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KSA-SM-08

The P/N formula for the log reduction when using a semi-quantitative disinfectant test of type SQ_1

[*Key Words*: LR, most probable number, MPN, positive/negative outcome]

Purpose: How to calculate the log reduction for a semi-quantitative test in which each treated carrier is positive or negative for viable microbes.

Nomenclature

Type SQ_1 test

P/N

Positive (P) carrier
Negative (N) carrier

n_{tr}, n_{un}
subscripts *tr*, *un*
N_{tr}, T_{un}
LD_{un}
MLD_{un}
LR

P/N formula

Numerical example

Interpretation

The previous knowledge sharing article (KSA-SM-07 The log reduction measure of disinfectant efficacy) discussed the log reduction (LR) measure in the context of a quantitative disinfectant test. For a type SQ_1 semi-quantitative disinfectant test, a different LR formula is required; namely, the so-called P/N formula, where P/N indicates the positive/negative outcome for each of the treated carriers. The purpose of this article is to present the P/N formula for calculating LR, a numerical example, and an explanation of the formula.

In a semi-quantitative test of type SQ_1, untreated carriers are enumerated as for a quantitative test, but for treated carriers, microbes are neither harvested nor enumerated (KSA-SM-02 Testing surface disinfectants: quantitative, semi-quantitative, qualitative, and alternative methods). Instead, a binary positive/negative (P/N) result is observed for each treated carrier. The outcome is "positive" if at least one microbe survived the disinfectant treatment and was able to replicate. The outcome is "negative" if the disinfectant treatment killed (or severely damaged) all microbes on the carrier. The AOAC Use-Dilution Method (AOAC 2006) is one of several standard disinfectant test methods of type SQ_1.

Let n_{tr} and n_{un} denote the number of carriers in the treated and untreated sets, respectively. Note that the subscripts *tr* and *un* are used to denote treated and untreated carriers. Let N_{tr}, denote the number of negative carriers among the n_{tr} treated carriers. Let T_{un} denote the viable cell density (cfu per carrier), LD_{un} denote the $\log_{10}$-transformed density for an untreated carrier ($LD_{un} = \log_{10}(T_{un})$), and MLD_{un} denote the mean log density for the n_{un} untreated carriers. The P/N formula for the log reduction (LR) is shown in equation (1) (Tomasino & Hamilton 2006).

$$\mathbf{LR = MLD_{un} - \log_{10}(-\log_e[(N_{tr} + 0.5) / (n_{tr} + 1)]).} \quad (1)$$

If the type SQ_1 test results were $MLD_{un} = 6.00$, $N_{tr} = 59$, and $n_{tr} = 60$, then $LR = 6.00 - \log_{10}[-\log_e(59.5/61)] = 6.00 - \log_{10}(0.025) = 7.60$. This LR = 7.60 indicates that, if 10^9 (= 1 billion) microbes were exposed to the disinfectant treatment, then only 25 ($=10^{9 - 7.60}$) microbes would survive on the average, corresponding to a percentage kill of 99.9999975% ($= [10^9 - 25)/ 10^9]\times 100\%$). This is exactly the same interpretation as for a quantitative test LR (KSA-SM-07).

Rationale

T_{tr}

LD_{tr}

MLD_{tr}

LR for a quantitative test is the difference between *MLD* values

Rationale for the P/N formula

In a quantitative test, viable microbes on each carrier are enumerated. For one untreated and one treated carrier, LR = $-\log_{10}(T_{tr}/T_{un}) = \log_{10}(T_{un}) - \log_{10}(T_{tr}) = LD_{un} - LD_{tr}$. This definition, when extended to a quantitative test using multiple carriers, becomes the difference in mean log densities as shown in equation (2).

$$\text{LR} = MLD_{un} - MLD_{tr} . \qquad (2)$$

For a type SQ_1 semi-quantitative test, the cells are not enumerated on treated carriers; therefore the MLD_{tr} part of equation (2) is unavailable. The challenge is to use P/N data to approximate the MLD_{tr} value. It is convenient to discuss this task in the context of a simple example, such as a test in which only 1 carrier was positive among 60 treated carriers. The tested disinfectant treatment would have to be quite active to kill all microbes on 59 of the carriers. It is reasonable to believe that, on the average, the single positive carrier would hold a very small number of viable microbes. Suppose that there were 3 cells on the positive carrier. Because the other 59 carriers held no viable cells, the viable cell density among the 60 treated carriers is 3 cfu per 60 carriers or 0.05 cfu per carrier, which is a log density of -1.30. Note that there are three ways to scale the same quantitative response: (i) the typical number of viable microbes per positive carrier, (ii) the typical viable cell density for all treated carriers, and (iii) the typical log density. Any two of (i), (ii), (iii) can be calculated from the third.

Probability theory provides an important tool for our task. Given any numerical value for the typical viable cell density such as our trial value of 0.05, it is possible to calculate the probability of the actual test outcome, which is 59 negative carriers for this example. Trial and error could be used to find the typical viable cell density that maximizes the probability of the actual outcome. The result is the so-called "maximum likelihood estimate (MLE)." When this approach is applied to our example, the MLE is 0.025 cfu per carrier. Note that the MLE for the typical number of cells on the positive carrier is 1.50 (= 60×0.025) and the MLE for the log density is -1.60 (=$\log_{10}(0.025)$). To use the MLE for calculating the LR, substitute -1.60 for MLD_{tr} in equation (2), resulting in LR = 7.60.

