

Fermentation tube test statistics for direct water sampling and comments on the Thomas formula

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ABSTRACT

This article describes a new interpretation of the Fermentation Tube Test (FTT) performed on water samples drawn from natural waters polluted by faecal bacteria. A novel general procedure to calculate the Most Probable Number of bacteria (MPN) in natural waters has been derived for the FTT for both direct and independent repetitive multiple water sampling. The generalization based on solving the newly proposed equation allows consideration of any a priori frequency distribution $g(n)$ of bacterial concentration in analysed water as opposed to the unbounded uniform a priori distribution $g(n)$ assumed in the standard procedures of the *Standard Methods of Examining Water and Wastewater* and *ISO 8199:1988*. Also a statistical analysis of the Thomas formula is presented. It is demonstrated that the Thomas formula is highly inaccurate. The authors propose, therefore, to remove the Thomas formula from the *Standard Methods of Examining Water and Wastewater* and *ISO 8199:1988* altogether and replace it with a solution of the proposed generalized equation.

Key words | bacteriological pollution, Bayesian interpretation, fermentation tube test, Thomas formula, water sampling

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INTRODUCTION

To assess bacterial pollution of raw water, treatment plants still use an old measuring technique – the Fermentation Tube Test (FTT) – that was developed during the First World War. This simple technique is based on the concept of repetitive sampling of water with a set of standard tubes, followed by addition of lactose to the water samples and counting samples from which fermentation gas is released. The gas resulting from consumption of lactose by bacteria is easily detectable, thus making the FTT an attractive measuring technique. In the ideal experimental set-up, if there are no bacteria in a water sample, no fermentation is observed (negative test result) and, conversely, the presence of even one bacterium in the tube initiates fermentation (positive test result). In the simplest case *one tube* of volume V_t is used *once* to draw a small water sample from a container of water taken at some location, such as a river or lake. When the bacteriological status of water at the

location is characterized by a local concentration of bacteria – n , the same constant concentration of bacteria n is assumed in the water in the container. For this purpose water in the container is well mixed thus assuring homogeneous distribution of bacteria within the container. Water in the container is considered to be the water source for the FTT sampling procedures. Equations to calculate the probability of getting positive ($p(+)$) or negative ($p(-)$) FTT results in a water sample drawn with the tube from a large and well mixed water source have been given in Nawalany (2000) and Nawalany & Loga (2004):

$$p(+) = 1 - e^{-nV_t} \quad (1a)$$

$$p(-) = p(0) = e^{-nV_t} \quad (1b)$$

It should be noted, that in cases for which an assumption of a “sufficiently large...water source” does not hold,

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multiple water sampling with tube of volume V_t cannot be considered independent sampling. Detailed discussion of such cases is presented by Nawalany (2000) and Nawalany & Loga (2004). In this paper, however, a large and well-mixed water source is assumed for the sake of consistency with real procedures applied in water quality monitoring. The FTT applied to r water samples drawn directly from the water source by sampling with just *one tube r-times* is denoted hereafter as FTT(r). Direct sampling from a sufficiently large and well-mixed water source leads to statistically *independent* outcomes of the FTT(r), i.e. the FTT outcome in any single sample of water does not depend on the FTT outcomes in other water samples. It should be noted also that in the case of large and well-mixed water sources, this type of direct sampling is equivalent of doing one-time multiple sampling, i.e. of taking once (and simultaneously) r water samples using r tubes of the same volume. The number of water samples from which fermentation gas is released after adding lactose is assigned m , ($m = 0, 1, \dots, r$). It is a measure of an outcome of the FTT(r). When the concentration of bacteria in the water source is equal to n and when the probability of getting a positive FTT outcome in a single water sample of volume $V_t - p(+)$, is given by Equation (1a), then the conditional probability of getting exactly m positive outcomes in r (independent) samples is equal to:

$$P(m|n) = \binom{r}{m} p(+)^m [1 - p(+)]^{r-m}$$

$$= \binom{r}{m} (1 - e^{-V_t n})^m e^{-V_t n(r-m)} \quad (m = 0, 1, \dots, r) \quad (2)$$

By equating a derivative of this probability to zero:

$$\frac{\partial P(m|n)}{\partial n} = 0 = \binom{r}{m} \frac{\partial p(+)}{\partial n} p(+)^{m-1} (1 - p(+))^{r-m-1} [m - r \cdot p(+)] \quad (3)$$

one can calculate “the most probable concentration of bacteria” in the sampled water using a standard equation:

$$n^* = \frac{1}{V_t} \ln \left(\frac{1}{1 - m/r} \right) \quad (4)$$

and, ultimately, the Most Probable Number of bacteria (MPN):

$$\text{MPN} = V_s n^* \quad (5)$$

where V_s is some standard volume of water, usually 100 ml.

