Science of the Total Environment 587-588 (2017) 102–107

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# The compartment bag test (CBT) for enumerating fecal indicator bacteria: Basis for design and interpretation of results

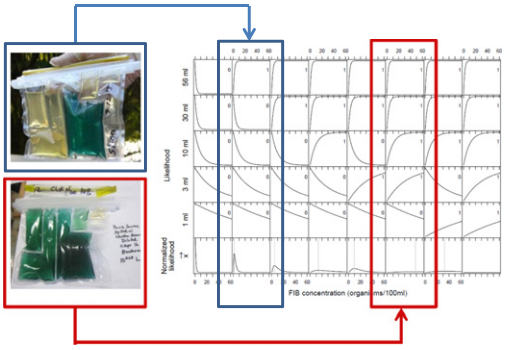
Andrew D. Gronewold[a, b, *], Mark D. Sobsey[c], Lanakila McMahan[d]

[a] NOAA, Great Lakes Environmental Research Laboratory, Ann Arbor, MI, USA
[b] Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, MI, USA
[c] Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina Chapel Hill, Chapel Hill, NC, USA
[d] United States Agency for International Development (USAID), Washington, D.C., USA

## HIGHLIGHTS

- The statistical basis for the compartment bag test is documented.
- Interpretation of test results reflects methodological uncertainty.
- Bayesian MCMC methods are employed to infer bacteria concentrations.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

*Article history:*
Received 11 January 2017
Received in revised form 6 February 2017
Accepted 6 February 2017
Available online 27 February 2017

Editor: D. Barcelo

*Keywords:*
Compartment bag test
Water quality
Drinking water
Human health
Statistical methods

## ABSTRACT

For the past several years, the compartment bag test (CBT) has been employed in water quality monitoring and public health protection around the world. To date, however, the statistical basis for the design and recommended procedures for enumerating fecal indicator bacteria (FIB) concentrations from CBT results have not been formally documented. Here, we provide that documentation following protocols for communicating the evolution of similar water quality testing procedures. We begin with an overview of the statistical theory behind the CBT, followed by a description of how that theory was applied to determine an optimal CBT design. We then provide recommendations for interpreting CBT results, including procedures for estimating quantiles of the FIB concentration probability distribution, and the confidence of compliance with recognized water quality guidelines. We synthesize these values in custom user-oriented 'look-up' tables similar to those developed for other FIB water quality testing methods. Modified versions of our tables are currently distributed commercially as part of the CBT testing kit.

Published by Elsevier B.V.

## 1. Introduction

Ensuring readily-available high quality drinking water is fundamental to human health and has important connections to socioeconomic status, commercial and industrial growth, and overall quality of life (Mekonnen and Hoekstra, 2016). The challenge of providing that ensurance is met in different ways around the world; in some communities, drinking water supplies are assumed protected if they are adequately separated from wastewater and other sources of contamination (George, 2008). In others, routine water quality testing is used to ensure compliance with recognized standards (Gleick, 1998; Novotny, 2003). Testing kits that support these assessments

* Corresponding author at: NOAA, Great Lakes Environmental Research Laboratory, Ann Arbor, MI, USA.
*E-mail address:* drew.gronewold@noaa.gov (A.D. Gronewold).

http://dx.doi.org/10.1016/j.scitotenv.2017.02.055
0048-9697/Published by Elsevier B.V.

often require a skilled technician to collect, analyze, and interpret results, as well as microbiological laboratory facilities. In regions of the world without these resources and where the time from water withdrawal (from its source) to consumption is short, alternative testing procedures are needed.

To address this gap in global water quality protection, researchers at the University of North Carolina Chapel Hill and Duke University developed a simple kit for enumerating FIB concentrations that is portable, relatively inexpensive, and provides easy-to-interpret results (Stauber et al., 2014). This kit, commonly referred to as the compartment bag test (or CBT), is currently manufactured and distributed by Aquagenx, LLC and has been tested and used in communities around the world (Murcott et al., 2015; Weiss et al., 2016). To date, however, the statistical basis for the design and recommended interpretation of results from the CBT have not been formally documented.

Here, following documentation for the development of similar water quality testing kits (McCrady, 1915; de Man, 1977; Tillett and Coleman, 1985; Haas, 1989; McBride et al., 2003), we begin with an overview of the statistical theory behind the CBT, followed by examples of how that theory was applied to determine an optimal CBT design. We then provide recommendations for interpreting CBT results, including procedures for estimating quantiles of the FIB concentration probability distribution, as well as procedures for calculating the confidence of compliance with World Health Organization (WHO) drinking water quality guidelines (McBride and Ellis, 2001; Borsuk et al., 2002; World Health Organization, 2004). We synthesize these values in custom user-oriented 'look-up' tables similar to those developed for other FIB testing kits (de Man, 1977). Finally, we explore the sensitivity of CBT results to departures from assumptions in the underlying statistical models, and from recommended protocols for sample collection and handling.

