Field Evaluation of a Low-cost Compartmentalized Bag MPN Method for the Detection and Quantification of *E. coli* in Stored Household Rainwater Samples Collected in Northeastern Thailand

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ABSTRACT

A large proportion of the population of northeastern Thailand relies on stored rainwater as its main source of drinking and household use water. Unfortunately, little data are available on the microbial quality of this water and its safety as a drinking water source is uncertain. This data gap is partly due to the high cost and technical difficulty of current water quality testing methods. The new, simple to use, low-cost compartmentalized bag test (CBT) may fill that gap. The CBT, which uses a chromogenic glucuronide substrate to signal the presence of *Escherichia coli* in a multi-compartment plastic bag, has been previously evaluated in controlled laboratory experiments with promising results and only a few field trials. The objective of this work was to evaluate the use of the CBT for the enumeration of *E. coli* in stored rainwater samples collected in a rural province of northeastern Thailand. A total of 189 100-mL drinking water samples were collected in duplicate from earthen and cement rainwater storage cisterns and plastic storage containers in 60 households during the dry (February – March) and wet (July) seasons of 2011. Samples were assayed in parallel by the CBT and the USEPA gold standard IDEXX Colisure/Colilert Quantitray 2000 method (QT). Dry season samples were incubated at 37°C for 24 hours and wet season samples incubated at ambient temperature (28-33°C) for 48 hours. Results from each method were obtained as Most Probable Number (MPN) *E. coli* concentrations and compared using the Wilcoxon matched-pairs signed rank test. Combined results from all 189 samples assayed demonstrated no significant difference in *E. coli* concentrations obtained from the CBT and the QT methods (p=0.74). Additionally, no significant difference between methods was found when the dry and wet season results were analyzed separately (p=0.53 and p=0.46, respectively). These results suggest that the CBT provides comparable results to the standard QT method for the detection and enumeration of *E. coli* in stored rainwater. Furthermore, this study provides evidence that the CBT can achieve reliable water quality assessment results in real-world practice and in resource poor settings where specialized laboratory resources are not readily available.

INTRODUCTION

Diarrheal disease, much of which is attributed to consumption of fecally contaminated water, claims the lives of over 1.8 million people annually. The WHO considers rainwater an improved source of drinking water, however there is little data to support this claim. For communities that rely on stored rainwater as their main source of drinking water, such as those in rural northeast Thailand, it is important that water is regularly tested to ensure its safety prior to use. In Thailand rainwater is often collected during the rainy season (June – July) and stored for up to nine months. Typically, large, cement rainwater containers are used to refill smaller daily use containers (Figure 1). Daily use containers often have wide-mouth openings, are low to the ground and accessible to children, and are dipped into with cups or buckets throughout the day as family members retrieve water. The combination of these storage and use practices puts the water source at constant risk of contamination. Regular testing of water supplies could inform health-based decisions that may aid in preventing much of the morbidity and mortality associated with the consumption of microbiologically poor quality water. Unfortunately, current water quality testing methods are often inaccessible in resource-limited settings due to their cost (often over 3 USD per sample) and technical difficulty to perform. The CBT, a novel, low-cost, field portable, and easy to use microbial water quality test, was developed to address these issues. The CBT is designed to detect and quantify fecal indicator bacteria *E. coli* in water samples without the requirement of specialized equipment or trained personnel, thus making it an attractive method for water quality testing in resource-limited areas.

OBJECTIVES

Determine if the CBT is a reliable, field-portable alternative to a current gold standard IDEXX Colisure/Colilert Quantitray method by evaluating:

1. If it achieves statistically similar results to Colisure/Colilert during a field survey of stored rainwater in Khon Kaen, Thailand.
2. If variations in microbial and physico-chemical water quality parameters affect performance of the CBT.

METHODS

Study Site

- Sixty households (HH) from the village of Wailum in the northeast province of Khon Kaen, Thailand were selected for this study.
- All HHs use stored rainwater as their primary source of drinking water.