The reason to consider n^* , i.e. value of n at which probability $P(m|n)$ assumes maximum, instead of the mean or median of binomial distribution, is dictated by the established practice in water monitoring of using procedures described in *Standard Methods of Examining Water and Wastewater (1998)* and in *ISO 8199:1988 (E) - Water Quality (International Organization for Standards 1988)*. There, values of MPN consistent with Equations (4) and (5) for different V_s , V_t and different ratios m/r can be found. In this article the two main sources of reference for the FTT procedures are abbreviated as SM and ISO, respectively.

As previously reported (Nawalany 2000; Nawalany & Loga 2004) the authors have interpreted the FTT outcomes in terms of the Bayesian statistics and explained discrepancies found in the test's statistical tables in SM and ISO. The authors proposed also a new definition of MPN based on the Bayes formula:

$$f(n|m) = \frac{P(m|n)g(n)}{\int_0^\infty P(m|n')g(n')dn'} = \frac{1}{C} P(m|n)g(n) \quad (6)$$

where $g(n)$ is the a priori probability distribution function (p.d.f.) of bacteria concentration in the water source;

$P(m|n)$ is the probability function of getting m FTT “successes” in r water samples when bacteria concentration in the water source is equal to n .

$$C = \int_0^\infty P(m|n')g(n')dn' \text{ is the normalization constant.} \quad (6a)$$

The a priori p.d.f. of bacteria concentration in the water source – $g(n)$, can, in general, be arbitrary, e.g. the Uniform or the Gamma distribution. For a short comment on $g(n)$ see the discussion of the example, given below. Function $f(n|m)$ is a posterior conditional p.d.f. of n being in the range $(n, n + dn)$ when the FTT(r) outcome is m . From a practical point of view, this function is much more interesting than $P(m|n)$ since it can be used in assessing the bacteriological status of water in the source provided that exactly m water

samples have fermented. According to the new definition of the MPN, instead of solving Equation (3) and using its solution (4) one should calculate the most probable concentration of bacteria by finding n^* that maximizes the a posteriori p.d.f. $f(n|m)$, i.e. by requiring:

$$\left. \frac{\partial f(n|m)}{\partial n} \right|_{n=n^*} = 0 \quad (7)$$

After applying the Bayes formula (6), Equation (7) can be written as:

$$\frac{\partial P(m|n)g(n)}{\partial n} = g(n) \frac{\partial P(m|n)}{\partial n} + P(m|n) \frac{dg(n)}{dn} = 0 \quad (7a)$$

or, equivalently, as:

$$\frac{(\partial P(m|n))/(\partial n)}{P(m|n)} = - \frac{(dg(n))/(dn)}{g(n)} \quad (8)$$

An important observation is to be made at this point. When the concentration of bacteria in the water source – n , can be considered as a random variable governed by the uniform probability distribution within the range $[0, n_{\max}]$ and $n_{\max} \rightarrow \infty$ then the a posteriori p.d.f. $f(n|m)$ does converge to the normalized p.d.f. function $\tilde{P}(m|n)$, i.e.

$$f(n|m) \rightarrow \frac{P(m|n)}{\int_0^\infty P(m|n')dn'} = \tilde{P}(m|n), \quad (m = 0, 1, \dots, r-1). \quad (9)$$

In this (and only this) case one is allowed to consider $\tilde{P}(m|n)$ as equivalent to the a posteriori p.d.f. $f(n|m)$ and, consequently, interpret concentration n^* calculated from Equation (3) as the one which has the highest probability of occurring when the outcome of the FTT(r) is m , ($m = 1, \dots, r-1$). The outcome $m = r$ does not allow any inference about bacterial concentration in the sampled water. Equation (8) is more general than Equation (3), as it provides a way of calculating MPN in cases when bacterial concentration in the water source is governed by an a priori $g(n)$ different from the uniform distribution. Equation (9) is valid for any finite r . An infinite number of samples is not considered here, being economically unrealistic (the cost of taking one water sample is always finite).