MLE

Estimate the "typical LD_{tr} value" and substitute it for MLD_{tr} in eq(2)

Fortunately, trial and error work is not necessary because statisticians have derived a formula for the MLE of MLD_{tr}, see equation (3). The associated method for calculating LR is the P/N formula of equation (1).

$$\text{MLE of } MLD_{tr} = \log_{10}(-\log_e[(N_{tr} + 0.5) / (n_{tr} + 1)]). \qquad (3)$$

Dispelling two common concerns

I have found that this P/N method for estimating LR is disconcerting to some lab specialists in two ways. First, the MLE formula is not intuitively appealing even though the maximum likelihood approach is acceptable. Laboratory specialists are much more comfortable working with actual cfu counts. However, the quantitative test cfu counts are just estimates, not the true viable cell density per carrier. Densities based on enumerations rely on a small sample of carriers and a dilution series that is affected by statistical sampling uncertainty. In fact, the maximum likelihood estimate based on 60 P/N carriers is as valid as the *MLD* based on cfu counts for a few carriers. The precisions of the two types of estimates will differ, but precision comparisons are beyond the scope of this article.

The second disconcerting aspect is illustrated by the example; the LR of 7.60 indicates that the microbial kill was greater than the number of microbes on one typical carrier (MLD_{un} = 6). This apparent paradox can be resolved by looking at what happens to the total number of microbes across the 60 treated carriers. The aggregate viable cell density per 60 untreated carriers is 60×10^6 and the aggregate density per 60 treated carriers is 60×0.025. The LR value is unaffected by this switch to the aggregated microbes viewpoint, and 7.60 seems quite reasonable;

$$\begin{aligned} LR &= \log_{10}(60\times10^6) - \log_{10}(60\times0.025) \\ &= \log_{10}(6.0\times10^7) - \log_{10}(1.50) = 7.778 - 0.176 = 7.60. \end{aligned}$$

Justification

Justification for the P/N formula

MPN

In microbiology, the MLE of the typical density per carrier is called the "most probable number (MPN)." The P/N formula uses the $\log_{10}$(MPN) in place of the unavailable MLD_{tr}. The MPN has a long history of effective use since it was first proposed in 1915 (McCrady 1915; Cochran 1950; Blodgett 2006). McCrady coined the term "most probable number" before the general theory of maximum likelihood estimation was developed by statisticians, hence the duplicate terminology. Garthright (1993) explained why $\log_{10}$(MPN) is a valid estimator of the MLD_{tr}.

Using the tools of conventional mathematical statistics, the MPN can be derived by constructing a plausible binomial probability model for relating the typical viable cell density per carrier to N_{tr} and n_{tr} (Blodgett and Garthright 1998; Garthright and Blodgett 2003). The binomial model is appropriate if the test method possesses good resemblance; that is, the inoculation protocol creates carriers closely resembling each other, all posing about the same microbial challenge to the disinfectant. Alternative probability models and estimation techniques have been explored (Hamilton and DeVries 1996; DeVries and Hamilton 1999). Hamilton & DeVries (1996 – equations 1 and 2) suggested a more complicated probability model that underlies the MPN and a method of moments estimation method. I have calculated both their estimate and the MPN side-by-side when analyzing hundreds of type SQ_1 tests and consistently found a negligible difference between the two estimates. The simpler MPN calculation produces practically the same numerical value as the more complicated calculation. Based on all these considerations, I recommend the P/N formula for calculating the LR.

Modification of MPN that provides a calculable LR even when N_{tr} = 0 or n_{tr}

Equation (1) provides a calculable LR for every possible test outcome. For a collaborative study or any other investigation that involves multiple disinfectant tests, it is important to record an LR value for every test, even for the occasional test that produces all negatives ($N_{tr} = n_{tr}$) or all positives (N_{tr} = 0). For this reason, equations (1) and (3) employ a minor modification to the conventional MPN, namely, $[(N_{tr} +0.5) / (n_{tr} +1)]$ is used in place of (N_{tr} / n_{tr}). The modification amounts to adding one fictitious carrier that is half negative and half positive, an accepted adjustment for binomial proportions (Wilson 1927; Vollset 1993; Hamilton and DeVries 1996).

Discussion

Limitations to responsiveness

Semi-quantitative tests are used primarily for testing higher efficacy disinfectants; that is, disinfectants capable of killing all microbes on a carrier. If a disinfectant

Type SQ_1 tests are not responsive to differences between low and medium efficacy disinfectants

has only medium or low efficacy, viable cells will occur on every treated carrier; that is, $N_{tr} = 0$ regardless of the exact level of efficacy. For this reason, type SQ_1 tests are ineffective at differentiating between low and medium efficacy treatments (Tomasino et al., 2008). The numerical value below which the type SQ_1 semi-quantitative test is incapable of responding to different efficacies is determined by the control carrier viable cell density. For example, consider a test method using 60 treated carriers and an inoculation protocol for which the MLD_{un} seldom exceeds 6.0. For that test, all low or medium efficacy disinfectant treatments would produce $N_{tr} = 0$ and an LR of approximately 5.3.

Type SQ_1 tests are not responsive to differences between exceedingly strong disinfectants

A type SQ_1 test will produce the same LR for all powerful disinfectants that consistently kill all microbes on all carriers, in which case $N_{tr} = n_{tr}$ for every test. A 60 carrier test with $MLD_{un} = 6.0$, will consistently produce LR = 8.0 for any powerful disinfectant treatment.

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