Sample Collection and Storage

- When available, samples were taken from each HH's main rainwater collection tank and from the refillable container used to store rainwater for daily consumption.
- Samples of 100 ml were collected using the same method (bucket, siphon) household members primarily used to retrieve water., stored on ice and processed within eight hours of collection.
- HHs were visited and sampled twice, once in the dry season (February – March) and once in the rainy season (July).
- Number of samples:
- Dry season: 105 samples collected in duplicate
- Rainy season: 84 samples collected in duplicate.
- Additionally: 59 samples from 35 HHs for H_2S-producing bacterial analysis.

Other water quality parameters: pH, temperature, total dissolved solids, dissolved oxygen, and turbidity measured in-situ during the dry season at the time of sample collection.

Figure 1. Typical earthen jar used to store rainwater for everyday use.

Sample Processing and analysis

E. coli test by two methods:
Gold-standard IDEXX Colisure/Colilert Quantitray 2000 method
Novel, low-cost compartmentalized bag test (CBT); see Figure 2, below

- **Colisure/Colilert Quantitray 2000:**
- Assayed per manufacturer's instructions; incubated at 37°C for 24 hours (dry season) or at ambient temp. (28-33°C) for 48 hours (rainy season).
- *E. coli* scored as numbers of large and small wells that fluoresced under a long-wave UV lamp.; MPN /100 ml from the IDEXX MPN table .
- Total coliform concentrations scored as numbers of large and small wells that turned visibly yellow; MPN /100 ml from the IDEXX MPN table
- **CBT:**
- Chromogenic (X-Gluc) *E. coli* broth culture medium added to 100 ml samples prior to adding the sample to the compartmentalized bag.
- Bag compartments filled to correct volumes by manipulating the bag.
- Bags incubated at 37°C for 24 hours (dry season) or at ambient temperature (28-33°C) for 48 hours (rainy season).
- *E. coli* scored numbers of compartments with a blue/green color change.
- MPN/100 ml based on MPN table formulated from the EPA MPN calculator spreadsheet for Microsoft Excel
- **H_2S-producing Bacteria:**
- Some (59) samples assayed for H_2S-producing bacteria using Pathoscreen medium (Hach Chemical) in Quantitray 2000 (IDEXX) tray to quantify.
- Motivation: H_2S-producing bacteria may be more environmentally persistent than *E. coli* , so a better indicator of pathogen survival in stored rainwater.

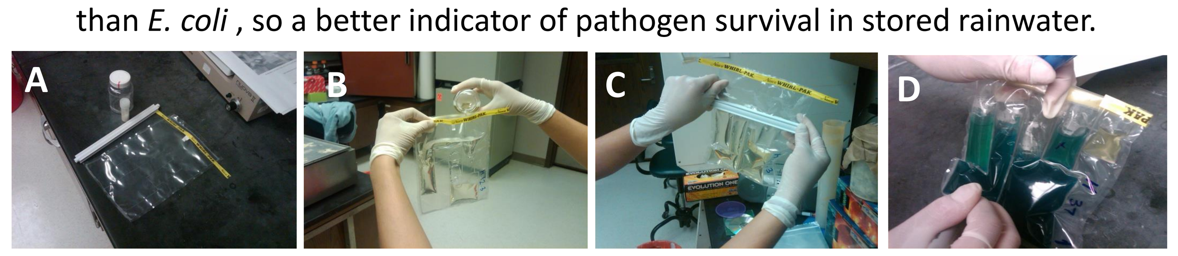

Figure 2. Compartment Bag Test (CBT).
A: Bag with 1, 3, 10, 30 and 56 ml compartments, clip seal, *E. coli* medium pack and sample bottle
B: CBT being filled with 100-ml water sample supplemented with *E. coli* medium
C: CBT with filled compartments being sealed with external plastic clip for incubation
D: CBT after incubation, with blue/blue-green *E. coli*- positive compartments

RESULTS

Microbiological Quality of Stored Rainwater in Khon Kaen, Thailand

Out of the 60 households that participated in the study:
Dry season:

- *E. coli* ≥1 /100 ml in at least one HH storage container = 39% (by Colisure).
- E. coli ≥1 /100 ml in all HH storage containers tested = 10.2%
- Rainy season:
- *E. coli* ≥1 /100 in at least one HH container = 82% (by Colilert).
- E. coli ≥1 /100 in all HH storage containers tested = 45.6%
- H2S-producing bacteria (35 households tested):

Out of the 35 households tested for H_2S-producing bacteria:

- H_2S bacteria ≥1 /100 in at least one HH storage container = 66%

CBT vs. Colisure/Colilert methods for *E. coli* detection and quantification

- Figure 3 and Table 1: Arithmetic mean *E. coli* MPN/100 ml and 95% CI by Colisure and CBT and by Colilert and CBT during dry and rainy seasons, respectively.
- Comparison of *E. coli* concentrations with Colisure/Colilert vs. CBT by Wilcoxon signed rank matched-pairs t-test:
  - No significant difference between Colisure and CBT during the dry season (p=0.53).
  - No significant difference between Colilert and CBT during the rainy season (p=0.46).
  - No significant difference between Colisure/Colilert and CBT for combined data of dry and rainy seasons (p=0.74).

Figure 3. mean *E. coli* concentrations and 95% CIs detected by Colisure and CBT (left) and Colilert and CBT (right) during dry and rainy seasons, respectively.

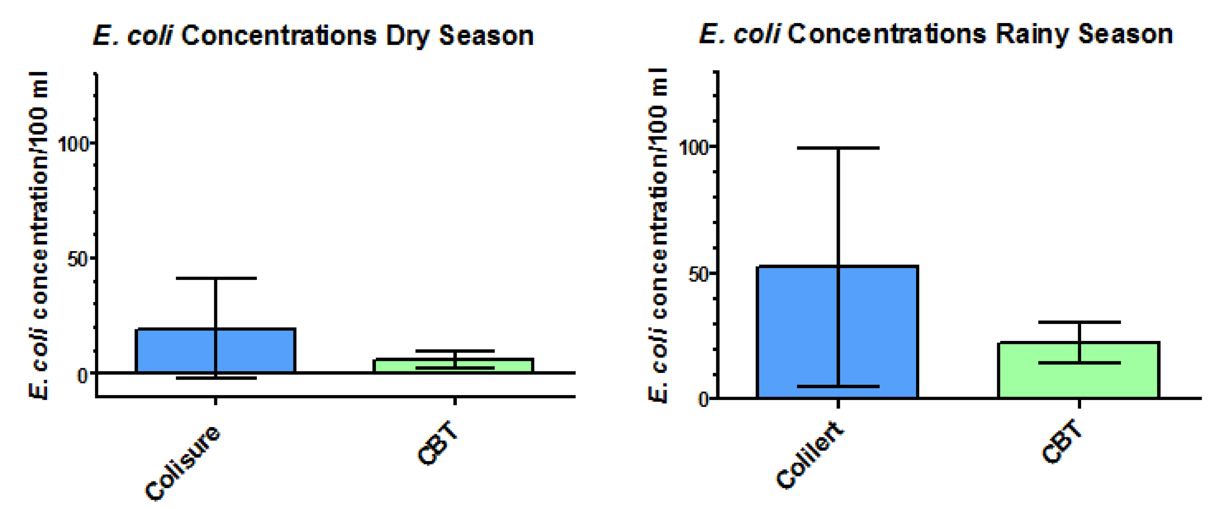

Table 1. Number of samples, median, mean and 95% CI *E. coli* concentrations by Colisure and CBT (dry season) and Colilert and CBT (rainy season) , respectively.

	Dry Season		Rainy Season	
	Colisure	**CBT**	**Colilert**	**CBT**
sample number	105	105	86	84
Median	0	0	2	1.19
Mean	19.57	6.205	52.3	22.44
95% CI*	[-2.29, 41.43]	[2.274, 10.14]	[4.94, 99.67]	[14.28, 30.6]

*95% Confidence interval around the mean

Associations between Colisure and/or CBT and other water quality parameters

Additional water quality parameters and bacteria measured during the dry season are presented in Table 2.