Equations (1)–(9) are presented here as an introduction and serve to make the entire text self-contained. In the following paragraphs the FTT outcomes are discussed for more complex modes of sampling, i.e. when water is

sampled repetitively and independently with a set of j_0 tubes ($j_0 > 1$) having different volumes. Essentially more complex cases of indirect sampling will be described in a separate article.

REPETITIVE MULTIPLE DIRECT SAMPLING

Repetitive multiple direct sampling is realized by taking water samples directly from the large and well-mixed water source with a collection of j_0 tubes having different volumes V_j , ($j = 1, \dots, j_0$). When a water sample is taken with a tube, it undergoes the FTT procedure; the corresponding outcome of the FTT for the sample can be either “positive (success)” or “negative (failure)”. Probability of a successful FTT outcome depends only on the concentration of bacteria in analysed water – n , and, parametrically, on volume V_j , of the given j -th tube – Equation (1). In the general case, sampling with j -th tube can be repeated r_j -times independently of repetitive sampling with other tubes. This type of the FTT test is assigned as FTT(r_1, r_2, \dots, r_{j_0}). A combined result of the multiple FTT testing is represented by integer numbers m_1, m_2, \dots, m_{j_0} and denoted as j_0 -tuple $(m_1, m_2, \dots, m_{j_0})$. Element m_j of the resultant j_0 -tuple $(m_1, m_2, \dots, m_{j_0})$ represents numbers of “successes” when sampling water with the j -th tube r_j -times, ($j = 1, \dots, j_0$). Hence, $m_j \in \{0, 1, \dots, r_j\}$, ($j = 1, \dots, j_0$). In the standard FTT procedures sampling is repeated r -times using all j_0 tubes at the time, i.e. $r_j = r$, ($j = 1, \dots, j_0$) – see Figure 1. In the following analysis the number of repetitions r_j , ($j = 1, \dots, j_0$), need not be necessarily the same. The mode of sampling described above is clearly independent sampling, since, for a given multiple set of samples, the chance of “success” (fermentation) in any water sample does not

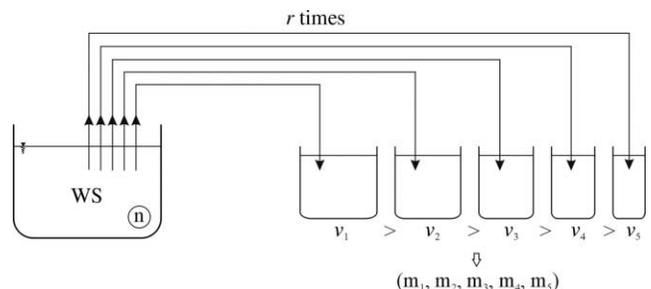


Figure 1 | Procedure of direct water sampling from large and well-mixed water source (WS) with $j_0 = 5$ tubes of different volume.

depend on whether or not water samples in other tubes are fermenting. Mutual independence of the FTT outcomes has its direct consequences for statistical interpretation of j_0 -tuples $(m_1, m_2, \dots, m_{j_0})$ – outcomes of FTT(r_1, r_2, \dots, r_{j_0}). Specifically, a chance of getting exactly m_j “successes” on the j -th position of the j_0 -tuple does not depend on number of successes on other positions of the tuple. For given j , ($j = 1, \dots, j_0$) it depends only on probability $p_j(+)$ and on number of sampling repetitions with j -th tube – r_j .

When water is sampled with j_0 tubes of volumes V_j , ($j = 1, \dots, j_0$) directly from a large and well-mixed water source characterized by concentration of bacteria n , the conditional probability of getting some fixed j_0 -tuple $(m_1, m_2, \dots, m_{j_0})$ is a product of probabilities of getting m_j positive FTT outcomes on the j -th position of the tuple, ($j = 1, \dots, j_0$), independently of the number of positive outcomes on the other positions, i.e.

$$P(m_1, \dots, m_{j_0} | n) = \prod_{j=1}^{j_0} P(m_j | n) \quad (10)$$

where

$$P(m_j | n) = \binom{r_j}{m_j} p_j(+)^{m_j} [1 - p_j(+)]^{r_j - m_j} \quad (11)$$

$$m_j \in \{1, \dots, r_j\}$$

$$p_j(+) = 1 - \exp[-V_j \cdot n] \quad \text{for } j = 1, \dots, j_0. \quad (12)$$

Equations (11) and (12) follow the similar Equations (2) and (1) for 1-tuple FTT(r) outcomes.

