KSA-SM-07

# The log reduction (LR) measure of disinfectant efficacy

[*Key Words*: viable cell density, log density, percentage kill, standard deviation of the log reduction, survival fraction]

The log reduction measure of disinfection efficacy was introduced in the second Knowledge Sharing Article (KSA-SM-02 Testing surface disinfectants: quantitative, semi-quantitative, qualitative, and alternative methods). Although the log reduction (denoted here by LR) is an important facet of quantitative disinfectant test methodology, only a succinct definition was provided in KSA-SM-02. The purpose of this article is to present calculation formulas, some interesting properties of the LR, a discussion the relationship between LR and two other popular measures of disinfectant efficacy (the survival fraction and the percentage killed), and procedures for calculating both the LR and the within-experiment standard deviation of LR. Numerical examples are provided.

This presentation will focus exclusively on surface disinfectant tests using vegetative bacteria. However, the discussion can be extended easily to other types of quantitative antimicrobial tests and to other microbes, including viruses. Methods for testing a disinfectant against surface-associated bacteria typically use easily manipulated carriers (e.g., glass disks) that hold the bacteria. In brief, the test is conducted as follows: Microbes are placed on carrier surfaces so that each carrier holds about the same number of cells. Some of the microbe-bearing carriers are treated with the disinfectant and others serve as untreated carriers. Treated carriers are exposed to the disinfectant and at the end of the designated contact time the disinfectant is neutralized to stop its activity. Untreated carriers usually receive the same manipulations and neutralization as the treated carriers, except that an inactive treatment, such as dilution water, is applied instead of the disinfectant.

The calculations discussed here start with the viable cell density on each carrier. The density on a carrier usually is determined by harvesting the cells from the carrier surface into suspension. Popular harvesting techniques include sonication, vortexing, and/or scraping. The suspension is disaggregated to create a suspension of randomly spaced single cells. Popular disaggregation techniques include sonication, vortexing, and/or homogenization. The number of viable cells in the suspension usually is estimated by creating a dilution series, spreading aliquots from the dilution tubes onto agar plates, incubating the plates, and counting the number of colonies of bacteria which have grown on each plate during incubation. The count is scaled-up to indicate the total number of colony forming units (cfu) in the suspension. Note that some technicians choose to record the counts in alternative ways; e.g., "cfu per mL of the suspension" or "cfu per $cm^2$ of coupon surface area." The efficacy measures discussed in this article are unaffected by the choice of units for the viable cell density, but the same units must be used for each and every coupon.

Because a disinfectant is formulated to kill microbes, the treated carriers should hold fewer viable microbes than the untreated carriers. Therefore, efficacy is measured by comparing the density of

viable microbes on treated carriers to the density on untreated carriers. Suppose for example that a treated carrier held $10^5$ viable cells when an untreated carrier held $10^7$ viable cells. The estimated fraction of cells surviving the treatment is shown in equation (1), where SF denotes the survival fraction.

$$\text{SF = treated carrier viable cell density / untreated carrier viable cell density} \qquad (1)$$
$$= 10^5/10^7 = 10^{-2} = 0.01.$$

If the SF is 0.01, then the percentage killed (denoted by PK) can be calculated as in equation (2).

$$\text{PK} = (1 - \text{SF}) \times 100\% = (1 - 0.01) \times 100\% = 99\%. \qquad (2)$$

Because the SF and PK calculations are based on the ratio of viable cell densities, treated carrier to untreated carrier, the density units cancel out.

