Aquagenx
Safe water for anyone, anywhere, anytime

Aquagenx Compartment Bag Test (CBT)
Performance Evaluation Data

I. Compartment Bag Test Evaluation Compared with Colilert

Lab Evaluation of the *E. coli* Chromogenic Medium of the CBT versus Colilert Medium in Compartment Bag MPN Test Format

Background

Colilert is a proprietary defined substrate medium for the detection of *E. coli* based on the hydrolysis of a fluorogenic Beta-D-Glucuronide substrate for which a hydrolysis product fluoresces blue under long wavelength UV light. Colilert has been extensively tested in presence-absence and most probable number formats and these methods are approved by US EPA and ISO. It was used as the "gold standard" method against which the CBT chromogenic Beta-D-Glucuronide medium could be compared for performance in the CBT compartment bag. Colilert has been documented previously to give good performance for *E. coli* detection in a presence-absence test format done in the same kind of clear polyethylene bag as is used for the CBT. The difference in this testing is that the bag for the CBT has been divided into 5 internal compartments of 1, 3, 10, 30 and 56 mL (100 mL total to provide quantitative MPN results. Split samples of water containing *E. coli* were analyzed simultaneously using the CBT with Colilert and the CBT with its chromogenic *E. coli* medium

Methods

Lab performance data were collected in a university lab by college students who were trained to conduct the Compartment Bag Test (CBT). The 600 samples of water in this dataset were split into groups tested as 100-mL sample volumes and analyzed using 1) the CBT and Hi-*E.coli* chromogenic medium and (2) the CBT and Colilert medium. Both tests reported *E. coli* concentration as an MPN/100 mL. Each sample was made by mixing an urban surface water impacted by upstream municipal wastewater discharges as well as local non-point sources of fecal contamination with deionized water to form uniform mixtures at one of two different dilution levels: 10mL of stream water to 90 mL of sterile diluent and 50 mL of stream water to 50 mL of sterile diluent. Within each test group, 180 samples were made at the 50 mL dilution and 120 samples were made at the 10mL dilution. For each test group and within each dilution level, the incubation temperature of the water samples was divided among the three temperatures of 27° C, 37° C, and 44° C. For each incubation temperature-sample dilution combination, the 10mL dilution had a sample size of 40 and the 50 mL dilution had a sample size of 60. Table 1 presents how samples were arrayed between the incubation temperatures and dilution levels for the CBT chromogenic medium test group and the Colilert medium test group. All samples were tested in CBT compartment bags, so MPN counts for Colilert are equivalent to MPN counts for CBTs.

Table 1. Numbers of samples tested with CBT for each temperature and dilution level.

	Number of Samples			
Sample Dilution/Temperature	27° C	37° C	44° C	Total
10 in 100 mL	40	40	40	120
50 in 100 mL	60	60	60	180
Total	100	100	100	300

Results and Data Analysis

In order to test whether CBT chromogenic medium and a standard medium, such as Colilert, report similar MPN values when both are conducted in a lab setting immediately after sample collection, a regression analysis was conducted. First a three-way ANOVA regression with log(MPN) as the dependent variable and categorical variables for test type, dilution, and temperature was performed.[1] This type of analysis compares the group means of log(MPN) between each level (i.e. each value) of each categorical variable while controlling for the other the variables. The coefficient of a given level for a given categorical variable measures the effect on log(MPN) of that level when compared to a reference level chosen to be the smallest value of the categorical variable. For example, the coefficient on CBT vs Colilert tells us the effect on log(MPN) of using CBT medium to measure *E. coli* concentration versus using the Colilert medium. In this example, Colilert is the reference level of the categorical variable test type. When coefficients are not statistically significant, we consider them to be no different from 0 so that there is no effect on log(MPN) of the level under consideration versus the reference level of a given categorical variable.