To calculate MPN from outcomes of the FTT(r_1, r_2, \dots, r_{j_0}) test, one should find the maximum of the a posteriori probability function $f(n | m_1, m_2, \dots, m_{j_0})$, i.e. solve the following equation (compare Equation (8)):

$$\frac{(\partial P(m_1, \dots, m_{j_0} | n)) / (\partial n)}{P(m_1, \dots, m_{j_0} | n)} = - \frac{(\partial g(n)) / (\partial n)}{g(n)}. \quad (13)$$

When substituting Equation (10) into Equation (13) one obtains:

$$\sum_{j=1}^{j_0} \frac{(\partial P(m_j | n)) / (\partial n)}{P(m_j | n)} = - \frac{(\partial g(n)) / (\partial n)}{g(n)} \quad (14)$$

Calculation of the corresponding derivatives in Equation (14):

$$\begin{aligned} \frac{\partial P(m_j | n)}{\partial n} &= \binom{r_j}{m_j} \frac{\partial p_j(+)}{\partial n} p_j(+)^{m_j-1} (1 - p_j(+))^{r_j - m_j - 1} \\ &\quad \times [m_j - r_j \cdot p_j(+)] \\ &= \binom{r_j}{m_j} \frac{(\partial p_j(+)) / (\partial n)}{p_j(+)(1 - p_j(+))} p_j(+)^{m_j} (1 - p_j(+))^{r_j - m_j} \\ &\quad \times [m_j - r_j \cdot p_j(+)] \\ &= \frac{(\partial p_j(+)) / (\partial n)}{p_j(+)(1 - p_j(+))} [m_j - r_j \cdot p_j(+)] \cdot P(m_j | n) \end{aligned} \quad (15)$$

and substituting them in (14) results in:

$$\sum_{j=1}^{j_0} \frac{(\partial p_j(+)) / (\partial n)}{p_j(+)(1 - p_j(+))} [m_j - r_j \cdot p_j(+)] = - \frac{(\partial g(n)) / (\partial n)}{g(n)} \quad (16)$$

where $m_j \in \{1, \dots, r_j\}$

$$p_j(+) = 1 - \exp[-V_j \cdot n] \quad (16a)$$

$$\frac{\partial p_j(+)}{\partial n} = V_j (1 - p_j(+)) \quad \text{for } j = 1, \dots, j_0. \quad (16b)$$

Substituting (16a) and (16b) to the left-hand side of Equation (16) results in:

$$\begin{aligned} \sum_{j=1}^{j_0} \frac{(\partial p_j(+)) / (\partial n)}{p_j(+)(1 - p_j(+))} [m_j - r_j \cdot p_j(+)] \\ = \sum_{j=1}^{j_0} V_j \cdot [m_j - r_j \cdot p_j(+)] / p_j(+) \end{aligned}$$

from which the ultimate equation for n^* can be written as follows:

$$\sum_{j=1}^{j_0} V_j \cdot [m_j - r_j \cdot p_j(+)] / p_j(+) = - \frac{(\partial g(n)) / (\partial n)}{g(n)} \quad (17)$$

where probabilities $p_j(+)$, ($j = 1, \dots, j_0$) are given by Equation (16a).

Hence, in the case when the frequency of bacteriological pollution of the water source is characterized by an arbitrary a priori p.d.f. $g(n)$, the most probable concentration of bacteria n^* corresponds to maximum

of a posteriori probability function $f(n|m_1, m_2, \dots, m_{j_0})$, i.e. it is a solution of a nonlinear Equation (17). The corresponding MPN is equal to $MPN = n^* \cdot V_s$. Clearly, in the special case, when $g(n)$ is assumed to be the unbounded uniform distribution, Equation (17) simplifies to:

$$\sum_{j=1}^{j_0} V_j \cdot [m_j - r_j \cdot p_j(+)] / p_j(+) = 0 \quad (17a)$$

This, for the special case of $j_0 = 1$, reduces to Equation (4).

