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Common statistical errors in logarithmic data of viral load

Suthon Vongsheree*

Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.

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ABSTRACT

Background: The mathematical operation of logarithm data follows the rules of logarithm as the numbers relate to specific meanings. The numbers prior to the decimal are the mantissa or the transformed power of 10 and the numbers after the decimal are the significant numbers of a given numeric data. Such operations result in errors in statistical calculations and lead to erroneous conclusions and eventual miss-interpretation of data.

Objectives: The principal aims of this study were to demonstrate and discuss several commonly made mistakes in calculation of logarithm data of viral load assays, and the subsequent errors in calculation of precision that affects the estimation of measurement uncertainty of the test.

Materials and methods: The study reviewed scholarly articles in 2017, using' viral load' as a keyword. Several sets of log data from inter-laboratory comparison of human immunodeficiency virus (HIV) load and multi-center evaluation of cytomegalovirus (CMV) load assays were used for this study to demonstrate the errors in calculation when the statistical calculations were performed using logarithm data.

Results: It was observed that presentation of average viral load data as arithmetic mean and calculation of other statistical measures directly from log viral load data was quite common, in spite of that fact that such average was log geometric mean of the viral load. Standard deviations (SD) calculated directly from log viral loads gave a new-undefined values that were irrelevant to the deviation of viral load from its mean. Such errors in SD calculation lead to extraordinarily low coefficient of variance and very low measurement uncertainty.

Conclusion: The SD, calculated from the log, and those SD calculated from the viral load per milliliter are different. Mathematically, such statistics should be calculated from the number of viruses per milliliter and could then be converted to a log scale for downstream use. Traditionally, the SD were calculated from the log which was very low. This study recommends that for all statistical measures, the absolute value is important, should use the viral load per milliliter in the calculations and then convert it into a log scale for correct usage.

* **Corresponding author.**
Author's Address: *Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.*
** **E-mail address:** suthon.v@dmsc.mail.go.th
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Introduction

It is mathematically obsolete to calculate statistical measures directly from logarithmic data, given the fact that such log data have very specific meaning, *i.e.*, all numbers prior to the decimal point are only the level or the mantissa and all numbers after the decimal points are characteristics or the significant figures.[1, 2]

The value of the numbers after the decimal point are limited in the range of 1 to 10, not 0 to 1 as the normal values whereas the numbers before the decimal points range from 10 to +infinity, and not 1 to + infinity. Mathematical operations typically comply with logarithmic rules, especially when the absolute values have certain meaning or are relevant to the subsequent interventions such as human immune deficiency virus (HIV) viral load which is one of the important surrogate markers for monitoring acquired immune deficiency syndrome (AIDS) progression, which in turn determines the therapeutic failure or success for a patient. A systematic review of the accuracy and precision of HIV viral load determination methods reveal that for treatment monitoring the average intra and inter assay precision of 3-5 % for the two US-FDA approved assays, Amplicore Monitor v 1.5 and Abbott Realtime HIV-1.[3] Such extremely low precision might relate to the direct calculation of the mean and standard deviations (SD) directly from the log values and thus, reflect a very low coefficient of variance (CV). Another study described the SD of cytomegalovirus (CMV) load assays from three laboratories and reported an overall SD of 0.18 $\log_{10}$ or 0.15 copies/mL which was too low for any real assay.[4] This study, therefore presented the common errors in calculation and presentation of statistics from logarithm (log) data. Thus, one needs to avoid these errors when using HIV load as a study model for further therapeutic interventions. Results reported from this study on CMV load assays were also included in the analysis, for the completeness of the raw data and for its relevance to the statistical treatment of the data. We found that the two calculated SD were different, *i.e*. the first method used SD of logarithmic data of HIV load and the second method used logarithm of SD that were calculated from absolute data of HIV load. These conditions easily lead to confusion in data interpretation prior to any further applications.

