



Statistical methods in microbial disinfection assays
by Todd Alan DeVries

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Statistics

Montana State University

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Abstract:

Disinfectant efficacy testing is performed by Federal regulatory agencies, disinfectant manufacturers, and consumer groups for the purposes of assessing the ability of a liquid chemical disinfectant to kill potentially harmful microorganisms, such as bacteria, spores and viruses, attached to inanimate surfaces. Carrier tests are microbial assays in which the disinfectant is applied to microbes that have been immobilized in a dried organic film on an impermeable surface such as glass. The goal of a carrier test is to provide a reproducible measure of disinfection efficacy. "Log Reduction" ("LR"), the reduction in numbers of viable microbes (log scale), is one conventional measure of efficacy.

The quantitative assay and the presence/absence assay are two microbial assays commonly used in disinfection efficacy testing. The quantitative assay is characterized by direct counts (quantitative data) of microbes surviving disinfection. The presence/absence assay is characterized by a series of presence/absence responses (qualitative data); a response of presence indicates one or more organisms have survived disinfection in a sample aliquot, while a response of absence indicates no organisms survived disinfection in a sample aliquot. Although the types of data collected from the quantitative assay and presence/absence assay are different, the goal of the two assays is the same - to estimate the "LR".

Very little attention has been devoted to the statistical analysis of carrier tests. In this dissertation, statistical models are developed for data arising from the quantitative assay and the presence/absence assay. Mathematical parameters defining disinfection efficacy (i.e. "LR") are presented, and various estimators and associated standard errors are derived for each parameter. Computer simulation and asymptotic efficiency results are used to document the small and large sample properties of the various estimators.

The simulation and theoretical results indicate that Method of Moments estimators are to be recommended over Maximum Likelihood Estimators for the quantitative assay. For the presence/absence assay, the form of the estimator to be recommended depends on the definition of "LR" used.

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TABLE OF CONTENTS

	Page
LIST OF TABLES	viii
LIST OF FIGURES	ix
ABSTRACT	x
1. Introduction	1
Description of the Quantitative Assay	3
Description of the Presence/Absence Assay	6
Common Measures of Disinfectant Efficacy	7
Specific Goals of this Dissertation	10
2. Quantitative Assay	11
Notation	11
Log Reduction (LR)	13
Two Simple Estimators of LR	14
Arguments Favoring the Average of Logs Method	15
Arguments Favoring the Log of Averages Method	19
Statistical Modeling Assumptions	19
Point Estimation of LR	23
Estimators of Υ_1 for the Model with no Over-dispersion	23
Estimators of Υ_1 for the Model with Over-dispersion	30
Estimators of Υ_2 for the Model with no Over-dispersion	31
Estimators of Υ_2 for the Model with Over-dispersion	32
Point Estimation of LR when Test Carrier Data are all Zeros	33
Standard Error Formulas for Estimators of LR	33
Standard Error Formulas for the Model with no Over-dispersion	33
Standard Error Formulas for the Model with Over-dispersion	35
Example	35
Example Calculations	37
Asymptotic Relative Efficiency Calculations	37
Computer Simulation Study	41

Computer Simulation Results	49
Comparison of $\hat{\Upsilon}_{1,MOM}$ to $\hat{\Upsilon}_{1,MLEOVER}$	50
Comparison of $\hat{\Upsilon}_{2,MOM}$ to $\hat{\Upsilon}_{2,MLEOVER}$	51
Comparison of Estimators of Υ_1 to Υ_2	52
Conclusions	54
Future Work	54
3. Presence/Absence Assay	57
Notation	57
Log Reduction	59
Statistical Modeling Assumptions	60
Strategies for Deriving Estimators of LR	64
Methods for Estimating μ_C, μ_X, μ_T , and μ_Y	64
Most Probable Number Estimator	66
Assay-wise Jackknife Estimator	67
Modified Score Function Estimator	67
Spearman-Kärber Estimator	68
Partial mle of μ_C and μ_X	69
Partial mle of μ_T and μ_Y	70
Point Estimation of LR	71
Maximum Likelihood Estimators of LR	71
Estimators of LR using the mpn, jack, msf, sk, or pmle Methods	72
Standard Error Formulas for Estimators of LR	73
Standard Error Formulas for mle of LR	73
SE Formulas the mpn, jack, msf, or sk Estimators of LR	74
Standard Error Formulas for the pmle of LR	75
Example	77
Computer Simulation Study	78
Comparison of Estimators of Υ_1	79
Comparison of Estimators of Υ_2	85
Comparison of Estimators of Υ_1 to Υ_2	91
Conclusions	91
Future Work	92
APPENDICES	93
APPENDIX A – Appendix Material for the Quantitative Assay	94
Derivation of the Negative Binomial Distribution.	95
Symbolic Descriptions of $f(\vec{\tau})$ and $H(\vec{\tau})$	96
Use of the Computer Package <i>Maple</i>	97
Symbolic Descriptions of $f^*(\vec{\tau}^*)$ and $H^*(\vec{\tau}^*)$	97
Inverting $H(\vec{\tau})$ or $H^*(\vec{\tau}^*)$	100
The Mean and Variance of the Log Gamma Distribution	101

Approximating a Log Negative Binomial with a Log Gamma	102
MLE of ϕ for the Model with no Over-dispersion	103
Asymptotic Distribution of Estimators of LR	103
Model with no Over-dispersion	104
Efficiency Calculations for the Model with no Over-dispersion	106
Model with Over-dispersion	107
Efficiency Calculations for the Model with Over-dispersion	109
APPENDIX B – Appendix Material for the Presence/Absence Assay	111
Derivation of the Marginal Distribution of $\vec{r}_i$	112
Applications of Theorem 1	113
Computing the MLE of $\vec{\tau}^*$	114
Functional Form of the First Partial Derivatives	116
Functional Form of the Second Partial Derivatives	117
Expressions for $V^*(\vec{\tau}^*)$, $\frac{\partial g_5(\vec{\tau}^*)}{\partial \vec{\tau}^*}$ and $\frac{\partial g_6(\vec{\tau}^*)}{\partial \vec{\tau}^*}$	122
Computation of $\hat{\mu}_C$, $\hat{\mu}_X$, and $V_C(\hat{\mu}_C, \hat{\eta}_C)$	123
First and Second Partial Derivatives	124
The Estimated Variance-covariance Matrix of $\hat{\mu}_C$ and $\hat{\eta}_C$	125
Computation of $\hat{\mu}_T$, $\hat{\mu}_Y$, and $V_C(\hat{\mu}_T, \hat{\eta}_T)$	125
First and Second Partial Derivatives	126
The Estimated Variance-covariance Matrix of $\hat{\mu}_T$ and $\hat{\eta}_T$	128
REFERENCES CITED	130

LIST OF TABLES

Table		Page
1	Example data from the quantitative assay	6
2	Example data from the presence/absence assay	9
3	Example of equivalent quantitative measures of disinfection efficacy	9
4	Check carrier data	36
5	Test carrier data	36
6	Asymptotic Relative Efficiency calculations	39
7	Parameter values used to generate Figure 6	43
8	Simulation parameters for the quantitative assay	44
9	Simulation results for $\hat{\Upsilon}_{1,MOM}$ and $\hat{\Upsilon}_{1,MLEOVER}$	45
10	Simulation results for $\hat{\Upsilon}_{2,MOM}$ and $\hat{\Upsilon}_{2,MLEOVER}$	46
11	Simulation results for $\hat{\Upsilon}_{1,MLENO}$ and $\hat{\Upsilon}_{2,MLENO}$	47
12	Observed ratios of $\frac{MSE(\hat{\Upsilon}_{1,MLEOVER})}{MSE(\hat{\Upsilon}_{1,MOM})}$ and $\frac{MSE(\hat{\Upsilon}_{2,MLEOVER})}{MSE(\hat{\Upsilon}_{2,MOM})}$	48
13	Observed ratios of $\frac{MSE^*(\hat{\Upsilon}_{2,MOM})}{MSE^*(\hat{\Upsilon}_{1,MOM})}$ and $\frac{MSE^*(\hat{\Upsilon}_{2,MLEOVER})}{MSE^*(\hat{\Upsilon}_{1,MLEOVER})}$	49
14	Example data from the presence/absence assay	77
15	Example calculations for estimators of Υ_1	78
16	Example calculations for estimators of Υ_2	78
17	Simulation parameters for the presence/absence assay	79
18	Simulation results for estimators of Υ_1 (cases 1-4)	80
19	Simulation results for estimators of Υ_1 (cases 5-8)	81
20	Simulation results for estimators of Υ_1 (cases 9-12)	82
21	Simulation results for estimators of Υ_1 (cases 13-16)	83
22	Simulation results for estimators of Υ_2 (cases 1-4)	86
23	Simulation results for estimators of Υ_2 (cases 5-8)	87
24	Simulation results for estimators of Υ_2 (cases 9-13)	88
25	Simulation results for estimators of Υ_2 (cases 13-16)	89
26	Observed ratios of $\widehat{MSE}^*$ s for the mle and mpn estimators	90

LIST OF FIGURES

Figure		Page
1	Schematic chart for test carriers in the quantitative assay	5
2	Schematic diagram for a serial-dilution assay	8
3	EDF for skewness coefficient of 195 check and test carrier data sets .	17
4	EDF for skewness coefficient of 195 log transformed data sets	18
5	Expectations of log negative binomial and log gamma random variables	28
6	$\text{ARE}(\hat{Y}_{1,\text{MOM}} : \hat{Y}_{2,\text{MOM}})$	42

ABSTRACT

Disinfectant efficacy testing is performed by Federal regulatory agencies, disinfectant manufacturers, and consumer groups for the purposes of assessing the ability of a liquid chemical disinfectant to kill potentially harmful microorganisms, such as bacteria, spores and viruses, attached to inanimate surfaces. Carrier tests are microbial assays in which the disinfectant is applied to microbes that have been immobilized in a dried organic film on an impermeable surface such as glass. The goal of a carrier test is to provide a reproducible measure of disinfection efficacy. "Log Reduction" ("LR"), the reduction in numbers of viable microbes (log scale), is one conventional measure of efficacy.

The quantitative assay and the presence/absence assay are two microbial assays commonly used in disinfection efficacy testing. The quantitative assay is characterized by direct counts (quantitative data) of microbes surviving disinfection. The presence/absence assay is characterized by a series of presence/absence responses (qualitative data); a response of presence indicates one or more organisms have survived disinfection in a sample aliquot, while a response of absence indicates no organisms survived disinfection in a sample aliquot. Although the types of data collected from the quantitative assay and presence/absence assay are different, the goal of the two assays is the same - to estimate the "LR".

Very little attention has been devoted to the statistical analysis of carrier tests. In this dissertation, statistical models are developed for data arising from the quantitative assay and the presence/absence assay. Mathematical parameters defining disinfection efficacy (i.e. "LR") are presented, and various estimators and associated standard errors are derived for each parameter. Computer simulation and asymptotic efficiency results are used to document the small and large sample properties of the various estimators.

The simulation and theoretical results indicate that Method of Moments estimators are to be recommended over Maximum Likelihood Estimators for the quantitative assay. For the presence/absence assay, the form of the estimator to be recommended depends on the definition of "LR" used.

CHAPTER 1

Introduction

Various microbiological assays have been developed for assessing the ability of a liquid chemical disinfectant to kill potentially harmful microorganisms such as bacteria, spores and viruses. The disinfectants are intended for application on nonporous surfaces in hospitals, restaurants, the home, etc. In environments of potentially high microbial contamination, such as hospitals, a measure of a disinfectant's efficacy is necessary if the product is to be used with any degree of confidence.

Establishing an index of efficacy for a particular disinfectant is done via a standardized laboratory test. Federal regulatory agencies, disinfectant manufacturers and consumer groups routinely test for efficacy of a disinfectant, and as such, require reliable testing methods. Depending on the intended use of a disinfectant, a specific standardized laboratory test must be passed before the United States Environmental Protection Agency (EPA) or the United States Food and Drug Administration (FDA) will approve the disinfectant for public usage. The tests are defined by standardized protocols that clearly state the microbial assays, microbial strains, inoculum sizes, culture conditions, testing materials, etc. Standardized tests are designed to be inexpensive and reproducible laboratory approximations of real world conditions. A standardized test is said to be reproducible if the within- and between- laboratory variability of the test are "low".

In the 1980's, environmental microbiologists expressed concern that the standardized tests used to register disinfectants were not reproducible, and that manufacturer label claims regarding germicidal activity were overstated. For

example, Rutala and Cole (1987) reported a collaborative study that assessed the reproducibility of the Use-Dilution-Method (UDM). Eighteen laboratories which were familiar with the UDM protocol tested six EPA-registered disinfectants, each of which had previously passed the performance criterion for the UDM. The results of the study by organisms were as follows: for 20%, 34%, and 62% of the trials, the disinfectants failed when tested against *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, respectively. These results call into question the manufacturers' product claims of germicidal activity for the six randomly chosen disinfectants used in the study. Also noted in the study was a high degree of variability in test results among the eighteen laboratories. Because of this inconsistency among laboratories, Rutala and Cole declared that the UDM was not reproducible, and therefore questioned the use of the UDM for registration purposes. They also hypothesized that other standardized tests were similarly not reproducible.

A government review of the statistical properties of standardized disinfectant tests concluded that up to 20% of the disinfectants on the market would not meet registration performance standards if they were retested; these disinfectants may be ineffective for their intended use (U.S. General Accounting Office 1990, as quoted in Bond et al. 1991). Bond et al. (1991) nicely summarize the necessity for reproducible standardized tests.

"The importance of establishing consistently appropriate and reproducible laboratory test methods to evaluate and compare the germicidal capabilities of liquid chemical agents cannot be overstated. If these test methods are not appropriate and verifiable, or if the resulting data are not reflective of in-use conditions, then the overall effectiveness of many generic recommendations and guidelines for selection and use of liquid chemical germicides will be weakened."

Since 1990, the EPA has adopted a multidisciplinary approach involving microbiologists, virologists, statisticians and other scientists to develop new

standardized tests which will be more reliable and reproducible than the preceding tests. Prior to the new tests, little attention was given to statistical analysis of antimicrobial assays. The EPA realized that statistical thinking was necessary to achieve the goal of developing improved standardized tests. Some important statistical tasks include developing statistical models for microbial assay data, mathematically defining parameters measuring a disinfectant's efficacy, identifying sources of variability in the tests, and suggesting designs that will reduce variability to acceptable levels. The general purpose of this dissertation is to describe statistical methodology for two microbial assays commonly used in disinfection efficacy testing; the quantitative assay and the presence/absence assay. A description of both assays will first be given before defining the specific goals of this dissertation.

Description of the Quantitative Assay

Central to the quantitative assay (as well as the presence/absence assay) is the concept of "check" and "test" "carriers". The carriers are often small stainless steel or glass penicylinders, or some other nonporous medium, which are inoculated with microorganisms for testing purposes. Figure 1 shows the major steps common in all quantitative assays for test carriers.

Step 1: A suspension of microbes is prepared according to a targeted density of microbes per unit volume. Included in the suspension are culture medium, which acts as a food source for the microbes, and possibly added "organic soil", in addition to the microorganisms.

Step 2: A sterilized carrier is selected.

Step 3: The carrier is placed in the prepared inoculum.

Step 4: The inoculum is dried on the carrier, leaving the microorganisms embedded in a protective organic film that represents a realistic challenge to the disinfectant.

After drying, the targeted inoculum size ranges between 10^5 and 10^8 viable organisms depending on whether bacteria, spores, or viruses are the test organism of interest.

Step 5: The carrier is treated with a purported disinfectant for a specified amount of time.

Step 6: Following neutralization of the disinfectant, the carrier is sonicated and/or vortexed, thus removing organisms from the carrier into a well-mixed liquid suspension.

Step 7: The suspension is serially diluted, plated or filtered, incubated, and colony forming units (cfu) are counted. The series of dilution levels, or synonymously, the fraction of total volume sampled, are recorded with the cfu counts so that cfu counts can be converted into a per carrier estimate of the number of viable organisms surviving disinfection. If a disinfectant is effective in killing organisms, few cfu counts will be observed for the test carriers.

In the absence of knowledge about the initial numbers of organisms exposed to disinfection, one cannot discern if low test carrier cfu counts are due to a strong disinfectant or to a weak disinfectant applied to carriers that contained few organisms. For this reason, a series of check carriers are run in addition to test carriers. Check carriers are treated in an identical manner as test carriers except an inert solution is applied to the carrier in lieu of an actual disinfectant. Check carrier cfu counts, along with the recorded dilution levels, provide an estimate of the number of viable organisms exposed to disinfection (on a per carrier basis). Thus in the quantitative assay, direct counts (quantitative data) are observed on both check and test carriers. Data from both check and test carriers are required in order to measure disinfectant efficacy. Because of inherent variability involved at each step in the test protocol, each assay requires multiple check and test carriers.

Figure 1: Schematic chart for test carriers in the quantitative assay

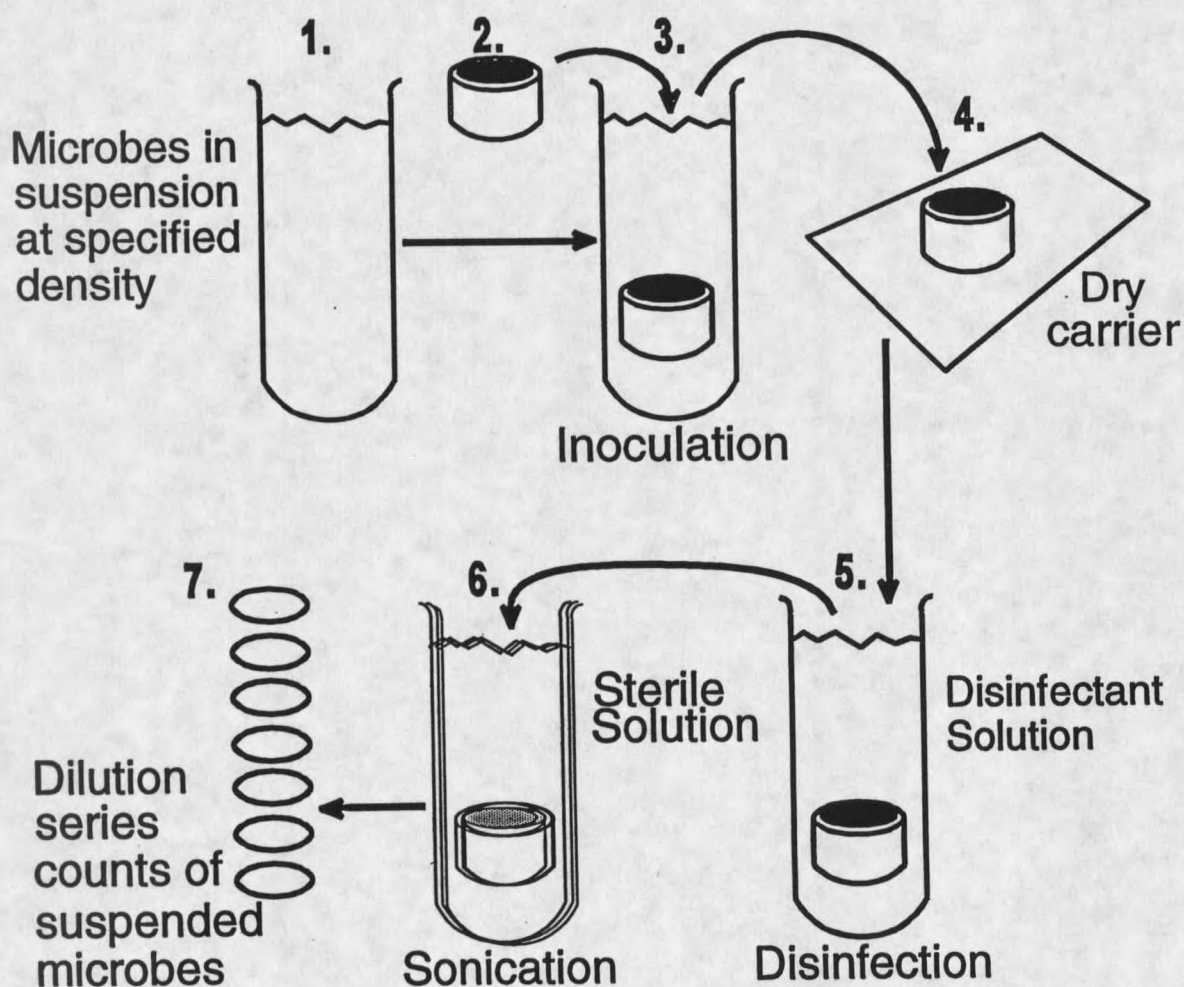


Table 1 gives an example of the type of data arising from a quantitative assay with three check carriers and ten test carriers. In this example, the check carriers contain approximately 10^8 organisms (check carrier counts divided by volume sampled for check carriers) and the test carriers contain approximately 1000 organisms (test carrier counts divided by volume sampled for test carriers) .

Table 1: Example data from the quantitative assay

Check Carriers			Test Carriers		
Carrier Number	Volume Sampled	Counts	Carrier Number	Volume Sampled	Counts
1	3×10^{-6}	189	1	0.33	267
2	3×10^{-6}	195	2	0.33	291
3	3×10^{-6}	219	3	0.33	99
			4	0.33	270
			5	0.33	318
			6	0.33	102
			7	0.33	297
			8	0.33	126
			9	0.33	222
			10	0.33	489

Description of the Presence/Absence Assay

The presence/absence assay (pa assay) is characterized by a series of presence/absence responses (qualitative data) for both check and test carriers. The pa assay can be thought of as a sequence of serial-dilution assays for test and check carriers. The serial-dilution assay (Loyer and Hamilton 1984) has proved valuable for determining the density of organisms in solutions in situations where colony-count methods are not effective (Eaton et al. 1992). For example, some viruses, such as the adenovirus, do not readily form colonies or plaques, hence the serial-dilution assay is commonly used in the field of virology.

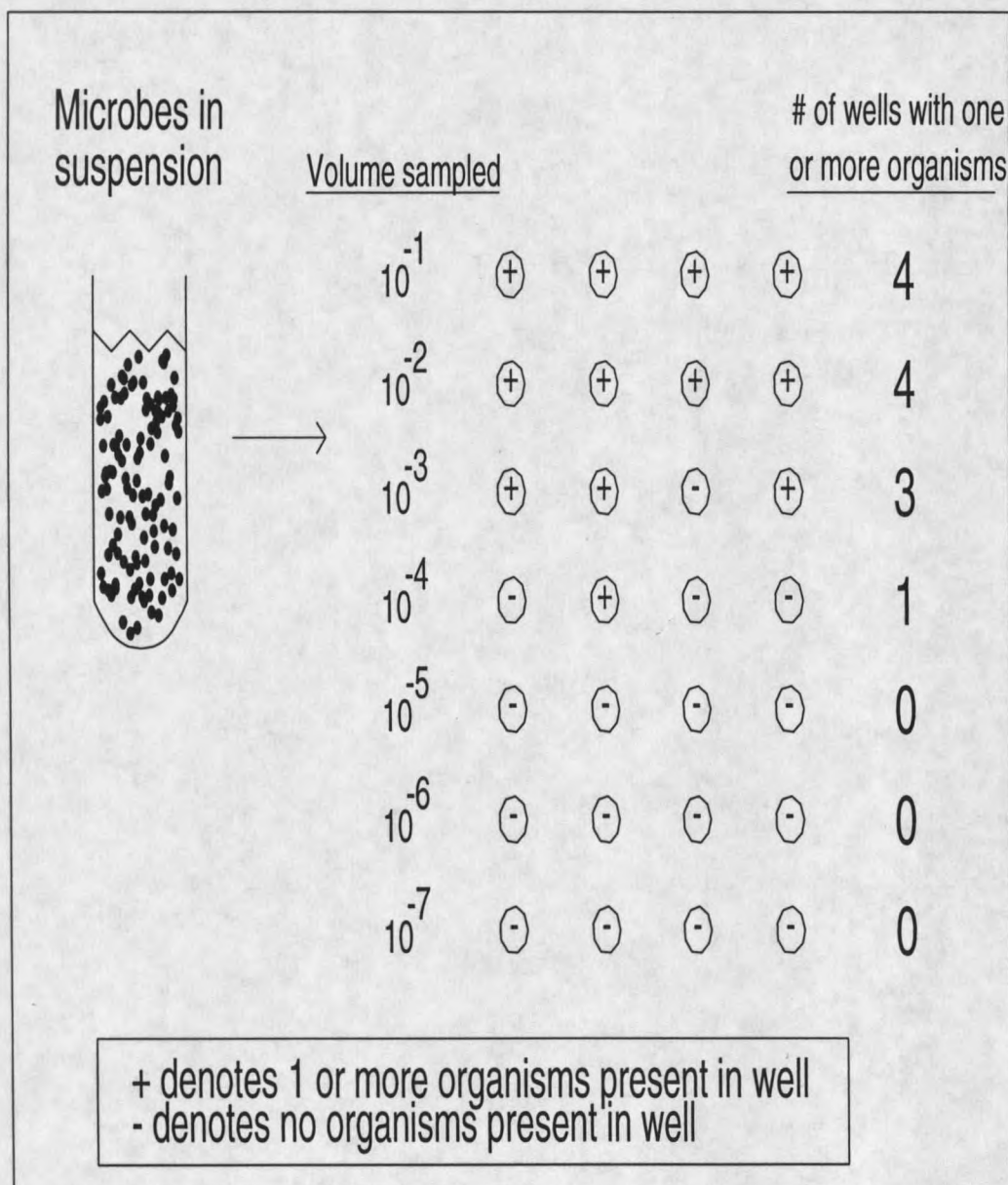
The main distinction between the pa and quantitative assays comes after the organisms are removed from a carrier into suspension via sonication. For the pa assay, the suspension is serially diluted. At each dilution stage, a number of sample aliquots are placed into tubes or wells and, following incubation, examined for a presence/absence response. It is assumed that a tube or well will exhibit a positive response (presence) if and only if one or more organisms in the sample survived disinfection. Otherwise it will exhibit a negative response (absence), indicating that no organisms in the sample survived disinfection. From the series of presence/absence responses and the associated dilution levels, an estimate of the number of viable organisms in suspension can be determined. Figure 2 shows the steps common to serial-dilution assays. A check carrier is treated in the same manner as a test carrier except that an inert solution is used in place of the disinfectant. The type of data recorded for a check or test carrier is then the number of wells exhibiting a positive response at each dilution stage. There is evidence to believe a disinfectant is effective if wells are negative at low dilutions for test carriers, but wells are positive at high dilutions for check carriers.

Table 2 gives an example of the data collected for a pa assay with four wells per dilution for each of three check and three test carriers. These data indicate that there are about 1000 times more viable organisms on check carriers than on test carriers. Notice that for check carriers, high dilutions of 10^{-7} or 10^{-8} are required to show absence of viable organisms in all four wells, whereas for test carriers, absence in all four wells occur at 10^{-4} or 10^{-5} dilutions.

Common Measures of Disinfectant Efficacy

As one can see from Tables 1 and 2, there are distinct differences in the types of data collected in the quantitative and presence/absence assays. The goal of each

Figure 2: Schematic diagram for a serial-dilution assay



Volume sampled denotes the fraction of the total suspension sampled per well

Table 2: Example data from the presence/absence assay

	Check Carriers			Test Carriers		
	Carrier 1	Carrier 2	Carrier 3	Carrier 1	Carrier 2	Carrier 3
Dilution	Number of wells positive			Number of wells positive		
10^{-1}	4	4	4	4	4	4
10^{-2}	4	4	4	4	4	4
10^{-3}	4	4	4	4	3	4
10^{-4}	4	4	4	0	1	4
10^{-5}	4	4	4	0	0	0
10^{-6}	4	3	3	0	0	0
10^{-7}	0	1	0	0	0	0
10^{-8}	0	0	0	0	0	0

of the two assays is the same: to measure how effective at killing microorganisms a disinfectant is; that is, to produce a quantitative summary of a disinfectant's efficacy. An example of a quantitative summary would be an estimate of the proportion of organisms surviving disinfection. Equivalent measures of efficacy are the percentage of organisms killed and the log reduction. For example, suppose it was known that N_1 of N_0 organisms would survive disinfection. Then $\frac{N_1}{N_0}$, $100 \times \left(1 - \frac{N_1}{N_0}\right) \%$, and $\log_{10}(N_0) - \log_{10}(N_1)$ are the survival fraction, the percentage killed, and the log reduction, respectively. Smaller survival fractions, or equivalently larger percentages killed and larger log reductions, indicate more active disinfectants. Table 3 gives numerical values for the survival fraction, percentage of organisms killed, and the log reduction assuming $N_0 = 1,000,000$ and $N_1 = 1$.

Table 3: Example of equivalent quantitative measures of disinfection efficacy

N_1	N_0	Survival fraction	Percentage killed	Log reduction
1	10^6	0.000001	99.9999%	6.0

Specific Goals of this Dissertation

The goals of this dissertation are to present statistical models for data arising from the quantitative assay and the presence/absence assay, to discuss alternative ways to quantify disinfection efficacy, to present methods I have derived for estimating these quantities and associated standard errors, and to compare alternative estimation methods. The comparisons are based on both theory and computer simulation. Separate chapters will be devoted to the quantitative assay and the presence/absence assay.

CHAPTER 2

Quantitative Assay

The goal of a quantitative assay is to estimate the Log Reduction (LR) for a disinfectant. Before defining LR it will be helpful if certain notation is listed.

Notation

A = Number of check carriers.

B = Number of test carriers.

D = Number of dilution stages for check carriers.

E = Number of dilution stages for test carriers. D does not have to equal E .

F = Number of samples per dilution for check or test carriers.

i = Subscript for check carriers; $i = 1, \dots, A$.

j = Subscript for dilution stage of a check carrier; $j = 1, \dots, D$.

k = Subscript for sample at a dilution stage for a check carrier; $k = 1, \dots, F$.

s = Subscript for test carriers; $s = 1, \dots, B$.

m = Subscript for dilution stage of a test carrier; $m = 1, \dots, E$.

n = Subscript for sample at a dilution stage for a test carrier; $n = 1, \dots, F$.

C_{ijk} = Random variable denoting the microbial count for check carrier i at dilution stage j and sample k .

c_{ijk} = Observed microbial count for check carrier i at dilution stage j and sample k .

$C_i = \sum_{j=1}^D \sum_{k=1}^F C_{ijk}$ = Random variable denoting the sum of microbial counts across dilutions and samples for check carrier i .

$c_i = \sum_{j=1}^D \sum_{k=1}^F c_{ijk}$ = Observed sum of microbial counts across dilutions and samples for check carrier i .

T_{smn} = Random variable denoting the microbial count for test carrier s at dilution stage m and sample n .

t_{smn} = Observed microbial count for test carrier s at dilution stage m and sample n .

$T_s = \sum_{m=1}^E \sum_{n=1}^F T_{smn}$ = Random variable denoting the sum of microbial counts across dilutions and samples for test carrier s .

$t_s = \sum_{m=1}^E \sum_{n=1}^F t_{smn}$ = Observed sum of microbial counts across dilutions and samples for test carrier s .

u_{ijk} = Fraction of total volume sampled for check carrier i at dilution j and sample k .

$u_i = \sum_{j=1}^D \sum_{k=1}^F u_{ijk}$ = Fraction of total volume sampled for check carrier i .

Notice that the total volume sampled may vary among check carriers.

v_{smn} = Fraction of total volume sampled for test carrier s at dilution m and sample n .

$v_s = \sum_{m=1}^E \sum_{n=1}^F v_{smn}$ = Fraction of total volume sampled for test carrier s .

Notice that the total volume sampled may vary among test carriers.

$X_i = \frac{C_i}{u_i}$ = Density for check carrier i ; $i = 1, \dots, A$.

$$X_i^* = \begin{cases} \log(X_i), & \text{if } X_i > 0 \\ \log\left(\frac{1}{u_i}\right), & \text{if } X_i = 0 \end{cases}$$

$\bar{X} = \frac{1}{A} \sum_{i=1}^A X_i$ = Average check carrier density.

$\bar{X}^* = \frac{1}{A} \sum_{i=1}^A X_i^*$ = Average log check carrier density.

$Y_s = \frac{T_s}{v_s}$ = Density for test carrier s ; $s = 1, \dots, B$.

$$Y_s^* = \begin{cases} \log(Y_s), & \text{if } Y_s > 0 \\ \log\left(\frac{1}{v_s}\right), & \text{if } Y_s = 0 \end{cases}$$

$\bar{Y} = \frac{1}{B} \sum_{s=1}^B Y_s$ = Average test carrier density.

$\bar{Y}^* = \frac{1}{B} \sum_{s=1}^B Y_s^*$ = Average log test carrier density.

P_s is the survival fraction for a randomly chosen microbe exposed to disinfection for test carrier s ; $s = 1, \dots, B$. For notational convenience, the subscript will be dropped

from P for the remainder of this chapter. P is a random variable that follows a distribution with mean ϕ and variance ζ^2 .

θ_{Ci} = Unobservable inoculum size for check carrier i ; $i = 1, \dots, A$.

θ_{Ts} = Unobservable inoculum size for test carrier s ; $s = 1, \dots, B$.

"Zero-adjusted" refers to the standard approach of adding 1 to zero counts before taking logs.

$\log(\cdot)$ is the base 10 logarithm.

$\ln(\cdot)$ is the natural logarithm.

$\hat{\cdot}$ is a symbol used to indicate "an estimator of".

Log Reduction (LR)

In my literature search of LR calculations for the quantitative assay, I have been able to find examples for the case of 1 check carrier and 1 test carrier, only.

LR is defined (Robison et al. 1988) as follows:

$$\text{LR} = \log(\text{number of organisms per carrier before exposure to disinfection}) - \log(\text{number of organisms per carrier after exposure to disinfection}).$$

Denote as c the check carrier count and its associated volume sampled as u . Denote as t the test carrier count and its associated volume sampled as v . Notice that $\frac{t}{v}$ is the estimated number of microbes per carrier surviving disinfection, while $\frac{c}{u}$ is the estimated number of microbes per carrier exposed to disinfection. The standard estimate of LR is: $\text{LR} = \log(\frac{c}{u}) - \log(\frac{t}{v})$. This quantity is sometimes called the microbicidal effect (ME) (Bloomfield et al. 1994) or the sterility assurance level (SAL) (Oxborrow and Berube 1991).