EXAMPLE

Direct (hence independent) sampling from a large and well-mixed water source has been performed using $j_0 = 3$ tubes of different volumes - $V_1 = 10$ ml, $V_2 = 1$ ml and $V_3 = 0.1$ ml. The FTT test has been repeated five times for each tube, i.e. $r_1 = r_2 = r_3 = r = 5$. Also the unbounded uniform a priori distribution $g(n) = \text{const.}$ has been assumed for bacterial concentration in the water source. Therefore, Equation (17a) has been used to calculate MPN assuming $V_s = 100$ ml of water. Two particular outcomes of the FTT have been considered and the corresponding MPN calculated:

1. $m_1 = 3, m_2 = 0, m_3 = 0 \Rightarrow MPN = 8$
2. $m_1 = 4, m_2 = 0, m_3 = 0 \Rightarrow MPN = 13$

Exactly the same values of MPN numbers are cited in SM, unsurprisingly as the SM standard procedures tacitly assume the unbounded uniform a priori distribution, $g(n) = \text{const.}$, as the correct one. Contrary to this, the authors claim that, in case of natural water sources it is Equation (17) which should be used to calculate n^* instead of Equations (17a) or (4) since the *assumption of the uniform a priori distribution of bacteria concentration hardly holds for real rivers, lakes or water reservoirs*. In most instances of sampling natural waters for sanitary purposes, the mode of operation of anthropogenic sources of bacteria (e.g. sewage inflows) superimposed on complex hydrodynamics of natural waters *do not* result in the same frequencies of occurrence of different bacteria concentrations in water at assumed sampling points. Therefore, prior to solving Equation (17) and calculating MPN from the FTT outcomes in a suite of independent samples, one

must estimate p.d.f. $g(n)$ at the sampling point. For this some (prior) measurements must be made using techniques that allow for direct assessment of bacteria concentration in water. This approach will be supported by analysis of a long series of bacteriological data from the Warsaw Waterworks and presented by the authors in future articles.

THE THOMAS FORMULA

When referring to the FTT procedures in *Standard Methods of Examining Water and Wastewater (1998)* and ISO 8199: 1988, the authors consider it appropriate to comment on the Thomas formula using notations introduced in this article. In its original form the Thomas formula states that: The MPN for combinations not appearing in the table, or other combinations of tubes or dilutions, may be estimated by the following ratio:

$$\begin{aligned} & \text{MPN/100 ml} \\ &= \frac{\text{no. of positive results} \times 100}{\sqrt{(\text{ml sample in negative tubes}) \times (\text{ml sample in all tubes})}} \end{aligned} \quad (18a)$$

After translating this formula into the notations of this article one may notice that the FTT considered by Thomas is the “one-time multiple-tube” test with different volumes of sampling tubes. This means that number of tubes is j_0 (in the example below $j_0 = 5$) and the number of repetitions $r_j = r = 1$. Consequently, the result m_j , ($j = 1, \dots, j_0$), on any position of the j_0 -tuple can be either 0 or 1. The phrase “ml sample in all tubes” means an integrated volume of all tubes used for (independent) sampling, i.e. $\sum_{j=1}^{j_0} V_j$, whereas “ml sample in negative tubes” means simply $\sum_{j=1}^{j_0} V_j - \sum_{j=1}^{j_0} m_j V_j$. Standard volume is assumed as $V_s = 100$ ml. When translated into the notations of this article, the Thomas formula for MPN can be written as follows:

$$MPN_{\text{TH}} = \frac{(\sum_{j=1}^{j_0} m_j) \times V_s}{\sqrt{(\sum_{j=1}^{j_0} V_j - \sum_{j=1}^{j_0} m_j V_j) \times (\sum_{j=1}^{j_0} V_j)}} \quad (18b)$$

Below, this equation is compared with equations derived in this article for two special cases.

Case A

In this case, all sampling tubes have the same volume, i.e. $V_1 = \dots = V_5 = V_t = 1$ ml and hence $\sum_{j=1}^{j_0} V_j = j_0 V_t$. Now, the Thomas formula can be rewritten as follows:

$$MPN_{TH} = \frac{(\sum_{j=1}^{j_0} (m_j)/(j_0)) \times ((V_s)/(V_t))}{\sqrt{1 - \sum_{j=1}^{j_0} (m_j)/(j_0)}} = \frac{x}{\sqrt{1-x}} \times \frac{V_s}{V_t} \quad (18c)$$

where $x = \sum_{j=1}^{j_0} (m_j)/(j_0) \leq 1$ as m_j are 0 or 1.

