubator, autoclave, NanoDrop spectrophotometer

Methodology

Vial Preparation:

1. HiCrome Coliform Agar w/ SLS prepared at 27 g/L in distilled water, brought to boil, and sterilized by autoclave along with 21 Erlenmeyer flasks
2. Tissue paper uniformly folded to absorb ~3 mL, dipped in liquid agar media for 2 seconds in a sterile biosafety cabinet
3. Tissue-loaded vials dried in hot air oven for 36 hours

Sample Preparation:

1. *E. coli* MG1655 cultured overnight in LB broth at 37°C with 200 RPM shaking
2. Culture density measured by OD600; cells pelleted at 16,000g for 10 minutes and resuspended in sterile PBS
3. Six 100 mL test samples prepared by serial 10-fold dilution and addition of 1 mL bacterial stock to 99 mL sterile water, yielding final concentrations of 100,000 / 10,000 / 1,000 / 100 / 10 / 1 CFU / 1 mL

Experimental Setup:

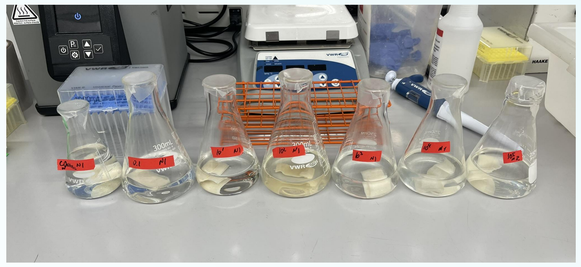
1. 100 mL of each sample added to prepared vials
2. All vials incubated at 37°C for 24 hours

Measurements:

1. OD600 (bacterial growth) and OD630 (chromogenic blue color development) measured by NanoDrop spectrophotometer at t = 0, 1, 2, and 24 hours
2. Visual color scored at each timepoint: no color change (negative), blue (*E. coli* positive), pink/red (coliform positive)

Cost Analysis

1. Cost per test was calculated from actual materials consumed in the 100 mL prototype, excluding shared equipment (incubator, autoclave, spectrophotometer)
2. Unit costs were derived from vendor pricing for each consumable at small-scale procurement quantities
3. Calculated cost was compared against the UNICEF TPP target threshold of under $6 per test (UNICEF TPP, 2019)



Results

Cost per test

- 1 Container (Whirlpak) ~ $0.43
- 1/ 21 * 2.7 g Media ~ $0.12
- Tissue paper (10 square inches) ~ $0.01

Total cost: $0.56

Container Evaluation

- Glass vials proved fragile during bulk incubation and are not recommended for scale-up
- Whirl-Pak sterile sample bags demonstrated adequate container integrity and are a viable alternative for the 100 mL format

Detection Results

- OD630 remained near zero across all concentrations and all timepoints, with no meaningful color development detected in any condition at 24 hours
- OD600 confirmed *E. coli* was present and viable across experimental conditions, ruling out culture failure as the cause of negative results

Conclusion

- No chromogenic color change was observed in any condition at any timepoint, despite OD600 confirming viable *E. coli* was present throughout incubation
- The most likely explanation is a **media scaling limitation**: the original ECC Vial is designed for 10 mL, where the tissue-embedded media is in direct, concentrated contact with the sample
- **Incubation duration** is a secondary factor: 24 hours may be insufficient at low concentrations in the 100 mL format
- At **$0.56 per test**, the 100 mL format is promising from a cost standpoint and well within the UNICEF TPP target of <$6 per test, assuming detection chemistry can be optimized
- **Future work**: increasing the quantity of media per vial proportionally to the volume scale-up, testing extended 48-hour incubation, and evaluating alternative media delivery formats beyond tissue paper

Citations

- UNICEF. Rapid *E. coli* Detection: Target Product Profile. 2019
- WHO. Guidelines for Drinking-Water Quality, Table 8.3. 2011
- ENPHO–EcoConcern. User's Guide for ECC Vial. 2019
- Eco Concern Pvt. Ltd. SOP: ECC Kit Production. 2019

Acknowledgements

We thank Libby Hsu and Mitali Chowdhury at MIT D-Lab for their mentorship and guidance throughout this project. We are grateful to the MIT BioMaker Space for providing lab space, equipment, and materials that made this work possible. Finally, we thank Professor Susan Murcott and all the D-Lab students who have contributed to growing and strengthening this project over the years.