The new quantitative assays require multiple check and test carriers. Microbiologists calculate LR in a number of different ways when analyzing a multiple check and test carrier assay. To my knowledge, no published paper defines

and compares the various estimators of LR. In the following sections of this chapter two different conceptualizations of LR for multiple carrier assays will be discussed, and an intuitive estimator will be presented for each definition of LR. The main purposes of this chapter are to present a method of moments (MOM) and maximum likelihood estimator (MLE) and associated standard errors for the two conceptualizations of LR. The standard error is important because it indicates inherent variability in the quantitative assay. Knowledge of the inherent variability will be useful as a benchmark for within- and between- laboratory variances that will be observed in forthcoming multi-laboratory collaborative studies of disinfectants on various microorganisms. Large sample asymptotic results will be derived for purposes of evaluating the estimators' efficiencies. Knowledge of the theoretical efficiencies of the estimators will be useful in providing recommendations as to which estimators should be used. Specifically, efficiency calculations will give guidelines as to whether the more easily calculated MOM estimators of LR can be justified in their use over the more laboriously calculated MLE of LR. Finally, results from a computer simulation study will be given for purposes of determining the small sample properties of the various estimators.

Two Simple Estimators of LR

One approach microbiologists have used to calculate LR is: (i) transform the check and test carrier densities to the log scale and (ii) subtract the average log test carrier density from the average log check carrier density. In notation, this estimator is $\bar{X}^* - \bar{Y}^*$. For obvious reasons I have decided to refer to this approach as the "average of logs". I denote the average of logs parameter as Υ_1 . Specifically, letting $E(\cdot)$ denote the expectation operator,

$$\Upsilon_1 = \frac{1}{A} \times E\left(\sum_{i=1}^A X_i^*\right) - \frac{1}{B} \times E\left(\sum_{s=1}^B Y_s^*\right). \quad (2.1)$$

By the Law of Large Numbers, the average of logs estimator is consistent for Υ_1 . Notice, for the single check and test carrier situation, the average of logs estimate is $\log(\frac{c}{u}) - \log(\frac{t}{v})$.

An alternative estimator of LR is as the negative logarithm of the survival fraction estimator, $\frac{\bar{Y}}{\bar{X}}$; i.e. $LR = -\log\left(\frac{\bar{Y}}{\bar{X}}\right)$. This estimator can also be written as $\log(\bar{X}) - \log(\bar{Y})$. Consequently, I refer to it as the log of averages approach. Notice, for the single check and test carrier situation, the survival fraction estimate is $\frac{t}{v} \div \frac{c}{u}$ and the log of averages estimate is simply $\log\left(\frac{c}{u}\right) - \log\left(\frac{t}{v}\right)$. Denote the log of averages parameter as Υ_2 . Specifically,

$$\Upsilon_2 = -\log(\phi), \quad (2.2)$$

where ϕ is the population mean of the distribution of survival fractions, P . By the Law of Large Numbers, the log of averages estimator is consistent for Υ_2 .

It should be emphasized that the average of logs and log of averages estimators are not estimating equivalent parameters. From Jensen's inequality it is known that $\log(\bar{X}) \geq \bar{X}^*$ and $\log(\bar{Y}) \geq \bar{Y}^*$, (assuming none of the test or check carrier densities are zero). However, no definitive statements can be made in terms of comparing the average of logs estimator to the log of averages estimator. For the single check and test carrier situation, however, the average of logs method and log of averages method yield identical results.

Arguments Favoring the Average of Logs Method

There are reasons to prefer the average of logs method to the log of averages method and vice versa. The average of logs estimator of LR is the log of the geometric mean of test carriers subtracted from the log of the geometric mean for check carriers. Because counts of microorganisms often exhibit positive skew, the preferred statistic for summarizing microbiological data is the geometric mean (Eaton et al. 1995).

For this reason, the average of logs method may be the method of choice by microbiologists. The assumption of microbial counts originating from distributions having positive skew appears to be reasonable for one particular application of the quantitative assay with which I am familiar. Figure 3 gives the Empirical Distribution Function (EDF) of the sample skewness coefficients from 195 check and test carrier data sets. Figure 4 gives the EDF of the sample skewness coefficients for the log transformed data. The data came from a multi-laboratory collaborative study (Springthorpe 1997) involving 15 laboratories each testing various disinfectant formulations. As seen from Figure 3, positive skew was evident in roughly 80% of the test carrier data sets. Positive skew does not seem to be as prevalent for the check carrier data sets. However, each check carrier data set consisted of only 3 observations (as opposed to 10 test carrier observations). Because the check carrier procedure should be constant from assay to assay within a laboratory, I felt it was appropriate to pool check carrier observations within laboratories. Results not shown here indicate that positive skew for the pooled check carrier data was evident in 12 of 15 laboratories. Thus it seems reasonable to believe that both check and test carrier data originate from distributions having positive skew.

From Figure 4 it is evident that the log test carrier counts are less skewed. There is some theoretical justification that the log transformation of microbiological data will induce less skew. For reasons to be presented later, I believe it is reasonable to assume negative binomial distributions for check and test carrier counts. The negative binomial distribution has positive skew and the log of a (zero-adjusted) negative binomial random variable has a more symmetric distribution. Some motivation for this statement can be provided by the gamma distribution. The log of a gamma has a more normal, and hence symmetric, distribution than the original gamma (Kotz, Johnson and Read 1985). Because the

Figure 3: EDF for skewness coefficient of 195 check and test carrier data sets

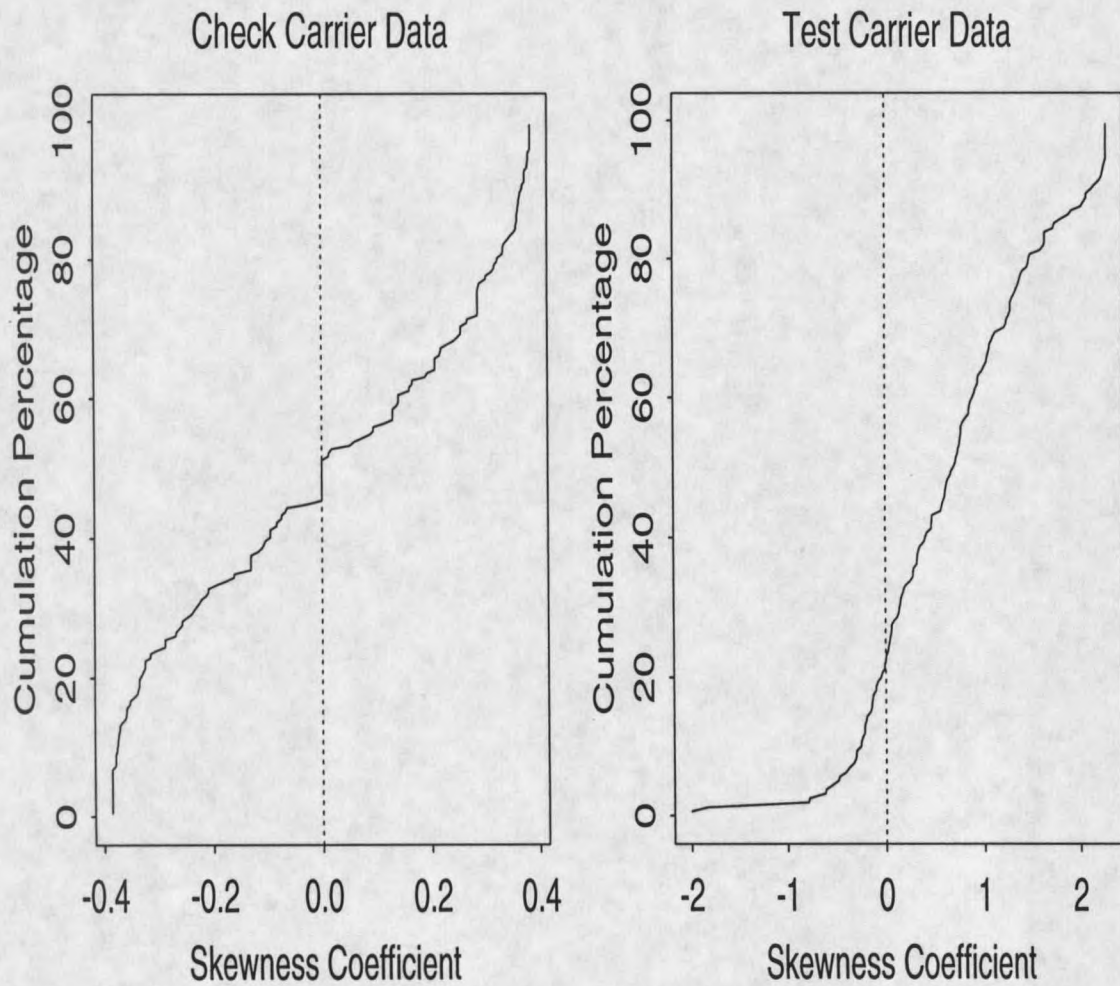
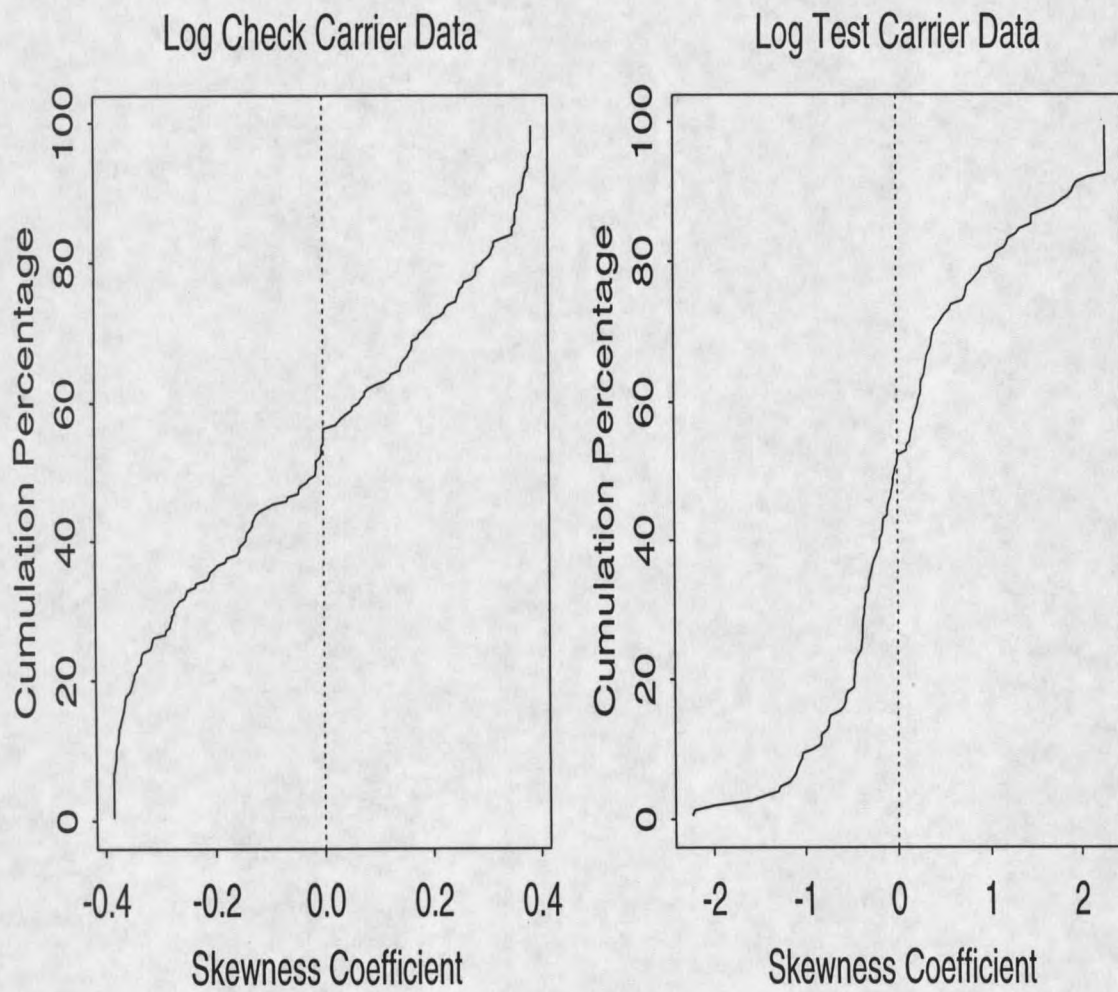


Figure 4: EDF for skewness coefficient of 195 log transformed check and test carrier data sets



negative binomial is well approximated by a gamma distribution, it is reasonable to conclude the log of a (zero-adjusted) negative binomial distribution has a more symmetric distribution.

Because the mean is a better estimator of central tendency when the underlying distribution is more symmetric, the average of logs method may be preferable to the log of averages. Also, statistical inference on the average of logs estimator of LR may be easier because of the movement towards normality due to the log transformation.

Arguments Favoring the Log of Averages Method

A reason to prefer using the log of averages estimator of LR is because it has a compact biological interpretation. Some microbiologists choose to quantify disinfection efficacy with the survival fraction, which is the antilog of Υ_2 . The survival fraction is not a simple function of Υ_1 , thus the average of logs estimator may be preferable from an interpretability argument. Also, the log of averages estimator is an appropriate estimator of the Oxborrow and Berube (1991) definition of SAL.

Statistical Modeling Assumptions

Define the inoculum size on a carrier as the number of viable organisms exposed to disinfection (or simulated disinfection).

Assumption 1: The unobservable inoculum sizes for the A check carriers and B test carriers, denoted as θ_{Ci} , $i = 1, \dots, A$ and θ_{Ts} , $s = 1, \dots, B$, respectively, are statistically independent and are approximately distributed as gamma (mean = $\alpha\beta$, variance = $\alpha\beta^2$) random variables; $\alpha > 0, \beta > 0$.

The inoculum size on a carrier is an integer-valued random variable which I believe can be reasonably well approximated by a negative binomial distribution. The negative binomial distribution can be derived as a mixture of Poisson distributions (McCullagh and Nelder 1989). The distribution of the inoculum sizes is conceivably a mixture of Poisson distributions because the pipette volumes will vary from sample to sample, and, when a pipette volume is removed from a well-mixed suspension, the number of organisms is a random variable following a Poisson distribution (Stearman 1955). Also, viable organisms are lost during the drying of the carriers; thus differences among carriers in both the length and intensity of the drying step will induce extra variability among the inoculum sizes. The additional sources of variability above and beyond Poisson sampling lead to the negative binomial distribution. Because the average inoculum size is so large (between 10^5 and 10^8), it is appropriate to act as though the inoculum sizes are a continuous random variable and the (continuous) gamma distribution closely approximates the (discrete) negative binomial distribution in these cases (Bartko 1961; Best and Gipps 1974; Guenther 1972; Mood, Graybill, and Boes 1974; Osaki and Li 1988; Pessin 1961).

Assumption 2: $C_{ijk} \mid \theta_{Ci}, u_{ijk}$, $i = 1, \dots, A$; $j = 1, \dots, D$; $k = 1, \dots, F$ are distributed independently as $\text{Poisson}(\theta_{Ci} \cdot u_{ijk})$.

Because the C_{ijk} , $j = 1, \dots, D$; $k = 1, \dots, F$ are independent Poisson random variables, $C_i \mid \theta_{Ci}, u_i$, are distributed independently as $\text{Poisson}(\theta_{Ci} \cdot u_i)$, $i = 1, \dots, A$.

Assumption 3: P is a random variable that follows a distribution with mean ϕ and variance ζ^2 . P and θ_{Ts} , $s = 1, \dots, B$ are statistically independent.

There are at least two reason to suspect that the survival fraction could vary among test carriers. First, variation in the amounts of disinfectant added to the

carriers and variation in the exposure period of disinfection could cause variable survival fractions. Second, the shape of the dried inoculum film could well affect disinfection rates, with thicker films yielding lower disinfection rates (Stewart 1996). It is conceivable that under ideal situations ϕ is constant among test carriers, in which case P is degenerate at ϕ . I will refer to the model with P having a non-degenerate distribution as the **over-dispersed model**, and the model with P degenerate as the **model with no over-dispersion**.

Assumption 4: $T_{smn} \mid \theta_{Ts}, v_{smn}, P$, $s = 1, \dots, B$; $m = 1, \dots, E$; $n = 1, \dots, F$ are distributed independently as $\text{Poisson}(\theta_{Ts} \cdot v_{smn} \cdot P)$. Thus, $T_s \mid \theta_{Ts}, v_s, P$, $s = 1, \dots, B$ are distributed independently as $\text{Poisson}(\theta_{Ts} \cdot v_s \cdot P)$.

It can be shown (see Appendix A) that the above assumptions imply that the marginal distributions of C_i are negative binomial ($r = \alpha$, $p = \frac{1}{\beta u_i + 1}$, $q = \frac{\beta u_i}{\beta u_i + 1}$), $i = 1, \dots, A$. The C_i are statistically independent and will be iid negative binomial if the sample volumes, u_i , are constant across check carriers. I have chosen to parameterize the negative binomial distribution to have mean $\frac{\alpha q}{p}$ and variance $\frac{\alpha q}{p^2}$. Thus,

$$P(C_i = c_i \mid \alpha, \beta, u_i) = \frac{\Gamma(c_i + \alpha)}{\Gamma(\alpha) c_i!} \left(\frac{\beta u_i}{\beta u_i + 1} \right)^{c_i} \left(\frac{1}{\beta u_i + 1} \right)^{\alpha} \quad c_i = 0, 1, 2, \dots$$

The mean and variance of the random variable C_i are $\alpha \beta u_i$ and $\alpha \beta u_i + \frac{1}{\alpha} (\alpha \beta u_i)^2$, respectively.

The above assumptions imply for the over-dispersed model that marginally, T_s , $s = 1, \dots, B$ are distributed independently with mean $= \alpha \beta \phi v_s$ and variance $\alpha \beta \phi v_s [1 + \alpha \beta \phi v_s (\frac{\phi^2 + \zeta^2 (\alpha + 1)}{\alpha \phi^2})]$. For convenience, denote $\frac{\phi^2 + \zeta^2 (\alpha + 1)}{\phi^2}$ as d ; $d \geq 1$. If $d = 1$, then $\zeta^2 = 0$ and the distribution of P is degenerate at ϕ . If $d > 1$, then $\zeta^2 > 0$ and the survival fraction varies among carriers. Because the distribution of P is unknown when $d > 1$, the marginal distribution of T_s cannot be derived. It is reasonable to assume, however, that the distribution of T_s is closely approximated

by a negative binomial ($r = \frac{\alpha}{d}$, $p = \frac{1}{\beta\phi v_s d + 1}$, $q = \frac{\beta\phi v_s d}{\beta\phi v_s d + 1}$), $s = 1, \dots, B$. The parameters for the negative binomial distribution for test carriers were chosen to match the exact mean and variance of test carriers. It can be shown (Appendix A) that if P is degenerate at ϕ , then T_s is distributed independently as negative binomial ($r = \alpha$, $p = \frac{1}{\beta\phi v_s + 1}$, $q = \frac{\beta\phi v_s}{\beta\phi v_s + 1}$), $s = 1, \dots, B$. Thus for the model with no over-dispersion, the mean and variance of T_s are $\alpha\beta\phi v_s$ and $\alpha\beta\phi v_s + \frac{1}{\alpha}(\alpha\beta\phi v_s)^2$, respectively. Note that the T_s will be iid negative binomial if the dilution levels, v_s , are constant across test carriers.

Assumption 5: The C_i , $i = 1, \dots, A$ and T_s , $s = 1, \dots, B$ are all statistically independent.

The joint probability mass function (pmf) of the observable data is then the product of A negative binomial check carrier count densities times the product of B negative binomial test carrier count densities. Notice that the form of the pmf will depend on whether P is degenerate at ϕ or P follows a distribution with mean ϕ and variance ζ^2 . Denote as $\vec{\tau}$ the vector parameter $(\phi, \alpha, \beta)'$. Let $\vec{c}$ and $\vec{t}$ denote the vectors of check and test carrier counts, respectively; and let $l(\vec{\tau} | \vec{c})$ and $l(\vec{\tau} | \vec{t})$ denote the associated log likelihoods. Then, when P is degenerate at ϕ ,

$$l(\vec{\tau} | \vec{c}) = \sum_{i=1}^A [\ln(\Gamma(c_i + \alpha)) - \ln(\Gamma(\alpha)) - \ln(c_i!) + \alpha \ln\left(\frac{1}{\beta u_i + 1}\right) + c_i \ln\left(\frac{\beta u_i}{\beta u_i + 1}\right)]$$

and

$$l(\vec{\tau} | \vec{t}) = \sum_{s=1}^B [\ln(\Gamma(t_s + \alpha)) - \ln(\Gamma(\alpha)) - \ln(t_s!) + \alpha \ln\left(\frac{1}{\beta\phi v_s + 1}\right) + t_s \ln\left(\frac{\beta\phi v_s}{\beta\phi v_s + 1}\right)].$$

The log likelihood function for the model with no over-dispersion, denoted by $l(\vec{\tau} | \vec{c}, \vec{t})$, is given in (2.3).

$$l(\vec{\tau} | \vec{c}, \vec{t}) = l(\vec{\tau} | \vec{c}) + l(\vec{\tau} | \vec{t}). \quad (2.3)$$

Similarly, denote as $\vec{\tau}^*$ the vector parameter $(\phi, \zeta, \alpha, \beta)'$. Let $l(\vec{\tau}^* | \vec{c})$ and $l(\vec{\tau}^* | \vec{t})$ denote the log likelihoods for the check and test carrier data, respectively. Note that

$$l(\vec{\tau}^* | \vec{c}) = \sum_{i=1}^A [\ln(\Gamma(c_i + \alpha)) - \ln(\Gamma(\alpha)) - \ln(c_i!) + \alpha \ln\left(\frac{1}{\beta u_i + 1}\right) + c_i \ln\left(\frac{\beta u_i}{\beta u_i + 1}\right)],$$

and, remembering that $d = \frac{\phi^2 + \zeta^2(\alpha+1)}{\alpha\phi^2}$, $l(\vec{\tau}^* | \vec{t}) =$

$$\sum_{s=1}^B [\ln\left(\Gamma(t_s + \frac{\alpha}{d})\right) - \ln\left(\Gamma(\frac{\alpha}{d})\right) - \ln(t_s!) + \frac{\alpha}{d} \ln\left(\frac{1}{\beta\phi v_s d + 1}\right) + t_s \ln\left(\frac{\beta\phi v_s d}{\beta\phi v_s d + 1}\right)].$$

Then the log likelihood function for the over-dispersed model, denoted by $l(\vec{\tau}^* | \vec{c}, \vec{t})$, is given in (2.4).

$$l(\vec{\tau}^* | \vec{c}, \vec{t}) = l(\vec{\tau}^* | \vec{c}) + l(\vec{\tau}^* | \vec{t}). \quad (2.4)$$

Point Estimation of LR

Estimators of Υ_1 for the Model with no Over-dispersion

The MOM estimator of the average of logs definition of LR is denoted as $\hat{\Upsilon}_{1, \text{MOM}}$, and is given by (2.5).

$$\hat{\Upsilon}_{1, \text{MOM}} = \overline{X^*} - \overline{Y^*}. \quad (2.5)$$

Notice that $\hat{\Upsilon}_{1, \text{MOM}}$ is calculable even if some or all of the test carrier counts are zero, and has the advantage of being easily calculated with the aid of a spreadsheet computer program.

Denote the maximum likelihood estimate of Υ_1 for the model with no over-dispersion as $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}$. Assuming that P is degenerate at ϕ then the computation of $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}$ involves finding the values of $\hat{\phi}, \hat{\alpha}, \hat{\beta}$ which maximize (2.3). Taking partial derivatives of (2.3) with respect to $\vec{\tau}$ and setting equal to $\vec{0}$, gives

three equations and three unknowns. However, these equations cannot be solved in a closed form and an iterative solution must be sought. I have chosen to use the Newton-Raphson method to obtain maximum likelihood estimates of $\vec{\tau}$. Specifically, the equations to solve are: $\frac{\partial l(\vec{\tau})}{\partial \vec{\tau}} = \vec{0}$. Taylor's approximation implies (2.6),

$$\frac{\partial l(\vec{\tau})}{\partial \vec{\tau}} = \vec{0} \doteq \frac{\partial l(\vec{\tau})}{\partial \vec{\tau}} \Big|_{\vec{\tau}=\hat{\vec{\tau}}} + \frac{\partial^2 l(\vec{\tau})}{\partial \vec{\tau} \partial \vec{\tau}'} \Big|_{\vec{\tau}=\hat{\vec{\tau}}} \times (\vec{\tau} - \hat{\vec{\tau}}). \quad (2.6)$$

Let,

$$\frac{\partial l(\vec{\tau})}{\partial \vec{\tau}} \Big|_{\vec{\tau}=\hat{\vec{\tau}}} = f(\hat{\vec{\tau}}) \quad \text{and} \quad \frac{\partial^2 l(\vec{\tau})}{\partial \vec{\tau} \partial \vec{\tau}'} \Big|_{\vec{\tau}=\hat{\vec{\tau}}} = H(\hat{\vec{\tau}}).$$

Solving (2.6) for $\vec{\tau}$ yields (2.7),

$$\vec{\tau} \doteq \hat{\vec{\tau}} + [-H(\hat{\vec{\tau}})]^{-1} \times f(\hat{\vec{\tau}}). \quad (2.7)$$

Symbolic descriptions of the vector of derivatives, $f_{3 \times 1}$, and the Hessian matrix $H_{3 \times 3}$, are given in Appendix A. Guidance on inverting $H(\hat{\vec{\tau}})$ is also given in Appendix A.

Jacoby et al. (1972) recommend using (2.8) in place of (2.7) to solve for $\vec{\tau}$; where (2.8) is a special case of the Levenberg-Marquardt algorithm (Seber and Wild 1989) .

$$\vec{\tau} \doteq \hat{\vec{\tau}} + \omega [-H(\hat{\vec{\tau}}) + \delta I]^{-1} \times f(\hat{\vec{\tau}}). \quad (2.8)$$

The ω and δ in (2.8) are constants; ω is chosen to modify the step sizes in the Newton-Raphson method, and δ is chosen to ensure the log likelihood function will be increased at each successive iteration. An initial guess for $\vec{\tau}$ is supplied in (2.8) and (2.8) is solved repeatedly until a convergence criteria for $\vec{\tau}$ has been met. Denote $\hat{\vec{\tau}}^{(t)}$ as the estimate of $\vec{\tau}$ at the t^{th} iteration, and $l(\hat{\vec{\tau}}^{(t)})$ as the value of equation (2.3) evaluated at $\hat{\vec{\tau}}^{(t)}$. The convergence criteria I used was to iterate until,

$$\left| \frac{l(\hat{\vec{\tau}}^{(t+1)}) - l(\hat{\vec{\tau}}^{(t)})}{l(\hat{\vec{\tau}}^{(t)})} \right| \leq 0.00001.$$

Jacoby et al. (1972) recommend the iteration scheme of (2.8) over (2.7) because (2.7) often generates $\hat{\tau}^{(t+1)}$ which has a smaller log likelihood value than $\hat{\tau}^{(t)}$. This can occur for two reasons. First, $-H(\hat{\tau}^{(t)})$ may not be positive definite, in which case (2.7) is not guaranteed to move the updated estimate towards a maximum. The δ in (2.8) is chosen such that $-H(\hat{\tau}) + \delta I$ is positive definite, where I is the identity matrix. This modification will generate updates that move in the direction of an increase in the log likelihood function. In my program I started with a value of $\delta = 10^{-10}$ and increased δ by a factor of 10 until $-H(\hat{\tau}) + \delta I$ was positive definite. Secondly, (2.7) often "overshoots" the true maximum. The ω in (2.8) is referred to as the step-length, and is useful for preventing overshooting. Rather than always taking a full step-length of $\omega = 1$, as in (2.7), it is advisable to choose a step-length such that the log likelihood is increased at the next iteration. In my program I chose eleven equally-spaced values of ω between 0.001 and 1.0 inclusive, and solved (2.8) for each value of ω . The ω which maximized $l(\hat{\tau} + \omega \times [-H(\hat{\tau}) + \delta I]^{-1} \times f(\hat{\tau}))$ among my choices of ω was chosen.

A nice by-product of using the Newton-Raphson method to find the mle of $\vec{\tau}$ is that the inverse of the negative of the matrix H , evaluated at the mle of $\vec{\tau}$, is an estimate of the variance-covariance matrix of $\hat{\vec{\tau}}$. Under suitable regularity conditions the information matrix of $\vec{\tau}$, denoted as $I(\vec{\tau})$, is $-E(H(\vec{\tau}))$ and the variance-covariance matrix of $\vec{\tau}$ is $[I(\vec{\tau})]^{-1}$. $E(H(\vec{\tau}))$ is not analytically tractable, as far as I can see, so for this reason I have decided to use the observed information, $-H(\vec{\tau})$, in the iteration scheme of (2.8). An estimate of the variance-covariance matrix of $\hat{\vec{\tau}}$ is then $[-H(\hat{\vec{\tau}})]^{-1}$. Denote as $V(\hat{\vec{\tau}})$ the estimated variance-covariance matrix of $\hat{\vec{\tau}}$; thus $V(\hat{\vec{\tau}}) = [-H(\hat{\vec{\tau}})]^{-1}$.

Once the value of $\hat{\vec{\tau}}$ has been found, $\hat{Y}_{1,MLE_{N0}}$ is given by (2.9),

$$\hat{Y}_{1,MLE_{N0}} = \left[-\frac{1}{A} \sum_{i=1}^A \log(u_i) + \frac{1}{A} \sum_{i=1}^A \sum_{c=1}^{\infty} \log(c) \frac{\Gamma(c+\hat{\alpha})}{\Gamma(\hat{\alpha})c!} \left(\frac{\hat{\beta} u_i}{\hat{\beta} u_i + 1} \right)^c \left(\frac{1}{\hat{\beta} u_i + 1} \right)^{\hat{\alpha}} \right] -$$

$$\left[-\frac{1}{B} \sum_{s=1}^B \log(v_s) + \frac{1}{B} \sum_{s=1}^B \sum_{t=1}^{\infty} \log(t) \frac{\Gamma(t+\hat{\alpha})}{\Gamma(\hat{\alpha})t!} \left(\frac{\beta \phi v_s}{\beta \phi v_s + 1} \right)^t \left(\frac{1}{\beta \phi v_s + 1} \right)^{\hat{\alpha}} \right] \quad (2.9)$$

Notice that,

$$E(X_i^*) = -\log(u_i) + \sum_{c=1}^{\infty} \log(c) \frac{\Gamma(c+\alpha)}{\Gamma(\alpha)c!} \left(\frac{\beta u_i}{\beta u_i + 1} \right)^c \left(\frac{1}{\beta u_i + 1} \right)^{\alpha} \quad (2.10)$$

The index on the summation starts at $c = 1$ because $x_i^* = \log(\frac{1}{u_i})$ if c_i , and hence x_i , are zero. Also notice that,

$$E(Y_s^*) = -\log(v_s) + \sum_{t=1}^{\infty} \log(t) \frac{\Gamma(t+\alpha)}{\Gamma(\alpha)t!} \left(\frac{\beta \phi v_s}{\beta \phi v_s + 1} \right)^t \left(\frac{1}{\beta \phi v_s + 1} \right)^{\alpha} \quad (2.11)$$

The summations in (2.10) and (2.11) cannot be evaluated to my knowledge; thus (2.9) is not calculable. However, good approximations to equations (2.10) and (2.11), and hence (2.9) are available. A brute force summation can be used to approximate (2.10) or (2.11) to a high degree of precision. To accurately approximate (2.10) choose a value N such that,

$$\left[\left(\sum_{c=1}^N c \times P(C_i = c) \right) - \alpha \beta u_i \right] \leq \epsilon \quad \text{where} \quad \epsilon \leq 10^{-5}.$$

Then $\left[\left(\sum_{c=1}^N c \times P(C_i = c) \right) \right]$ is within ϵ of the true expectation of C_i . By a similar argument, I believe that (2.12) will be a very accurate approximation of (2.10).

$$E(X_i^*) \doteq -\log(u_i) + \sum_{c=1}^N \log(c) \frac{\Gamma(c+\alpha)}{\Gamma(\alpha)c!} \left(\frac{\beta u_i}{\beta u_i + 1} \right)^c \left(\frac{1}{\beta u_i + 1} \right)^{\alpha} \quad (2.12)$$

Similarly, to accurately approximate (2.11) choose a value N ($N = 500$ often works) such that,

$$\left[\left(\sum_{t=1}^N t \times P(T_s = t) \right) - \alpha \beta \phi v_s \right] \leq \epsilon.$$

Then (2.13) is an accurate approximation of (2.11),

$$E(Y_s^*) \doteq -\log(v_s) + \sum_{t=1}^N \log(t) \frac{\Gamma(t+\alpha)}{\Gamma(\alpha)t!} \left(\frac{\beta \phi v_s}{\beta \phi v_s + 1} \right)^t \left(\frac{1}{\beta \phi v_s + 1} \right)^{\alpha} \quad (2.13)$$

The summations in (2.12) and (2.13) are a bit tedious to work with. It should be pointed out that if the variances of C_i and T_s are "big", then a large number of

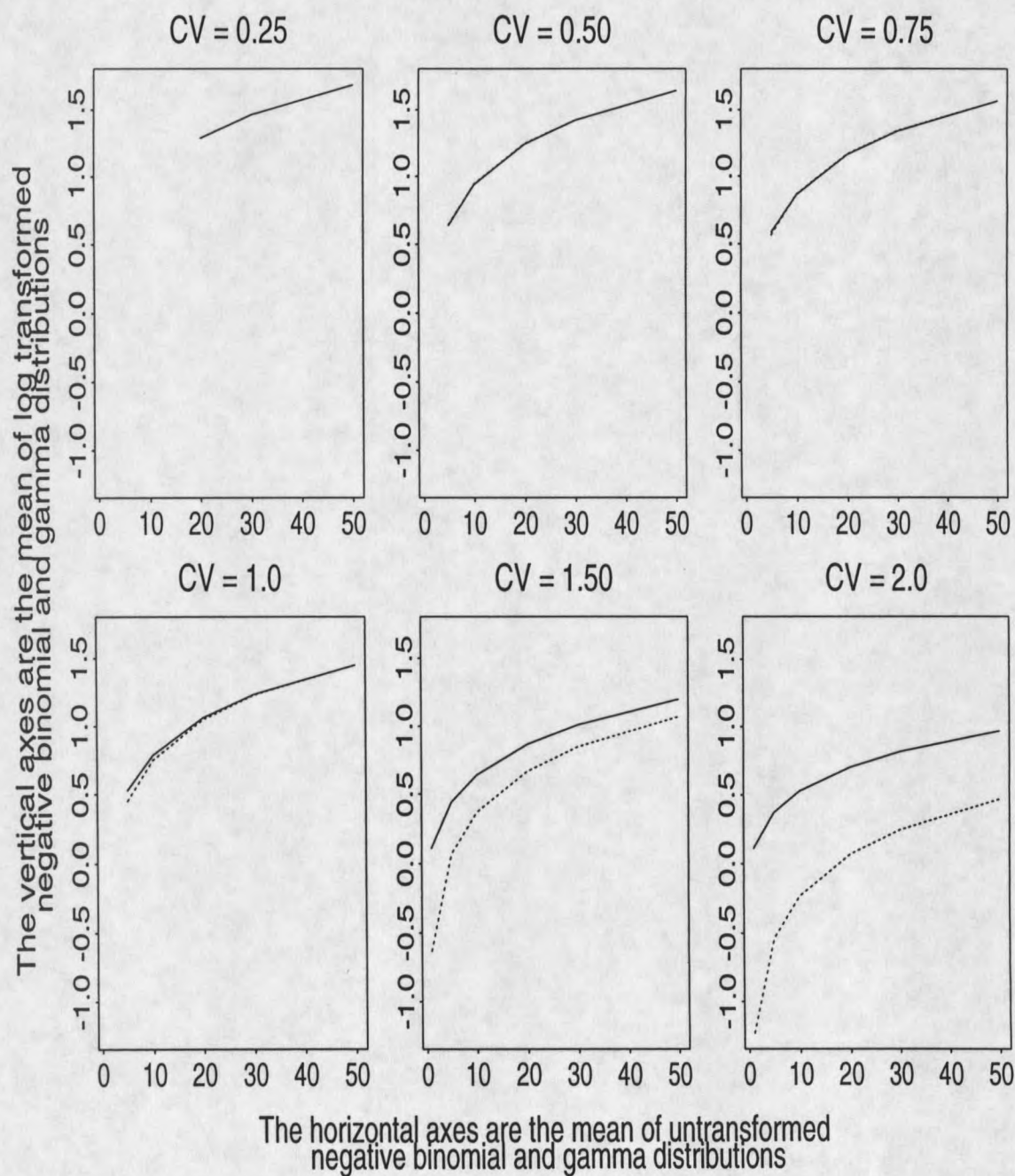
terms (say greater than 10,000) will have to be evaluated in the summations of (2.12) and (2.13). More compact approximations to (2.10) and (2.11) are obtainable, however, providing the expectations of C_i and T_s are sufficiently removed from zero. Because the negative binomial can be well approximated by a gamma, the log of a (zero-adjusted) negative binomial can be well approximated by the log of a gamma under certain situations. Figure 5 illustrates conditions when the log of a negative binomial is well approximated by the log of a gamma. For each separate plot of Figure 5, a coefficient of variation for the untransformed negative binomial and gamma distributions is fixed, and the expectations of a log negative binomial (solid line) and log gamma (dashed line) are plotted for an untransformed mean of 1, 5, 10, 20, 30, and 50. Whenever the dashed line is "close" to the solid line, then the expectation of a log gamma closely approximates the expectation of a log negative binomial. One can see from Figure 5 that as the mean decreases and as the coefficient of variation increases, the log gamma approximation becomes poorer. As a conservative rule, I recommend approximating the log of a negative binomial with the log of a gamma when the expected value of a carrier (check or test) is greater than 10 and the corresponding coefficient of variation is less than or equal to unity.