## 2. Experimental

### 2.1. Statistical basis for interpreting CBT results

The CBT is a manufactured clear plastic multi-compartment bag into which 100 ml of a water sample is distributed (Stauber et al., 2014). Each compartment contains a growth substrate designed to detect groups of FIB (such as hydrogen sulphide producers), or specific bacteria such as *Escherichia coli* (EC), by turning a distinctive color (e.g. blue-green) indicating growth of "target" (e.g. FIB or EC) bacteria during an incubation period. The CBT will yield a pattern of 'positive' and 'negative' compartments from which a user can infer the FIB concentration of the original sample following the common assumption (Greenwood and Yule, 1917; Cochran, 1950; Woodward, 1957; El-Shaarawi et al., 1981; Hurley and Roscoe, 1983; de Man, 1983; Haas and Heller, 1988; Woomer et al., 1990; Briones and Reichardt, 1999) that, for a given sample, the number of target bacteria ($y_i$) in compartment $i$ ($i \in [1, m]$ and $m$ is the total number of compartments) with volume $v_i$ (assuming a well-mixed sample) is well-represented by a Poisson probability distribution $y_i \sim \text{Po}(\lambda_i = cv_i/100)$ with FIB concentration $c$ (in organisms per 100 ml), and mean and variance $\lambda_i$. The probability of a positive compartment of volume $v_i$ is $1-exp(-cv_i/100)$. The joint probability of any pattern of positive and negative compartments $\vec{x}$ (where the over-arrow superscript denotes a row vector, $x_i \in [0, 1]$ and $x = 1$ indicates a positive compartment) is then expressed as the product of a series of $m$ independent Bernoulli trials:

$$f(\vec{x} \mid \vec{v}, c) \propto \prod_{i=1}^{m} \left(1 - e^{-cv_i/100}\right)^{x_i} \left(e^{-cv_i/100}\right)^{1-x_i} \quad (1)$$

Conventional interpretations of presence/absence test kits for FIB often focus on a deterministic solution to $c$ from Eq. (1). This value is commonly referred to as the "most probable number" (or MPN) and can be calculated as (Hurley and Roscoe, 1983; McBride, 2005; Gronewold and Wolpert, 2008)

$$\text{MPN} = \underset{c}{\text{argmax}} \left[ \prod_{i=1}^{m} \left(1 - e^{-cv_i/100}\right)^{x_i} \left(e^{-cv_i/100}\right)^{1-x_i} \right] \quad (2)$$

We implement this formulation using the `uniroot` function in the `R` statistical software package (R Core Team, 2014). Corresponding code is included in the Supplementary Information.

Multiple methods have been developed for expressing uncertainty in the MPN, however most do not explicitly acknowledge that the probability distribution of the MPN for a given pattern of positive and negative compartments is typically discrete and multimodal, while the probability distribution of the FIB concentration is almost always unimodal and continuous (Klee, 1993; Gronewold and Wolpert, 2008). Therefore, in addition to reporting conventional MPN values, we propose two interpretations of CBT results that allow for a more robust understanding of the uncertainty in the FIB concentration and how that uncertainty affects the confidence of compliance with water quality guidelines (McBride and Ellis, 2001; Gronewold and Borsuk, 2009, 2010). The first is based on calculating quantiles of the likelihood function of the FIB concentration (Eq. (1), written as a function of $c$ for given $\vec{x}$ and $\vec{v}$), as well as the probability that the FIB concentration exceeds 1, 10, 100, or 1000 organisms per 100 ml.

The second interpretation is based on a Bayesian analysis of CBT results (Bernardo and Ramon, 1998; Press, 2003; Bolstad, 2004) where the posterior probability distribution of the FIB concentration $c$ is proportional to the product of the likelihood function (Eq. (1)) and prior probability distribution $\pi(c)$:

$$f(c \mid \vec{x}, \vec{v}) \propto \pi(c) f(\vec{x} \mid \vec{v}, c) \quad (3)$$

One advantage of this approach is that it allows for expression of *a priori* assumptions about the potential range of the FIB concentration in a water sample. Methods based on the likelihood function alone, in contrast, implicitly assume *a priori* that FIB concentrations ranging from 0 to $\infty$ are equally likely; an assumption analogous to a belief that gross contamination is just as likely as a FIB concentration within a few orders of magnitude of (or even well below) WHO water quality guidelines. This *a priori* belief is just one of many a CBT user might have about water quality at a particular sampling location (Press, 2003). Here, we present calculations based on a lognormal prior $\pi(c) = \text{LN}(\mu = 0, \sigma^2 = 100)$, with log-concentration mean $\mu$ and variance $\sigma^2$, intended to represent an *a priori* belief that the FIB concentration is most likely low, but that extreme FIB concentrations are possible. We view further investigation of impacts of alternative priors on CBT results as an important area for future research.

It is informative to note that previous studies have explored alternative probability models for interpreting multiple-compartment water quality analysis results, including the negative binomial model and variations of the Poisson model that account for thinning and dispersion (Christian and Pipes, 1983; El-Shaarawi et al., 1981; Messner and Wolpert, 2002; Crainiceanu et al., 2003). Recent research, however (see Gronewold et al., 2008; Wu et al., 2014), indicates that only extreme and persistent violations of the Poisson probability model would justify application of an alternative probability model.

Finally, following Eq. (1), we calculate the relative likelihood of each possible combination of positive and negative compartments. Results of this calculation provide an indication of CBT outcomes that are most likely, and those that (because they are extremely unlikely) might indicate contamination or thinning of individual