Table 2. Median, arithmetic mean, and 95% CI around the mean for microbial and physico-chemical water quality measurements in HH water samples

	Total Coliforms (MPN/100ml)	H_2S[1] (MPN/100ml)	temp. (°C)	pH	TDS[2] (ppm)	DO[3] (mg/L)	Turbidity (NTU)
Median	88.9	1	27.6	7.79	83	0.7	0.18
Mean	486.9	28.1	27.8	7.75	90.4	1.13	0.437

[1]H_2S-producing bacteria [2]Total Dissolved Solids [3]Dissolved Oxygen

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RESULTS (CONTINUED)

Associations between Colisure and/or CBT and other water quality parameters

Correlations between additional water quality parameters and *E. coli as* measured during the dry season are presented in Table 3.

Table 3. Spearman correlation coefficients for *E. coli* concentrations measured by Colisure and CBT methods at significance of p=0.05.

	Coliform MPN/ 100ml	Colisure *E. coli* MPN/100 ml	CBT *E. coli* MPN/100 ml	H_2S MPN/ 100 ml	temp. (°C)	pH	Turb. (NTU)
Colisure *E. coli* MPN/100 ml	0.53*	NA**	0.64*	0.41*	-0.31*	-0.14	0.22*
CBT *E. coli* MPN/100 ml	0.40*	0.64*	NA	0.23	-0.27*	-0.21*	0.070

*Spearman correlation coefficient significant at p=0.05 **Not applicable

Calculated Spearman correlation to determine if variations in *E. coli* concentrations obtained from Colisure and the CBT were significantly correlated (p=0.05) with changes in the water quality parameters (Table 3).

- *E. coli* concentrations by Colisure and CBT were positively correlated with each other.
- *E. coli* concentrations by both Colisure and CBT are positively correlated with concentration of total coliforms
- *E. coli* concentrations by Colisure and CBT are inversely correlated with temperature.
- *E. coli* concentrations by Colisure were positively correlated with concentrations of H_2S-producing bacteria and with turbidity.
- *E. coli* concentrations by CBT were inversely correlated with pH.
- *E. coli* concentrations by Colisure and CBT did not correlate with TDS or DO.

CONCLUSIONS

- Stored rainwater may not be a microbiologically safe source of drinking water, as *E. coli* was present in drinking water storage containers in 39% and 82% of surveyed households in the dry and rainy seasons, respectively.
- *E. coli* concentration in HH water seemed to increase during the rainy season.
- The results obtained from the CBT did not significantly differ from results obtained by Colisure when incubated at 37°C for 24 hours during the dry season or from Colilert when incubated at ambient temperature for 48 hours during the wet season. These results are in agreement with previous laboratory studies[1].
- *E. coli* concentrations measured by the CBT method may be affected by differences in pH in stored water. This correlation was not observed between for *E. coli* measured by Colisure and pH. Additional studies should be done to further investigate these correlations and the possible reasons for them.
- Because *E. coli* were detected in collected rainwater and stored household water at levels considered unsafe, larger scale water quality surveys should be performed to confirm this finding and investigate causes and interventions.
- The detection of *E. coli* in harvested rainwater, an improved water, has implications for the achievement of the UN safe water access target of the Millennium Development Goals (MDGs) because this "improved" water was often microbiologically unsafe.
- The CBT may be a valuable tool for doing studies on the microbial quality of household and community water in resource-limited areas to track MDG progress , such as in the stored rainwater of this study.
- The CBT may be a reliable alternative to expensive, cumbersome and less portable methods, such as the Colisure/Colilert Quantitray method used here.

REFERENCES

1. Pierson, K., Casanova, L., Sobsey, M. (2010) Evaluation of a Low-Cost Compartmentalized Bag MPN Method for the Detection and Quantification of *E. coli* in Fecally Contaminated Drinking Water. ASM, San Diego, May 2008.

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