Denote $\Psi(w) = \frac{d[\ln \Gamma(w)]}{dw}$ as the well-known Psi function, and $\Psi'(w)$ as the derivative of $\Psi(w)$. Assuming the expectations of check carrier i and test carrier s are greater than 10, and the coefficients of variation are less than or equal to unity, then (2.10) is well approximated by (2.14) and (2.11) is well approximated by (2.15) (see Appendix A).

$$E(X_i^*) \doteq \frac{\Psi\left(\frac{\alpha\beta u_i}{\beta u_i + 1}\right) + \ln(\beta u_i + 1)}{\ln(10)} - \log(u_i). \quad (2.14)$$

$$E(Y_s^*) \doteq \frac{\Psi\left(\frac{\alpha\beta\phi v_s}{\beta\phi v_s + 1}\right) + \ln(\beta\phi v_s + 1)}{\ln(10)} - \log(v_s). \quad (2.15)$$

Figure 5: Comparison of the Expectation of a Log Negative Binomial (solid line) and a Log Gamma (dashed line) for various combinations of the Mean and Coefficient of Variation (CV) of untransformed negative binomial and gamma distributions.



Assuming (2.14) and (2.15) are appropriate, then an approximation to (2.9), denoted as $\hat{\Upsilon}_{1',MLE_{N_0}}$, is (2.16),

$$\hat{\Upsilon}_{1',MLE_{N_0}} \doteq \frac{1}{A} \sum_{i=1}^A \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u_i}{\hat{\beta}u_i+1}\right) + \ln(\hat{\beta}u_i+1)}{\ln(10)} - \frac{1}{A} \sum_{i=1}^A \log(u_i) - \frac{1}{B} \sum_{s=1}^B \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}\hat{\phi}v_s}{\hat{\beta}\hat{\phi}v_s+1}\right) + \ln(\hat{\beta}\hat{\phi}v_s+1)}{\ln(10)} + \frac{1}{B} \sum_{s=1}^B \log(v_s). \quad (2.16)$$

From experience with analyzing data from the quantitative assay, I have found that the check carrier counts are greater than 10 and almost always have sample coefficients of variation less than 1.0; hence (2.14) is an appropriate approximation to (2.10). However, if the disinfectant is very active then the mean of the test carrier counts will be less than 10 and (2.15) will sometimes be a poor approximation to (2.11). For these situations I recommend approximating equation (2.11) with (2.13). Then an approximation to (2.9), denoted as $\hat{\Upsilon}_{1'',MLE_{N_0}}$, is (2.17)

$$\hat{\Upsilon}_{1'',MLE_{N_0}} \doteq \frac{1}{A} \sum_{i=1}^A \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u_i}{\hat{\beta}u_i+1}\right) + \ln(\hat{\beta}u_i+1)}{\ln(10)} - \frac{1}{A} \sum_{i=1}^A \log(u_i) - \frac{1}{B} \sum_{s=1}^B \sum_{t=1}^N \log(t) \frac{\Gamma(t+\hat{\alpha})}{\Gamma(\hat{\alpha})t!} \left(\frac{\hat{\beta}\hat{\phi}v_s}{\hat{\beta}\hat{\phi}v_s+1}\right)^t \left(\frac{1}{\hat{\beta}\hat{\phi}v_s+1}\right)^{\hat{\alpha}} + \frac{1}{B} \sum_{s=1}^B \log(v_s). \quad (2.17)$$

Throughout the remainder of this chapter, I will be using the single prime notation to indicate that (2.16) is appropriate to use for the MLE of Υ_1 . The double prime notation will be used to indicate that (2.17) is appropriate to use for the mle of Υ_1 . Notice that (2.16) and (2.17) are somewhat simplified if the dilutions sampled are constant among check carriers and constant among test carriers; i.e. $u_i = u$ for $i = 1, \dots, A$; $v_s = v$ for $s = 1, \dots, B$. Then (2.16) and (2.17) can be written as (2.18) and (2.19), respectively.

$$\hat{\Upsilon}_{1',MLE_{N_0}} \doteq \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u}{\hat{\beta}u+1}\right) + \ln(\hat{\beta}u+1)}{\ln(10)} - \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}\hat{\phi}v}{\hat{\beta}\hat{\phi}v+1}\right) + \ln(\hat{\beta}\hat{\phi}v+1)}{\ln(10)} + \log\left(\frac{v}{u}\right). \quad (2.18)$$

$$\hat{\Upsilon}_{1'',MLE_{N_0}} \doteq \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u}{\hat{\beta}u+1}\right) + \ln(\hat{\beta}u+1)}{\ln(10)} + \log\left(\frac{v}{u}\right) -$$

$$\sum_{t=1}^N \log(t) \frac{\Gamma(t + \hat{\alpha})}{\Gamma(\hat{\alpha})t!} \left(\frac{\hat{\beta}\hat{\phi}_v}{\hat{\beta}\hat{\phi}_v + 1} \right)^t \left(\frac{1}{\hat{\beta}\hat{\phi}_v + 1} \right)^{\hat{\alpha}}. \quad (2.19)$$

Estimators of Υ_1 for the Model with Over-dispersion

The MOM estimator of Υ_1 does not depend on whether the model is with or without over-dispersion. Thus (2.5) is the appropriate formula to use for the MOM approach.

Denote as $\hat{\Upsilon}_{1,MLE_{OVER}}$ the estimator of Υ_1 when the model with over-dispersion is believed to be true. Formulas (2.18) and (2.19) are not appropriate estimators of Υ_1 when the model is over-dispersed. Assuming P is not degenerate at ϕ , then the computation of $\hat{\Upsilon}_{1,MLE_{OVER}}$ involves finding the values of $\hat{\phi}, \hat{\zeta}, \hat{\alpha}, \hat{\beta}$ which maximize (2.4). Taking partial derivatives of (2.4) with respect to $\vec{\tau}^*$ and setting them equal to $\vec{0}$, gives four equations and four unknowns. Again, these equations cannot be solved in a closed form and an iterative solution must be sought. Specifically, the equations to solve are: $\frac{\partial l(\vec{\tau}^*)}{\partial \vec{\tau}^*} = \vec{0}$. Let,

$$\frac{\partial l(\vec{\tau}^*)}{\partial \vec{\tau}^*} \Big|_{\vec{\tau}^* = \hat{\vec{\tau}}^*} = f^*(\hat{\vec{\tau}}^*), \quad \text{and} \quad \frac{\partial^2 l(\vec{\tau}^*)}{\partial \vec{\tau}^* \partial \vec{\tau}^{*T}} \Big|_{\vec{\tau}^* = \hat{\vec{\tau}}^*} = H^*(\hat{\vec{\tau}}^*).$$

An iteration scheme similar to (2.8) was followed to get the maximum likelihood estimate of $\vec{\tau}^*$. Notice that $-H^*(\hat{\vec{\tau}}^*)$ is the observed information matrix. Denote $V^*(\hat{\vec{\tau}}^*)$ as the estimated variance-covariance matrix of $\hat{\vec{\tau}}^*$. $V^*(\hat{\vec{\tau}}^*)$ is the inverse of the observed information matrix, i.e. $V^*(\hat{\vec{\tau}}^*) = [-H^*(\hat{\vec{\tau}}^*)]^{-1}$. Symbolic descriptions of $f^*_{4 \times 1}$ and $H^*_{4 \times 4}$ are given in Appendix A. Guidance on inverting H^* is also given in Appendix A.

For sake of brevity, equations for $\hat{\Upsilon}_{1,MLE_{OVER}}$ will only be given assuming a constant volume sampled, u , for check carriers and a constant volume sampled, v , for test carriers. Let $\hat{d}$ denote the estimated value of d . Specifically, $\hat{d} = \frac{\hat{\phi}^2 + \hat{\zeta}^2(\hat{\alpha} + 1)}{\hat{\phi}^2}$.

Then, if $E(T_s) \geq 10$ and the coefficient of variation is less than or equal to unity, the maximum likelihood estimator of Υ_1 , denoted as $\hat{\Upsilon}_{1',MLE_{OVER}}$ is given by (2.20); otherwise (2.21) is the maximum likelihood estimator and is denoted as $\hat{\Upsilon}_{1'',MLE_{OVER}}$.

$$\hat{\Upsilon}_{1',MLE_{OVER}} \doteq \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u}{\hat{\beta}u+1}\right) + \ln(\hat{\beta}u + 1)}{\ln(10)} - \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}\hat{\phi}v}{\hat{\beta}\hat{\phi}dv+1}\right) + \ln(\hat{\beta}\hat{\phi}dv + 1)}{\ln(10)} + \log\left(\frac{v}{u}\right). \quad (2.20)$$

$$\hat{\Upsilon}_{1'',MLE_{OVER}} \doteq \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u}{\hat{\beta}u+1}\right) + \ln(\hat{\beta}u + 1)}{\ln(10)} + \log\left(\frac{v}{u}\right) - \sum_{t=1}^N \log(t) \frac{\Gamma(t + \frac{\hat{\alpha}}{d})}{\Gamma(\frac{\hat{\alpha}}{d})t!} \left(\frac{\hat{\beta}\hat{\phi}dv}{\hat{\beta}\hat{\phi}dv + 1}\right)^t \left(\frac{1}{\hat{\beta}\hat{\phi}dv + 1}\right)^{\frac{\hat{\alpha}}{d}}. \quad (2.21)$$

Note that N is chosen so that,

$$\left[\left(\sum_{t=1}^N t \times P(T_s = t)\right) - \hat{\alpha}\hat{\beta}\hat{\phi}v\right] \leq \epsilon \quad \text{where} \quad \epsilon \leq 10^{-5}.$$

Estimators of Υ_2 for the Model with no Over-dispersion

The expectation of Y_s is $\alpha\beta\phi$, and the expectation of X_i is $\alpha\beta$. The ratio of expectations of test and check carrier densities is then ϕ . Thus the negative logarithm of the ratio of expectations for the average test and average check carrier densities is $-\log \phi$, which is the definition of Υ_2 . The MOM estimator of Υ_2 , given by (2.22) is, $\hat{\Upsilon}_{2,MOM}$.

$$\hat{\Upsilon}_{2,MOM} = -\log\left(\frac{\bar{Y}}{\bar{X}}\right). \quad (2.22)$$

Equation (2.22) can be rewritten as (2.23).

$$\hat{\Upsilon}_{2,MOM} = \log(\bar{X}) - \log(\bar{Y}). \quad (2.23)$$

Notice that (2.23) is easily computed with the aid of a computer spreadsheet or a calculator.

Denote the maximum likelihood estimate of Υ_2 as $\hat{\Upsilon}_{2,\text{MLE}_{\text{NO}}}$ when the model with no over-dispersion is believed to be true. Assuming P is degenerate at ϕ , then equation (2.8) can be used to find the values of $\hat{\phi}, \hat{\alpha}, \hat{\beta}$ which maximize (2.3). $\hat{\Upsilon}_{2,\text{MLE}_{\text{NO}}}$ is then given by (2.24).

$$\hat{\Upsilon}_{2,\text{MLE}_{\text{NO}}} = -\log(\hat{\phi}). \quad (2.24)$$

It can be shown for the model with no over-dispersion, and constant volumes sampled u and v , that the MOM and MLE estimators of Υ_2 are identical. The derivation of this result is given in Appendix A.

Estimators of Υ_2 for the Model with Over-dispersion

The MOM estimator of Υ_2 does not depend on whether the model is with or without over-dispersion, thus (2.22) is the appropriate formula to use for the MOM. Formula (2.24) is not an appropriate estimator of Υ_2 , however, when the model is over-dispersed. Assume that the values $\hat{\phi}, \hat{\zeta}, \hat{\alpha}, \hat{\beta}$ have been found which maximize (2.4). Denote as $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$ the MLE of Υ_2 for the over-dispersed model. The formula for $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$ is given in (2.25).

$$\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}} = -\log(\hat{\phi}). \quad (2.25)$$

Notice that (2.24) and (2.25) are identical equations symbolically, but that the value $\hat{\phi}$ may be different depending on whether the over-dispersed model or the model with no over-dispersion is chosen. Thus, different maximum likelihood point estimators for Υ_2 are obtained depending on which model is chosen. It is my experience that the values obtained using (2.24) and (2.25) differ very little from a practical standpoint.

Point Estimation of LR when Test Carrier Data are all Zeros

The estimators $\hat{\Upsilon}_{1,MLE_{NO}}$, $\hat{\Upsilon}_{1,MLE_{OVER}}$, $\hat{\Upsilon}_{2,MOM}$, $\hat{\Upsilon}_{2,MLE_{NO}}$, and $\hat{\Upsilon}_{2,MLE_{OVER}}$ are infinite when all test carrier counts are zero. To eliminate this deficiency, I propose that the formulas for the point estimates of Υ_1 and Υ_2 and associated standard errors be calculated as if one microbial count was observed for the test carrier with the largest volume sampled. This rule will provide conservative estimates for Υ_1 and Υ_2 . For all real data I have seen, this rule does not make a practical difference in the log reduction interpretation.

Standard Error Formulas for Estimators of LR

Denote as $SE(\cdot)$, the standard error of the estimator.

Standard Error Formulas for the Model with no Over-dispersion

Let $S_{X^*}^2$ and $S_{Y^*}^2$ denote the sample variance for the log densities of check and test carriers, respectively. The sample variance is calculated in the standard way, thus,

$$S_{X^*}^2 = \frac{1}{A-1} \sum_{i=1}^A (X_i^* - \bar{X}^*)^2 \quad \text{and} \quad S_{Y^*}^2 = \frac{1}{B-1} \sum_{s=1}^B (Y_s^* - \bar{Y}^*)^2.$$

The standard error for $\hat{\Upsilon}_{1,MOM}$ has a simple form and is given in (2.26).

$$SE(\hat{\Upsilon}_{1,MOM}) = \sqrt{\frac{S_{X^*}^2}{A} + \frac{S_{Y^*}^2}{B}}. \quad (2.26)$$

Denote as CV_X and CV_Y as the sample coefficients of variation for check and test carrier densities, respectively; i.e.

$$CV_X = \frac{\sqrt{S_X^2}}{\bar{X}} \quad \text{and} \quad CV_Y = \frac{\sqrt{S_Y^2}}{\bar{Y}}.$$

The delta method (Bishop, Fienberg, and Holland 1975) was used to derive formula (2.27) for $SE(\hat{\Upsilon}_{2,MOM})$;

$$SE(\hat{\Upsilon}_{2,MOM}) = \log(e) \times \sqrt{\frac{CV_X^2}{A} + \frac{CV_Y^2}{B}} \quad (2.27)$$

where e is base of the natural logarithm; i.e. $e \doteq 2.7182818$ and $\log(e) \doteq 0.4342945$.

Because the computation of $\hat{\Upsilon}_{1,MLE_{NO}}$ is dependent upon the mean and coefficient of variation of test carrier counts, two formulas are necessary for $SE(\hat{\Upsilon}_{1,MLE_{NO}})$. For sake of brevity, I will assume that the dilutions sampled for check and test carriers are u and v , respectively, and therefore (2.18) and (2.19) are the appropriate formulas for $\hat{\Upsilon}_{1,MLE_{NO}}$. Both of the standard error formulas given below for $\hat{\Upsilon}_{1,MLE_{NO}}$ are obtained by use of the multivariate delta method as described in Bishop, Fienberg, and Holland (1975). Some justification is required for use of the multivariate delta method to derive standard errors and that justification is given in Appendix A. Briefly, denote as $g_1(\hat{\tau})$ and $g_2(\hat{\tau})$ the expressions on the right hand side of equations (2.18) and (2.19), respectively. Notice, that $g_1(\hat{\tau})$ and $g_2(\hat{\tau})$ are simply functions of $\hat{\tau}$ equating to $\hat{\Upsilon}_{1,MLE_{NO}}$. Then formulas for $SE(\hat{\Upsilon}_{1,MLE_{NO}})$ are given in (2.28) and (2.29).

$$SE(\hat{\Upsilon}_{1',MLE_{NO}}) = \sqrt{\left(\frac{\partial g_1(\hat{\tau})}{\partial \hat{\tau}}\right)' [-H(\hat{\tau})]^{-1} \left(\frac{\partial g_1(\hat{\tau})}{\partial \hat{\tau}}\right)}. \quad (2.28)$$

$$SE(\hat{\Upsilon}_{1'',MLE_{NO}}) = \sqrt{\left(\frac{\partial g_2(\hat{\tau})}{\partial \hat{\tau}}\right)' [-H(\hat{\tau})]^{-1} \left(\frac{\partial g_2(\hat{\tau})}{\partial \hat{\tau}}\right)}. \quad (2.29)$$

The partial derivatives, $\frac{\partial g_1(\hat{\tau})}{\partial \hat{\tau}}$ and $\frac{\partial g_2(\hat{\tau})}{\partial \hat{\tau}}$, are given in Appendix A. It should be noted that the standard errors formulas for $\hat{\Upsilon}_{1,MLE_{NO}}$, when $\hat{\Upsilon}_{1,MLE_{NO}}$ is defined by equations (2.16) or (2.17), are derived in a similar manner as equations (2.28) and (2.29) by choosing the appropriate function $g(\hat{\tau})$.

The delta method was used to derive formula (2.30) for $SE(\hat{\Upsilon}_{2,MLE_{NO}})$. In (2.30), $V_{[1,1]}$ denotes the one-one element of the estimated variance-covariance matrix for $\hat{\tau}$. Note that $V_{[1,1]}$ is the estimated variance of $\hat{\phi}$.

$$SE(\hat{\Upsilon}_{2,MLE_{NO}}) = \log(e) \times \sqrt{\frac{V_{[1,1]}}{\hat{\phi}^2}}. \quad (2.30)$$

Standard Error Formulas for the Model with Over-dispersion

Because $\hat{\Upsilon}_{1,\text{MOM}}$ and $\hat{\Upsilon}_{2,\text{MOM}}$ are the same for the model with and without over-dispersion, equations (2.26) and (2.27) are the appropriate formulas for $\text{SE}(\hat{\Upsilon}_{1,\text{MOM}})$ and $\text{SE}(\hat{\Upsilon}_{2,\text{MOM}})$, respectively, for the model with over-dispersion.

Let $g_3(\hat{\tau}^*)$ and $g_4(\hat{\tau}^*)$ denote the expressions on the right hand side of equations (2.20) and (2.21), respectively. The multivariate delta method was used to derive formulas (2.31) and (2.32) for $\text{SE}(\hat{\Upsilon}_{1,\text{MLEOVER}})$.

$$\text{SE}(\hat{\Upsilon}_{1',\text{MLEOVER}}) = \sqrt{\left(\frac{\partial g_3(\hat{\tau}^*)}{\partial \hat{\tau}^*} \right)' [-H^*(\hat{\tau}^*)]^{-1} \left(\frac{\partial g_3(\hat{\tau}^*)}{\partial \hat{\tau}^*} \right)}. \quad (2.31)$$

$$\text{SE}(\hat{\Upsilon}_{1'',\text{MLEOVER}}) = \sqrt{\left(\frac{\partial g_4(\hat{\tau}^*)}{\partial \hat{\tau}^*} \right)' [-H^*(\hat{\tau}^*)]^{-1} \left(\frac{\partial g_4(\hat{\tau}^*)}{\partial \hat{\tau}^*} \right)}. \quad (2.32)$$

I used the delta method to derive formula (2.33) for $\text{SE}(\hat{\Upsilon}_{2,\text{MLEOVER}})$. In (2.33), $V^*_{[1,1]}$ denotes the one-one element of the estimated variance-covariance matrix for $\hat{\tau}^*$. Notice that $V^*_{[1,1]}$ is the estimated variance of $\hat{\phi}$ for the model with over-dispersion.

$$\text{SE}(\hat{\Upsilon}_{2,\text{MLEOVER}}) = \log(e) \times \sqrt{\frac{V^*_{[1,1]}}{\hat{\phi}^2}}. \quad (2.33)$$

Example

An example will be considered for purposes of illustrating the use of the formulas for the calculation of the estimated LR and associated standard errors. The data for check carriers is in Table 4, while the data for test carriers is in Table 5.

Let $A = 3$ check carriers.

Let $B = 10$ test carriers.

Let $D = 1$; i.e. use data at one dilution for check carriers.

Let $E = 2$; i.e. use data at two dilutions for test carriers.

Let $F = 3$ samples (plates or filters) per dilution.

Table 4: Check carrier data

Carrier number	Dilution volume per plate	Plate counts	Total volume sampled	Total colony counts	Density	Log density
1	10^{-6}	(63,60,66)	3×10^{-6}	189	6.3×10^{-7}	7.799
2	10^{-6}	(59,65,71)	3×10^{-6}	195	6.5×10^{-7}	7.813
3	10^{-6}	(76,79,64)	3×10^{-6}	219	7.3×10^{-7}	7.863

Table 5: Test carrier data

Car. no.	Diln 1	Plate counts diln 1	Diln 2	Plate counts diln 2	Total vol. samp.	Total colony counts	Dens.	Log dens.
1	10^{-1}	(60,91,92)	10^{-2}	(6,8,10)	0.33	267	809.09	2.908
2	10^{-1}	(69,104,94)	10^{-2}	(7,10,7)	0.33	291	881.18	2.945
3	10^{-1}	(32,37,30)	10^{-2}	(0,0,0)	0.33	99	300.0	2.477
4	10^{-1}	(72,88,89)	10^{-2}	(7,8,6)	0.33	270	818.18	2.913
5	10^{-1}	(65,150,79)	10^{-2}	(5,12,7)	0.33	318	963.64	2.984
6	10^{-1}	(29,26,44)	10^{-2}	(1,0,2)	0.33	102	309.09	2.490
7	10^{-1}	(75,91,107)	10^{-2}	(5,9,10)	0.33	297	900.0	2.954
8	10^{-1}	(35,47,32)	10^{-2}	(4,4,4)	0.33	126	381.82	2.582
9	10^{-1}	(66,77,58)	10^{-2}	(6,7,8)	0.33	222	672.73	2.828
10	10^{-1}	(145,138,161)	10^{-2}	(14,14,17)	0.33	489	1481.82	3.171

$$\bar{X}^* = 7.825,$$

$$\bar{Y}^* = 2.825,$$

$$\bar{X} = 6.7 \times 10^7,$$

$$\bar{Y} = 751.818,$$

$$S_{X^*}^2 = 0.001136513,$$

$$S_{Y^*}^2 = 0.05366018,$$

$$CV_X = 0.07897765, \quad \text{and}$$

$$CV_Y = 0.4790878$$

Example Calculations

$$\hat{T}_{1,MOM} = 7.825 - 2.825 = 5.000.$$

$$SE(\hat{T}_{1,MOM}) = \sqrt{\frac{0.001136513}{3} + \frac{0.05366018}{10}} = 0.07579.$$

$$\hat{T}_{2,MOM} = -\log\left(\frac{751.818}{6.7 \times 10^7}\right) = 4.950.$$

$$SE(\hat{T}_{2,MOM}) = 0.4342945 \times \sqrt{\frac{(0.07897765)^2}{3} + \frac{(0.4790878)^2}{10}} = 0.06871.$$

$$\hat{T}_{1',MLE_{NO}} = 4.950, \quad SE(\hat{T}_{1',MLE_{NO}}) = 0.1196.$$

$$\hat{T}_{2,MLE_{NO}} = 4.950, \quad SE(\hat{T}_{2,MLE_{NO}}) = 0.1193.$$

$$\hat{T}_{1',MLE_{OVER}} = 5.00, \quad SE(\hat{T}_{1',MLE_{OVER}}) = 0.07143.$$

$$\hat{T}_{2,MLE_{OVER}} = 4.950, \quad SE(\hat{T}_{2,MLE_{OVER}}) = 0.06755.$$

It should be noted that the average of the test carrier counts is greater than 10 and the estimated coefficient of variation was less than 1.0. Thus $\hat{T}_{1',MLE_{NO}}$ and $\hat{T}_{1',MLE_{OVER}}$ are appropriate estimators of T_1 .

Asymptotic Relative Efficiency Calculations

Let R be a constant denoting the ratio of sample sizes, $\frac{B}{A}$. Let κ_1 and κ_2 be estimators of $h_1(\bar{\tau}^*)$ and $h_2(\bar{\tau}^*)$, respectively. Huber (1981) defines the asymptotic

relative efficiency (ARE) of κ_1 to κ_2 as

$$\text{ARE}(\kappa_1 : \kappa_2) = \lim_{A, B \rightarrow \infty} \frac{\text{var}(\kappa_{2:R}) / (E(\kappa_{2:R}))^2}{\text{var}(\kappa_{1:R}) / (E(\kappa_{1:R}))^2}$$

where $\text{var}(\kappa_{1:R})$ and $\text{var}(\kappa_{2:R})$ are the theoretical variances of κ_1 and κ_2 , respectively, assuming the underlying parameter is $\bar{\tau}^*$ and the ratio of sample sizes is R . If the expectations of κ_1 and κ_2 are equal in the limit, then

$$\text{ARE}(\kappa_1 : \kappa_2) = \lim_{A, B \rightarrow \infty} \frac{\text{var}(\kappa_{2:R})}{\text{var}(\kappa_{1:R})}$$

which is the definition of asymptotic relative efficiency commonly seen in mathematical statistics texts. Notice, however, that Huber's definition of ARE allows one to compare estimators of different parameters. Thus Huber's definition will allow me to calculate the $\text{ARE}(\hat{\Upsilon}_{1, \text{MOM}} : \hat{\Upsilon}_{2, \text{MOM}})$, and, $\text{ARE}(\hat{\Upsilon}_{1, \text{MLE}} : \hat{\Upsilon}_{2, \text{MLE}})$.

The ARE of $\hat{\Upsilon}_{1, \text{MOM}}$ to $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}(\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}})$ and ARE of $\hat{\Upsilon}_{2, \text{MOM}}$ to $\hat{\Upsilon}_{2, \text{MLE}_{\text{NO}}}(\hat{\Upsilon}_{2, \text{MLE}_{\text{OVER}}})$ can be calculated given parameter values for ϕ , ζ , α , and β along with the volumes sampled for check and test carriers. Appendix A gives the asymptotic distributions of $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}$ and $\hat{\Upsilon}_{2, \text{MLE}_{\text{NO}}}$ assuming the sample sizes A and B tend to infinity with constant ratio R , for the model with no over-dispersion. When over-dispersion is present the asymptotic variances for $\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}}$ and $\hat{\Upsilon}_{2, \text{MLE}_{\text{OVER}}}$ are not easily written down in closed form. They can, however, be well approximated. The asymptotic distributions for $\hat{\Upsilon}_{1, \text{MOM}}$ and $\hat{\Upsilon}_{2, \text{MOM}}$ are also given in Appendix A. It should be noted that the parameter values are specified for ARE calculations, hence it is known whether the model has over-dispersion or not. Thus the subscripts of "NO" and "OVER" will be dropped for notational convenience during efficiency calculations of the MLE estimators.

Calculations of $\text{ARE}(\hat{\Upsilon}_{1, \text{MOM}} : \hat{\Upsilon}_{1, \text{MLE}})$, $\text{ARE}(\hat{\Upsilon}_{1, \text{MLE}} : \hat{\Upsilon}_{2, \text{MLE}})$, and $\text{ARE}(\hat{\Upsilon}_{1, \text{MOM}} : \hat{\Upsilon}_{2, \text{MOM}})$ when $R = \frac{10}{3}$ are given in Table 6 for the twelve cases

defined in Table 8. The same twelve cases were investigated in a simulation study, to be described in the following section. Calculations of $\text{ARE}(\hat{\Upsilon}_{2,\text{MOM}} : \hat{\Upsilon}_{2,\text{MLE}})$ were equal to 1.0 for all cases and therefore $\text{ARE}(\hat{\Upsilon}_{2,\text{MOM}} : \hat{\Upsilon}_{2,\text{MLE}})$ is excluded from Table 6. An efficiency of 1.0 for $\text{ARE}(\hat{\Upsilon}_{2,\text{MOM}} : \hat{\Upsilon}_{2,\text{MLE}})$ was to be expected when the model does not have over-dispersion because the MOM and MLE estimators of ϕ are identical. However, it was a surprise to me to see an efficiency of 1.0 for $\text{ARE}(\hat{\Upsilon}_{2,\text{MOM}} : \hat{\Upsilon}_{2,\text{MLE}})$ when the model was over-dispersed. This result suggests that the MOM and MLE estimators of ϕ have the same asymptotic variance when the model is over-dispersed. Further, results in Appendix A show that the MOM and MLE estimators of Υ_2 are normally distributed with the same mean, hence the ARE calculations presented in this section indicate that the MOM and MLE estimators of Υ_2 have the same asymptotic distribution when the model is over-dispersed.

Table 6: Asymptotic Relative Efficiency calculations of $\hat{\Upsilon}_{1,\text{MOM}}$ to $\hat{\Upsilon}_{1,\text{MLE}}$; $\hat{\Upsilon}_{1,\text{MLE}}$ to $\hat{\Upsilon}_{2,\text{MLE}}$; and $\hat{\Upsilon}_{1,\text{MOM}}$ to $\hat{\Upsilon}_{2,\text{MOM}}$ for the cases defined in Table 8.

1. Case	2. $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{1,\text{MLE}})$	3. $\text{ARE}(\hat{\Upsilon}_{1,\text{MLE}} : \hat{\Upsilon}_{2,\text{MLE}})$	4. $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{2,\text{MOM}})$
1	0.990	0.990	0.981
2	0.987	0.715	0.706
3	0.950	0.990	0.941
4	0.999	0.759	0.758
5	0.735	0.988	0.727
6	0.991	0.732	0.725
7	0.938	0.940	0.882
8	0.931	1.509	1.405
9	0.915	0.962	0.880
10	0.940	1.433	1.347
11	0.766	0.965	0.740
12	0.963	1.048	1.009

The results in column 2 of Table 6 show that MOM is more than 90%

efficient except for cases 5 and 11. Cases 5,6,11, and 12 represent levels of variability in the inoculum size that I expect will seldom occur in practice. Also, cases 5 and 11 represent the less realistic situation where the test carriers are not over-dispersed. Cases 1-4 and 7-10 represent levels of variability in the inoculum size that can be reasonably expected for the quantitative assays used in practice. For these cases, the MOM estimator of Υ_1 has a reasonably high efficiency compared to MLE.

From column 3 of Table 6 it is seen that the MLE of Υ_2 is more efficient than the MLE of Υ_1 except for cases 8, 10, and 12. The same statement can be made for the MOM of Υ_2 compared to the MOM of Υ_1 . Cases 8, 10, and 12 represent cases where the mean and coefficient of variation of the test carrier distribution are 10 and 1.5, respectively. The distributions of test carriers for cases 8,10, and 12 are highly skewed to the right. Thus it is plausible that the mean of the logs may be a more easily estimated parameter than the log of the means. It is possible, however, that the higher efficiency of Υ_1 estimators is due to my arbitrary rule of adding 1 to a zero count before taking the log. A zero count has a relatively high probability of occurring for cases 8,10, and 12. I chose to zero-adjust the data by adding a 1 to the zero counts because, among all constants in the interval $(0,1]$ that could be added to the zeros, adding one minimizes the variance of the log negative binomial random variable.

I created Table 7 and Figure 6 to investigate why $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{2,\text{MOM}})$ is greater than 1.0 for some cases. For the calculations in Table 7, I fixed α at 100 and β at 10^6 , thus setting the mean and coefficient of variation for the inoculum size at 10^8 and 0.10, respectively. The corresponding coefficient of variation for check carriers was 0.14. I then fixed an index of dispersion, d , to be either 1.0, 72.33, 143.67, or 215. The dispersion indices represent no over-dispersion ($d = 1.0$) to heavy over-dispersion ($d = 215$). Within each level of dispersion I fixed ϕ to be

either 10^{-6} , $10^{-6.25}$, $10^{-6.5}$, $10^{-6.75}$, 10^{-7} , $10^{-7.25}$ or $10^{-7.5}$. Denote as $E[\text{test}]$, $CV(\text{test})$, $\text{skew}(\text{test})$ and $P(\text{test}=0)$ the expected value of test carrier counts, coefficient of variation of test carrier counts, skewness coefficient of test carrier counts, and probability a test carrier count equals zero, respectively. Table 7 gives $E[\text{test}]$, $CV(\text{test})$, $\text{skew}(\text{test})$, $P(\text{test}=0)$, and $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{2,\text{MOM}})$ when ϕ and d are fixed.