The LR is the reciprocal of SF when transformed to a logarithmic scale; see equation (3).

$$\text{LR} = \log_{10}(1/\text{SF}) = \log_{10}(1/0.01) = \log_{10}(10^2) = 2. \qquad (3)$$

An alternative method for calculating LR is shown in equation (4). The second line in equation (4) uses a mathematical rule for logarithms; viz., the log of a ratio is the difference of logs.

$$\text{LR} = \log_{10}(\text{untreated carrier viable cell density / treated carrier viable cell density}) \qquad (4)$$
$$= \log_{10}(\text{untreated carrier viable cell density}) - \log_{10}(\text{treated carrier viable cell density})$$
$$= \log_{10}(10^7) - \log_{10}(10^5) = 7 - 5 = 2.$$

Each whole number increase in LR is equivalent to a tenfold decrease in SF. Because the SF is "unitless," so is the LR. A small value for SF, a large value for LR, and a large value for PK all indicate good disinfectant efficacy. For a completely inactive treatment, the density on the treated carrier would be the same as the density on the untreated carrier, in which case, SF = 1, LR = 0, PK = 0%.

Most disinfectant tests utilize multiple carriers; therefore, we must extend the calculation formulas for application to multiple carriers. Using the notation of KSA-SM-06 ("Enumerating viable cells by pooling counts for several dilutions"), let $T$ denote the estimated density (total cfu per carrier). The subscripts $Tr$ and $Un$ will be used to indicate whether the carrier was Treated with the disinfectant or was Untreated. Suppose the test utilizes $n_{Tr}$ treated carriers and $n_{Un}$ untreated carriers. Using this notation, the formula in equation (4) becomes $\text{LR} = \log_{10}(T_{Un}) - \log_{10}(T_{Tr})$. Note that the LR is the difference between log densities, untreated minus treated. Let $LD$ denote the log density, $LD = \log_{10}(T)$ so that $LD_{Un}$ and $LD_{Tr}$ denote the log densities for untreated and treated carriers, respectively. Next, let $MLD_{Un}$ denote the mean of $LD_{Un}$ values across the $n_{Un}$ untreated carriers and $MLD_{Tr}$ denote the mean of $LD_{Tr}$ values across the $n_{Tr}$ treated carriers. Then LR is the difference in mean log densities as shown in equation (5) (Zelver et al. 2002).

$$\text{LR} = MLD_{Un} - MLD_{Tr}. \qquad (5)$$

If one wants to present the results using either SF or PK, then the equations (6) or (7) can be used to convert the calculated LR to the desired measure.

$$\text{SF} = 10^{-\text{LR}}. \qquad (6)$$

$$PK = (1 - 10^{-LR}) \times 100\%. \tag{7}$$

The LR value for a disinfectant test is affected by many small perturbations and it is important to calculate a measure of uncertainty for the LR. The usual measure of uncertainty for a single experiment is the standard deviation of LR, denoted by $S_{LR}$. Let $S_{Un}$ and $S_{Tr}$ denote the sample standard deviations of the log reduction values for the untreated carriers and the treated carriers, respectively. Then the formula for calculating $S_{LR}$ is shown in equation (8) (Zelver et al. 2001).

$$S_{LR} = [(S^2_{Un} / n_{Un}) + (S^2_{Tr} / n_{Tr})]^{\frac{1}{2}}. \tag{8}$$

## Calculation steps for LR, S$_{LR}$, SF, and PK

1. For each carrier, calculate the log density.
2. Calculate the mean and standard deviation of the log density values for the untreated carriers.
3. Calculate the mean and standard deviation of the log density values for the treated carriers
4. Calculate the log reduction using equation (5).
5. Calculate the standard deviation of the log reduction using equation (8).
6. Calculate SF and PK using equations (6) and (7).

## Numerical example

The data in Table 1 illustrate the calculations.

**Table 1.** Observed viable cell density (CFU/carrier) for each carrier, the associated *LD*, the mean and standard deviation of *LD* for $n_{Un} = 2$ untreated carriers, the mean and standard deviation of *LD* for $n_{Tr} = 3$ treated carriers, the LR, and the standard deviation of LR.