A notable observation from the ANOVA regression results in Table 2 is that the estimated change in MPN from using CBT medium versus Colilert medium is 0 as the p-value on the coefficient is 0.18 and insignificant. This result provides evidence that CBT medium performs similarly to Colilert medium in quantifying *E. coli* concentration in water samples. As expected, using a dilution of 50ml versus 10ml significantly increases MPN by an estimated 8.67 units. This is an expected result as the more diluted surface water should have less bacteria in it and the change of 8.67 units is significant at the 0.1% level. Maintaining a sample at 44° F versus 27° F is associated with a significant (at the 5% level) increase of 1.22 in MPN value, which is only a small difference in MPN concentration. Though this change is statistically significant, the magnitude of the effect on MPN is not large. The effect of maintaining samples at 37° F versus 27° F is not statistically significant, indicating that the media give equivalent MPN results at these temperatures.

Table 2. Three-way ANOVA regression results with log(MPN) as the dependent variable.

	Coefficient	Estimated unit change on MPN	Significance level	p-value
CBT vs Colilert	-0.09	0		0.18
Dilution 50ml vs 10ml	2.16	8.67	***	<0.0001
37° vs 27°	0.10	0		0.21
44° vs 27°	0.20	1.22	*	0.02

Significance level key: ~ 10%; * 5%; ** 1%; *** 0.1%

[1] Very similar results were found using sqrt(MPN) as the transformed dependent variable.

In addition to testing the effect of the explanatory variables on log(MPN) concentration, the variables' effects on determining if the reported MPN was less than 48.3 or greater than or equal to 48.3 was also measured (as this is the upper uncensored detection limit of the CBT MPN test). Two other logistic regressions were performed using less than 100 MPN or greater than or equal to 100 (as the assigned uncensored upper detection limit of the test) as well as less than 1.5 MPN or greater than or equal to 1.5 MPN as the uncensored lower detection limit of the test). In addition, these three sets of MPN cutoffs are of interest as they represent the smallest and largest MPN values in the dataset that correspond to the World Health Organization recommended decimal categories of drinking water risk. No *E. coli*/100 ml or <1.5 MPN/100 mL in the CBT is considered "safe", MPN values between 1-10 MPN/100 mL are considered intermediation risk, 10-100 MPN per 100 mL are considered high risk and >100/100 mL is considered very high risk. The results for all of the regressions were similar to the results reported in Table 3.

Through logistic regressions the effect of variables on the odds of having unsafe drinking water can be measured. Similar to the ANOVA regression, logistic regression measures the effect of the independent variables on the dependent variable. However, because in the logistic case the response is a dummy variable, the coefficients are interpreted as the multiplicative effect on the log odds of having an MPN greater than or equal to 48.3 for a unit change in the explanatory variable while holding all other variables constant.[2] Taking the exponential of the coefficient provides the multiplicative effect on the odds of having an MPN greater than or equal to 48.3 for a unit change in the explanatory variable. Just as in the ANOVA regression, when coefficients are not statistically significant, there is no effect on the log odds of having MPN greater than or equal to 48.3 with a unit change in the independent variable.

The results in Table 3 indicate that most of the coefficients are not significant. The odds of having an MPN greater than or equal to 48.3 when the temperature is 44° F are 1.63 times larger than the odds when the temperature is 27° F, however that increase is only significant at the 10% level. All other coefficients have p-values greater than 0.10 and are associated with explanatory variables that do not significantly affect the odds of having an MPN greater than or equal to 48.3.

Table 3. Logistic regression results comparing MPN < 48.3 vs >=48.3 as the dependent variable

	Coefficient	Estimated change in odds of MPN>=48.3	Significance level	p-value
CBT vs Colilert	-0.17	0		0.45
Dilution 50ml vs 10ml	20.03	0		0.98
37° vs 27°	0.34	0		0.19
44° vs 27°	0.49	1.63	~	0.07

Significance level key: ~ 10%; * 5%; ** 1%; *** 0.1%

Both the ANOVA regression and the logistic regression above suggest that using CBT medium versus Colilert medium does not significantly affect MPN concentrations of *E. coli* in water. The regressions report no effect of medium type or temperature used on log(MPN) nor on the odds of reporting MPN values greater than or equal to 100, 48.3, or 1.5 per 100 mL.

[2] Note, the odds of event A are the probability of event A happening divided by the probability of event A not happening.