When $d = 215$, the skewness coefficient is near 2.93 for all values of ϕ . For $d = 215$, $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{2,\text{MOM}}) < 1.0$ when $-\log(\phi) \leq 6.5$, but $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{2,\text{MOM}}) > 1.0$ when $-\log(\phi) \geq 6.75$. Thus the skewness coefficient by itself does not help explain why $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{2,\text{MOM}}) > 1.0$.

In general, Figure 6 indicates that as $-\log(\phi)$ increases and hence $E[\text{test}]$ decreases, $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{2,\text{MOM}})$ tends to increase. I believe this relationship may well be attributable to the add 1 rule to zero-adjust rather than to superior performance of estimators of Υ_1 versus estimators of Υ_2 .

Computer Simulation Study

I conducted twelve computer simulation experiments to assess the properties of the various estimators of Υ_1 and Υ_2 , and the associated standard errors. Each of the twelve experiments consisted of 1000 simulated quantitative assay data sets. All simulations used $A = 3$ check carriers and $B = 10$ test carriers. The parameter values were varied to encompass a wide range of possibilities likely to be encountered in practice. I choose to fix ϕ at either 10^{-7} or $10^{-3.5}$, representing an active and moderately active disinfectant, respectively. I choose values for the mean (μ) and coefficient of variation (CV) for the inoculum sizes, as well as the index of over-dispersion, d , after analyzing 195 quantitative assay data sets taken from a multi-laboratory collaborative study. Most of the estimated inoculum sizes from the

Figure 6: $\text{ARE}(\hat{\Upsilon}_{1,\text{MOM}} : \hat{\Upsilon}_{2,\text{MOM}})$ for various combinations of the dispersion index, d .

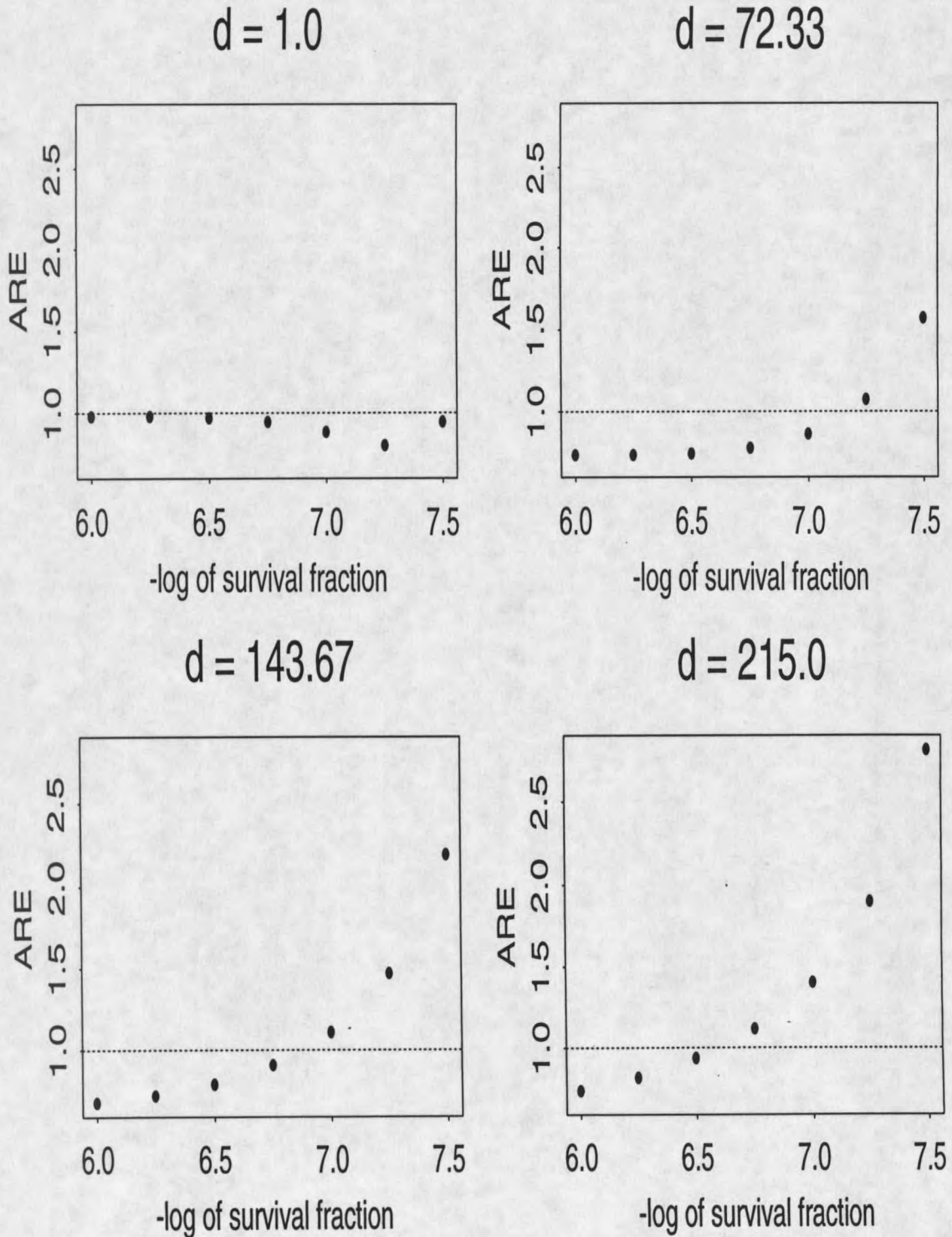


Table 7: Parameter values used to generate Figure 6

ϕ	d	E[test]	CV(test)	skew(test)	P(test)	ARE
10^{-6}	1.0	100	0.14	0.21	7.89×10^{-31}	0.980
10^{-6}	72.33	100	0.86	1.70	2.64×10^{-3}	0.734
10^{-6}	143.67	100	1.20	2.40	3.14×10^{-2}	0.679
10^{-6}	215.0	100	1.47	2.93	8.21×10^{-2}	0.739
$10^{-6.25}$	1.0	56.23	0.17	0.23	4.19×10^{-20}	0.977
$10^{-6.25}$	72.33	56.23	0.86	1.70	5.76×10^{-3}	0.732
$10^{-6.25}$	143.67	56.23	1.21	2.40	4.66×10^{-2}	0.724
$10^{-6.25}$	215.0	56.23	1.47	2.93	1.07×10^{-1}	0.819
$10^{-6.50}$	1.0	31.62	0.20	0.25	1.17×10^{-12}	0.968
$10^{-6.50}$	72.33	31.62	0.87	1.70	1.24×10^{-2}	0.740
$10^{-6.50}$	143.67	31.62	1.21	2.40	6.92×10^{-2}	0.795
$10^{-6.50}$	215.0	31.62	1.48	2.93	1.40×10^{-1}	0.938
$10^{-6.75}$	1.0	17.78	0.26	0.30	7.79×10^{-8}	0.944
$10^{-6.75}$	72.33	17.78	0.88	1.70	2.64×10^{-2}	0.773
$10^{-6.75}$	143.67	17.78	1.22	2.40	1.02×10^{-1}	0.911
$10^{-6.75}$	215.0	17.78	1.49	2.93	1.81×10^{-1}	1.12
10^{-7}	1.0	10	0.33	0.36	7.26×10^{-5}	0.882
10^{-7}	72.33	10	0.91	1.70	5.42×10^{-2}	0.862
10^{-7}	143.67	10	1.24	2.40	1.49×10^{-1}	1.109
10^{-7}	215.0	10	1.50	2.93	2.35×10^{-1}	1.405
$10^{-7.25}$	1.0	5.62	0.43	0.46	4.21×10^{-3}	0.801
$10^{-7.25}$	72.33	5.62	0.95	1.71	1.06×10^{-1}	1.072
$10^{-7.25}$	143.67	5.62	1.27	2.40	2.15×10^{-1}	1.472
$10^{-7.25}$	215.0	5.62	1.53	2.93	3.02×10^{-1}	1.898
$10^{-7.5}$	1.0	3.16	0.57	0.59	4.45×10^{-2}	0.945
$10^{-7.5}$	72.33	3.16	1.02	1.73	1.93×10^{-1}	1.573
$10^{-7.5}$	143.67	3.16	1.32	2.41	3.04×10^{-1}	2.194
$10^{-7.5}$	215.0	3.16	1.57	2.94	3.85×10^{-1}	2.819

Table 8: Simulation parameters for the quantitative assay

Case	ϕ	d	u	v	CV inoc.	CV check	CV test	Υ_1	Υ_2
1	$10^{-3.5}$	1.0	10^{-6}	10^{-2}	0.10	0.141	0.115	3.498	3.5
2	$10^{-3.5}$	99.68	10^{-6}	10^{-2}	0.10	0.141	1.0	3.747	3.5
3	$10^{-3.5}$	1.0	10^{-6}	10^{-2}	0.30	0.316	0.305	3.498	3.5
4	$10^{-3.5}$	11.08	10^{-6}	10^{-2}	0.30	0.316	1.0	3.729	3.5
5	$10^{-3.5}$	1.0	10^{-6}	10^{-2}	0.75	0.757	0.752	3.497	3.5
6	$10^{-3.5}$	1.77	10^{-6}	10^{-2}	0.75	0.757	1.0	3.614	3.5
7	10^{-7}	1.0	10^{-6}	1.0	0.10	0.141	0.332	7.022	7.0
8	10^{-7}	215	10^{-6}	1.0	0.10	0.141	1.5	7.356	7.0
9	10^{-7}	1.0	10^{-6}	1.0	0.30	0.316	0.436	7.024	7.0
10	10^{-7}	23.89	10^{-6}	1.0	0.30	0.316	1.5	7.338	7.0
11	10^{-7}	1.0	10^{-6}	1.0	0.75	0.757	0.814	7.017	7.0
12	10^{-7}	3.82	10^{-6}	1.0	0.75	0.757	1.5	7.222	7.0

collaborative study were close to the target inoculum size of 10^8 organisms per carrier. Thus I decided to set the mean inoculum size at 10^8 for all simulations, implying $\alpha\beta = 10^8$. Cases with small, medium, and large CV for the inoculum size were simulated. Note that $\alpha = (CV)^{-2}$ and $\beta = \mu \times (CV)^2$ were completely determined by μ and CV. I set d to represent either no over-dispersion ($d=1.0$), or relatively high over-dispersion. Once d was fixed it was easy to backsolve to determine the value of ζ for the simulations. The parameter settings for each of the twelve simulated cases are given in Table 8. Also included in Table 8 are the volumes sampled, u and v, the CV values for inoculum, check and test carriers, as well as the true values of Υ_1 and Υ_2 . Results for $\hat{\Upsilon}_{1,MOM}$ and $\hat{\Upsilon}_{1,MLE_{OVER}}$ are presented in Tables 9 and 12. Results for $\hat{\Upsilon}_{2,MOM}$ and $\hat{\Upsilon}_{2,MLE_{NO}}$ are presented in Tables 10 and 12, and results for $\hat{\Upsilon}_{1,MLE_{NO}}$ and $\hat{\Upsilon}_{2,MLE_{NO}}$ are presented in Table 11. Because there are two ways of defining LR, one may also be interested in comparing estimators of Υ_1 to estimators of Υ_2 . Results of such comparisons are presented in Table 13.

Table 9: Simulation results for $\hat{T}_{1,MOM}$ and $\hat{T}_{1,MLE_{OVER}}$ for the cases defined in Table 8.

1.	Results for $\hat{T}_{1,MOM}$				Results for $\hat{T}_{1,MLE_{OVER}}$			
	2.	3.	4.	5.	6.	7.	8.	9.
Case	Bias $\hat{T}_1$	Mean SE($\hat{T}_1$)	SD($\hat{T}_1$)	$\widehat{MSE}$ $\hat{T}_1$	Bias $\hat{T}_1$	Mean SE($\hat{T}_1$)	SD($\hat{T}_1$)	$\widehat{MSE}$ $\hat{T}_1$
1	0.000	0.03689	0.03850	0.00148	0.001	0.03400	0.03836	0.00147
2	-0.002	0.1716	0.1749	0.0306	-0.002	0.1684	0.1774	0.0315
3	0.000	0.08660	0.09108	0.00829	0.006	0.07032	0.08964	0.00806
4	0.001	0.1885	0.1987	0.0394	0.003	0.1821	0.2023	0.0409
5	0.010	0.2284	0.2462	0.0606	0.035	0.1779	0.2315	0.0547
6	-0.002	0.2677	0.2664	0.0709	0.012	0.2320	0.2589	0.0671
7	0.001	0.05915	0.05930	0.00351	0.006	0.05978	0.05984	0.00361
8	0.000	0.1835	0.1768	0.0312	0.009	0.1666	0.1768	0.0313
9	-0.006	0.1012	0.1117	0.0125	0.001	0.08667	0.1102	0.0121
10	0.002	0.1959	0.2000	0.0400	0.007	0.1754	0.1971	0.0388
11	0.005	0.2375	0.2585	0.0668	0.036	0.1882	0.2403	0.0590
12	-0.004	0.2749	0.2838	0.0805	0.004	0.2400	0.2775	0.0769

Table 10: Simulation results for $\hat{\Upsilon}_{2,\text{MOM}}$ and $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$ for the cases defined in Table 8.

1. Case	Results for $\hat{\Upsilon}_{2,\text{MOM}}$				Results for $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$			
	2. Bias $\hat{\Upsilon}_2$	3. Mean $\text{SE}(\hat{\Upsilon}_2)$	4. $\text{SD}(\hat{\Upsilon}_2)$	5. $\widehat{\text{MSE}}$ $\hat{\Upsilon}_2$	6. Bias $\hat{\Upsilon}_2$	7. Mean $\text{SE}(\hat{\Upsilon}_2)$	8. $\text{SD}(\hat{\Upsilon}_2)$	9. $\widehat{\text{MSE}}$ $\hat{\Upsilon}_2$
1	-0.001	0.03665	0.03822	0.00146	-0.001	0.03381	0.03819	0.00146
2	0.020	0.1316	0.1436	0.0210	0.020	0.1323	0.1436	0.0210
3	-0.005	0.08351	0.08816	0.00780	-0.003	0.06939	0.08820	0.00780
4	0.013	0.1497	0.1609	0.0261	0.014	0.1463	0.1608	0.0260
5	-0.023	0.1911	0.2253	0.0512	-0.015	0.1646	0.2273	0.0518
6	-0.017	0.2117	0.2329	0.0545	-0.015	0.1974	0.2333	0.0546
7	0.002	0.05596	0.05632	0.00317	0.002	0.05626	0.05633	0.00317
8	0.052	0.1830	0.2187	0.0505	0.052	0.2012	0.2187	0.0505
9	-0.009	0.09374	0.1056	0.0112	-0.009	0.08134	0.1057	0.0112
10	0.046	0.1987	0.2369	0.0582	0.046	0.2064	0.2360	0.0578
11	-0.023	0.1977	0.2293	0.0531	-0.022	0.1752	0.2298	0.0533
12	0.004	0.2501	0.2837	0.0804	0.006	0.2564	0.2834	0.0803

Table 11: Simulation results for $\hat{\Upsilon}_{1,MLE_{NO}}$ and $\hat{\Upsilon}_{2,MLE_{NO}}$ for the cases defined in Table 8.

1. Case	Results for $\hat{\Upsilon}_{1,MLE_{NO}}$				Results for $\hat{\Upsilon}_{2,MLE_{NO}}$			
	2. Bias $\hat{\Upsilon}_1$	3. Mean SE($\hat{\Upsilon}_1$)	4. SD($\hat{\Upsilon}_1$)	5. MSE $\hat{\Upsilon}_1$	6. Bias $\hat{\Upsilon}_2$	7. Mean SE($\hat{\Upsilon}_2$)	8. SD($\hat{\Upsilon}_2$)	9. MSE $\hat{\Upsilon}_2$
1	-0.001	0.03642	0.03839	0.00147	-0.001	0.03625	0.03822	0.00146
2	-0.229	0.2390	0.1440	0.0732	0.020	0.2378	0.1436	0.0210
3	-0.005	0.08201	0.08857	0.00786	-0.005	0.08164	0.08816	0.00780
4	-0.218	0.2425	0.1615	0.0734	0.013	0.2412	0.1609	0.0261
5	-0.022	0.1962	0.2268	0.0519	-0.023	0.1951	0.2253	0.0512
6	-0.133	0.2525	0.2344	0.0725	-0.017	0.2509	0.2329	0.0545
7	0.002	0.05703	0.05916	0.00350	0.002	0.05471	0.05632	0.00317
8	-0.407	0.3401	0.2464	0.2262	0.052	0.3239	0.2187	0.0505
9	-0.010	0.08601	0.1089	0.0120	-0.009	0.08357	0.1056	0.0112
10	-0.400	0.3434	0.2633	0.2290	0.046	0.3263	0.2369	0.0582
11	-0.023	0.1999	0.2321	0.0543	-0.023	0.1971	0.2293	0.0531
12	-0.356	0.3661	0.2980	0.2156	0.004	0.3443	0.2837	0.0804

Table 12: Observed ratios of $\widehat{\text{MSE}}_s$, MLE_{over} to MOM, and p-values for testing H_0 : $\frac{\widehat{\text{MSE}}(\hat{T}_{1,\text{MLE}_{\text{OVER}}})}{\widehat{\text{MSE}}(\hat{T}_{1,\text{MOM}})} = 1.0$; and H_0 : $\frac{\widehat{\text{MSE}}(\hat{T}_{2,\text{MLE}_{\text{OVER}}})}{\widehat{\text{MSE}}(\hat{T}_{2,\text{MOM}})} = 1.0$ for the cases defined in Table 8.

1.	2.	3.	4.	5.
Case	$\frac{\widehat{\text{MSE}}(\hat{T}_{1,\text{MLE}_{\text{OVER}}})}{\widehat{\text{MSE}}(\hat{T}_{1,\text{MOM}})}$	p-value	$\frac{\widehat{\text{MSE}}(\hat{T}_{2,\text{MLE}_{\text{OVER}}})}{\widehat{\text{MSE}}(\hat{T}_{2,\text{MOM}})}$	p-value
1	0.993	0.149	0.999	0.194
2	1.029	0.0003	1.000	0.102
3	0.972	0.0059	0.999	0.786
4	1.038	$< 10^{-4}$	1.000	0.631
5	0.903	$< 10^{-4}$	1.012	0.066
6	0.946	0.0006	1.002	0.584
7	1.028	0.0094	1.000	0.965
8	1.003	0.860	1.000	0.619
9	0.968	0.0090	1.001	0.502
10	0.970	0.0245	0.993	0.100
11	0.883	$< 10^{-7}$	1.003	0.515
12	0.955	0.0003	0.998	0.649

Table 13: Observed ratios of $\widehat{MSE}^*$ s and p-values for testing $H_0: \frac{MSE^*(\hat{T}_{2,MOM})}{MSE^*(\hat{T}_{1,MOM})} = 1.0$; and $H_0: \frac{MSE^*(\hat{T}_{2,MLEOVER})}{MSE^*(\hat{T}_{1,MLEOVER})} = 1.0$ for the cases defined in Table 8.

1.	2.	3.	4.	5.
Case	$\frac{\widehat{MSE}^*(\hat{T}_{2,MOM})}{\widehat{MSE}^*(\hat{T}_{1,MOM})}$	p-value	$\frac{\widehat{MSE}^*(\hat{T}_{2,MLEOVER})}{\widehat{MSE}^*(\hat{T}_{1,MLEOVER})}$	p-value
1	0.986	0.020	0.991	$< 10^{-4}$
2	0.787	$< 10^{-9}$	0.765	$< 10^{-10}$
3	0.939	$< 10^{-5}$	0.965	0.0002
4	0.750	$< 10^{-10}$	0.723	$< 10^{-10}$
5	0.843	$< 10^{-8}$	0.946	0.0059
6	0.819	$< 10^{-7}$	0.868	$< 10^{-5}$
7	0.909	$< 10^{-8}$	0.885	$< 10^{-10}$
8	1.786	$< 10^{-10}$	1.781	$< 10^{-10}$
9	0.903	$< 10^{-9}$	0.932	$< 10^{-8}$
10	1.601	$< 10^{-10}$	1.636	$< 10^{-10}$
11	0.798	$< 10^{-10}$	0.906	$< 10^{-4}$
12	1.063	0.067	1.111	0.0008

Computer Simulation Results

It is clear from Table 11 that $\hat{T}_{1,MLE_{NO}}$ had large bias when test carrier counts were over-dispersed (the even numbered cases defined in Table 8). For these cases, the estimated MSE, denoted as $\widehat{MSE}$, for $\hat{T}_{1,MLE_{NO}}$ was much larger than the

corresponding $\widehat{\text{MSE}}$ of $\hat{\Upsilon}_{1,\text{MOM}}$ and $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$. Table 11 shows that $\text{SE}(\hat{\Upsilon}_{2,\text{MLE}_{\text{NO}}})$ is a biased estimate of $\text{SD}(\hat{\Upsilon}_{2,\text{MLE}_{\text{NO}}})$ when the model is over-dispersed. Because it seems plausible to believe over-dispersion will be present in real world applications, neither $\hat{\Upsilon}_{1,\text{MLE}_{\text{NO}}}$ nor $\hat{\Upsilon}_{2,\text{MLE}_{\text{NO}}}$ should be used in my opinion. Thus I shall focus primarily on comparisons of $\hat{\Upsilon}_{1,\text{MOM}}$ to $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ (Table 9), and, $\hat{\Upsilon}_{2,\text{MOM}}$ to $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$ (Table 10).

Comparison of $\hat{\Upsilon}_{1,\text{MOM}}$ to $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$

$\hat{\Upsilon}_{1,\text{MOM}}$ is an unbiased estimator of Υ_1 and the simulation results presented in column 2 of Table 9 do not contradict this statement. Asymptotically, $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ is an unbiased estimator of Υ_1 and the results in column 6 of Table 9 suggest the small sample bias, if any, of $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ is of little practical concern.

The $\widehat{\text{MSE}}$ of $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ was smaller than the $\widehat{\text{MSE}}$ of $\hat{\Upsilon}_{1,\text{MOM}}$ for cases 1,3,5,6, and 9-12 (see Table 12 for the ratio of $\widehat{\text{MSE}}$ s). The biggest discrepancies between the $\widehat{\text{MSE}}$ occurred in cases 5,6,11, and 12. These are cases with the highest variability of inoculum size ($\text{CV} > 0.75$ for both check and test carrier counts). I believe that real assays will seldom produce such high variability. However, when such high variability does occur, the results in Table 12 suggest $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ should be preferred over $\hat{\Upsilon}_{1,\text{MOM}}$ on the basis of a mean squared error comparison. For the cases 1-4 and 7-10 that are more typical of situations in practice, neither $\hat{\Upsilon}_{1,\text{MOM}}$ nor $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ is superior to the other on the basis of mean squared error comparisons.

Results of a formal significance test of the difference (or equivalently, the ratio) of MSE for $\hat{\Upsilon}_{1,\text{MOM}}$ and $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ are presented in the third column of Table 12. I tested

$$H_0 : \text{MSE}(\hat{\Upsilon}_{1,\text{MOM}}) - \text{MSE}(\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}) = 0 \quad \equiv \quad H_0 : \frac{\text{MSE}(\hat{\Upsilon}_{1,\text{MOM}})}{\text{MSE}(\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}})} = 1$$

Versus

$$H_a : \text{MSE}(\hat{\Upsilon}_{1,\text{MOM}}) - \text{MSE}(\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}) \neq 0 \quad \equiv \quad H_a : \frac{\text{MSE}(\hat{\Upsilon}_{1,\text{MOM}})}{\text{MSE}(\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}})} \neq 1$$

for each of the twelve simulated cases. Because both estimators of Υ_1 were calculated on the same data sets, I used a paired Z-test procedure with 1000 paired observations for each test. The p-values are recorded in Table 12 so that the reader can judge for themselves as to whether an observed practical difference in MSE between $\hat{\Upsilon}_{1,\text{MOM}}$ and $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ can be attributed to sampling variability.

The standard error formula for $\hat{\Upsilon}_{1,\text{MOM}}$ appears to be less biased than that of $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$. For all cases simulated, the mean of the 1000 standard errors was less than the standard deviation of the point estimates for $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$. For cases 5 and 11 $\text{SE}(\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}})$ badly underestimated $\text{SD}(\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}})$, but these are cases of excessive variability.

All things considered, I would recommend using $\hat{\Upsilon}_{1,\text{MOM}}$ over $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ because the MOM method is easy to calculate, is unbiased, has a reliable standard error formula, and is comparable in terms of MSE to $\hat{\Upsilon}_{1,\text{MLE}_{\text{OVER}}}$ for situations likely to be met in practice.

Comparison of $\hat{\Upsilon}_{2,\text{MOM}}$ to $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$

It is clear from Table 10 that both $\hat{\Upsilon}_{2,\text{MOM}}$ and $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$ have negligible bias for the cases simulated. As seen in Table 10 and the fourth column of Table 12, the $\widehat{\text{MSE}}$ for both of these estimators are nearly identical for each case simulated.

For sake of completeness, the p-values for formal significance tests of the differences (or equivalently, the ratios) of MSE's for $\hat{\Upsilon}_{2,\text{MOM}}$ and $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$ are presented in the last column of Table 12. I tested

$$H_o : \text{MSE}(\hat{\Upsilon}_{2,\text{MOM}}) - \text{MSE}(\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}) = 0 \quad \equiv \quad H_o : \frac{\text{MSE}(\hat{\Upsilon}_{2,\text{MOM}})}{\text{MSE}(\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}})} = 1$$

Versus

$$H_a : \text{MSE}(\hat{\Upsilon}_{2,\text{MOM}}) - \text{MSE}(\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}) \neq 0 \quad \equiv \quad H_a : \frac{\text{MSE}(\hat{\Upsilon}_{2,\text{MOM}})}{\text{MSE}(\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}})} \neq 1$$

for each of the twelve simulated cases using a paired Z test procedure. Most p-values were larger than 0.10, except for case 5 where $p = 0.066$. At a 0.05 significance level, for all twelve cases the $\widehat{\text{MSE}}$ for $\hat{\Upsilon}_{2,\text{MOM}}$ is not statistically significantly different than the $\widehat{\text{MSE}}$ for $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$. Thus from a MSE criterion neither $\hat{\Upsilon}_{2,\text{MOM}}$ nor $\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}}$ is superior to the other.

Table 10 shows how well the standard error formulas performed. For both estimators and for each of the twelve cases, the mean of the 1000 standard errors was less than the empirical standard deviation of the point estimates. Thus the standard error formulas for both estimators appear to be biased downwards. In general, $\text{SE}(\hat{\Upsilon}_{2,\text{MOM}})$ was less biased than $\text{SE}(\hat{\Upsilon}_{2,\text{MLE}_{\text{OVER}}})$ when the distribution of P was degenerate at ϕ (odd numbered cases), but slightly more biased when the model was over-dispersed; the latter situation more likely to exist in practice. However, $\hat{\Upsilon}_{2,\text{MOM}}$ and $\text{SE}(\hat{\Upsilon}_{2,\text{MOM}})$ are far easier to compute than their MLE counterparts. Overall, I recommend using $\hat{\Upsilon}_{2,\text{MOM}}$ as the estimator of Υ_2 . The estimators have about the same statistical characteristics but I believe microbiologists will feel much more comfortable using a procedure they can compute with the aid of a spreadsheet rather than a somewhat complicated maximum likelihood program.

Comparison of Estimators of Υ_1 to Υ_2

Huber's (1981) definition of asymptotic relative efficiency gives us a basis for determining which of Υ_1 or Υ_2 is more reliably estimated. In the section on *Asymptotic Relative Efficiency calculations* of this chapter, the asymptotic analyses showed that estimators of Υ_2 were generally superior to estimators of Υ_1 . The sample sizes likely to be seen in practice (3 check carriers and 10 test carriers) are

far from being large samples. Thus, it is of interest to compare estimators of different parameters when the sample sizes are small. A reasonable criteria is the (relative) mean squared error. I define the relative mean square error of an estimator, say $\hat{\Upsilon}$, of a parameter, say Υ , as:

$$\text{MSE}^*(\hat{\Upsilon}) = \frac{E[(\hat{\Upsilon} - \Upsilon)^2]}{\Upsilon^2} = \frac{\text{MSE}(\hat{\Upsilon})}{\Upsilon^2}.$$

It should be noted that calculations of the relative mean square error need to be made at identical sample sizes. Ratios of relative $\widehat{\text{MSE}}$ s are shown in Table 13. Those ratios are very similar to the Asymptotic Relative Efficiencies shown in Table 6, and indicate practical differences exist between relative mean squared errors for estimators of Υ_1 versus estimators of Υ_2 .

Results of a formal significance test of the difference between the relative MSE of $\hat{\Upsilon}_{1,\text{MOM}}$ and $\hat{\Upsilon}_{2,\text{MOM}}$, and between the relative MSE of $\hat{\Upsilon}_{1,\text{MLEOVER}}$ and $\hat{\Upsilon}_{2,\text{MLEOVER}}$ are presented in Table 13 for sake of completeness. Column 3 is the p-values for

$$H_0 : \text{MSE}^*(\hat{\Upsilon}_{2,\text{MOM}}) - \text{MSE}^*(\hat{\Upsilon}_{1,\text{MOM}}) = 0 \quad \equiv \quad H_0 : \frac{\text{MSE}^*(\hat{\Upsilon}_{2,\text{MOM}})}{\text{MSE}^*(\hat{\Upsilon}_{1,\text{MOM}})} = 1$$

Versus

$$H_a : \text{MSE}^*(\hat{\Upsilon}_{2,\text{MOM}}) - \text{MSE}^*(\hat{\Upsilon}_{1,\text{MOM}}) \neq 0 \quad \equiv \quad H_a : \frac{\text{MSE}^*(\hat{\Upsilon}_{2,\text{MOM}})}{\text{MSE}^*(\hat{\Upsilon}_{1,\text{MOM}})} \neq 1$$

using a paired Z test procedure. Column 5 is the p-values for

$$H_0 : \text{MSE}^*(\hat{\Upsilon}_{2,\text{MLEOVER}}) - \text{MSE}^*(\hat{\Upsilon}_{1,\text{MLEOVER}}) = 0 \quad \equiv \quad H_0 : \frac{\text{MSE}^*(\hat{\Upsilon}_{2,\text{MLEOVER}})}{\text{MSE}^*(\hat{\Upsilon}_{1,\text{MLEOVER}})} = 1$$

Versus

$$H_a : \text{MSE}^*(\hat{\Upsilon}_{2,\text{MLEOVER}}) - \text{MSE}^*(\hat{\Upsilon}_{1,\text{MLEOVER}}) \neq 0 \quad \equiv \quad H_a : \frac{\text{MSE}^*(\hat{\Upsilon}_{2,\text{MLEOVER}})}{\text{MSE}^*(\hat{\Upsilon}_{1,\text{MLEOVER}})} \neq 1$$

using a paired Z test procedure.

Only results for the tests between the MOM estimators will be discussed because similar results hold for the comparisons between the MLE estimators. From Table 13 it is seen that $\hat{\Upsilon}_{2,MOM}$ has a smaller relative $\widehat{MSE}$ than $\hat{\Upsilon}_{1,MOM}$ for all cases except 8,10, and 12. The results are highly statistically significant for most cases. For reasons presented in the *Asymptotic Relative Efficiency* section of this chapter, I believe the "superior" performance of $\hat{\Upsilon}_{1,MOM}$ over $\hat{\Upsilon}_{2,MOM}$ for cases 8,10, and 12 is due more so to my arbitrary rule of adding 1 to a zero test carrier count before taking the log, than to Υ_1 being more easily estimated than Υ_2 .

Conclusions

The results indicate that Υ_2 is the more easily estimated parameter. For most of the cases considered, the estimators of Υ_2 were superior to the estimators of Υ_1 on the basis of a relative mean square error argument. On average, $\hat{\Upsilon}_2$ will be "closer" to Υ_2 than $\hat{\Upsilon}_1$ will be to Υ_1 , where "closer" is defined in terms of a relative squared error criterion.

The MOM estimators are recommended over the corresponding MLE estimators regardless as to which log reduction parameter, Υ_1 or Υ_2 , is chosen as the parameter of interest. Although the MLE estimators of Υ_1 had smaller MSE for most of the simulated cases, $\hat{\Upsilon}_{1,MOM}$ performs better when the balance between MSE, standard error formula performance, and ease of computability are all taken into account. The statistical characteristics of $\hat{\Upsilon}_{2,MOM}$ and $\hat{\Upsilon}_{2,MLEOVER}$ are very similar; however, $\Upsilon_{2,MOM}$ is recommended on the basis of ease of computation.

Future Work

Two key issues should be considered in future work: improved standard error formulas and tests for over-dispersion of test carrier counts. The standard error

formula for $\hat{T}_{2,MOM}$ is biased downward when over-dispersion is present, and a bias adjustment for the standard error formula would be a useful improvement.

In practice one is faced with the difficulty of not knowing whether the test carrier counts are over-dispersed; that is whether P is degenerate at ϕ or P has a distribution with mean ϕ and positive variance ζ^2 . Thus one might wish to test the hypothesis that $\zeta = 0$. It appears feasible to use a likelihood ratio test for testing such hypotheses.

Let $\vec{\tau}^* = (\phi, \zeta, \alpha, \beta)'$ and let $\vec{T}^*$ denote the parameter space for $\vec{\tau}^*$. Let $\vec{\tau}_0^* = (\phi, 0, \alpha, \beta)'$ and let $\vec{T}_0^*$ denote the parameter space for $\vec{\tau}_0^*$. The hypothesis of interest in this case is,

$$H_0 : \vec{\tau}^* = \vec{\tau}_0^*.$$

The likelihood ratio test is valid in this situation because the usual regularity conditions, as defined in Lehmann (1991), are satisfied for the negative binomial model (Swanson 1997). Let

$$\sup_{\vec{\tau}^* \in \vec{T}_0^*} L(\vec{\tau}^*) \quad \text{and} \quad \sup_{\vec{\tau}^* \in \vec{T}^*} L(\vec{\tau}^*)$$

denote the maximized likelihood values under the null and unrestricted parameter spaces, respectively. Let,

$$\Lambda = \frac{\sup_{\vec{\tau}^* \in \vec{T}_0^*} L(\vec{\tau}^*)}{\sup_{\vec{\tau}^* \in \vec{T}^*} L(\vec{\tau}^*)}.$$

Then the usual likelihood ratio statistic, $-2 \ln(\Lambda)$, has a $\chi^2(1)$ distribution, asymptotically. Let λ denote the computed value of Λ for a particular data set. Then one would reject the hypothesis $H_0 : \zeta = 0$ at some designated level of significance, say α^* , if $-2 \ln(\Lambda)$ is greater than the $1 - \alpha^*$ quantile of a $\chi^2(1)$ distribution.