Equation (18c) can be directly compared with Equation (5) after substituting to the latter - j_0 for r and $\sum_{j=1}^{j_0} m_j$ for m , which results in:

$$MPN = \frac{V_s}{V_t} \ln \left(1 - \sum_{j=1}^{j_0} \frac{m_j}{j_0} \right)^{-1} = \frac{V_s}{V_t} \ln \left(\frac{1}{1-x} \right). \quad (18d)$$

After assuming that the negatives show only on the last positions of the tuples, Equation (18c) has been applied to calculate MPN_{TH} and Equation (18d) for calculating MPN. If all FTT results are negative ($x = 0$) then $MPN = MPN_{TH} = 0$. And this is the only case when MPN and MPN_{TH} coincide. If all FTT results are positive ($x = 1$) then both MPN and MPN_{TH} are infinite. Values of MPN_{TH} and MPN for number of negatives different than 0 and 5 are presented in Table 1 together with the relative error δ of the Thomas formula.

In this case the Thomas formula ensures reasonable accuracy although for small number of negatives it is becoming slightly inaccurate. The reason of introducing the formula (in 1942) was lack of means for accurate computations. Instead of calculating $\ln(1/(1-x))$ Thomas has proposed calculation of $x/(\sqrt{1-x})$ which has a similar Taylor expansion: $-x - ((x^2)/2) + O(x^3)$. Naturally, today this kind of approximation is not necessary.

Table 1 | Comparison of actual MPN with MPN_{TH} calculated from the Thomas formula - Case A

No. of positives	No. of negatives	MPN_{TH}	MPN	δ (%)
1	4	22.36	22.31	0.2
2	3	51.64	51.08	1.1
3	2	94.87	91.63	3.5
4	1	178.89	160.94	11.1

Table 2 | Comparison of actual MPN with MPN_{TH} calculated from the Thomas formula - Case B

No. of positives	No. of negatives	MPN_{TH}	MPN	δ (%)
1	4	284.62	230.00	+23.75
2	3	1800.91	2500.00	-27.96
3	2	8581.21	25000.00	-65.68
4	1	37947.52	300000.00	-87.35

Case B

In this case volumes of sampling tubes represent a descending series, i.e. $V_1 = 1$ ml, $V_2 = 0.1$ ml, $V_3 = 0.01$ ml, $V_4 = 0.001$ ml and $V_5 = 0.0001$ ml and $\sum_{j=1}^5 V_j = 1.1111$ ml. As in Case A it has been assumed that the negatives show only in the last positions of the tuples. Equation (18b) has been applied to calculate MPN_{TH} . For calculating MPN Equation (17a) has been solved. The resultant MPN_{TH} and MPN are presented in Table 2 together with the relative error δ of the Thomas formula.

Although not all possible arrangements of the “negatives” in the tuples have been analysed, the comparison of MPN_{TH} and MPN shown in Table 2 is conclusive: the Thomas formula is highly inaccurate.

CONCLUSIONS

A new and general procedure of calculating the most probable concentrations of bacteria (and MPN) in natural waters has been derived for the Fermentation Tube Test in case of direct and independent repetitive multiple water sampling. The generalization based on solving Equation (17) does allow considering *any* a priori frequency distribution $g(n)$ of bacteria concentration in analysed water as opposed to the unbounded uniform a priori distribution $g(n)$ assumed in the present standard procedures of SM and ISO. Since the assumption of the uniform distribution of bacteria concentration hardly holds in rivers, lakes or water reservoirs, prior to applying Equation (17) one must estimate p.d.f. $g(n)$ of bacteria concentration at the assumed sampling point. Additionally, the present tables of the MPN need to be replaced by simple software solving nonlinear Equation (17) for different suites

of sampling tubes, different repetition modes and different estimated p.d.f. $g(n)$.

Also, as a concomitant result, strong comment can be made on the Thomas formula. The latter is recommended by SM and ISO for calculating MPN for the cases of using sampling sets (sets of tubes) that are not referenced in the standard MPN tables. By comparing MPN calculated from the Thomas formula given in the SM and ISO with values of MPN calculated from Equation (17a), the high inaccuracy of the Thomas formula has been demonstrated even for the unbounded uniform a priori distribution. The authors, therefore, propose the removal of the Thomas formula from the [Standard Methods of Examining Water and Wastewater \(1998\)](#) and [ISO 8199:1988](#) altogether and its replacement with a solution of Equation (17a) or, in the case when $g(n)$ is different from the unbounded uniform a

priori distribution, with the solution of the proposed generalized Equation (17).

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