| Untreated carriers | | | Treated carriers | | | | |
|---|---|---|---|---|---|---|---|
| **No.** | **density** | **log density (*LD*)** | **No.** | **density** | **log density (*LD*)** | | |
| 1 | $5.763 \times 10^6$ | 6.76065 | 1 | $1.005 \times 10^3$ | 3.00217 | | |
| 2 | $8.018 \times 10^6$ | 6.90407 | 2 | $1.965 \times 10^3$ | 3.29336 | | |
| | | | 3 | $1.076 \times 10^3$ | 3.03181 | | |
| | | **Mean (*MLD$_{Un}$*)** | | | **Mean (*MLD$_{Tr}$*)** | **LR** | |
| | | 6.83236 | | | 3.10911 | 3.72324 | |
| | | **Standard Deviation (*S$_{Un}$*)** | | | **Standard Deviation (*S$_{Tr}$*)** | **Standard Deviation (S$_{LR}$)** | |
| | | 0.10141 | | | 0.16025 | 0.11706 | |

The calculated means and standard deviations are entered into equations (5) and (8) as follows:

$$LR = 6.83236 - 3.10911 = 3.72324 \text{ and}$$

$$S_{LR} = [((0.10141)^2 / 2) + ((0.16025)^2 / 3)]^{\frac{1}{2}} = 0.11706.$$

The LR result often is presented as LR $\pm$ S$_{LR}$ or $3.72 \pm 0.12$ for this example. The SF and PK values, found by applying equations (6) and (7), are

$$SF = 10^{-3.72324} = 1.89128 \times 10^{-4} \text{ and}$$
$$PK = (1 - 10^{-3.72324}) \times 100\% = 99.981\%.$$

The small $S_{LR}$ of 0.12 indicates that, in this experiment, the log densities varied little within the two groups, untreated carriers and treated carriers. Notice that the standard deviation for the treated carriers (0.16) was larger than the standard deviation for the untreated carriers (0.10) as is typical for disinfectant tests. There are various steps in a disinfectant test experiment that could cause more variability for treated carriers; e.g., slight variations in the exposure concentrations and contact times would affect viable cell densities on the treated carriers but not on the untreated carriers.

NOTE: The standard deviations of SF and PK <u>cannot</u> be found simply by applying equations (6) and (7) to $S_{LR}$. Further discussion about calculating uncertainty values for SF and PK is beyond the scope of this article.

## Technical issues

### *The mean of log densities is not the same as the log of mean densities*
For microbial viable cell counts on carriers, the geometric mean of carrier densities better indicates the density on the typical carrier than does the conventional arithmetic mean (Eaton et al. 1995). The mean of log densities is the log-transformed geometric mean of densities, which is different from the log-transformed (arithmetic) mean of the densities (Jarvis 2008). In fact, except when the density is the same for each carrier in a set, the mean of the log densities across the set will always be smaller than the log of the mean of densities. One might not anticipate that the order in which the log and average operations are applied is important; hence, this admonition.

### *Rounding*
We recommend carrying at least 4 significant digits (at least 5 decimal places on log-scale values) through all calculations. At the end, the LR result should be rounded to 1 or 2 decimal places. If the LR is one of many LR values in a large study, retain 5 or more significant digits on each LR through the final set of calculations, then round the final numbers for presentation purposes.

### *What to do when an observed density is zero*
Because the log of zero is mathematically undefined, the *LD* cannot be calculated for a carrier when the cfu counts were zero on all observed agar plates. To calculate the LR, some adjustment is necessary. Generally, it is appropriate to substitute an artificial count of ½ for the observed zero on just one agar plate at the first counted dilution.

## References

Eaton, A.D., et al., editors (1995) *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association: Washington, DC (see the last part of Section 9020 - Quality Assurance, which ends with "Therefore, although regulations or tradition may require or cause microbiological data to be reported as the arithmetic mean or median, the preferred statistic for summarizing microbiological data is the geometric mean."

Jarvis, B. (2008) *Statistical Aspects of the Microbiological Analysis of Foods – Second Edition*. Elsevier: Amsterdam (especially chapter 2).

Zelver, N., Hamilton, M., Goeres, D., and Heersink, J. (2001) Development of a standardized antibiofilm test, *Methods in Enzymology - Biofilms II*, R.J. Doyle, editor, 337:363-376.

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