It should be noted that the sample sizes likely to be encountered in practice for the quantitative assay will be rather small. Nevertheless, it is hoped that

$-2\ln(\Lambda)$ will have an approximate $\chi^2(1)$ distribution for the samples sizes likely to be encountered in practice. If it is found that the $\chi^2(1)$ approximation is not satisfactory, one could use Bartlett's correction to improve the χ^2 assumption (McCullagh and Nelder, Appendix C, 1989). Investigation of the likelihood ratio test, and simpler alternative test procedures, is left for future work.

CHAPTER 3

Presence/Absence Assay

The basic goal of the presence/absence assay (pa assay) is to determine if a disinfectant under study is efficacious. A disinfectant is judged to be effective if its "Log Reduction" (LR) is "large". Although data obtained from the pa assay is qualitative in nature, it will be shown in the following sections that the LR (which is a quantitative summary) can be estimated. Before discussing LR for the pa assay in further detail, some notation will be presented.

Notation

A = Number of check carriers.

B = Number of test carriers.

i = Subscript for check carriers; $i = 1, \dots, A$.

D_i = Number of dilution stages for check carrier i .

j = Subscript for dilution stage of a check carrier; $j = 1, \dots, D_i$.

n_{ij} = Number of samples at dilution j of check carrier i .

k = Subscript for sample at a dilution stage for a check carrier; $k = 1, \dots, n_{ij}$.

u_{ij} = Fraction of volume sampled for each sample at dilution j of check carrier i .

$u_{ij} < u_{i(j+1)} \quad \forall \quad j = 1, \dots, D_i - 1$.

$\vec{u}_i = (u_{i1}, u_{i2}, \dots, u_{iD_i})$. $\vec{u}_i$ is the vector of dilutions for check carrier i .

$y_{ijk} =$

$\begin{cases} 1, & \text{if 1 or more organisms are present in sample } k \text{ of dilution } j \text{ of check carrier } i \\ 0, & \text{Otherwise.} \end{cases}$

$r_{ij} = \sum_{k=1}^{n_{ij}} y_{ijk}$.

$\vec{r}_i = (r_{i1}, r_{i2}, \dots, r_{iD_i})$. $\vec{r}_i$ is the vector of the number of positive samples for check carrier i .

s = Subscript for test carriers; $s = 1, \dots, B$.

E_s = Number of dilution stages for test carrier s .

m = Subscript for dilution stage of a test carrier; $m = 1, \dots, E_s$.

w_{sm} = Number of samples at dilution m of test carrier s .

h = Subscript for sample at a dilution stage for a test carrier; $h = 1, \dots, w_{sm}$.

v_{sm} = Fraction of volume sampled for each sample at dilution m of test carrier s .

$v_{sm} < v_{s(m+1)} \quad \forall \quad m = 1, \dots, E_s - 1$.

$\vec{v}_s = (v_{s1}, v_{s2}, \dots, v_{sE_s})$. $\vec{v}_s$ is the vector of dilutions for test carrier s .

$z_{smh} =$

$\begin{cases} 1, & \text{if 1 or more organisms are present in sample } h \text{ of dilution } m \text{ of test carrier } s \\ 0, & \text{Otherwise.} \end{cases}$

$g_{sm} = \sum_{h=1}^{w_{sm}} z_{smh}$.

$\vec{g}_s = (g_{s1}, g_{s2}, \dots, g_{sE_s})$. $\vec{g}_s$ is the vector of the number of positive samples for test carrier s .

θ_i = Unobservable inoculum size for check carrier i .

θ_s = Unobservable inoculum size for test carrier s .

P_s is the survival fraction for a randomly chosen microbe exposed to disinfection for test carrier s ; $s = 1, \dots, B$. For notational convenience, the subscript will be dropped from P for the remainder of this chapter. P is a random variable that follows a distribution with mean ϕ and variance ζ^2 .

C_i = Number of organisms surviving the simulated disinfection steps for check carrier i .

μ_C = Expected number of organisms surviving the simulated disinfection steps for check carriers.

T_s = Number of organisms surviving the disinfection steps for test carrier s .

μ_T = Expected number of organisms surviving the disinfection steps for test carriers.

$$X_i = \begin{cases} \log(C_i), & \text{if } C_i > 0 \\ 0, & \text{if } C_i = 0 \end{cases}$$

$$\mu_X = E(X_i).$$

$$Y_s = \begin{cases} \log(T_s), & \text{if } T_s > 0 \\ 0, & \text{if } T_s = 0 \end{cases}$$

$$\mu_Y = E(Y_s).$$

$\hat{}$ is a symbol used to indicate "an estimator of".

Log Reduction

The pa assay is most frequently used to test disinfectants against viruses. Chen (1991) discusses a number of tests commonly used to assess the effectiveness of a disinfectant against viruses. Chen outlines the protocol for the calculation of LR from data obtained from the the pa assay for a single check carrier and a single test carrier. Define as ID_{50} the volume sampled (on a negative logarithm scale) such that there is a 50 percent probability a sample at that dilution will contain 1 or more organisms. Then LR is often calculated as the ID_{50} for the test carrier data subtracted from the ID_{50} for the check carrier data.

It is likely that future standardized tests for disinfectants involving the pa assay will require multiple check and test carriers be used. The question is then raised as to which is the "most appropriate" definition of LR. As with the quantitative assay I will give two plausible definitions of LR. The "mean of the logs" and the "log of the means", denoted as Υ_1 and Υ_2 , respectively. Specifically,

$$\Upsilon_1 = \mu_X - \mu_Y, \tag{3.1}$$

and

$$\Upsilon_2 = -\log(\phi), \tag{3.2}$$

where ϕ is the population mean of the distribution of survival fractions, P . Let $\hat{\mu}_X = \frac{1}{A} \sum_{i=1}^A \hat{X}_i$, and $\hat{\mu}_Y = \frac{1}{B} \sum_{s=1}^B \hat{Y}_s$. Then a simple estimator of Υ_1 is given in (3.3),

$$\hat{\Upsilon}_1 = \hat{\mu}_X - \hat{\mu}_Y. \quad (3.3)$$

Let $\hat{\mu}_C = \frac{1}{A} \sum_{i=1}^A \hat{C}_i$, and $\hat{\mu}_T = \frac{1}{B} \sum_{s=1}^B \hat{T}_s$. Because $\phi = \frac{\mu_T}{\mu_C}$, a reasonable estimator of ϕ is $\frac{\hat{\mu}_T}{\hat{\mu}_C}$. Accordingly, a reasonable estimator of Υ_2 is given in (3.4),

$$\hat{\Upsilon}_2 = -\log\left(\frac{\hat{\mu}_T}{\hat{\mu}_C}\right) = \log(\hat{\mu}_C) - \log(\hat{\mu}_T). \quad (3.4)$$

Reasons for possibly preferring Υ_1 to Υ_2 as the definition of LR and vice-versa have been discussed in the quantitative assay setting and will not be repeated here.

The main purposes of this chapter are to present various estimators and associated standard errors for both Υ_1 and Υ_2 . Results from a computer simulation study will show the small sample properties of the various estimators. Finally, some conclusions will be drawn as to which, if any, of the estimators are satisfactory. Before presenting the various estimators of LR, some modeling assumptions will first be stated.

Statistical Modeling Assumptions

Because the pa and quantitative assays are similar in the inoculation and disinfection steps, the modeling assumptions for these steps will be very similar for the two assays.

Assumption 1: The inoculum sizes for the A check carriers and B test carriers, denoted as $\theta_i, i = 1, \dots, A$ and $\theta_s, s = 1, \dots, B$ respectively, are statistically independent and are distributed approximately as gamma (mean = $\alpha\beta$, variance = $\alpha\beta^2$) random variables; $\alpha > 0, \beta > 0$.

The inoculum size on a carrier, check or test, is really a discrete random variable. However, because the inoculum sizes are so large, their distribution is closely approximated by the continuous gamma distribution.

Assumption 2: All organisms survive the simulated disinfection steps involved for check carrier i . Thus C_i are approximately iid gamma (α, β) random variables; $i = 1, \dots, A$.

Assumption 3: The random variable P follows a distribution with mean ϕ and variance ζ^2 ; P and θ_s , $s = 1, \dots, B$ are statistically independent.

It is reasonable to believe that the same circumstances causing the survival fraction to vary from carrier to carrier for the quantitative assay are present in the pa assay. Under ideal situations, ϕ is constant among test carriers, in which case, P is degenerate at ϕ . I will refer to the model with P having a non-degenerate distribution as the **over-dispersed model**, and the model with P degenerate as the **model with no over-dispersion**.

Assumption 4: $T_s \mid \theta_s, P$ are iid Poisson($\theta_s \cdot P$) random variables, $s = 1, \dots, B$.

Technically, $T_s \mid \theta_s, P$ is distributed as a binomial random variable with θ_s trials and probability of "success" P . Because θ_s is large and P is relatively small, the Poisson approximation to the binomial is appropriate (Feller 1968).

Assumptions 1, 3, and 4 imply that marginally, T_s is distributed iid with mean $= \alpha\beta\phi$ and variance $\alpha\beta\phi[1 + \alpha\beta\phi(\frac{\phi^2 + \zeta^2(\alpha+1)}{\alpha\phi^2})]$. For convenience, denote $\frac{\phi^2 + \zeta^2(\alpha+1)}{\alpha\phi^2}$ as d ; $d \geq 1$. If $d = 1$, then $\zeta^2 = 0$ and the distribution of P is degenerate at ϕ . If $d > 1$, then $\zeta^2 > 0$ and the survival fraction varies among carriers. Instead of attempting to model the distribution of P , I have chosen to model the distribution of T_s .

Assumption 5: The marginal distribution of T_s can be reasonably approximated

by a negative binomial distribution with parameters ($\rho = \frac{\alpha}{d}$, $p = \frac{1}{\beta\phi d+1}$, $q = \frac{\beta\phi d}{\beta\phi d+1}$), $s = 1, \dots, B$. It is assumed that the T_s are iid.

The parameters ρ , p , and q were chosen so that the expectation and variance of T_s are $\alpha\beta\phi$ and $\alpha\beta\phi[1 + \alpha\beta\phi(\frac{\phi^2 + \zeta^2(\alpha+1)}{\alpha\phi^2})]$, respectively. In Appendix A it is shown that the marginal distribution of T_s is exactly negative binomial when $d = 1$.

Assumption 6: The C_i and T_s are all statistically independent; $i = 1, \dots, A$; $s = 1, \dots, B$.

Assumption 7: Assume the "single hit Poisson model" (Cyr et al. 1990). Then $Y_{ijk} \mid C_i, u_{ij}$, $i = 1, \dots, A$; $j = 1, \dots, D_i$ are distributed as independent Bernoulli random variables, where $\Pr(Y_{ijk} = 1) = 1 - e^{-C_i \cdot u_{ij}}$ for $k = 1, \dots, n_{ij}$; and

$Z_{smh} \mid T_s, v_{sm}$, $s = 1, \dots, B$; $m = 1, \dots, E_s$ are distributed as independent Bernoulli random variables, where $\Pr(Z_{smh} = 1) = 1 - e^{-T_s \cdot v_{sm}}$ for $h = 1, \dots, w_{sm}$.

Assumption 8: Assume independence among replicates. Thus $r_{ij} \mid C_i, u_{ij}$ are distributed independently as $\text{binomial}(n_{ij}, 1 - e^{-C_i \cdot u_{ij}})$ random variables; $i = 1, \dots, A$; $j = 1, \dots, D_i$.

$g_{sm} \mid T_s, v_{sm}$ are distributed independently as $\text{binomial}(w_{sm}, 1 - e^{-T_s \cdot v_{sm}})$ random variables; $s = 1, \dots, B$; $m = 1, \dots, E_s$.

The joint conditional probability mass function (pmf) of the vector of positive samples for check carriers, given the number of organisms surviving the simulated disinfection, is given by (3.5).

$$\Pr(\vec{r}_1, \dots, \vec{r}_A \mid C_1, \dots, C_A) = \prod_{i=1}^A \left[\prod_{j=1}^{D_i} \binom{n_{ij}}{r_{ij}} (1 - e^{-C_i \cdot u_{ij}})^{r_{ij}} (e^{-C_i \cdot u_{ij}})^{n_{ij} - r_{ij}} \right]. \quad (3.5)$$

The joint conditional pmf of the vector of positive samples for test carriers, given the number of organisms surviving the disinfection steps, is given by (3.6).

$$\Pr(\vec{g}_1, \dots, \vec{g}_B \mid T_1, \dots, T_B) = \prod_{s=1}^B \left[\prod_{m=1}^{E_s} \binom{w_{sm}}{g_{sm}} (1 - e^{-T_s \cdot v_{sm}})^{g_{sm}} (e^{-T_s \cdot v_{sm}})^{w_{sm} - g_{sm}} \right]. \quad (3.6)$$

Because the C_i and T_s are statistically independent, the joint conditional pmf of the vector of positive samples for check and test carriers, given the C_i and T_s , is just the product of the pmf's as given by (3.7).

$$\Pr(\vec{r}_1, \dots, \vec{g}_B \mid C_1, \dots, T_B) = \Pr(\vec{r}_1, \dots, \vec{r}_A \mid C_1, \dots, C_A) \times \Pr(\vec{g}_1, \dots, \vec{g}_B \mid T_1, \dots, T_B). \quad (3.7)$$

Equation (3.7) is of little practical use however, because the C_i and T_s are never actually observed. Thus to perform inference on either Υ_1 or Υ_2 using a maximum likelihood approach, it is necessary to derive the joint marginal distribution of $\vec{r}_1, \dots, \vec{g}_B$. To derive the joint marginal distribution, the unobservable C_i and T_s must be integrated and summed out of the joint distribution of $\vec{r}_1, \dots, \vec{g}_B$ and $C_1, \dots, T_s$. The details of this derivation are rather lengthy and, accordingly, are presented in Appendix B. Denote as $\vec{\tau}^*$ the vector parameter $(\phi, \zeta, \alpha, \beta)'$ and let $l(\vec{\tau}^* \mid \vec{r}_1, \dots, \vec{r}_A)$ and $l(\vec{\tau}^* \mid \vec{g}_1, \dots, \vec{g}_B)$ be the log likelihood functions of the observable data for check and test carriers, respectively. Then $l(\vec{\tau}^* \mid \vec{r}_1, \dots, \vec{r}_A)$ and $l(\vec{\tau}^* \mid \vec{g}_1, \dots, \vec{g}_B)$ are given in (3.8) and (3.9), respectively.

$$l(\vec{\tau}^* \mid \vec{r}_1, \dots, \vec{r}_A) = \sum_{i=1}^A \ln \left[\prod_{j=1}^{D_i} \binom{n_{ij}}{r_{ij}} \left(\sum_{L_i=1}^{M_i} \gamma_{L_i} \left(\frac{1}{1 + \beta \xi_{L_i}} \right)^\alpha \right) \right]. \quad (3.8)$$

$$l(\vec{\tau}^* \mid \vec{g}_1, \dots, \vec{g}_B) = \sum_{s=1}^B \ln \left[\prod_{m=1}^{E_s} \binom{w_{sm}}{g_{sm}} \left(\sum_{L_s=1}^{M_s} \gamma_{L_s} \left(\frac{1}{1 + \beta \phi d(1 - e^{-\xi_{L_s}})} \right)^{\frac{\alpha}{d}} \right) \right]. \quad (3.9)$$

Details of the computations of the constants ξ, γ , and M in (3.8) and (3.9) are explained in Appendix B. The log likelihood function of the observable check and test carrier data is given by (3.10).

$$l(\vec{\tau}^* \mid \vec{r}_1, \dots, \vec{g}_B) = l(\vec{\tau}^* \mid \vec{r}_1, \dots, \vec{r}_A) + l(\vec{\tau}^* \mid \vec{g}_1, \dots, \vec{g}_B). \quad (3.10)$$

Strategies for Deriving Estimators of LR

I will discuss two general strategies for deriving estimators of Υ_1 and/or Υ_2 . The first strategy is to use maximum likelihood estimation techniques. Equation (3.10) is the likelihood function for the observable check and test carrier data. The maximum likelihood approach (mle) involves maximizing (3.10) with respect to $\vec{\tau}^*$.

Because the maximization of (3.10) is complicated, I sought a second (and computationally easier) strategy for deriving estimators of LR. The second strategy involves estimating μ_C (or μ_X) and μ_T (or μ_Y) separately from the check and test carrier data, respectively. Then $\hat{\Upsilon}_1$ and $\hat{\Upsilon}_2$ are given by (3.3) and (3.4), respectively. It should be noted that the second strategy is an approach I have seen microbiologists use, although the reasons for its use appear to be based on intuition rather than any theoretical justification. Several methods for estimating μ_C (or μ_X) and μ_T (or μ_Y) will be discussed in the next section.

Methods for Estimating μ_C, μ_X, μ_T , and μ_Y

There are various estimators of μ_C (μ_X , μ_T , μ_Y) an analyst could choose from. I will discuss the five estimators:

- 1) Most probable number (mpn) (Loyer and Hamilton 1984).
- 2) mpn jackknife estimator (jack) (Does et al. 1988).
- 3) mpn modified score function (msf) (Mehrabani and Matthews 1995).
- 4) Spearman-Kärber (sk) (Finney 1978).
- 5) partial maximum likelihood estimator (pmle; theoretical foundations not previously described).

It should be noted that virologists often use the technique of Reed and Muench (1938) to calculate the ID_{50} . Miller (1973) showed that the Reed-Muench

estimator is an estimator of the mean of a tolerance distribution and not the median as previously thought. Thus in virology, the Reed-Muench estimator is estimating the average sample volume (on a negative logarithm scale) that will contain 1 or more organisms. Because the Reed-Muench estimator is estimating a mean and not a median, the Reed-Muench estimator is estimating a parameter that is different than the conceptual definition of ID_{50} . Miller also showed that the Reed-Muench estimator is equivalent asymptotically to both the Spearman-Kärber and Dragstedt-Behrens (Finney 1978) estimators. Numerous small sample studies (Finney 1978) have showed the superiority of the Spearman-Kärber estimator over the Reed-Muench and Dragstedt-Behrens estimators. Finney emphatically states that the Reed-Muench and Dragstedt-Behrens estimators ought never be used. For these reasons, the Reed-Muench and Dragstedt-Behrens methods for estimating μ_C (μ_X , μ_T , μ_Y), will not be carried out in this dissertation.

The term "partial maximum likelihood estimator (pmle)" deserves some attention because, to my knowledge, it is not standard in the statistical literature. A pmle of μ_C (μ_X) will be obtained, using a maximum likelihood approach, from the check carrier data only. Similarly, a pmle of μ_T (μ_Y) will be obtained from the test carrier data only. Notice from equations (3.8) and (3.9) that the α and β parameters occur in both the check and test carrier portions of the log likelihood functions. Thus the pmle approach is ignoring, in some sense, the model under which the data is assumed to have originated. It is my intuition that the pmle estimators of LR will ultimately be less efficient than mle. However, the maximizations required using a pmle approach are simpler than the standard mle approach, and thus make the pmle approach worth considering.

Because the methods for estimating μ_C (μ_X) and μ_T (μ_Y) are similar, I will discuss only the estimation of μ_C (μ_X) for all methods except pmle.

Most Probable Number Estimator

Suppose C_i organisms survived the simulated disinfection process for check carrier i . Denote as $L(C_i | \vec{r}_i)$ the likelihood function, for check carrier i , as implied by assumptions 7 and 8. Then $L(C_i | \vec{r}_i)$ is given by (3.11).

$$L(C_i | \vec{r}_i) = \prod_{j=1}^{D_i} \binom{n_{ij}}{r_{ij}} (1 - e^{-C_i \cdot u_{ij}})^{r_{ij}} (e^{-C_i \cdot u_{ij}})^{n_{ij} - r_{ij}}. \quad (3.11)$$

The value $\hat{C}_{i,mpn}$ which maximizes (3.11), for the observed data $\vec{r}_i$, is called the mpn estimate of C_i . Details of computing the mpn are given in (Taswell 1981). It should be noted that if all replicate samples are positive at every dilution stage (i.e. $r_{ij} = n_{ij}$ for $j = 1, \dots, D_i$) then (3.11) is unbounded in C_i . To overcome this obstacle it is common to change r_{i1} from n_{i1} to $n_{i1} - 1$ (Mehrabani and Matthews 1995), a convention I will use here.

$$\text{Let } \hat{X}_{i,mpn} = \begin{cases} \log(\hat{C}_{i,mpn}), & \text{if } \hat{C}_{i,mpn} > 0 \\ 0, & \text{if } \hat{C}_{i,mpn} = 0. \end{cases}$$

For the test carrier data, define $\hat{T}_{s,mpn}$ as the mpn estimate for test carrier s . Let

$$\hat{Y}_{s,mpn} = \begin{cases} \log(\hat{T}_{s,mpn}), & \text{if } \hat{T}_{s,mpn} > 0 \\ 0, & \text{if } \hat{T}_{s,mpn} = 0. \end{cases}$$

Denote the mpn estimates of μ_C and μ_X as $\hat{\mu}_{C,mpn}$ and $\hat{\mu}_{X,mpn}$, respectively. Then

$$\hat{\mu}_{C,mpn} = \frac{1}{A} \sum_{i=1}^A \hat{C}_{i,mpn} \quad \text{and} \quad \hat{\mu}_{X,mpn} = \frac{1}{A} \sum_{i=1}^A \hat{X}_{i,mpn}.$$

Similarly,

$$\hat{\mu}_{T,mpn} = \frac{1}{B} \sum_{s=1}^B \hat{T}_{s,mpn} \quad \text{and} \quad \hat{\mu}_{Y,mpn} = \frac{1}{B} \sum_{s=1}^B \hat{Y}_{s,mpn}.$$

Although the mpn is unbiased asymptotically (Cochran 1950), it is well known that for small replicate sizes, n_{ij} , the mpn has a significant positive bias (Mehrabani and Matthews 1995). Because the replicate sizes will likely be small when the pa assay is used in practice, it is wise to consider less biased alternatives to the mpn estimator. Garthright (1993) showed that the mpn has a much smaller bias

when transformed to the log scale. Thus $\hat{\mu}_{X,mpn}$ and $\hat{\mu}_{Y,mpn}$ are nearly unbiased estimators, which may ultimately prove favorable for estimating Υ_1 .

Mehrabi and Matthews (1995) showed that the assay-wise jackknife and modified score function estimators, described below, have significantly reduced bias over the mpn. No studies to my knowledge have compared the assay-wise jackknife, modified score function, and mpn estimators on the log scale.

Assay-wise Jackknife Estimator

Following Does et al. (1988), define

$$\hat{C}_{i,jack} = \frac{1}{N_i} \sum_{L=1}^{N_i} (N_i \times \hat{C}_{i,mpn} - (N_i - 1) \times \hat{C}_{i,mpn(-L)})$$

where $N_i = \sum_j n_{ij}$ and $\hat{C}_{i,mpn(-L)}$ is the mpn estimate of C_i based on the data with assay L (a single y_{ijk}) omitted. It should be noted that $\hat{C}_{i,jack}$ can sometimes be negative which is unsatisfying. For these cases replace $\hat{C}_{i,jack}$ with $\hat{C}_{i,mpn}$.

Let $\hat{X}_{i,jack} = \begin{cases} \log(\hat{C}_{i,jack}), & \text{if } \hat{C}_{i,jack} > 0 \\ 0, & \text{if } \hat{C}_{i,jack} = 0. \end{cases}$

Define $\hat{T}_{s,jack}$ and $\hat{Y}_{s,jack}$ in a similar manner. Then

$$\hat{\mu}_{C,jack} = \frac{1}{A} \sum_{i=1}^A \hat{C}_{i,jack} \quad \text{and} \quad \hat{\mu}_{X,jack} = \frac{1}{A} \sum_{i=1}^A \hat{X}_{i,jack}$$

and,

$$\hat{\mu}_{T,jack} = \frac{1}{B} \sum_{s=1}^B \hat{T}_{s,jack} \quad \text{and} \quad \hat{\mu}_{Y,jack} = \frac{1}{B} \sum_{s=1}^B \hat{Y}_{s,jack}.$$

Modified Score Function Estimator

Mehrabi and Matthews (1995) applied the modified score function to the serial-dilution assay, as defined by the likelihood function given in (3.11). The modified score function is a general bias reducing technique of maximum likelihood

estimators derived by Firth (1993). Let $I(C_i)$ denote Fisher's information assuming (3.11),

$$I(C_i) = \sum_j \frac{u_{ij}^2}{(1 - e^{-C_i \cdot u_{ij}})} \times n_{ij} \times e^{-C_i \cdot u_{ij}}.$$

Let $\hat{C}_{i,\text{msf}}$ be the msf estimator of C_i . Then $\hat{C}_{i,\text{msf}}$ is the solution of:

$$\sum_j \left[r_{ij} - (n_{ij} + \frac{1}{2} h_{ij})(1 - e^{-C_i \cdot u_{ij}}) \right] \times \frac{u_{ij}}{(1 - e^{-C_i \cdot u_{ij}})} = 0 \quad (3.12)$$

where $h_{ij} = \frac{u_{ij}^2 \times n_{ij} \times e^{-C_i \cdot u_{ij}}}{(1 - e^{-C_i \cdot u_{ij}})} \times I(C_i)^{-1}$. If all $r_{ij} = 0$, then there is no solution to (3.12), however it is reasonable to define $\hat{C}_{i,\text{msf}}$ as zero for these cases. It should be noted that there is a solution to (3.12) when all replicates are positive.

$$\text{Let } \hat{X}_{i,\text{msf}} = \begin{cases} \log(\hat{C}_{i,\text{msf}}), & \text{if } \hat{C}_{i,\text{msf}} > 0 \\ 0, & \text{if } \hat{C}_{i,\text{msf}} = 0. \end{cases}$$

Define $\hat{T}_{s,\text{msf}}$ and $\hat{Y}_{s,\text{msf}}$ in a similar manner. Then

$$\hat{\mu}_{C,\text{msf}} = \frac{1}{A} \sum_{i=1}^A \hat{C}_{i,\text{msf}} \quad \text{and} \quad \hat{\mu}_{X,\text{msf}} = \frac{1}{A} \sum_{i=1}^A \hat{X}_{i,\text{msf}}$$

and,

$$\hat{\mu}_{T,\text{msf}} = \frac{1}{B} \sum_{s=1}^B \hat{T}_{s,\text{msf}} \quad \text{and} \quad \hat{\mu}_{Y,\text{msf}} = \frac{1}{B} \sum_{s=1}^B \hat{Y}_{s,\text{msf}}.$$

Spearman-Kärber Estimator

The sk estimator is often used to estimate the mean of a tolerance distribution in bioassay (Finney 1978). When the sk estimator is applied to data obtained from a serial-dilution assay, the mean sample volume (on a negative logarithm scale) which will contain 1 or more organisms is being estimated. For notational convenience in discussing the sk estimator, I will assume for the sk estimator that the volumes sampled (say u_{ij}) are equally spaced on a log scale. This assumption is valid for serial-dilution assays. Let the difference (on the log scale) between successive volumes sampled be denoted as D . Then the sk estimator of X_i , denoted as $\hat{X}_{i,\text{sk*}}$, is

$$\hat{X}_{i,\text{sk*}} = -\log(u_{i1}) + \frac{D}{2} - D \sum_j \frac{r_{ij}}{n_{ij}}.$$

Johnson and Brown (1961) showed that $\hat{X}_{i,sk*}$ is a biased estimator of X_i under Assumptions 7 and 8. I will denote as $\hat{X}_{i,sk}$ the unbiased sk estimator of X_i , given by Johnson and Brown, as

$$\hat{X}_{i,sk} = \frac{\Psi(1)}{\ln(10)} + \hat{X}_{i,sk*}$$

where $\Psi(\cdot)$ is the well known Psi function; $\frac{\Psi(1)}{\ln(10)} \doteq -0.2507$. Let $\hat{C}_{i,sk} = 10^{\hat{X}_{i,sk}}$.

Define $\hat{T}_{s,sk}$ and $\hat{Y}_{s,sk}$ in a similar manner. Then

$$\hat{\mu}_{C,sk} = \frac{1}{A} \sum_{i=1}^A \hat{C}_{i,sk} \quad \text{and} \quad \hat{\mu}_{X,sk} = \frac{1}{A} \sum_{i=1}^A \hat{X}_{i,sk}$$

and,

$$\hat{\mu}_{T,sk} = \frac{1}{B} \sum_{s=1}^B \hat{T}_{s,sk} \quad \text{and} \quad \hat{\mu}_{Y,sk} = \frac{1}{B} \sum_{s=1}^B \hat{Y}_{s,sk}.$$

Partial mle of μ_C and μ_X

The likelihood function for the observable check carrier data is given by (3.8). I have chosen to reparameterize (3.8) in terms of μ_C and η_C ; where η_C is the coefficient of variation of inoculum sizes for check carriers. Expressed in terms of μ_C and η_C ,

$$\alpha = \frac{1}{\eta_C^2} \quad \text{and} \quad \beta = \mu_C \eta_C^2.$$

Substituting μ_C and η_C into (3.8) gives (3.13).

$$l(\mu_C, \eta_C \mid \vec{r}_1, \dots, \vec{r}_A) = \sum_{i=1}^A \ln \left[\prod_{j=1}^A \binom{n_{ij}}{r_{ij}} \left(\sum_{L_i=1}^{M_i} \gamma_{L_i} \left(\frac{1}{1 + \mu_C \eta_C^2 \xi_{L_i}} \right)^{\frac{1}{\eta_C^2}} \right) \right]. \quad (3.13)$$

Denote as $\hat{\mu}_C$ and $\hat{\eta}_C$ the values of μ_C and η_C that jointly maximize (3.13).

Then $\hat{\mu}_C$ is the pmle of μ_C . The expectation of X_i is given in Appendix A, where e^{X_i} follows a gamma distribution. Thus the pmle of μ_X , denoted as $\hat{\mu}_X$, is given as,

$$\hat{\mu}_X = \frac{\Psi\left(\frac{1}{\hat{\eta}_C^2}\right) + \ln(\hat{\mu}_C \times \hat{\eta}_C^2)}{\ln(10)}.$$

I used the modified Newton-Raphson method as suggested by Jacoby et al. (1972) for calculating $\hat{\mu}_C$ and $\hat{\eta}_C$; details are given in Appendix B.

Partial mle of μ_T and μ_Y

The likelihood function for the observable test carrier data is given by (3.9).

Because it may be of interest to determine the mle for μ_T (μ_Y) from the test carrier data alone, I will reparameterize (3.9) in terms of

$$\mu_T = \frac{\rho q}{p} \quad \text{and} \quad \eta_T = \frac{1}{\sqrt{r q}}; \quad \text{where}$$

η_T is the coefficient of variation for the number of organisms surviving the disinfection steps for test carriers. Expressed in terms of α , β , ϕ , and d ,

$$\rho = \frac{\alpha}{d}, \quad p = \frac{1}{1 + \beta \phi d} \quad \text{and} \quad q = 1 - p.$$

Substituting μ_T and η_T into (3.9) and performing some algebraic manipulations gives (3.14).

$$l(\mu_T, \eta_T \mid \vec{g}_1, \dots, \vec{g}_B) = \sum_{s=1}^B \ln \left[\prod_{s=1}^B \binom{w_{sm}}{g_{sm}} \left(\sum_{L_s=1}^{M_s} \gamma_{L_s} \left[\mu_T \times \eta_T^2 \times (1 - e^{-\xi_{L_s}}) + e^{-\xi_{L_s}} \right]^{\left(\frac{-\mu_T}{\mu_T \times \eta_T^2 - 1} \right)} \right) \right]. \quad (3.14)$$

Denote as $\hat{\mu}_T$ and $\hat{\eta}_T$ the values of μ_T and η_T that jointly maximize (3.14). Then $\hat{\mu}_T$ is the pmle of μ_T .

Computing the pmle of μ_Y is slightly more complicated. Let $\hat{\rho}_T = \frac{\hat{\mu}_T}{\hat{\mu}_T \times \hat{\eta}_T^2 - 1}$, $\hat{p}_T = \frac{1}{\hat{\mu}_T \times \hat{\eta}_T^2}$, $\hat{q}_T = 1 - \hat{p}_T$. The expectation of Y_s is

$$E(Y_s) = \sum_{t=1}^{\infty} \log(t) \times \frac{\Gamma(t + \rho)}{\Gamma(\rho)t!} q^t p^\rho. \quad (3.15)$$

To find the mle of μ_Y , one would substitute $\hat{\rho}_T$, $\hat{p}_T$ and $\hat{q}_T$ into (3.15). The summation in (3.15) cannot be exactly evaluated to my knowledge, however good approximations exist. Drawing on my work presented in the quantitative assay chapter, the log gamma approximation to (3.15) is suitable if $\mu_T > 10$ and $\eta_T \leq 1.0$; in which case $\hat{\mu}_Y$ is given by (3.16),

$$\hat{\mu}_Y = \frac{\Psi\left(\frac{1}{\hat{\eta}_T^2}\right) + \ln(\hat{\mu}_T \times \hat{\eta}_T^2)}{\ln(10)}. \quad (3.16)$$

When $\hat{\mu}_T < 10$ and/or $\hat{\eta}_T > 1.0$, a brute force summation can be used to approximate (3.15) to a high degree of precision. Choose a value N such that,

$$\left[\left(\sum_{t=1}^N t \times \frac{\Gamma(t + \hat{\rho})}{\Gamma(\hat{\rho})t!} \right) (\hat{q}^t) (\hat{p}^{\rho}) - \hat{\mu}_T \right] \leq \epsilon \quad \text{where} \quad \epsilon \leq 10^{-5}.$$

Then (3.17) is a good approximation to (3.15),

$$E(Y_s) \doteq \sum_{t=1}^N \log(t) \times \frac{\Gamma(t + \rho)}{\Gamma(\rho)t!} (q^t) (p^{\rho}). \quad (3.17)$$

The value of $\hat{\mu}_Y$ is calculated substituting $\hat{\rho}_T, \hat{p}_T$ and $\hat{q}_T$ into (3.17). Details for obtaining $\hat{\mu}_T$ and $\hat{\eta}_T$ are given in Appendix B.