Materials and methods

In this study, we reviewed published scholarly articles from 2017 by searching key terms such as "levels of HIV-1" using Google Scholar as a literature database to observe the presentation of the average levels of HIV loads; e.g. arithmetic and/or geometric mean. The tables of the CMV load [5] were checked for the errors in statistical calculations, using Microsoft Excel ((Microsoft Corporation, Redmond, WA). Robust statistical measures was applied for inter-laboratory comparisons of HIV loads reported from 11 medical laboratories, using AMC Robstat as a Microsoft Excel add-in program.[6]

Results

Errors in calculation and presentation of statistics from log data

From a Google Scholar search conducted on June 12, 2017, there were 2,190 research articles displaying mean or average HIV load, and 1,470 articles providing geometric mean HIV loads, whereas only 222 articles displaying arithmetic mean HIV loads. The arithmetic and geometric means were not comparable. Indeed, the arithmetic mean of log HIV load was just the log geometric mean of HIV load, if this mean was calculated directly from the summation of log HIV load divided by number of observation, as simply proved by the following calculation.

Let x_i was HIV copies/mL, and n as number of observations and geometric mean $=(x1+x2+...+xn)^{1/n}$ Then, $\log_{10}$ geometric mean of log HIV equaled $1/n(\Sigma \log x_i)$ or was equivalent to what most publications stated as mean or arithmetic mean of log HIV load.

It is obsolete to calculate and present HIV load as arithmetic mean if the study directly calculated the arithmetic mean HIV load from log HIV load, given that such an arithmetic mean is just the log geometric mean and both values are not always comparable. Such errors are demonstrated in Table 1.

Table 1 A hypothetical HIV load from an inter-laboratory comparison consensus HIV load, log copies/mL=5.85.

Laboratory	A. log HIV	B. copies/mL	C. convert B. to log	Robust Z from log	Robust Z from copies/mL
1	5.99	9.77E+05	5.99	0.256	0.573
2	5.85	7.08E+05	5.85	0.000	0.000
3	5.04	1.10E+05	5.04	-1.481	-1.273
4	5.03	1.07E+05	5.03	-1.499	-1.278
5	5.97	9.33E+05	5.97	0.219	0.479
6	5.96	9.12E+05	5.96	0.201	0.434
7	5.01	1.02E+05	5.01	-1.536	-1.288
8	5.99	9.77E+05	5.99	0.256	0.573
9	5.04	1.10E+05	5.04	-1.481	-1.273
10	5.00	1.00E+05	5.00	-1.554	-1.293
11	5.91	8.13E+05	5.91	0.110	0.233

Table 1 A hypothetical HIV load from an inter-laboratory comparison consensus HIV load, log copies/mL= 5.85. (continues)

Statistics	A. from log	B. from copies/mL	C. convert B. to log
Mean	5.526	5.318E+05	5.726
Geometric mean	5.507	3.360E+05	5.526
Median	5.850	7.079E+05	5.850
SD	0.483	4.148E+05	5.618
CV.%	8.73	78.00	78.00

Difference of arithmetic mean and geometric mean of HIV load

The information collected from the literature showed that if the data were quite closed or clustered in a narrow range, the arithmetic and geometric mean were same. This characteristic was also observed for blood pH by Boutilier and Shelton 1979.[7] Rare HIV load data sets might behave like those of blood pH variation; e.g. the HIV load from inter-laboratory comparison based on the same specimens, using the same analytical system and performed by personnel with comparable skills and competencies. The arithmetic and the geometric means were not much different when the study dealt with the variation of the last 3 digits of log data. This trend was similar to blood pH which was strictly controlled at the physiological range which is very narrow. It is clear that such conditions are not applicable to viral load data where the analytical values are far more variable.