It is concluded from this analysis that the determination of MPN concentrations of *E. coli* bacteria in water are detected as well with CBT chromogenic medium as they are with Colilert chromogenic medium.

II. Field Evaluation of the Compartment Bag Test (CBT) against the Colisure Quanti-tray to Detect and Quantify *E. coli* in Drinking Waters and their Sources in Rural Communities of Laos and Thailand

In order to further evaluate the performance of the CBT in comparison to a standard MPN test for *E. coli* detection in the field, household and source water samples from selected communities in Laos and Thailand were analyzed in parallel by the CBT and the IDEXX Quanti-tray method using Colisure defined substrate medium. The CBT uses a chromogenic coliform medium containing X-Gluc as the basis for *E. coli* detection as visible blue hydrolysis product. The IDEXX Quanti-Tray system was used with Colisure medium, which detects *E. coli* as UV light fluorescence from MUG hydrolysis. Water samples were from sites in Laos having largely under-served areas where water sources were predominantly unimproved dug wells and sanitation facilities were largely lacking. Study sites in Thailand had more improved services for both water supply and sanitation and water sources were predominantly household harvested rainwater. At household point of use, collected water was stored in plastic gallon jugs, thermos canisters, small earthen jars or plastic buckets and these waters were primarily accessed by scooping with a cup, likely resulting in hand contact with the water.

Water quality analysis was done at field sites May through August 2012, and consisted of duplicate samples from household containers from which drinking water is fetched, from all corresponding sources. Repeat samples were collected three days after the initial sampling. At larger water bodies, multiple samples from different locations were collected. A total of 130 samples were analyzed in Laos and 119 in Thailand.

In the initial analysis, samples were scored for *E. coli* presence or absence per 100 mL because the World Health Organization Guidelines consider absence of *E. coli* per 100 mL as "safe" water. As shown in the table below, many of the water samples (93 of 248 or 37.5%) were positive for *E. coli* by both methods of analysis. When the CBT and IDEXX Quanti-Tray results were compared by Fishers exact test, there was an extremely significant association ($p<0.0001$), indicating that the two methods give similar results with a sensitivity of 81%, a specificity of 89%, a positive predictive value of 86% and a negative predictive value of 84%.

Methods	**CBT**	
Quanti-tray 2000	**+**	**-**
+	93	15
-	22	118

The number of samples in the different WHO health risk *E. coli* concentration categories, 0, 1-10, 10-100 and >100 MPN/100 mL, depending on whether measured by CBT or IDEXX Quanti-Tray were compared with Wilcoxon matched-pairs signed-ranks tests at the $\alpha = 0.05$ significance level. For the data collected from Laos water sources, the number of samples in the different *E. coli* concentration categories between CBT results and IDEXX Quanti-Tray results were not significantly different ($p = 0.4615$). For the data collected from Thailand water sources, the number of samples in the different *E. coli* concentration categories between CBT results and IDEXX Quanti-Tray results were also not significantly different ($p =$

0.6250). These results document that CBT results are equivalent to IDEXX Quanti-Tray Colisure results in providing information about the safety of water based on *E. coli* presence or absence and *E. coli* concentrations are reliably predicted by the CBT, equivalent to that of a standard method.

	Thailand Samples		Laos Samples	
WHO range	**CBT**	**QT**	**CBT**	**QT**
<0	148	165	123	154
1 through 10	47	51	45	48
10+ through 100	20	18	40	37
>100	9	6	30	31
Eliminated due to leakage	16	0	34	2
Total number of samples	**240**	**240**	**272**	**272**

When the CBT and Quanti-Tray data for *E. coli* concentrations in water samples from Laos were examined by the Mann-Whitney Test to determine if medians of the CBT and IDEXX Quanti-Tray analysis differ significantly, the two-tailed P value was 0.4594, considered not significant. Likewise, when the CBT and Quanti-Tray data for *E. coli* concentrations in samples from Thailand were similarly examined by the Mann-Whitney test, the two-tailed P value was 0.7904, also considered not significant.

Overall, these results indicate that the CBT MPN method for *E. coli* in water provides equivalent results to a standard MPN method, the IDEXX Quanti-Tray procedure using Colisure medium.