Point Estimation of LR

Maximum Likelihood Estimators of LR

The log likelihood function for the observable data is given in (3.10) and is the sum of the log likelihoods for the check and test carrier data as given in (3.8) and (3.9), respectively. Notice that the α and β parameters show up in both the check and test carrier likelihoods, thus the maximum likelihood approach needs to estimate ϕ, ζ, α and β from both the check and test carrier data.

Denote as $\hat{\tau}^*$ the value of $\bar{\tau}^*$ which maximizes (3.10). I chose to use the modified Newton-Raphson method as suggested by Jacoby et al. (1972) to solve for $\hat{\tau}^*$. Remembering that $d = \frac{\phi^2 + \zeta^2(\alpha+1)}{\phi^2}$, then the mle estimator of Υ_1 will be formed by substituting the components of $\hat{\tau}^*$ into the formulas for the expectation of a log negative binomial (with parameters $\rho = \frac{\alpha}{d}, p = \frac{1}{\beta\phi d+1}, q = 1 - p$) and a log gamma (with parameters α and β). Formally, the mle of Υ_1 is given in (3.18),

$$\hat{\Upsilon}_{1,mle} = \frac{\Psi(\hat{\alpha}) + \ln(\hat{\beta})}{\ln(10)} - \sum_{t=1}^{\infty} \log(t) \times \frac{\Gamma(t + \hat{\rho})}{\Gamma(\hat{\rho})t!} (\hat{q}^t) (\hat{p}^{\hat{\rho}}) \quad (3.18)$$

where $\hat{\rho} = \frac{\hat{\alpha}}{\hat{d}}, \hat{p} = \frac{1}{\hat{\beta}\hat{\phi}\hat{d}+1}$, and $\hat{q} = 1 - \hat{p}$.

Because I have two approximations to the expectation of a log negative binomial random variable, I will have two formulas for computing the mle of Υ_1 , denoted as $\hat{\Upsilon}_{1',\text{mle}}$ and $\hat{\Upsilon}_{1'',\text{mle}}$. Define $\hat{\Upsilon}_{1',\text{mle}}$ as in (3.19)

$$\hat{\Upsilon}_{1',\text{mle}} = \frac{\Psi(\hat{\alpha}) + \ln(\hat{\beta})}{\ln(10)} - \frac{\Psi(\hat{\rho}\hat{q}) + \ln(\frac{1}{\hat{p}})}{\ln(10)}. \quad (3.19)$$

Equation (3.19) is appropriate when both $\frac{\hat{\rho}\hat{q}}{\hat{p}} > 10$ (the estimated mean of the test carriers is greater than 10) and $\frac{1}{\sqrt{\hat{\rho}\hat{q}}} \leq 1.0$ (the estimated coefficient of variation for test carriers is less than 1.0).

When the estimated mean of the test carriers is too small or the estimated coefficient of variation is too large, then $\hat{\Upsilon}_{1'',\text{mle}}$ will be defined as the appropriate mle estimator of Υ_1 .

$$\hat{\Upsilon}_{1'',\text{mle}} = \frac{\Psi(\hat{\alpha}) + \ln(\hat{\beta})}{\ln(10)} - \sum_{t=1}^N \log(t) \times \frac{\Gamma(t + \hat{\rho})}{\Gamma(\hat{\rho})t!} (\hat{q}^t) (\hat{p}^{\hat{\rho}}) \quad (3.20)$$

where N is chosen such that $\left[\sum_{t=1}^N t \times \frac{\Gamma(t + \hat{\rho})}{\Gamma(\hat{\rho})t!} (\hat{q}^t) (\hat{p}^{\hat{\rho}}) - \frac{\hat{\rho}\hat{q}}{\hat{p}} \right] \leq 10^{-5}$.

The mle estimator of Υ_2 , denoted as $\Upsilon_{2,\text{mle}}$, is given in (3.21).

$$\hat{\Upsilon}_{2,\text{mle}} = -\log(\hat{\phi}). \quad (3.21)$$

Estimators of LR using the mpn, jack, msf, sk, or pmle Methods

Denote as $\hat{\Upsilon}_{1,\text{mpn}}$ and $\hat{\Upsilon}_{2,\text{mpn}}$ the estimators of Υ_1 and Υ_2 , respectively, using the mpn approach for estimating the mean number of organisms surviving the disinfection steps for check and test carriers. Denote the jackknife, modified score function, Spearman-Kärber, or partial mle estimators of Υ_1 and Υ_2 using similar notation. Then equations (3.22) - (3.31) are the appropriate estimators of Υ_1 and Υ_2 using the mpn, jack, msf, sk, and pmle approaches, respectively.

$$\hat{\Upsilon}_{1,\text{mpn}} = \hat{\mu}_{X,\text{mpn}} - \hat{\mu}_{Y,\text{mpn}}. \quad (3.22)$$

$$\hat{\Upsilon}_{2,\text{mpn}} = \log(\hat{\mu}_{C,\text{mpn}}) - \log(\hat{\mu}_{T,\text{mpn}}) . \quad (3.23)$$

$$\hat{\Upsilon}_{1,\text{jack}} = \hat{\mu}_{X,\text{jack}} - \hat{\mu}_{Y,\text{jack}} . \quad (3.24)$$

$$\hat{\Upsilon}_{2,\text{jack}} = \log(\hat{\mu}_{C,\text{jack}}) - \log(\hat{\mu}_{T,\text{jack}}) . \quad (3.25)$$

$$\hat{\Upsilon}_{1,\text{msf}} = \hat{\mu}_{X,\text{msf}} - \hat{\mu}_{Y,\text{msf}} . \quad (3.26)$$

$$\hat{\Upsilon}_{2,\text{msf}} = \log(\hat{\mu}_{C,\text{msf}}) - \log(\hat{\mu}_{T,\text{msf}}) . \quad (3.27)$$

$$\hat{\Upsilon}_{1,\text{sk}} = \hat{\mu}_{X,\text{sk}} - \hat{\mu}_{Y,\text{sk}} . \quad (3.28)$$

$$\hat{\Upsilon}_{2,\text{sk}} = \log(\hat{\mu}_{C,\text{sk}}) - \log(\hat{\mu}_{T,\text{sk}}) . \quad (3.29)$$

$$\hat{\Upsilon}_{1,\text{pmle}} = \hat{\mu}_X - \hat{\mu}_Y . \quad (3.30)$$

$$\hat{\Upsilon}_{2,\text{pmle}} = \log(\hat{\mu}_C) - \log(\hat{\mu}_T) . \quad (3.31)$$

Standard Error Formulas for Estimators of LR

Standard Error Formulas for mle of LR

Because the computation of $\hat{\Upsilon}_{1,\text{mle}}$ is dependent upon the mean and coefficient of variation of test carrier counts, two formulas are necessary for $\text{SE}(\hat{\Upsilon}_{1,\text{mle}})$. Let $V^*(\hat{\tau}^*)$ denote the estimated variance-covariance matrix of $\hat{\tau}^*$. Let $g_5(\hat{\tau}^*)$ and $g_6(\hat{\tau}^*)$ denote the expressions on the right hand sides of equations (3.19) and (3.20),

respectively. The multivariate delta method was used to derive formulas (3.32) and (3.33) for $SE(\hat{\Upsilon}_{1,\text{mle}})$.

$$SE(\hat{\Upsilon}_{1',\text{mle}}) = \sqrt{\left(\frac{\partial g_5(\hat{\tau}^*)}{\partial \hat{\tau}^*}\right)' V^*(\hat{\tau}^*) \left(\frac{\partial g_5(\hat{\tau}^*)}{\partial \hat{\tau}^*}\right)}. \quad (3.32)$$

$$SE(\hat{\Upsilon}_{1'',\text{mle}}) = \sqrt{\left(\frac{\partial g_6(\hat{\tau}^*)}{\partial \hat{\tau}^*}\right)' V^*(\hat{\tau}^*) \left(\frac{\partial g_6(\hat{\tau}^*)}{\partial \hat{\tau}^*}\right)}. \quad (3.33)$$

I used the delta method to derive formula (3.34) for $SE(\hat{\Upsilon}_{2,\text{mle}})$. In (3.34), $V^*_{[1,1]}$ denotes the one-one element of the estimated variance-covariance matrix for $\hat{\tau}^*$. Notice that $V^*_{[1,1]}$ is the estimated variance of $\hat{\phi}$.

$$SE(\hat{\Upsilon}_{2,\text{mle}}) = \log(e) \times \sqrt{\frac{V^*_{[1,1]}}{\hat{\phi}^2}}. \quad (3.34)$$

Expressions for $V^*(\hat{\tau}^*)$, $\frac{\partial g_5(\hat{\tau}^*)}{\partial \hat{\tau}^*}$, and $\frac{\partial g_6(\hat{\tau}^*)}{\partial \hat{\tau}^*}$, are given in Appendix B.

SE Formulas the mpn, jack, msf, or sk Estimators of LR

Let $S^2_{X,\text{mpn}}$ and $S^2_{Y,\text{mpn}}$ denote the sample variance for the log mpn estimates of the number of organisms surviving disinfection for the check and test carriers, respectively. The sample variance is calculated in the standard way, thus,

$$S^2_{X,\text{mpn}} = \frac{1}{A-1} \sum_{i=1}^A (\hat{X}_{i,\text{mpn}} - \hat{\mu}_{X,\text{mpn}})^2 \quad \text{and} \quad S^2_{Y,\text{mpn}} = \frac{1}{B-1} \sum_{s=1}^B (\hat{Y}_{s,\text{mpn}} - \hat{\mu}_{Y,\text{mpn}})^2.$$

I will use the capital S notation for the sample variances throughout the remainder of this chapter unless otherwise noted.

Denote as $CV_{C,\text{mpn}}$ and $CV_{T,\text{mpn}}$ the sample coefficients of variation for the mpn estimates of organisms surviving disinfection for the check and test carriers, respectively; i.e.

$$CV_{C,\text{mpn}} = \frac{\sqrt{S^2_{C,\text{mpn}}}}{\hat{\mu}_{C,\text{mpn}}} \quad \text{and} \quad CV_{T,\text{mpn}} = \frac{\sqrt{S^2_{T,\text{mpn}}}}{\hat{\mu}_{T,\text{mpn}}}.$$

I will use the capital CV notation for the sample coefficient of variation throughout the remainder of this chapter unless otherwise noted.

Equations (3.35) - (3.42) give the standard errors for the estimators of Υ_1 and Υ_2 using the mpn, jack, msf, and sk approaches, respectively. The delta method was used to derive standard error formulas for estimators of Υ_2 .

$$SE(\hat{\Upsilon}_{1,mpn}) = \sqrt{\frac{S_{X,mpn}^2}{A} + \frac{S_{Y,mpn}^2}{B}}. \quad (3.35)$$

$$SE(\hat{\Upsilon}_{2,mpn}) = \frac{1}{\ln(10)} \times \sqrt{\frac{CV_{X,mpn}^2}{A} + \frac{CV_{Y,mpn}^2}{B}}. \quad (3.36)$$

$$SE(\hat{\Upsilon}_{1,jack}) = \sqrt{\frac{S_{X,jack}^2}{A} + \frac{S_{Y,jack}^2}{B}}. \quad (3.37)$$

$$SE(\hat{\Upsilon}_{2,jack}) = \frac{1}{\ln(10)} \times \sqrt{\frac{CV_{X,jack}^2}{A} + \frac{CV_{Y,jack}^2}{B}}. \quad (3.38)$$

$$SE(\hat{\Upsilon}_{1,msf}) = \sqrt{\frac{S_{X,msf}^2}{A} + \frac{S_{Y,msf}^2}{B}}. \quad (3.39)$$

$$SE(\hat{\Upsilon}_{2,msf}) = \frac{1}{\ln(10)} \times \sqrt{\frac{CV_{X,msf}^2}{A} + \frac{CV_{Y,msf}^2}{B}}. \quad (3.40)$$

$$SE(\hat{\Upsilon}_{1,sk}) = \sqrt{\frac{S_{X,sk}^2}{A} + \frac{S_{Y,sk}^2}{B}}. \quad (3.41)$$

$$SE(\hat{\Upsilon}_{2,sk}) = \frac{1}{\ln(10)} \times \sqrt{\frac{CV_{X,sk}^2}{A} + \frac{CV_{Y,sk}^2}{B}}. \quad (3.42)$$

Standard Error Formulas for the pmle of LR

Let $\widehat{\text{Var}}(\cdot)$ denote the estimated variance of the argument in parentheses. Let

$V_C(\hat{\mu}_C, \hat{\eta}_C)$ denote the estimated variance-covariance matrix of $\hat{\mu}_C$ $\hat{\eta}_C$, and let

$$g_7(\mu, \eta) = \frac{\Psi(\frac{1}{\eta^2}) + \ln(\mu\eta^2)}{\ln(10)}.$$

The multivariate delta method was used to derive an approximate formula for $\widehat{\text{Var}}(\hat{\mu}_X)$.

$$\widehat{\text{Var}}(\hat{\mu}_X) \doteq \left(\frac{\partial g_7}{\partial \mu} \Big|_{\mu=\hat{\mu}_C}, \frac{\partial g_7}{\partial \eta} \Big|_{\eta=\hat{\eta}_C} \right) V_C(\hat{\mu}_C, \hat{\eta}_C) \left(\frac{\partial g_7}{\partial \mu} \Big|_{\mu=\hat{\mu}_C}, \frac{\partial g_7}{\partial \eta} \Big|_{\eta=\hat{\eta}_C} \right)'.$$

Let $V_{C[1,1]}$ denote the one-one element of the estimated variance-covariance matrix of $\hat{\mu}_C$ and $\hat{\eta}_C$; i.e. $V_{C[1,1]}$ is the estimated variance of $\hat{\mu}_C$. I used the delta method to derive the formula approximating $\widehat{\text{Var}}(\log(\hat{\mu}_C))$,

$$\widehat{\text{Var}}(\log(\hat{\mu}_C)) \doteq \frac{1}{[\ln(10)]^2} \times \frac{V_{C[1,1]}}{\hat{\mu}_C^2}.$$

Let $V_T(\hat{\mu}_T, \hat{\eta}_T)$ denote the estimated variance-covariance matrix of $\hat{\mu}_T$ and $\hat{\eta}_T$. Let

$$g_8(\mu, \eta) = \sum_{t=1}^N \log(t) \times \frac{\Gamma\left(t + \frac{\mu}{\mu\eta^2 - 1}\right)}{\Gamma\left(\frac{\mu}{\mu\eta^2 - 1}\right) t!} \left[\left(\frac{1}{\mu\eta^2} \right)^{\frac{\mu}{\mu\eta^2 - 1}} \right] \left[\left(\frac{\mu\eta^2 - 1}{\mu\eta^2} \right)^t \right].$$

The multivariate delta method was used to derive an approximate formula for $\widehat{\text{Var}}(\hat{\mu}_Y)$. Because the computation of $\hat{\mu}_Y$ is dependent upon the mean and coefficient of variation of test carrier counts, two formulas are necessary for $\widehat{\text{Var}}(\hat{\mu}_Y)$. Let (i) be the condition that $\hat{\mu}_T > 10$ and $\hat{\eta}_T \leq 1$. Let (ii) be the converse of (i). Then

$$\widehat{\text{Var}}(\hat{\mu}_Y) \doteq \begin{cases} \left(\frac{\partial g_7}{\partial \mu} \Big|_{\mu=\hat{\mu}_T}, \frac{\partial g_7}{\partial \eta} \Big|_{\eta=\hat{\eta}_T} \right) V_T(\hat{\mu}_T, \hat{\eta}_T) \left(\frac{\partial g_7}{\partial \mu} \Big|_{\mu=\hat{\mu}_T}, \frac{\partial g_7}{\partial \eta} \Big|_{\eta=\hat{\eta}_T} \right)' & \text{if (i)} \\ \left(\frac{\partial g_8}{\partial \mu} \Big|_{\mu=\hat{\mu}_T}, \frac{\partial g_8}{\partial \eta} \Big|_{\eta=\hat{\eta}_T} \right) V_T(\hat{\mu}_T, \hat{\eta}_T) \left(\frac{\partial g_8}{\partial \mu} \Big|_{\mu=\hat{\mu}_T}, \frac{\partial g_8}{\partial \eta} \Big|_{\eta=\hat{\eta}_T} \right)', & \text{if (ii).} \end{cases}$$

Let $V_{T[1,1]}$ denote the one-one element of the estimated variance-covariance matrix of $(\hat{\mu}_T, \hat{\eta}_T)$; i.e. $V_{T[1,1]}$ is the estimated variance of $\hat{\mu}_T$. I used the delta method to derive the formula approximating $\widehat{\text{Var}}(\log(\hat{\mu}_T))$,

$$\widehat{\text{Var}}(\log(\hat{\mu}_T)) \doteq \frac{1}{[\ln(10)]^2} \times \frac{V_{T[1,1]}}{\hat{\mu}_T^2}.$$

The formulas for computing $\text{SE}(\hat{\Upsilon}_{1,\text{pmle}})$ and $\text{SE}(\hat{\Upsilon}_{1,\text{pmle}})$ are given in (3.43) and (3.44), respectively.

$$\text{SE}(\hat{\Upsilon}_{1,\text{pmle}}) = \sqrt{\widehat{\text{Var}}(\hat{\mu}_X) + \widehat{\text{Var}}(\hat{\mu}_Y)}. \quad (3.43)$$

$$\text{SE}(\hat{\Upsilon}_{2,\text{pmle}}) = \sqrt{\widehat{\text{Var}}(\log(\hat{\mu}_C)) + \widehat{\text{Var}}(\log(\hat{\mu}_T))}. \quad (3.44)$$

Details for computing $\frac{\partial g_7}{\partial \mu}$, $\frac{\partial g_7}{\partial \eta}$, $\frac{\partial g_8}{\partial \mu}$, $\frac{\partial g_8}{\partial \eta}$, and the variance-covariance matrices for $(\hat{\mu}_C, \hat{\eta}_C)$ and $(\hat{\mu}_T, \hat{\eta}_T)$ are given in Appendix B.

Example

Table 14 is an example data set with $A = 3$ check carriers and $B = 3$ test carriers. Ten-fold dilutions were performed between 10^{-1} and 10^{-8} , inclusive. Four wells were tested at each dilution stage, and the number of wells responding positive at each dilution stage are recorded in Table 14. The estimated LR values for Υ_1 , and associated standard errors, were calculated for each of the methods described in the preceding sections and are given in Table 15. The estimated LR values and associated standard errors for Υ_2 are given in Table 16. Notice the difference of roughly 0.50 logs between the estimates of Υ_1 and Υ_2 . This difference is due in part to the rather large variability among test carriers.

Table 14: Example data from the presence/absence assay

	Check Carriers			Test Carriers		
	Carrier 1	Carrier 2	Carrier 3	Carrier 1	Carrier 2	Carrier 3
Dilution	Number of wells positive			Number of wells positive		
10^{-1}	4	4	4	4	4	4
10^{-2}	4	4	4	4	4	4
10^{-3}	4	4	4	4	2	4
10^{-4}	4	4	4	0	1	4
10^{-5}	4	4	4	0	0	2
10^{-6}	4	3	3	0	0	0
10^{-7}	0	1	0	0	0	0
10^{-8}	0	0	0	0	0	0

Table 15: Example calculations for estimators of Υ_1

	Method					
	mle	pmle	mpn	jack	msf	sk
Estimate of Υ_1	2.52	2.52	2.50	2.40	2.54	2.50
Standard error	0.6014	0.6849	0.5607	0.5687	0.5246	0.5528

Table 16: Example calculations for estimators of Υ_2

	Method					
	mle	pmle	mpn	jack	msf	sk
Estimate of Υ_2	1.96	1.96	1.89	1.91	1.99	1.89
Standard error	0.4778	0.4778	0.4119	0.4395	0.4019	0.4104

Computer Simulation Study

I conducted 16 computer simulation experiments, denoted as cases 1-16, to assess the statistical properties of the various estimators of Υ_1 and Υ_2 , and the associated standard errors. Each of the 16 experiments consisted of 1000 simulated pa assay data sets. For all simulations, $n_{ij} = w_{sm} = 4$ for all i and j , and for all s and m . The fractions of volume sampled (u_{ij} and v_{sm}) were constant across simulations and were the same for both check and test carriers; the sampling scheme utilized was $\vec{u}_i = \vec{v}_s =$

$$(5 \times 10^{-2}, 5 \times 10^{-3}, 5 \times 10^{-4}, 5 \times 10^{-5}, 5 \times 10^{-6}, 5 \times 10^{-7}, 5 \times 10^{-8}, 5 \times 10^{-9}),$$

for all $i = 1, \dots, A$ and $s = 1, \dots, B$. The mean inoculum size was fixed at 10^8 , implying $\alpha\beta = 10^8$. The parameters ϕ , CV check, and d were varied to cover a range of conditions likely to be encountered in real-world applications of the pa assay. Once

the values of α , β , ϕ and d are set, Υ_1 and Υ_2 are completely specified. Finally, I used $A = 3$ check carriers for all simulations, while the number of test carriers, B , was set at either 3 or 10. The parameter settings for the 16 experiments are given in Table 17. Results of the simulations are presented in Tables 18-26.

Table 17: Simulation parameters for the presence/absence assay

Case	A	B	ϕ	d	CV check	CV test	Υ_1	Υ_2
1	3	3	$10^{-3.5}$	1.0	0.10	0.102	3.500	3.5
2	3	3	$10^{-3.5}$	56.22	0.10	0.750	3.631	3.5
3	3	3	$10^{-3.5}$	1.0	0.40	0.400	3.500	3.5
4	3	3	$10^{-3.5}$	3.51	0.40	0.750	3.598	3.5
5	3	10	$10^{-3.5}$	1.0	0.10	0.102	3.500	3.5
6	3	10	$10^{-3.5}$	56.22	0.10	0.750	3.631	3.5
7	3	10	$10^{-3.5}$	1.0	0.40	0.400	3.500	3.5
8	3	10	$10^{-3.5}$	3.51	0.40	0.750	3.598	3.5
9	3	3	10^{-5}	1.0	0.10	0.141	5.002	5.0
10	3	3	10^{-5}	24.0	0.10	0.500	5.055	5.0
11	3	3	10^{-5}	1.0	0.40	0.412	5.003	5.0
12	3	3	10^{-5}	1.50	0.40	0.500	5.021	5.0
13	3	10	10^{-5}	1.0	0.10	0.141	5.002	5.0
14	3	10	10^{-5}	24.0	0.10	0.500	5.055	5.0
15	3	10	10^{-5}	1.0	0.40	0.412	5.003	5.0
16	3	10	10^{-5}	1.50	0.40	0.500	5.021	5.0

Comparison of Estimators of Υ_1

Tables 18 (cases 1-4), 19 (cases 5-8), 20 (cases 9-12) and 21 (cases 13-16) contain the results for the estimators of Υ_1 . As can be seen from column 3 of the aforementioned tables, each estimator is essentially unbiased. No estimator is clearly superior to the others when comparisons of MSE are made (column 6 of Tables 18-21). In general, the sk estimator had one of the largest MSE for all 16 cases. The jackknife estimator had the lowest MSE for cases 9-16 and performed reasonably well for cases 1-8. The MSE for mle and pmle were the lowest for cases

Table 18: Simulation results for estimators of Υ_1 (cases 1-4)

1.	2.	3.	4.	5.	6.
Case	method	Bias $\hat{\Upsilon}_1$	Mean SE($\hat{\Upsilon}_1$)	SD($\hat{\Upsilon}_1$)	$\widehat{\text{MSE}}$ $\hat{\Upsilon}_1$
1	mle	-0.0017	0.2270	0.2136	0.0456
	pmle	-0.0005	0.2484	0.2130	0.0454
	mpn	-0.0082	0.2059	0.2151	0.0463
	jack	0.0175	0.2207	0.2320	0.0541
	msf	-0.0372	0.2074	0.2177	0.0488
	sk	-0.0256	0.2145	0.2260	0.0517
2	mle	0.0049	0.2760	0.2941	0.0865
	pmle	0.0044	0.3093	0.2935	0.0862
	mpn	-0.0062	0.2808	0.2959	0.0876
	jack	0.0041	0.2782	0.2980	0.0888
	msf	-0.0249	0.2859	0.2998	0.0905
	sk	-0.0147	0.2910	0.3016	0.0912
3	mle	-0.0119	0.2462	0.2547	0.0650
	pmle	-0.0135	0.2774	0.2540	0.0647
	mpn	-0.0162	0.2481	0.2538	0.0647
	jack	0.0011	0.2599	0.2694	0.0726
	msf	-0.0343	0.2502	0.2577	0.0676
	sk	-0.0276	0.2536	0.2602	0.0684
4	mle	-0.0004	0.2818	0.3100	0.0961
	pmle	-0.0006	0.3196	0.3100	0.0961
	mpn	-0.0012	0.2961	0.3113	0.0971
	jack	0.0001	0.3005	0.3132	0.0981
	msf	-0.0242	0.2991	0.3160	0.1005
	sk	-0.0018	0.3012	0.3208	0.1032

Table 19: Simulation results for estimators of Υ_1 (cases 5-8)

1. Case	2. method	3. Bias $\hat{\Upsilon}_1$	4. Mean SE($\hat{\Upsilon}_1$)	5. SD($\hat{\Upsilon}_1$)	6. $\widehat{\text{MSE}}$ $\hat{\Upsilon}_1$
5	mle	0.0100	0.1817	0.1742	0.0304
	pmle	0.0118	0.2110	0.1741	0.0305
	mpn	-0.0036	0.1610	0.1762	0.0311
	jack	0.0228	0.1541	0.1687	0.0290
	msf	-0.0338	0.1656	0.1826	0.0345
	sk	-0.0192	0.1697	0.1850	0.0346
6	mle	0.0024	0.2141	0.2249	0.0506
	pmle	0.0019	0.2753	0.2251	0.0507
	mpn	-0.0148	0.1961	0.2247	0.0507
	jack	0.0005	0.1854	0.2134	0.0456
	msf	-0.0340	0.2008	0.2313	0.0546
	sk	-0.0238	0.2046	0.2330	0.0549
7	mle	0.0100	0.1919	0.2137	0.0458
	pmle	0.0107	0.2468	0.2118	0.0450
	mpn	0.0007	0.1891	0.2094	0.0438
	jack	0.0150	0.1885	0.2130	0.0456
	msf	-0.0177	0.1915	0.2134	0.0459
	sk	-0.0103	0.1954	0.2136	0.0457
8	mle	-0.0037	0.2208	0.2354	0.0554
	pmle	-0.0050	0.2877	0.2354	0.0554
	mpn	-0.0154	0.2191	0.2324	0.0543
	jack	-0.0094	0.2150	0.2306	0.0532
	msf	-0.0277	0.2218	0.2368	0.0569
	sk	-0.0238	0.2251	0.2392	0.0578

Table 20: Simulation results for estimators of Υ_1 (cases 9-12)

1. Case	2. method	3. Bias $\hat{\Upsilon}_1$	4. Mean SE($\hat{\Upsilon}_1$)	5. SD($\hat{\Upsilon}_1$)	6. $\widehat{\text{MSE}}$ $\hat{\Upsilon}_1$
9	mle	0.0049	0.2337	0.2175	0.0473
	pmle	0.0082	0.2482	0.2176	0.0474
	mpn	0.0079	0.1934	0.2129	0.0454
	jack	0.0051	0.1542	0.1801	0.0325
	msf	0.0080	0.2035	0.2239	0.0520
	sk	0.0048	0.2108	0.2304	0.0531
10	mle	0.0156	0.2508	0.2665	0.0712
	pmle	0.0173	0.2677	0.2664	0.0713
	mpn	0.0089	0.2267	0.2597	0.0675
	jack	0.0095	0.2023	0.2418	0.0586
	msf	-0.0037	0.2306	0.2638	0.0696
	sk	0.0025	0.2360	0.2703	0.0731
11	mle	-0.0005	0.2502	0.2738	0.0750
	pmle	-0.0014	0.2756	0.2727	0.0743
	mpn	0.0004	0.2368	0.2630	0.0691
	jack	-0.0088	0.2171	0.2505	0.0628
	msf	0.0000	0.2407	0.2676	0.0716
	sk	-0.0014	0.2470	0.2754	0.0759
12	mle	0.0147	0.2592	0.2760	0.0764
	pmle	0.0147	0.2861	0.2770	0.0769
	mpn	0.0138	0.2522	0.2672	0.0716
	jack	0.0131	0.2341	0.2589	0.0672
	msf	0.0083	0.2540	0.2692	0.0725
	sk	0.0093	0.2606	0.2742	0.0753

Table 21: Simulation results for estimators of Υ_1 (cases 13-16)

1. Case	2. method	3. Bias $\hat{\Upsilon}_1$	4. Mean SE($\hat{\Upsilon}_1$)	5. SD($\hat{\Upsilon}_1$)	6. $\widehat{\text{MSE}}$ $\hat{\Upsilon}_1$
13	mle	0.0038	0.1862	0.1744	0.0304
	pmle	0.0071	0.2151	0.1739	0.0303
	mpn	0.0003	0.1589	0.1686	0.0284
	jack	-0.0041	0.1298	0.1484	0.0220
	msf	-0.0027	0.1669	0.1775	0.0315
	sk	-0.0036	0.1694	0.1770	0.0313
14	mle	0.0209	0.1976	0.1902	0.0366
	pmle	0.0212	0.2395	0.1890	0.0362
	mpn	0.0039	0.1737	0.1837	0.0338
	jack	0.0082	0.1520	0.1636	0.0268
	msf	-0.0094	0.1786	0.1889	0.0358
	sk	-0.0036	0.1822	0.1932	0.0373
15	mle	0.0131	0.1961	0.2128	0.0455
	pmle	0.0148	0.2479	0.2112	0.0448
	mpn	0.0038	0.1898	0.2024	0.0410
	jack	0.0021	0.1757	0.1924	0.0370
	msf	0.0011	0.1927	0.2069	0.0428
	sk	-0.0006	0.1975	0.2064	0.0426
16	mle	0.0090	0.2005	0.2128	0.0454
	pmle	0.0096	0.2532	0.2112	0.0447
	mpn	-0.0023	0.1933	0.2028	0.0411
	jack	-0.0026	0.1786	0.1970	0.0388
	msf	-0.0094	0.1960	0.2056	0.0424
	sk	-0.0068	0.2012	0.2107	0.0445

1-4. It is interesting to note how similar the MSE are for mle and pmle for all cases. Results not shown in this paper indicate that the point estimates for the mle and pmle methods are nearly identical for most cases.

Performance of the standard error formula can be determined by comparing the mean of the 1000 standard errors (column 4) with the standard deviation of the point estimates (column 5) in Tables 18-21. Let ESE (expected standard error) denote the mean of the 1000 SEs for a particular case and TSD (true standard deviation) denote the standard deviation of the 1000 point estimates. Generally speaking, the standard error formulas tend to underestimate the true standard deviation, the exception being the formula for pmle which consistently overestimated the standard deviation. I computed the absolute percent relative bias of the standard error formula, $\left|100 \times \left(\frac{\text{ESE}-\text{TSD}}{\text{TSD}}\right)\right| \%$, for each of the 16 cases and for each of the methods. The average, over all 16 cases, of the absolute percent relative bias of standard error was 6.4%, 13.9%, 7.1%, 9.4%, 7.3% and 6.7% for the mle, pmle, mpn, jack, msf and sk methods, respectively.

It should be mentioned that the sk method is the simplest to compute and can be done with the aid of a spreadsheet program. The remaining estimators all require iterative solutions, and as such, require more sophisticated computer programs than the sk method. The mpn method is slightly simpler to program than the jack and msf methods, and much easier to program than the mle and pmle methods.

Overall, it is my opinion that there is little practical difference among the various estimators of Υ_1 explored in this simulation study. All estimators have little bias and, with the exception of pmle, have standard error formulas which appear to work reasonably well. The sk estimator has the advantage of being calculable in closed form. However, the sk estimator appears to be the most variable of the

estimators considered in this dissertation. The mpn estimator appears to have reasonably good statistical properties in comparison with the other estimators, and has the advantage of being the easiest estimator to compute aside from sk. Because there is a precedent for using the most probable number estimator to obtain estimates of the density of viruses in suspension (Myers et al. 1994; Payment et al. 1985), virologists may feel more comfortable using $\hat{\Upsilon}_{1,\text{mpn}}$ over other estimators of Υ_1 .

Comparison of Estimators of Υ_2

Results for the estimators are given in Tables 22 (cases 1-4), 23 (cases 5-8), 24 (cases 9-12) and 25 (cases 13-16). The bias of all estimators appears to be negligible (column 3 of Tables 22-25).

Comparisons of MSE indicate that mle is the best estimator of Υ_2 , as mle had the smallest MSE for all cases except 9 and 10; for cases 9 and 10 mle had the second smallest MSE. The msf method had the largest MSE for cases 5-16 which was somewhat surprising to me. Results not presented in this dissertation indicate that the msf method is superior, in terms of MSE, to mpn and jack for estimating the density of organisms in suspension via a serial-dilution experiment. This conclusion is also documented in Mehrabi and Matthews (1995). The performance of the msf method in this study leads me to believe that the msf estimator is not as well suited on the log scale as are other estimators.