A previous study reported on the failure in statistical calculations from log values[8] using data from a multi-centered evaluation of CMV load. This study reported that the study calculated arithmetic means from log CMV load data from 23 participants and obtained the mean CMV loads in $\log_{10}$ as 2.96, 3.81, 4.77 and 5.66 copies/mL. In contrast, the mean CMV load calculated from copies/mL and converted into $\log_{10}$ were 3.33, 4.21, 5.14 and 6.19 copies/mL for the samples of known CMV load of 2.7, 3.7, 4.7 and 5.7 copies/mL respectively. These errors are described in details in Table 2.

Table 2 Mean CMV load in log scales from 23 laboratories participating the multi-center evaluation of CMV load assays.

	Known CMV Viral DNA Panel			
Laboratory	**2.70 copies/mL**	**3.70 copies/mL**	**4.70 copies/mL**	**5.70 copies/mL**
1	4.12	4.37	5.08	5.97
2	*	*	4	4.78
3	3.27	4.04	5.18	5.74
4	2.92	3.89	5.01	5.75
5	2.54	3.17	4.47	5.02
6	3.02	3.64	4.79	5.94
7	3.53	4.72	5.77	6.58
8	3.06	3.98	4.97	6.02
9	2.79	3.59	4.76	5.7
10	3.36	4.31	5.27	6.25
11	2.77	3.84	4.92	5.93
12	2.98	3.76	4.63	5.68
13	2.59	3.8	4.71	5.66
14	4.08	5.2	6.1	7.25
15	2.04	3.2	4.26	5.56
16	2.71	3.68	4.73	5.67
17	*	3.45	4.14	4.75
18	2.51	3.5	4.62	5.63
19	*	3.29	4.3	4.97
20	2.6	3.68	4.72	5.68
21	2.53	3.54	4.46	5.47
22	2.3	2.95	3.74	4.83

*(*CMV loads were less than 50 copies/mL, statistics are calculated directly from log CMV load values with the values calculated from copies/mL in parenthesis after each statistics)*

Table 2 Mean CMV load in log scales from 23 laboratories participating the multi-center evaluation of CMV load assays. (continues)

	Known CMV Viral DNA Panel			
Laboratory	**2.70 copies/mL**	**3.70 copies/mL**	**4.70 copies/mL**	**5.70 copies/mL**
23	3.41	4.3	5.04	5.45
Mean*	2.96 (3.33)	3.81 (4.21)	4.77 (5.14)	5.66 (6.19)
SD*	0.54 (3.57)	0.53 (4.53)	0.53 (5.43)	0.58 (6.56)
%CV*	18.40 (171)	13.86 (209)	11.17 (196)	10.20 (256)

*(*CMV loads were less than 50 copies/mL, statistics are calculated directly from log CMV load values with the values calculated from copies/mL in parenthesis after each statistics)*

Most seriously affected statistics were calculated directly from log values of SD

The SD reported in the articles were a new terminology since mathematical operations do not conform to logarithmic rules. The SD from log viral load were irrelevant to the SD of viral load, and thus, could not be converted back to the SD since it was SD of the log values. The common characteristics of these special SD were extraordinarily low SD, typically as low as 0.5 or lower. From the same study on CMV load, very low SD of four samples as log CMV as 0.54, 0.53, 0.53 and 0.58 copies/mL were reported, which when converted to copies/mL were 3.5, 3.4, 3.4, and 3.8 copies/mL, respectively. When SD is computed from copies/mL, the SD after conversion into log scales are 3.57, 4.53, 5.43, and 6.56 which when converted into copies/mL were 3.7×10^3, 3.4×10^4, 2.7×10^5, 3.6×10^6 copies/mL, or 10^3 to 10^6 folds to those calculated from the log viral load data, respectively. Such irrelevant SD showed lower deviation and claimed higher precision of the assay as very low SD.