The performance of the standard error formulas can be determined by comparing the mean of the 1000 standard errors (column 4) with the standard deviation of the point estimates (column 5) in Tables 22-25. It is clear that the standard error formulas for mpn, jack, msf and sk underestimate the true standard deviation by 20%-30%. Such bias is not acceptable and limits the usefulness of these

Table 22: Simulation results for estimators of Υ_2 (cases 1-4)

1. Case	2. method	3. Bias $\hat{\Upsilon}_2$	4. Mean SE($\hat{\Upsilon}_2$)	5. SD($\hat{\Upsilon}_2$)	6. $\widehat{\text{MSE}}$ $\hat{\Upsilon}_2$
1	mle	-0.0145	0.2310	0.2158	0.0468
	pmle	-0.0067	0.2397	0.2193	0.0481
	mpn	-0.0174	0.1925	0.2334	0.0548
	jack	-0.0339	0.2015	0.2557	0.0665
	msf	-0.0361	0.1924	0.2364	0.0572
	sk	-0.0296	0.1984	0.2439	0.0604
2	mle	0.0629	0.2770	0.2934	0.0900
	pmle	0.0672	0.2807	0.2944	0.0912
	mpn	0.0397	0.2384	0.3046	0.0943
	jack	0.0218	0.2348	0.3157	0.1002
	msf	0.0272	0.2423	0.3084	0.0959
	sk	0.0339	0.2454	0.3093	0.0968
3	mle	-0.0358	0.2504	0.2602	0.0690
	pmle	-0.0198	0.2601	0.2627	0.0694
	mpn	-0.0270	0.2201	0.2727	0.0751
	jack	-0.0420	0.2267	0.2896	0.0856
	msf	-0.0397	0.2203	0.2779	0.0788
	sk	-0.0346	0.2231	0.2777	0.0783
4	mle	0.0260	0.2821	0.3133	0.0988
	pmle	0.0368	0.2877	0.3159	0.1011
	mpn	0.0199	0.2498	0.3287	0.1084
	jack	0.0089	0.2477	0.3411	0.1164
	msf	0.0115	0.2518	0.3332	0.1112
	sk	0.0177	0.2527	0.3319	0.1105

Table 23: Simulation results for estimators of Υ_2 (cases 5-8)

1.	2.	3.	4.	5.	6.
Case	method	Bias $\hat{\Upsilon}_2$	Mean SE($\hat{\Upsilon}_2$)	SD($\hat{\Upsilon}_2$)	$\widehat{\text{MSE}}$ $\hat{\Upsilon}_2$
5	mle	0.0007	0.1837	0.1757	0.0309
	pmle	0.0094	0.2046	0.1809	0.0328
	mpn	-0.0326	0.1570	0.1970	0.0399
	jack	-0.0583	0.1485	0.1916	0.0401
	msf	-0.0489	0.1590	0.2092	0.0462
	sk	-0.0429	0.1632	0.2030	0.0431
6	mle	0.0319	0.2171	0.2283	0.0532
	pmle	0.0324	0.2457	0.2299	0.0539
	mpn	-0.0271	0.1837	0.2419	0.0592
	jack	-0.0413	0.1718	0.2323	0.0557
	msf	-0.0390	0.1864	0.2551	0.0666
	sk	-0.0337	0.1902	0.2489	0.0631
7	mle	-0.0139	0.1967	0.2170	0.0473
	pmle	-0.0001	0.2310	0.2196	0.0482
	mpn	-0.0435	0.1785	0.2305	0.0550
	jack	-0.0664	0.1727	0.2310	0.0578
	msf	-0.0548	0.1787	0.2415	0.0613
	sk	-0.0527	0.1834	0.2340	0.0575
8	mle	-0.0020	0.2250	0.2433	0.0592
	pmle	0.0024	0.2536	0.2461	0.0606
	mpn	-0.0421	0.1996	0.2567	0.0676
	jack	-0.0610	0.1896	0.2483	0.0654
	msf	-0.0486	0.2013	0.2692	0.0748
	sk	-0.0484	0.2044	0.2623	0.0712

Table 24: Simulation results for estimators of Υ_2 (cases 9-13)

1.	2.	3.	4.	5.	6.
Case	method	Bias $\hat{\Upsilon}_2$	Mean SE($\hat{\Upsilon}_2$)	SD($\hat{\Upsilon}_2$)	$\widehat{\text{MSE}}$ $\hat{\Upsilon}_2$
9	mle	-0.0020	0.2353	0.2205	0.0486
	pmle	0.0104	0.2420	0.2245	0.0505
	mpn	0.0115	0.1840	0.2359	0.0558
	jack	0.0053	0.1473	0.2136	0.0457
	msf	0.0123	0.1968	0.2623	0.0690
	sk	0.0077	0.1967	0.2503	0.0627
10	mle	0.0372	0.2536	0.2671	0.0727
	pmle	0.0460	0.2566	0.2704	0.0752
	mpn	0.0291	0.2046	0.2790	0.0787
	jack	0.0211	0.1776	0.2651	0.0707
	msf	0.0218	0.2133	0.3003	0.0907
	sk	0.0256	0.2133	0.2896	0.0845
11	mle	-0.0177	0.2532	0.2762	0.0766
	pmle	0.0040	0.2604	0.2833	0.0803
	mpn	0.0065	0.2135	0.2909	0.0847
	jack	-0.0006	0.1893	0.2771	0.0768
	msf	0.0060	0.2213	0.3121	0.0974
	sk	0.0021	0.2210	0.3036	0.0922
12	mle	0.0018	0.2615	0.2764	0.0764
	pmle	0.0205	0.2681	0.2813	0.0796
	mpn	0.0189	0.2242	0.2884	0.0835
	jack	0.0175	0.2016	0.2852	0.0816
	msf	0.0157	0.2310	0.3083	0.0953
	sk	0.0124	0.2312	0.2959	0.0877

Table 25: Simulation results for estimators of Υ_2 (cases 13-16)

1. Case	2. method	3. Bias $\hat{\Upsilon}_2$	4. Mean SE($\hat{\Upsilon}_2$)	5. SD($\hat{\Upsilon}_2$)	6. $\widehat{\text{MSE}}$ $\hat{\Upsilon}_2$
13	mle	-0.0023	0.1878	0.1763	0.0311
	pmle	0.0113	0.2092	0.1830	0.0336
	mpn	-0.0187	0.1575	0.1983	0.0397
	jack	-0.0208	0.1339	0.1938	0.0380
	msf	-0.0274	0.1708	0.2192	0.0488
	sk	-0.0272	0.1645	0.2009	0.0411
14	mle	0.0348	0.2032	0.1966	0.0398
	pmle	0.0392	0.2268	0.1987	0.0410
	mpn	-0.0104	0.1693	0.2121	0.0451
	jack	-0.0125	0.1502	0.2021	0.0410
	msf	-0.0235	0.1813	0.2316	0.0542
	sk	-0.0194	0.1759	0.2194	0.0485
15	mle	-0.0098	0.2016	0.2145	0.0461
	pmle	0.0094	0.2326	0.2210	0.0489
	mpn	-0.0259	0.1806	0.2309	0.0540
	jack	-0.0266	0.1654	0.2278	0.0526
	msf	-0.0322	0.1899	0.2468	0.0620
	sk	-0.0323	0.1864	0.2306	0.0542
16	mle	-0.0049	0.2064	0.2157	0.0465
	pmle	0.0096	0.2352	0.2204	0.0487
	mpn	-0.0295	0.1833	0.2305	0.0540
	jack	-0.0304	0.1677	0.2298	0.0537
	msf	-0.0389	0.1928	0.2450	0.0615
	sk	-0.0367	0.1903	0.2372	0.0576

methods. The mean, over all 16 cases, of the absolute percent relative bias of the standard error formula for mle was 6.2%. Thus the standard error formula for mle appears to be reliable.

Clearly, mle is the superior estimator of Υ_2 when MSE and performance of the standard error formula are considered. The pmle method would also be a reasonable choice. Both pmle and mle are fairly difficult to program, however. Among the more easily computable methods, no estimator stands out as being superior on the basis of MSE comparisons.

Table 26: Observed ratios of $\widehat{\text{MSE}}^*$'s for the mle and mpn estimators for the cases defined in Table 17.

1.	2.	3.
Case	$\frac{\widehat{\text{MSE}}^*(\hat{\Upsilon}_{1,\text{mle}})}{\widehat{\text{MSE}}^*(\hat{\Upsilon}_{2,\text{mle}})}$	$\frac{\widehat{\text{MSE}}^*(\hat{\Upsilon}_{1,\text{mpn}})}{\widehat{\text{MSE}}^*(\hat{\Upsilon}_{2,\text{mpn}})}$
1	0.975	0.845
2	0.893	0.863
3	0.942	0.861
4	0.920	0.848
5	0.986	0.779
6	0.884	0.795
7	0.968	0.796
8	0.886	0.759
9	0.972	0.813
10	0.959	0.839
11	0.978	0.816
12	0.991	0.850
13	0.977	0.716
14	0.899	0.732
15	0.986	0.758
16	0.967	0.755

Comparison of Estimators of Υ_1 to Υ_2

Ratios of relative $\widehat{\text{MSE}}$ s for $\hat{\Upsilon}_1$ to $\hat{\Upsilon}_2$ are given in Table 26 for mle and mpn, only. The ratio of relative $\widehat{\text{MSE}}$ s for pmle are very similar to mle, and ratios of relative $\widehat{\text{MSE}}$ s for jack, msf and sk are very similar to mpn. The ratios of relative $\widehat{\text{MSE}}$ s are less than 1.0 for all cases indicating that Υ_1 is more easily estimated than Υ_2 ; particularly for the mpn, jack, msf, and sk methods. This result was somewhat of a surprise to me, as it is a reversal from most of the results seen for the quantitative assay. My own suspicion is that when estimating the mean density of organisms from multiple serial-dilution experiments, it is more efficient to work on the log scale. Garthright's (1993) results give some weight to this argument as he showed that the log of the mpn from a serial-dilution experiment is less biased and more normally distributed than the mpn.

Conclusions

Υ_1 is more easily estimated than Υ_2 . Among estimators of Υ_1 , no single estimator of Υ_1 stood out as being superior, however, as all estimators of Υ_1 are essentially unbiased, have reasonable standard error formulas, and, for the most part, are comparable in terms of MSE. The sk estimator of Υ_1 appears to have a slightly higher mse than the other estimators considered in this study, and as such, should perhaps be avoided. The mpn estimator of Υ_1 has reasonably good statistical properties and may be the preferred estimator of choice on the basis of precedent.

The mle of Υ_2 was deemed superior to all other methods on the basis of MSE comparisons and performance of standard errors. The mpn, jack, msf, and sk estimators of Υ_2 had standard error formulas which seriously underestimate the true standard deviation.

Future Work

Improved standard error formulas, tests for over-dispersion among test carrier data, and investigation into the uniqueness of mle and pmle may all be fruitful areas of research. The standard error formulas for $\hat{\Upsilon}_{2,mpn}$, $\hat{\Upsilon}_{2,jack}$, $\hat{\Upsilon}_{2,msk}$, and $\hat{\Upsilon}_{2,sk}$ are biased downwards by as much as 30% and a bias adjustment for these methods would be a significant contribution.

The likelihood ratio test is an obvious method for determining if over-dispersion is present among the test carrier data. Programming the computation of the test statistic should not be too difficult, however, justification of the use of the likelihood ratio test may prove more challenging.

The computation of the mle proved to be fairly difficult. One problem I often encountered was an unstable hessian matrix during the modified Newton-Raphson iterations. Often times the updated estimates of α would exceed 10^6 , while estimates of β would be driven towards zero. Such values of α and β would cause numerical instability in the inverse of the hessian matrix, which directly influences the standard error calculations of the mle for Υ_1 and Υ_2 . To remedy this situation, I would not allow estimates of α to exceed 10^6 . This remedy kept the hessian from becoming too unstable numerically, but is an ad-hoc solution. For cases such as these, I wonder if there is a unique mle solution. Similar problems were encountered with the pmle method.

APPENDICES

APPENDIX A

Appendix Material for the Quantitative Assay

Derivation of the Negative Binomial Distribution.

Let T denote the number of microbes surviving disinfection in a sample volume of size v . Let θ be the unknown inoculum size on a carrier, and let ϕ denote the survival fraction. Assume θ is distributed as gamma(mean = $\alpha\beta$, variance = $\alpha\beta^2$). Assume $T \mid \theta, \phi, v$ is Poisson($\theta\phi v$). Then the marginal distribution of T is negative binomial with parameters $r = \alpha$, $p = \frac{1}{\beta\phi v + 1}$, $q = \frac{\beta\phi v}{\beta\phi v + 1}$. The derivation (Mood, Graybill, and Boes 1974) is as follows:

$$\begin{aligned}
 P(T = t) &= \int_0^\infty \frac{e^{-\theta\phi v} (\theta\phi v)^t}{t!} \times \frac{\theta^{\alpha-1} e^{-\frac{\theta}{\beta}}}{\beta^\alpha \Gamma(\alpha)} d\theta \\
 &= \frac{(\phi v)^t}{t! \beta^\alpha \Gamma(\alpha)} \times \int_0^\infty \theta^{(t+\alpha-1)} e^{-\theta/(\frac{\beta}{\beta\phi v + 1})} d\theta \\
 &= \frac{(\phi v)^t \Gamma(t + \alpha) \left(\frac{\beta}{\beta\phi v + 1}\right)^{t+\alpha}}{t! \beta^\alpha \Gamma(\alpha)} \times \int_0^\infty \frac{\theta^{(t+\alpha-1)} e^{-\theta/(\frac{\beta}{\beta\phi v + 1})}}{\Gamma(t + \alpha) \left(\frac{\beta}{\beta\phi v + 1}\right)^{t+\alpha}} d\theta \\
 &= \frac{\Gamma(t + \alpha)}{\Gamma(\alpha) t!} \left(\frac{\beta\phi v}{\beta\phi v + 1}\right)^t \left(\frac{1}{\beta\phi v + 1}\right)^\alpha \times 1, \quad t = 0, 1, 2, \dots
 \end{aligned}$$

which is the pmf of a negative binomial with parameters r , p , and q .

Notice that the marginal distribution of check carriers can be derived in a similar fashion with $\phi = 1$, and the check carrier dilution u replaced for v . It then follows that the check carrier count, C , taken from a sampled volume of size u , follows a negative binomial with parameters $r = \alpha$, $p = \frac{1}{\beta u + 1}$, $q = \frac{\beta u}{\beta u + 1}$. Thus,

$$P(C = c) = \frac{\Gamma(c + \alpha)}{\Gamma(\alpha) c!} \left(\frac{\beta u}{\beta u + 1}\right)^c \left(\frac{1}{\beta u + 1}\right)^\alpha, \quad c = 0, 1, 2, \dots$$

Symbolic Descriptions of $f(\vec{\tau})$ and $H(\vec{\tau})$.

Denote by $l(\vec{\tau})$ the log likelihood function for the models with no over-dispersion as given in (2.3). Let

$$f(\vec{\tau}) = \begin{pmatrix} \frac{\partial l(\vec{\tau})}{\partial \phi} \\ \frac{\partial l(\vec{\tau})}{\partial \alpha} \\ \frac{\partial l(\vec{\tau})}{\partial \beta} \end{pmatrix}, \quad \text{where}$$

$$\frac{\partial l(\vec{\tau})}{\partial \phi} = \sum_{s=1}^B \frac{T_s - \alpha \beta \phi v_s}{\phi(\beta \phi v_s + 1)},$$

$$\frac{\partial l(\vec{\tau})}{\partial \alpha} =$$

$$\sum_{i=1}^A \left(\Psi(C_i + \alpha) - \Psi(\alpha) - \ln(\beta u_i + 1) \right) + \sum_{s=1}^B \left(\Psi(T_s + \alpha) - \Psi(\alpha) - \ln(\beta \phi v_s + 1) \right), \text{ and}$$

$$\frac{\partial l(\vec{\tau})}{\partial \beta} = \sum_{i=1}^A \frac{C_i - \alpha \beta u_i}{\beta(\beta u_i + 1)} + \sum_{s=1}^B \frac{T_s - \alpha \beta \phi v_s}{\beta(\beta \phi v_s + 1)}.$$

The Hessian is,

$$H(\vec{\tau}) = \begin{pmatrix} \frac{\partial^2 l(\vec{\tau})}{\partial \phi^2} & \frac{\partial^2 l(\vec{\tau})}{\partial \phi \partial \alpha} & \frac{\partial^2 l(\vec{\tau})}{\partial \phi \partial \beta} \\ \frac{\partial^2 l(\vec{\tau})}{\partial \alpha \partial \phi} & \frac{\partial^2 l(\vec{\tau})}{\partial \alpha^2} & \frac{\partial^2 l(\vec{\tau})}{\partial \alpha \partial \beta} \\ \frac{\partial^2 l(\vec{\tau})}{\partial \beta \partial \phi} & \frac{\partial^2 l(\vec{\tau})}{\partial \beta \partial \alpha} & \frac{\partial^2 l(\vec{\tau})}{\partial \beta^2} \end{pmatrix}, \quad \text{where}$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \phi^2} = \sum_{s=1}^B \frac{\alpha \beta^2 \phi^2 v_s^2 - T_s (2\beta \phi v_s + 1)}{\phi^2 (\beta \phi v_s + 1)^2},$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \phi \partial \alpha} = \sum_{s=1}^B -\frac{\beta v_s}{\beta \phi v_s + 1},$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \phi \partial \beta} = \sum_{s=1}^B \frac{-v_s (T_s + \alpha)}{(\beta \phi v_s + 1)^2},$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \alpha \partial \phi} = \frac{\partial^2 l(\vec{\tau})}{\partial \phi \partial \alpha},$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \alpha^2} = \sum_{i=1}^A (\Psi'(C_i + \alpha) - \Psi'(\alpha)) + \sum_{s=1}^B (\Psi'(T_s + \alpha) - \Psi'(\alpha)),$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \alpha \partial \beta} = \sum_{i=1}^A -\frac{u_i}{\beta u_i + 1} + \sum_{s=1}^B -\frac{\phi v_s}{\beta \phi v_s + 1},$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \beta \partial \phi} = \frac{\partial^2 l(\vec{\tau})}{\partial \phi \partial \beta},$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \beta \partial \alpha} = \frac{\partial^2 l(\vec{\tau})}{\partial \alpha \partial \beta}, \text{ and}$$

$$\frac{\partial^2 l(\vec{\tau})}{\partial \beta^2} = \sum_{i=1}^A \frac{\alpha \beta^2 u_i^2 - C_i(2\beta u_i + 1)}{\beta^2(\beta u_i + 1)^2} + \sum_{s=1}^B \frac{\alpha \beta^2 \phi^2 v_s^2 - T_s(2\beta \phi v_s + 1)}{\beta^2(\beta \phi v_s + 1)^2}.$$

Use of the Computer Package *Maple*

The computer package *Maple* (Waterloo Maple Software, 1994) can be used to perform symbolic differentiation, and as such, is particularly well suited for finding first and second partial derivatives of complicated functions such as $l(\vec{\tau})$. *Maple* can also be used to evaluate a function of several variables, such as $f(\vec{\tau})$, under specific numerical settings of the variables. This feature of *Maple* proved invaluable to me as a programming check. For these reasons, all derivatives defined in this dissertation, including components of $f(\vec{\tau})$ and $H(\vec{\tau})$, were obtained using *Maple*.

Symbolic Descriptions of $f^*(\vec{\tau}^*)$ and $H^*(\vec{\tau}^*)$.

Denote by $l(\vec{\tau}^*)$ the log likelihood function for the model with over-dispersion as given by (2.4). Because the equations for $f^*(\vec{\tau}^*)$ and $H^*(\vec{\tau}^*)$ are long, some notation will be adopted for ease of presentation. Define k_1 through k_8 as follows:

$$k_1 = \beta \phi^2 v_s + \beta \zeta^2(\alpha + 1)v_s + \phi;$$

$$k_2 = \phi^2 + \zeta^2(\alpha + 1);$$

$$k_3 = \frac{1}{\phi} - \frac{2\beta \phi v_s + 1}{k_1};$$

$$k_4 = \frac{2\alpha \phi \zeta^2(\alpha + 1)}{k_2^2};$$

$$k_5 = \frac{\alpha \phi^2}{k_2};$$

$$k_6 = T_s + k_5;$$

$$k_7 = \frac{2\phi \zeta^2}{k_2^2} + \frac{4\alpha \phi^3 \zeta^2}{\%c2^3}; \text{ and}$$

$$k_8 = \frac{\phi^4 + \phi^2 \zeta^2}{\%c2^2}.$$

Let

$$f^*(\vec{\tau}^*) = \begin{pmatrix} \frac{\partial l(\vec{\tau}^*)}{\partial \phi} \\ \frac{\partial l(\vec{\tau}^*)}{\partial \zeta} \\ \frac{\partial l(\vec{\tau}^*)}{\partial \alpha} \\ \frac{\partial l(\vec{\tau}^*)}{\partial \beta} \end{pmatrix}, \quad \text{where}$$

$$\frac{\partial l(\tau^*)}{\partial \phi} = \sum_{s=1}^B [(\Psi(k_6) - \Psi(k_5)) \times \frac{2\alpha\phi\zeta^2(\alpha+1)}{k_2^2} + \frac{2\alpha\phi\zeta^2(\alpha+1)(\ln(\phi)-\ln(k_1))}{k_2^2} + \frac{\alpha\phi^2(1/\phi - (2\beta\phi v_s + 1)/k_1)}{k_2} + T_s \times (\frac{2\beta\phi v_s}{k_1 - \phi} - \frac{2\beta\phi v_s + 1}{k_1})],$$

$$\frac{\partial l(\tau^*)}{\partial \zeta} = \sum_{s=1}^B [(\Psi(k_5) - \Psi(k_6)) \times \frac{2\phi^2\zeta\alpha(\alpha+1)}{k_2^2} - \frac{2\phi^2\zeta(\alpha^2 + \alpha)(\ln(\phi)-\ln(k_1))}{k_2^2} - \frac{2\phi^2\beta\zeta(\alpha^2 + \alpha)v_s}{k_2 k_1} + T_s \times (\frac{2\beta\zeta(\alpha+1)v_s}{k_1 - \phi} - \frac{2\beta\zeta(\alpha+1)v_s}{k_1})],$$

$$\frac{\partial l(\tau^*)}{\partial \alpha} = \sum_{i=1}^A [\Psi(C_i + \alpha) - \Psi(\alpha) - \ln(\beta u_i + 1)] + \sum_{s=1}^B [(\Psi(k_6) - \Psi(k_5)) \times (\frac{\phi^2}{k_2} - \frac{\alpha\phi^2\zeta^2}{k_2^2}) + \frac{\phi^2(\ln(\phi)-\ln(k_1))}{k_2} - \frac{\alpha\phi^2\zeta^2(\ln(\phi)-\ln(k_1))}{k_2^2} - \frac{\alpha\beta\phi^2\zeta^2 v_s}{k_2 k_1} + T_s \times (\frac{\beta\zeta^2 v_s}{k_1 - \phi} - \frac{\beta\zeta^2 v_s}{k_1})], \text{ and}$$

$$\frac{\partial l(\tau^*)}{\partial \beta} = \sum_{i=1}^A [\frac{-\alpha u_i}{\beta u_i + 1} + \frac{C_i}{\beta(\beta u_i + 1)}] + \sum_{s=1}^B [\frac{-\alpha\phi^2(\phi^2 v_s + \zeta^2(\alpha+1)v_s)}{k_2 k_1} + T_s \times (\frac{\phi^2 v_s + \zeta^2(\alpha+1)v_s}{k_1 - \phi} - \frac{\phi^2 v_s + \zeta^2(\alpha+1)v_s}{k_1})].$$

Let

$$H^*(\tau^*) = \begin{pmatrix} \frac{\partial^2 l(\tau^*)}{\partial \phi^2} & \frac{\partial^2 l(\tau^*)}{\partial \phi \partial \zeta} & \frac{\partial^2 l(\tau^*)}{\partial \phi \partial \alpha} & \frac{\partial^2 l(\tau^*)}{\partial \phi \partial \beta} \\ \frac{\partial^2 l(\tau^*)}{\partial \zeta \partial \phi} & \frac{\partial^2 l(\tau^*)}{\partial \zeta^2} & \frac{\partial^2 l(\tau^*)}{\partial \zeta \partial \alpha} & \frac{\partial^2 l(\tau^*)}{\partial \zeta \partial \beta} \\ \frac{\partial^2 l(\tau^*)}{\partial \alpha \partial \phi} & \frac{\partial^2 l(\tau^*)}{\partial \alpha \partial \zeta} & \frac{\partial^2 l(\tau^*)}{\partial \alpha^2} & \frac{\partial^2 l(\tau^*)}{\partial \alpha \partial \beta} \\ \frac{\partial^2 l(\tau^*)}{\partial \beta \partial \phi} & \frac{\partial^2 l(\tau^*)}{\partial \beta \partial \zeta} & \frac{\partial^2 l(\tau^*)}{\partial \beta \partial \alpha} & \frac{\partial^2 l(\tau^*)}{\partial \beta^2} \end{pmatrix}, \text{ where}$$

$$\frac{\partial^2 l(\tau^*)}{\partial \phi^2} = \sum_{s=1}^B [(\Psi'(k_6) - \Psi'(k_5)) \times k_4^2 + (\Psi(k_6) - \Psi(k_5)) \times (\frac{2\alpha}{k_2} - \frac{10\alpha\phi^2}{k_2^2} + \frac{8\alpha\phi^4}{k_2^3}) + \frac{2\alpha(\ln(\phi)-\ln(k_1))}{k_2} - \frac{10\alpha\phi^2(\ln(\phi)-\ln(k_1))}{k_2^2} + \frac{4\alpha\phi k_3}{k_2} + \frac{8\alpha\phi^4(\ln(\phi)-\ln(k_1))}{k_2^3} - \frac{4\alpha\phi^3 k_3}{k_2^2} + \frac{(\alpha\phi^2) \times (-1/\phi^2 - 2\beta v_s/k_1 + (2\beta\phi v_s + 1)^2/k_1^2)}{k_2} + T_s \times (\frac{2\beta v_s}{k_1 - \phi} - \frac{4(\beta\phi v_s)^2}{(k_1 - \phi)^2} - \frac{2\beta v_s}{k_1} + \frac{(2\beta\phi v_s + 1)^2}{k_1^2})],$$

$$\frac{\partial^2 l(\tau^*)}{\partial \phi \partial \zeta} = \sum_{s=1}^B [(\Psi'(k_5) - \Psi'(k_6)) \times (\frac{2k_4\phi^2\zeta\alpha(\alpha+1)}{k_2^2}) + (\Psi(k_5) - \Psi(k_6)) \times (\frac{4\phi\zeta\alpha(\alpha+1)}{k_2^2}) + (\Psi(k_6) - \Psi(k_5)) \times (\frac{8\phi^3\zeta\alpha(\alpha+1)}{k_2^3} - \frac{4\phi\zeta\alpha(\alpha+1)(\ln(\phi)-\ln(k_1))}{k_2^2} + \frac{8\phi^3\zeta\alpha(\alpha+1)(\ln(\phi)-\ln(k_1))}{k_2^3} - \frac{2\phi^2\zeta\alpha(\alpha+1)k_3}{k_2^2} - \frac{4\phi\zeta\alpha(\alpha+1)\beta v_s}{k_1 k_2} + \frac{4\phi^3\zeta\alpha(\alpha+1)\beta v_s}{k_1 k_2^2} + \frac{2\phi^2\zeta\alpha(\alpha+1)\beta v_s(2\beta\phi v_s + 1)}{k_1^2 k_2} + T_s \times (\frac{-4\phi\zeta(\alpha+1)\beta^2 v_s^2}{(k_1 - \phi)^2} + \frac{2\zeta(\alpha+1)\beta v_s(2\beta\phi v_s + 1)}{k_1^2})],$$

$$\frac{\partial^2 l(\tau^*)}{\partial \phi \partial \alpha} = \sum_{s=1}^B [(\Psi'(k_6) - \Psi'(k_5))k_4 k_8 + (\Psi(k_6) - \Psi(k_5))k_7 + \frac{2\phi(\ln(\phi)-\ln(k_1))}{k_2} - \frac{2\phi^3(\ln(\phi)-\ln(k_1))}{k_2^2} + \frac{\phi^2 k_3}{k_2} - \frac{2\phi\zeta^2\alpha(\ln(\phi)-\ln(k_1))}{k_2^2} + \frac{4\phi^3\zeta^2\alpha(\ln(\phi)-\ln(k_1))}{k_2^3} - \frac{\phi^2\zeta^2\alpha k_3}{k_2^2} - \frac{2\phi\zeta^2\alpha\beta v_s}{k_1 k_2} + \frac{2\phi^3\zeta^2\alpha\beta v_s}{k_1 k_2^2} + \frac{\phi^2\zeta^2\alpha\beta v_s(2\beta\phi v_s + 1)}{k_1^2 k_2} +$$

$$T_s \times \left(\frac{-2\phi\zeta^2\beta^2v_s^2}{(k_1-\phi)^2} + \frac{\zeta^2\beta v_s(2\phi\beta v_s+1)}{k_1^2} \right)],$$

$$\begin{aligned} \frac{\partial^2 l(\tau^*)}{\partial \phi \partial \beta} = \sum_{s=1}^B & \left[\frac{-2\phi\alpha v_s(\phi^2+\zeta^2(\alpha+1))}{k_1 k_2} + \frac{2\phi^3\alpha v_s(\phi^2+\zeta^2(\alpha+1))}{k_1 k_2^2} - \frac{2\phi^3\alpha v_s}{k_1 k_2} + \right. \\ & \frac{\phi^2\alpha v_s(\phi^2+\zeta^2(\alpha+1))(2\phi\beta v_s+1)}{k_1^2 k_2} + T_s \times \left(\frac{2\phi v_s}{k_1-\phi} - \frac{2\phi\beta v_s^2(\phi^2+\zeta^2(\alpha+1))}{(k_1-\phi)^2} - \frac{2\phi v_s}{k_1} + \right. \\ & \left. \left. \frac{v_s(2\phi\beta v_s+1)(\phi^2+\zeta^2(\alpha+1))}{k_1^2} \right) \right], \end{aligned}$$

$$\frac{\partial^2 l(\tau^*)}{\partial \zeta \partial \phi} = \frac{\partial^2 l(\tau^*)}{\partial \phi \partial \zeta},$$

$$\begin{aligned} \frac{\partial^2 l(\tau^*)}{\partial \zeta^2} = \sum_{s=1}^B & \left[(\Psi'(k_6) - \Psi'(k_5)) \times \left(\frac{4\phi^4\zeta^2\alpha^2(\alpha+1)^2}{k_2^4} \right) + (\Psi(k_6) - \Psi(k_5)) \times \left(\frac{8\phi^2\zeta^2\alpha(\alpha+1)^2}{k_2^3} \right) + \right. \\ & (\Psi(k_5) - \Psi(k_6)) \times \left(\frac{2\phi^2\alpha(\alpha+1)}{k_2^2} \right) + \frac{8\phi^2\zeta^2\alpha(\alpha+1)^2(\ln(\phi)-\ln(k_1))}{k_2^3} + \frac{8\phi^2\zeta^2\alpha(\alpha+1)^2\beta v_s}{k_1 k_2^2} - \\ & \frac{2\phi^2\alpha(\alpha+1)(\ln(\phi)-\ln(k_1))}{k_2^2} - \frac{2\phi^2\alpha(\alpha+1)\beta v_s}{k_1 k_2} + \frac{4\phi^2\zeta^2\alpha(\alpha+1)^2\beta^2 v_s^2}{k_1^2 k_2} + \\ & \left. T_s \times \left(\frac{2(\alpha+1)\beta v_s}{k_1-\phi} - \frac{4\zeta^2(\alpha+1)^2\beta^2 v_s^2}{(k_1-\phi)^2} - \frac{2(\alpha+1)\beta v_s}{k_1} + \frac{4\zeta^2(\alpha+1)^2\beta^2 v_s^2}{k_1^2} \right) \right], \end{aligned}$$

$$\begin{aligned} \frac{\partial^2 l(\tau^*)}{\partial \zeta \partial \alpha} = \sum_{s=1}^B & \left[(\Psi'(k_5) - \Psi'(k_6)) \times \left(\frac{2\phi^2\zeta\alpha(\alpha+1)k_8}{k_2^2} \right) + \right. \\ & (\Psi(k_6) - \Psi(k_5)) \times \left(\frac{-2\phi^2\zeta(\alpha+1)}{k_2^2} + \frac{4\phi^2\zeta^3\alpha(\alpha+1)}{k_2^3} - \frac{2\phi^2\zeta\alpha}{k_2^2} \right) - \frac{2\phi^2\zeta(\alpha+1)(\ln(\phi)-\ln(k_1))}{k_2^2} - \\ & \frac{2\phi^2\zeta(\alpha+1)\beta v_s}{k_1 k_2} + \frac{4\phi^2\zeta^3\alpha(\alpha+1)(\ln(\phi)-\ln(k_1))}{k_2^3} + \frac{4\phi^2\zeta^3\alpha(\alpha+1)\beta v_s}{k_1 k_2^2} - \frac{2\phi^2\zeta\alpha(\ln(\phi)-\ln(k_1))}{k_2^2} - \\ & \left. \frac{2\phi^2\zeta\alpha\beta v_s}{k_1 k_2} + \frac{2\phi^2\zeta^3\alpha(\alpha+1)\beta^2 v_s^2}{k_1^2 k_2} + T_s \times \left(\frac{2\zeta\beta v_s}{k_1-\phi} - \frac{2\zeta^3(\alpha+1)\beta^2 v_s^2}{(k_1-\phi)^2} - \frac{2\zeta\beta v_s}{k_1} + \frac{2\zeta^3(\alpha+1)\beta^2 v_s^2}{k_1^2} \right) \right], \end{aligned}$$

$$\begin{aligned} \frac{\partial^2 l(\tau^*)}{\partial \zeta \partial \beta} = \sum_{s=1}^B & \left[\frac{2\phi^2\zeta\alpha(\alpha+1)v_s(\phi^2+\zeta^2(\alpha+1))}{k_1 k_2^2} - \frac{2\phi^2\zeta\alpha(\alpha+1)v_s}{k_1 k_2} + \frac{2\phi^2\zeta\alpha(\alpha+1)\beta v_s^2(\phi^2+\zeta^2(\alpha+1))}{k_1^2 k_2} + \right. \\ & \left. T_s \times \left(\frac{2\zeta(\alpha+1)v_s}{k_1-\phi} - \frac{2\zeta(\alpha+1)\beta v_s^2(\phi^2+\zeta^2(\alpha+1))}{(k_1-\phi)^2} - \frac{2\zeta(\alpha+1)v_s}{k_1} + \frac{2\zeta(\alpha+1)\beta v_s^2(\phi^2+\zeta^2(\alpha+1))}{k_1^2} \right) \right], \end{aligned}$$

$$\frac{\partial^2 l(\tau^*)}{\partial \alpha \partial \phi} = \frac{\partial^2 l(\tau^*)}{\partial \phi \partial \alpha},$$

$$\frac{\partial^2 l(\tau^*)}{\partial \alpha \partial \zeta} = \frac{\partial^2 l(\tau^*)}{\partial \zeta \partial \alpha},$$

$$\begin{aligned} \frac{\partial^2 l(\tau^*)}{\partial \alpha^2} = \sum_{i=1}^A & \left[\Psi'(C_i + \alpha) - \Psi'(\alpha) \right] + \\ & \sum_{s=1}^B \left[(\Psi'(k_6) - \Psi'(k_5)) \times k_8^2 + (\Psi(k_6) - \Psi(k_5)) \times \left(\frac{-2\phi^2\zeta^2}{k_2^2} + \frac{2\phi^2\zeta^4\alpha}{k_2^3} \right) - \right. \\ & \frac{2\phi^2\zeta^2(\ln(\phi)-\ln(k_1))}{k_2^2} - \frac{2\phi^2\zeta^2\beta v_s}{k_1 k_2} + \frac{2\phi^2\zeta^4\alpha(\ln(\phi)-\ln(k_1))}{k_2^3} + \frac{2\phi^2\zeta^4\alpha\beta v_s}{k_1 k_2^2} + \frac{\phi^2\zeta^4\alpha\beta^2 v_s^2}{k_1^2 k_2} + \\ & \left. T_s \times \left(\frac{\zeta^4\beta^2 v_s^2}{k_1^2} - \frac{\zeta^4\beta^2 v_s^2}{(k_1-\phi)^2} \right) \right], \end{aligned}$$

$$\frac{\partial^2 l(\tau^*)}{\partial \alpha \partial \beta} = \sum_{i=1}^A \left[\frac{-u_i}{\beta u_i + 1} \right] +$$

$$\sum_{s=1}^B \left[\frac{-\phi^2 v_s (\phi^2 + \zeta^2 (\alpha+1))}{k_1 k_2} + \frac{\phi^2 \zeta^2 \alpha v_s (\phi^2 + \zeta^2 (\alpha+1))}{k_1 k_2^2} - \frac{\phi^2 \zeta^2 \alpha v_s}{k_1 k_2} + \frac{\phi^2 \zeta^2 \alpha \beta v_s^2 (\phi^2 + \zeta^2 (\alpha+1))}{k_1^2 k_2} \right] +$$

$$T_s \times \left(\frac{\zeta^2 v_s}{k_1 - \phi} - \frac{\zeta^2 \beta v_s^2 (\phi^2 + \zeta^2 (\alpha+1))}{(k_1 - \phi)^2} - \frac{\zeta^2 v_s}{c1} + \frac{\zeta^2 \beta v_s^2 (\phi^2 + \zeta^2 (\alpha+1))}{k_1^2} \right)],$$

$$\frac{\partial^2 l(\vec{\tau}^*)}{\partial \beta \partial \phi} = \frac{\partial^2 l(\vec{\tau}^*)}{\partial \phi \partial \beta},$$

$$\frac{\partial^2 l(\vec{\tau}^*)}{\partial \beta \partial \zeta} = \frac{\partial^2 l(\vec{\tau}^*)}{\partial \zeta \partial \beta},$$

$$\frac{\partial^2 l(\vec{\tau}^*)}{\partial \beta \partial \alpha} = \frac{\partial^2 l(\vec{\tau}^*)}{\partial \alpha \partial \beta}, \quad \text{and}$$

$$\frac{\partial^2 l(\vec{\tau}^*)}{\partial \beta^2} = \sum_{i=1}^A \left[\frac{\alpha u_i^2}{(\beta u_i + 1)^2} + \dot{C}_i \times \left(\frac{u_i^2}{(\beta u_i + 1)^2} - \frac{1}{\beta^2} \right) \right] +$$

$$\sum_{s=1}^B \left[\frac{\phi^2 \alpha v_s^2 k_2}{k_1^2} + T_s \times \left(-\frac{1}{\beta^2} + \frac{v_s^2 k_2^2}{k_1^2} \right) \right].$$

Inverting $H(\vec{\tau})$ or $H^*(\vec{\tau}^*)$

The Newton-Raphson method for obtaining maximum likelihood estimates requires that $H(\vec{\tau})$ (or $H^*(\vec{\tau}^*)$) be inverted at each iteration step. During my simulations, the matrix inversion routine used in the statistical package *Splus* would sometimes fail to produce the inverse of $H(\vec{\tau})$ (or $H^*(\vec{\tau}^*)$). To remedy this situation, I found the formula for inverting a partitioned matrix (Myers 1990) to be useful. To invert $H(\vec{\tau})$ using partitioned matrices let,

$$X_1'X_1 = \begin{pmatrix} \frac{\partial^2 l(\vec{\tau})}{\partial \phi^2} & \frac{\partial^2 l(\vec{\tau})}{\partial \phi \partial \alpha} \\ \frac{\partial^2 l(\vec{\tau})}{\partial \alpha \partial \phi} & \frac{\partial^2 l(\vec{\tau})}{\partial \alpha^2} \end{pmatrix}, \quad X_1'X_2 = \left(\frac{\partial^2 l(\vec{\tau})}{\partial \phi \partial \beta}, \frac{\partial^2 l(\vec{\tau})}{\partial \alpha \partial \beta} \right)', \quad X_2'X_2 = \frac{\partial^2 l(\vec{\tau})}{\partial \beta^2}.$$

Let, $C_{11} = X_1'X_1 - X_1'X_2(X_2'X_2)^{-1}X_2'X_1$ and $C_{22} = X_2'X_2 - X_2'X_1(X_1'X_1)^{-1}X_1'X_2$.