One suggestion for handling these log SD viral load was that simple addition and subtraction of anti log SD should not be performed, but multiplication and division are acceptable. This approach does not have a sound basis for this specific special treatment of SD, given that SD is a measure of deviation from mean. For example, mean CMV load and SD in log scale were 2.7 and 0.5, respectively, where mean+SD should have been 2.7+0.5 or anti log 3.2 which corresponds to only 1,585 copies/mL. However, the general operation resulted in antilog 2.7 + antilog 0.5 equal to 504 copies/mL. Both are irrelevant to the SD of the test assay of 3.57 log CMV load which corresponded to 4,217 copies/mL.

The use of plasma HIV RNA levels as surrogate markers[9] for monitoring the progression of AIDS and efficacy of therapy, as a threefold change or 0.5 log scale change was derived from another study cohort. This three-fold change was one of the strongest predictor, but the three-fold change was not derived from the precision or corresponding SD of any laboratories, except that the same laboratory produced this data.

Erroneous calculation of mean and SD resulted in the estimation of measurement of uncertainty of the test method

At least two types of uncertainties add up to represent the measurement of uncertainty of a quantitative evaluation system. Type A uncertainties are those of quality control material used in routine works and type B uncertainties relate to the calibrators provided from the manufacturers. From the CMV data mentioned earlier, if mean and SD were both calculated directly from log CMV as 2.7 and 0.54, the relative type A uncertainty would be 3.5/501 or 0.007 copies/mL and the standard uncertainty of type B was calculated to be 3 %. According to the policy and requirement on estimation of uncertainty measurement and traceability N0715007 of Thailand Accreditation Body,[10] the combined relative uncertainty is defined as the square root of summation of (relative uncertainty) 2 which equals to 0.031 and the expanded uncertainty is only 0.062 copies/mL. However, the corrected mean and SD of 3.33 and 3.57 expanded the uncertainty to 3.48 copies/mL or 56 times more than those calculated from logarithm values. For log CMV of 2.8, a physician might get wrong uncertainty and would predict the result as 631±39 copies/mL for the measurement of uncertainty using direct calculation from the logarithm value while the corrected one would be 631±2194 or 0-2825 copies/mL.

Algorithm A for robust z-score analysis of the proficiency testing result.

There was no indication that such log values could be directly analyzed by this robust z-score statistics. Fortunately, robust statistics used median as the average and the final grading of participant laboratory results of log viral load was still the comparable to using copies/mL. However, the value of each z-score has a specific meaning, not just satisfied or not, but also how the values were far from the consensus ones, thus, helping the participant laboratories to improve their testing quality. Some robust statistics are illustrated in Table 1.

Discussion

Direct calculation of arithmetic mean from log viral load data yields a false arithmetic mean as it is just a log geometric mean of the HIV load. Direct calculation of standard deviation provides a new undefined statistical measure which tends to over express extremely low SD and hence provide extraordinarily high precision as well as very low measurement uncertainty. The robust z-scores for scoring the laboratory-specific performance of the participants in proficiency testing programs using log data certainly provide imprecise and unreliable results. These errors may affect the clinical usage of the viral load when such precision

is low and might alter the treatment by misleading lower precision of the assay. This study has reviewed such errors and has suggested calculation of statistics directly from the viral copies/mL and then performing a log-transformation for further applications. The measurement of uncertainty may also apply the mean and SD from the copies/mL of internal quality control (QC) as type A uncertainty and acquire type B uncertainty from the manufacturer that is derived from the copies/mL. However, this study only showed that SD calculated by both methods, one using log of SD and the other was SD of log, were not the same at all, and are neither comparable nor convertible. More exhaustive mathematical studies shall verify this claim in future studies. However, the QC of laboratory report will be confusing for actual SD transformed to log scale which is different from standard methods using SD of log HIV load values. This study therefore reminds investigators dealing with log data if its absolute value would be interpreted to some comparison such as the viral load assays. The paper does not lower the value of logarithm for other purposes that apply relative log values in risks modeling or logistic regression. More exhaustive approaches may be needed to gather insights on suitable application of these data.

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