Then,

$$H^{-1}(\vec{\tau}) = \begin{pmatrix} (C_{11})^{-1} & -(X_1'X_1)^{-1}X_1'X_2(C_{22})^{-1} \\ -(C_{22})^{-1}X_2'X_1(X_1'X_1)^{-1} & (C_{22})^{-1} \end{pmatrix}.$$

To invert $H^*(\vec{\tau}^*)$ let,

$$\begin{aligned}
X_1'X_1 &= \begin{pmatrix} \frac{\partial^2 l(\vec{\tau}^*)}{\partial \phi^2} & \frac{\partial^2 l(\vec{\tau}^*)}{\partial \phi \partial \zeta} \\ \frac{\partial^2 l(\vec{\tau}^*)}{\partial \zeta \partial \phi} & \frac{\partial^2 l(\vec{\tau}^*)}{\partial \zeta^2} \end{pmatrix}, & X_1'X_2 &= \begin{pmatrix} \frac{\partial^2 l(\vec{\tau}^*)}{\partial \phi \partial \alpha} & \frac{\partial^2 l(\vec{\tau}^*)}{\partial \phi \partial \beta} \\ \frac{\partial^2 l(\vec{\tau}^*)}{\partial \zeta \partial \alpha} & \frac{\partial^2 l(\vec{\tau}^*)}{\partial \zeta \partial \beta} \end{pmatrix}, \\
X_2'X_1 &= \begin{pmatrix} \frac{\partial^2 l(\vec{\tau}^*)}{\partial \alpha \partial \phi} & \frac{\partial^2 l(\vec{\tau}^*)}{\partial \alpha \partial \zeta} \\ \frac{\partial^2 l(\vec{\tau}^*)}{\partial \beta \partial \phi} & \frac{\partial^2 l(\vec{\tau}^*)}{\partial \beta \partial \zeta} \end{pmatrix}, & X_2'X_2 &= \begin{pmatrix} \frac{\partial^2 l(\vec{\tau}^*)}{\partial \alpha^2} & \frac{\partial^2 l(\vec{\tau}^*)}{\partial \alpha \partial \beta} \\ \frac{\partial^2 l(\vec{\tau}^*)}{\partial \beta \partial \alpha} & \frac{\partial^2 l(\vec{\tau}^*)}{\partial \beta^2} \end{pmatrix}.
\end{aligned}$$

Let C_{11} and C_{22} be defined as above. Then,

$$[H^*(\vec{\tau}^*)]^{-1} = \begin{pmatrix} (C_{11})^{-1} & -(X_1'X_1)^{-1}X_1'X_2(C_{22})^{-1} \\ -(C_{22})^{-1}X_2'X_1(X_1'X_1)^{-1} & (C_{22})^{-1} \end{pmatrix}.$$

The Mean and Variance of the Log Gamma Distribution

Result: Let θ be distributed gamma(mean = α , variance = $\alpha\beta^2$). Then,

$$E(\ln(\theta)) = \Psi(\alpha) + \ln(\beta) \quad \text{and} \quad \text{Var}(\ln(\theta)) = \Psi'(\alpha).$$

The derivation of the expectation and variance of the natural log of a gamma random variable is given below (R. Boik, personal communication, 1996) :

Proof: Because $\Gamma(\alpha) = \int_0^\infty \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} d\theta$,

$$\begin{aligned}
\Psi(\alpha) &= \frac{\partial \ln(\Gamma(\alpha))}{\partial \alpha} = \frac{1}{\Gamma(\alpha)} \int_0^\infty \frac{\partial}{\partial \alpha} \left(\frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} \right) d\theta \\
&= \int_0^\infty \left[\ln(\theta) \times \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\Gamma(\alpha)\beta^\alpha} + \ln\left(\frac{1}{\beta}\right) \times \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\Gamma(\alpha)\beta^\alpha} \right] d\theta \\
&= E(\ln(\theta)) - \ln(\beta) \\
&\Rightarrow E(\ln(\theta)) = \Psi(\alpha) + \ln(\beta).
\end{aligned}$$

$$\begin{aligned}
\Psi'(\alpha) &= \frac{\partial \Psi(\alpha)}{\partial \alpha} = \frac{\partial (E(\ln(\theta)) - \ln(\beta))}{\partial \alpha} \\
&= \frac{\partial}{\partial \alpha} \frac{1}{\Gamma(\alpha)} \int_0^\infty \ln(\theta) \times \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} d\theta
\end{aligned}$$

$$\begin{aligned}
&= \frac{1}{\Gamma(\alpha)} \left[\int_0^\infty [(\ln(\theta))^2 \times \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} + \ln(\theta) \ln\left(\frac{1}{\beta}\right) \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha}] d\theta \right] \\
&\quad \left[\int_0^\infty \ln(\theta) \times \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} d\theta \right] \times \frac{\partial \Gamma(\alpha) / \partial \alpha}{(\Gamma(\alpha))^2} (-1). \\
\text{But, } \frac{\partial \Gamma(\alpha)}{\partial \alpha} &= \frac{\partial}{\partial \alpha} \left[\int_0^\infty \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} d\theta \right] \\
&= \left[\int_0^\infty \frac{\partial}{\partial \alpha} \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} d\theta \right] \\
&= \int_0^\infty \left[\ln(\theta) \times \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} + \ln\left(\frac{1}{\beta}\right) \times \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\beta^\alpha} \right] d\theta \\
&= \Gamma(\alpha) [E(\ln(\theta)) - \ln(\beta)]. \\
\text{Thus, } \Psi'(\alpha) &= E[(\ln(\theta))^2] - \ln(\beta) E[\ln(\theta)] - \frac{E[\ln(\theta)] \Gamma(\alpha) (E[\ln(\theta)] - \ln(\beta))}{\Gamma(\alpha)} \\
&= E[(\ln(\theta))^2] - (E[\ln(\theta)])^2 = \text{Var}[\ln(\theta)].
\end{aligned}$$

It should be noted that the derivation above required the differentiation under the integral sign in numerous instances. The differentiation under the integral sign is valid because the gamma distribution is a member of the exponential family in which case, for any integrable function, h , the integral

$$\int_0^\infty h(\theta) \frac{e^{-\frac{\theta}{\beta}} \theta^{\alpha-1}}{\Gamma(\alpha) \beta^\alpha} d\theta$$

is continuous and has derivatives of all orders with respect to α or β , and these can be obtained by differentiating under the integral sign (Lehmann 1991).

Approximating a Log Negative Binomial with a Log Gamma

To obtain an approximation to the log of a negative binomial (adjusting for zeros), one can match the first two moments of a negative binomial and a gamma. For a negative binomial distribution with mean $\frac{rq}{p}$ and variance $\frac{rq}{p^2}$, set $\alpha = rq$, and $\beta = \frac{1}{p}$. Then the expectation and variance of the log of a negative binomial are

approximated by $\frac{\Psi(rq) + \ln(\frac{1}{p})}{\ln(10)}$ and $\frac{\Psi'(rq)}{(\ln(10))^2}$, respectively.

MLE of ϕ for the Model with no Over-dispersion

Result: Assume that the dilution sampled, u , is constant across check carriers and the dilution sampled, v , is constant across test carriers. Then the mle of ϕ is $\frac{\bar{Y}}{\bar{X}}$.

Proof (R. Boik, personal communication, 1994):

$$\begin{aligned} \frac{\partial l(\bar{\tau})}{\partial \phi} = 0 &\Rightarrow \frac{\sum_{s=1}^B [T_s - \phi \alpha \beta v]}{\phi(\phi \beta v + 1)} = 0 \Rightarrow \sum_{s=1}^B T_s = B \phi \alpha \beta v \\ &\Rightarrow \phi = \frac{\sum_{s=1}^B T_s}{B \alpha \beta v} = \frac{\bar{Y}}{\alpha \beta}, \\ \frac{\partial l(\bar{\tau})}{\partial \beta} = 0 &\Rightarrow \frac{\sum_{i=1}^A [C_i - \alpha \beta u]}{\beta(\beta u + 1)} + \frac{\sum_{s=1}^B [T_s - \phi \alpha \beta v]}{\beta(\phi \beta v + 1)} = 0. \end{aligned}$$

Substituting $\frac{\bar{Y}}{\alpha \beta}$ for ϕ implies that $\phi \beta v = \frac{\bar{Y}_v}{\alpha}$. Thus,

$$\begin{aligned} \frac{\sum_{i=1}^A [C_i - \alpha \beta u]}{\beta(\beta u + 1)} + \frac{\sum_{s=1}^B [T_s - \bar{Y}_v]}{\beta(\bar{Y}_v / \alpha + 1)} = 0 &\Rightarrow \frac{\sum_{i=1}^A [C_i - \alpha \beta u]}{\beta(\beta u + 1)} + 0 = 0 \\ &\Rightarrow \sum_{i=1}^A C_i = A \alpha \beta u \Rightarrow \beta = \frac{\bar{X}}{\alpha}. \end{aligned}$$

$$\text{Plugging } \beta = \frac{\bar{X}}{\alpha} \text{ into } \phi = \frac{\bar{Y}}{\alpha \beta} \Rightarrow \phi = \frac{\bar{Y}}{\bar{X}} \text{ as claimed.}$$

Asymptotic Distribution of Estimators of LR

For convenience, assume that the dilution sampled, u , is constant across check carriers and the dilution sampled, v , is constant across test carriers. Let $R = \frac{B}{A}$ be the ratio of the sample sizes; B to A , and let A and B tend to infinity with constant ratio, R . A few results will be needed to derive the asymptotic distributions of the estimators of LR.

Theorem 1.5 page 337, (Lehmann 1991) states that if

$$\sqrt{n}[T_n - \theta] \xrightarrow{L} N(0, \gamma^2), \text{ then } \sqrt{n}[g(T_n) - g(\theta)] \xrightarrow{L} N(0, \gamma^2[g'(\theta)]^2)$$

assuming $g'(\theta)$ exists and is not zero.

Theorem 14.6-2 (Multivariate delta method; page 493, Bishop, Fienberg, and Holland 1975) states that, assuming the usual regularity conditions on g hold, if

$$\sqrt{n}[\hat{\tau} - \bar{\tau}] \xrightarrow{L} \text{MVN}(0, \Sigma) \quad \text{then} \quad \sqrt{n}[g(\hat{\tau}) - g(\bar{\tau})] \xrightarrow{L} \text{MVN}\left(0, \left(\frac{\partial g}{\partial \bar{\tau}}\right)' \Sigma \left(\frac{\partial g}{\partial \bar{\tau}}\right)\right).$$

Model with no Over-dispersion

Denote the probability that a check carrier takes on the value c as $p_1(c)$. Denote the probability that a test carrier takes on the value t as $p_2(t)$. By the Central Limit Theorem (CLTH),

$$\sqrt{A} [\bar{X}^* - E(\bar{X}^*)] \xrightarrow{L} N(0, \sigma_{X^*}^2) \quad \text{and} \quad \sqrt{A} [\bar{Y}^* - E(\bar{Y}^*)] \xrightarrow{L} N\left(0, \frac{\sigma_{Y^*}^2}{R}\right).$$

The formulas for $\sigma_{X^*}^2$ and $\sigma_{Y^*}^2$ are given below:

$$\sigma_{X^*}^2 = \sum_{c=1}^{\infty} (\log(c))^2 p_1(c) - [\sum_{c=1}^{\infty} \log(c) p_1(c)]^2 \quad \text{and}$$

$$\sigma_{Y^*}^2 = \sum_{t=1}^{\infty} (\log(t))^2 p_2(t) - [\sum_{t=1}^{\infty} \log(t) p_2(t)]^2. \quad \text{Thus,}$$

$$\sqrt{A} [\hat{\Upsilon}_{1, \text{MOM}} - \Upsilon_1] \xrightarrow{L} N\left(0, \sigma_{X^*}^2 + \frac{\sigma_{Y^*}^2}{R}\right).$$

By the CLTH,

$$\sqrt{A} [\bar{X} - E(\bar{X})] \xrightarrow{L} N\left(0, \frac{\alpha\beta(\beta u + 1)}{u}\right) \quad \text{and}$$

$$\sqrt{A} [\bar{Y} - E(\bar{Y})] \xrightarrow{L} N\left(0, \frac{\phi\alpha\beta(\phi\beta v + 1)}{Rv}\right).$$

Applying theorem 1.5 from (Lehmann 1991) and using the fact that the sum of independent normal random variables is again normal gives the following result,

$$\sqrt{A} [\hat{\Upsilon}_{2, \text{MOM}} - \Upsilon_2] \xrightarrow{L} N\left(0, [\log(e)]^2 \times \left[\frac{R\phi v(\beta u + 1) + u(\phi\beta v + 1)}{R\phi\alpha\beta uv}\right]\right).$$

Before establishing the asymptotic distributions of $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}$ and $\hat{\Upsilon}_{2, \text{MLE}_{\text{NO}}}$ some background work is helpful. Denote by Σ the variance-covariance matrix of $\hat{\tau}$.

Because the usual regularity conditions hold, $\Sigma = -E[H(\bar{\tau})]^{-1}$. Let

$$K = A [(R+1)\Psi'(\alpha) - E(\Psi'(C+\alpha)) - R \times E(\Psi'(T+\alpha))],$$

and let

$$k_9 = K\alpha - \frac{\beta u}{\beta u + 1} - \frac{R\phi\beta b}{\phi\beta v + 1}.$$

Then,

$$\Sigma = \frac{1}{A} \times \begin{pmatrix} \frac{(\phi u)(\phi\beta v + 1) + R\phi^2 v(\beta u + 1)}{R\alpha\beta uv} & 0 & \frac{-\phi(\beta u + 1)}{\alpha u} \\ 0 & \frac{\alpha}{k_9} & \frac{-\beta}{k_9} \\ \frac{-\phi(\beta u + 1)}{\alpha u} & \frac{-\beta}{k_9} & \frac{\beta(k_9(\beta u + 1) + \beta u(\phi\beta v + 1))}{k_9(\phi\beta v + 1)\alpha u} \end{pmatrix}, \quad \text{and}$$

$$\sqrt{A} [\hat{\tau} - \bar{\tau}] \xrightarrow{L} \text{MVN}(0, A\Sigma).$$

The multivariate delta method is applicable to determine the asymptotic distribution of $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}$ because $\hat{\tau}$ is asymptotically multivariate normal and g_1 and g_2 are continuous functions. If equation (2.18) is used to calculate $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}$ then $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}} = g_1(\hat{\phi}, \hat{\alpha}, \hat{\beta})$ where,

$$g_1(\hat{\phi}, \hat{\alpha}, \hat{\beta}) = \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u}{\hat{\beta}u+1}\right) + \ln(\hat{\beta}u+1)}{\ln(10)} - \frac{\Psi\left(\frac{\hat{\phi}\hat{\alpha}\hat{\beta}v}{\hat{\phi}\hat{\beta}v+1}\right) + \ln(\hat{\phi}\hat{\beta}v+1)}{\ln(10)} + \log\left(\frac{v}{u}\right).$$

The asymptotic distribution of $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}$ is (assuming (2.18)),

$$\sqrt{A} [\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}} - \Upsilon_1] \xrightarrow{L} \text{MVN}\left(0, \left(\frac{\partial g_1}{\partial \bar{\tau}}\right)' A\Sigma \left(\frac{\partial g_1}{\partial \bar{\tau}}\right)\right), \quad \text{where}$$

$$\frac{\partial g_1}{\partial \phi} = \log(e) \times \left(-\left[\Psi'\left(\frac{\phi\alpha\beta v}{\phi\beta v+1}\right)\right]\left(\frac{\alpha\beta v}{(\phi\beta v+1)^2}\right) - \frac{\beta v}{\phi\beta v+1}\right),$$

$$\frac{\partial g_1}{\partial \alpha} = \log(e) \times \left(\frac{\beta u \Psi'\left(\frac{\alpha\beta u}{\beta u+1}\right)}{\beta u+1} - \frac{\phi\beta v \Psi'\left(\frac{\phi\alpha\beta v}{\phi\beta v+1}\right)}{\phi\beta v+1}\right), \quad \text{and}$$

$$\frac{\partial g_1}{\partial \beta} = \log(e) \times \left(\frac{\alpha u \Psi'\left(\frac{\alpha\beta u}{\beta u+1}\right)}{(\beta u+1)^2} + \frac{u}{\beta u+1} - \frac{\phi\alpha v \Psi'\left(\frac{\phi\alpha\beta v}{\phi\beta v+1}\right)}{(\phi\beta v+1)^2} - \frac{\phi v}{\phi\beta v+1}\right).$$

If equation (2.19) is used to calculate $\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}}$ then,

$$\hat{\Upsilon}_{1, \text{MLE}_{\text{NO}}} = g_2(\hat{\phi}, \hat{\alpha}, \hat{\beta}) = \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u}{\hat{\beta}u+1}\right) + \ln(\hat{\beta}u+1)}{\ln(10)} + \log\left(\frac{v}{u}\right) -$$

$$\sum_{t=1}^N \log(t) \left(\frac{\Gamma(t+\hat{\alpha})}{\Gamma(\hat{\alpha})t!}\right) + \left(\frac{\hat{\phi}\hat{\beta}v}{\hat{\phi}\hat{\beta}v+1}\right)^t + \left(\frac{1}{\hat{\phi}\hat{\beta}v+1}\right)^{\hat{\alpha}}.$$

Denote the probability that a test carrier takes on the value t as $p_2(t)$. The asymptotic distribution of $\hat{\Upsilon}_{1,MLE_{NO}}$ is (assuming (2.19)),

$$\sqrt{A} [\hat{\Upsilon}_{1,MLE_{NO}} - \Upsilon_1] \xrightarrow{L} MVN \left(0, \left(\frac{\partial g_2}{\partial \vec{\tau}} \right)' A \Sigma \left(\frac{\partial g_2}{\partial \vec{\tau}} \right) \right), \text{ where}$$

$$\frac{\partial g_2}{\partial \phi} = - \sum_{t=1}^N \log(t) p_2(t) \times \left[\frac{\phi \alpha \beta v - t}{\phi(\phi \beta v + 1)} \right],$$

$$\frac{\partial g_2}{\partial \alpha} =$$

$$\log(e) \times \left(\frac{\beta u \Psi' \left(\frac{\alpha \beta u}{\beta u + 1} \right)}{\beta u + 1} \right) - \left(\sum_{t=1}^N \log(t) p_2(t) \times \left[\Psi(t + \alpha) - \Psi(\alpha) + \ln \left(\frac{1}{\phi \beta v + 1} \right) \right] \right), \text{ and}$$

$$\frac{\partial g_2}{\partial \beta} = \log(e) \times \left(\frac{\alpha u \Psi' \left(\frac{\alpha \beta u}{\beta u + 1} \right)}{(\beta u + 1)^2} + \frac{u}{\beta u + 1} \right) - \left(\sum_{t=1}^N \log(t) p_2(t) \times \left[\frac{\phi \alpha \beta v - t}{\beta(\phi \beta v + 1)} \right] \right).$$

The multivariate delta method was used to determine the asymptotic distribution of $\hat{\Upsilon}_{2,MLE_{NO}}$,

$$\sqrt{A} [\hat{\Upsilon}_{2,MLE_{NO}} - \Upsilon_2] \xrightarrow{L} N \left(0, [\log(e)]^2 \times \left(\frac{R \phi v (\beta u + 1) + u (\phi \beta v + 1)}{R \phi \alpha \beta u v} \right) \right).$$

Efficiency Calculations for the Model with no Over-dispersion

$$ARE(\hat{\Upsilon}_{1,MOM} : \hat{\Upsilon}_{1,MLE_{NO}}) = \begin{cases} \frac{\left(\frac{\partial g_1}{\partial \vec{\tau}} \right)' A \Sigma \left(\frac{\partial g_1}{\partial \vec{\tau}} \right)}{\sigma_{X^*}^2 + \sigma_{Y^*}^2 / R}, & \text{when (2.18) is used for } \hat{\Upsilon}_{1,MLE_{NO}} \\ \frac{\left(\frac{\partial g_2}{\partial \vec{\tau}} \right)' A \Sigma \left(\frac{\partial g_2}{\partial \vec{\tau}} \right)}{\sigma_{X^*}^2 + \sigma_{Y^*}^2 / R}, & \text{when (2.19) is used for } \hat{\Upsilon}_{1,MLE_{NO}}, \end{cases}$$

$$ARE(\hat{\Upsilon}_{2,MOM} : \hat{\Upsilon}_{2,MLE_{NO}}) = \frac{[\log(e)]^2 \times \left(\frac{R \phi v (\beta u + 1) + u (\phi \beta v + 1)}{R \phi \alpha \beta u v} \right)}{[\log(e)]^2 \times \left(\frac{R \phi v (\beta u + 1) + u (\phi \beta v + 1)}{R \phi \alpha \beta u v} \right)} = 1,$$

$$ARE(\hat{\Upsilon}_{1,MOM} : \hat{\Upsilon}_{2,MOM}) = \frac{\frac{\sigma_{X^*}^2 + \sigma_{Y^*}^2 / R}{\tau_1^2}}{[\log(e)]^2 \times \left(\frac{R \phi v (\beta u + 1) + u (\phi \beta v + 1)}{(R \phi \alpha \beta u v) \times \tau_2^2} \right)}, \text{ and}$$

$$ARE(\hat{\Upsilon}_{1,MLE_{NO}} : \hat{\Upsilon}_{2,MLE_{NO}}) = \begin{cases} \frac{\frac{\left(\frac{\partial g_1}{\partial \vec{\tau}} \right)' A \Sigma \left(\frac{\partial g_1}{\partial \vec{\tau}} \right)}{\tau_1^2}}{[\log(e)]^2 \times \left(\frac{R \phi v (\beta u + 1) + u (\phi \beta v + 1)}{(R \phi \alpha \beta u v) \times \tau_2^2} \right)}, & \text{assuming (2.18)} \\ \frac{\frac{\left(\frac{\partial g_2}{\partial \vec{\tau}} \right)' A \Sigma \left(\frac{\partial g_2}{\partial \vec{\tau}} \right)}{\tau_1^2}}{[\log(e)]^2 \times \left(\frac{R \phi v (\beta u + 1) + u (\phi \beta v + 1)}{(R \phi \alpha \beta u v) \times \tau_2^2} \right)}, & \text{assuming (2.19)}. \end{cases}$$

It should be noted that σ_{X*}^2 , σ_{Y*}^2 , and Σ all had to be approximated for efficiency calculations. For example, to approximate σ_{X*}^2 I determined a value N such that, $\left[\sum_{c=1}^N c \times p_1(c)\right] - \alpha\beta u < 0.00001$. Then σ_{X*}^2 was approximated by the following: $\sum_{c=1}^N (\log(c))^2 p_1(c) - \left[\sum_{c=1}^N \log(c) p_1(c)\right]^2$. A similar strategy was used to approximate σ_{Y*}^2 and Σ .

Model with Over-dispersion

The asymptotic distributions of the estimators of LR are derived in a similar manner for the model with over-dispersion as for the model with no over-dispersion. For sake of brevity most of the derivations will not be repeated here. Denote the probability that a check carrier takes on the value c as $p_1(c)$. Denote the probability that a test carrier takes on the value t as $p_2^*(t)$. Note that the pmf for check carriers is the same regardless if the model with or without over-dispersion is used, however the pmf for test carriers is dependent upon which model is chosen. The asymptotic distribution of $\hat{\Upsilon}_{1,MOM}$ is,

$$\sqrt{A} \left[\hat{\Upsilon}_{1,MOM} - \Upsilon_1 \right] \xrightarrow{L} N \left(0, \sigma_{X*}^2 + \frac{\sigma_{Y*}^2}{R} \right).$$

Note that σ_{X*}^2 , which is a function of $p_1(c)$, is calculated as in the model with no over-dispersion, but $\sigma_{Y*}^2 = \sum_{t=1}^{\infty} [\log(t)]^2 p_2^*(t) - \left[\sum_{t=1}^{\infty} \log(t) p_2^*(t) \right]^2$. The asymptotic distribution of $\hat{\Upsilon}_{2,MOM}$ is given as follows: $\sqrt{A} \left[\hat{\Upsilon}_{2,MOM} - \Upsilon_2 \right] \xrightarrow{L}$

$$N \left(0, [\log(e)]^2 \times \left(\frac{R\phi v (\beta u + 1) + u (\phi \beta v (1 + \zeta^2 (\alpha + 1) / \phi^2) + 1)}{R\phi \alpha \beta u v} \right) \right).$$

Denote as Σ^* the variance-covariance matrix of $\hat{\tau}^*$. Because the usual regularity conditions hold, $\sqrt{A} \left[\hat{\tau}^* - \tau^* \right] \xrightarrow{L} MVN(0, A\Sigma^*)$, where $\Sigma^* = -[E(H^*(\tau))]^{-1}$. The form of Σ^* is not easily written down symbolically, but it can be well approximated. If equation (2.20) is used to calculate $\hat{\Upsilon}_{1,MLEOVER}$ then,

$\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}} = g_3(\hat{\phi}, \hat{\alpha}, \hat{\beta}, \hat{\zeta})$ where,

$$g_3(\hat{\phi}, \hat{\alpha}, \hat{\beta}, \hat{\zeta}) = \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u}{\hat{\beta}u+1}\right) + \ln(\hat{\beta}u+1)}{\ln(10)} - \frac{\Psi\left(\frac{\hat{\phi}\hat{\alpha}\hat{\beta}v}{\hat{\phi}\hat{\beta}\hat{d}v+1}\right) + \ln(\hat{\phi}\hat{\beta}\hat{d}v+1)}{\ln(10)} + \log\left(\frac{v}{u}\right).$$

The asymptotic distribution of $\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}}$ can be determined using the multivariate delta method, and is, (assuming (2.20)),

$$\sqrt{A} [\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}} - \Upsilon_1] \xrightarrow{L} \text{MVN} \left(0, \left(\frac{\partial g_3}{\partial \vec{\tau}^*} \right)' A \Sigma^* \left(\frac{\partial g_3}{\partial \vec{\tau}^*} \right) \right).$$

For notational convenience, let

$$k_1 = \beta v (\phi^2 + \zeta^2 (\alpha + 1)) + \phi, \quad \text{and}$$

$$k_2 = \phi^2 + \zeta^2 (\alpha + 1). \quad \text{Then}$$

$$\frac{\partial g_3}{\partial \phi} = \log(e) \times \left[-\Psi' \left(\frac{\phi^2 \alpha \beta v}{k_1} \right) \left(\frac{2\phi \alpha \beta v}{k_1} - \frac{\phi^2 \alpha \beta v (2\phi \beta v + 1)}{k_1^2} \right) - \frac{2\phi \beta v + 1 - k_1/\phi}{k_1} \right],$$

$$\frac{\partial g_3}{\partial \zeta} = \log(e) \times \left[\frac{2\phi^2 \zeta \alpha (\alpha + 1) \beta^2 v^2 \Psi' \left(\frac{\phi^2 \alpha \beta v}{k_1} \right)}{k_1^2} - \frac{2\zeta \beta (\alpha + 1) v}{k_1} \right],$$

$$\frac{\partial g_3}{\partial \alpha} = \log(e) \times \left[\frac{\beta u \Psi' \left(\frac{\alpha \beta u}{\beta u + 1} \right)}{\beta u + 1} - \left(\frac{\phi^2 \beta v}{k_1} - \frac{\phi^2 \zeta^2 \alpha \beta^2 v^2}{k_1^2} \right) \Psi' \left(\frac{\phi^2 \alpha \beta v}{k_1} \right) - \frac{\zeta^2 \beta v}{k_1} \right], \quad \text{and}$$

$$\frac{\partial g_3}{\partial \beta} = \log(e) \times \left[\frac{\alpha u \Psi' \left(\frac{\alpha \beta u}{\beta u + 1} \right)}{(\beta u + 1)^2} + \frac{u}{\beta u + 1} - \left(\frac{\phi^2 \alpha v}{k_1} - \frac{\phi^2 \alpha v [k_1 - \phi]}{k_1^2} \right) \Psi' \left(\frac{\phi^2 \alpha \beta v}{k_1} \right) - \frac{\phi^2 v + \zeta^2 (\alpha + 1) v}{k_1} \right].$$

If equation (2.21) is used to calculate $\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}}$ then

$$\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}} = g_4(\hat{\phi}, \hat{\alpha}, \hat{\beta}, \hat{\zeta}); \quad \text{where } g_4(\hat{\phi}, \hat{\alpha}, \hat{\beta}, \hat{\zeta}) = \log\left(\frac{v}{u}\right) + \frac{\Psi\left(\frac{\hat{\alpha}\hat{\beta}u}{\hat{\beta}u+1}\right) + \ln(\hat{\beta}u+1)}{\ln(10)} - \sum_{t=1}^N \log(t) \left(\frac{\Gamma\left(t + \frac{\hat{\alpha}}{d}\right)}{\Gamma\left(\frac{\hat{\alpha}}{d}\right)} \right) \left(\frac{\hat{\phi}\hat{\beta}\hat{d}v}{\hat{\phi}\hat{\beta}\hat{d}v+1} \right)^t \left(\frac{1}{\hat{\phi}\hat{\beta}\hat{d}v+1} \right)^{\frac{\hat{\alpha}}{d}}.$$

The asymptotic distribution of $\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}}$ is, (assuming (2.21)),

$$\sqrt{A} [\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}} - \Upsilon_1] \xrightarrow{L} \text{MVN} \left(0, \left(\frac{\partial g_4}{\partial \vec{\tau}^*} \right)' A \Sigma^* \left(\frac{\partial g_4}{\partial \vec{\tau}^*} \right) \right).$$

$$\frac{\partial g_4}{\partial \phi} = -\sum_{t=1}^N \log(t) p_2^*(t) [(\Psi(t + \frac{\alpha}{d}) - \Psi(\frac{\alpha}{d}) + \ln(\frac{\phi}{k_1})) \times (\frac{2\phi\alpha}{k_2} - \frac{2\phi^3\alpha}{k_2^2}) +$$

$$\frac{\phi\alpha(1-\phi(2\phi\beta v+1)/k_1)}{k_2} + t\left(\frac{2\phi}{k_2} - \frac{2\phi\beta v+1}{k_1}\right)],$$

$$\frac{\partial g_4}{\partial \zeta} = -2 \times \sum_{t=1}^N \log(t) p_2^*(t) \left[\left(\Psi\left(\frac{\alpha}{d}\right) - \Psi\left(t + \frac{\alpha}{d}\right) \right) \times \frac{\zeta\alpha(\alpha+1)}{d \times k_2} - \frac{2\zeta\alpha(\alpha+1)}{d} \times \left(\frac{\ln(\phi/k_1)}{k_2} + \frac{\beta v}{k_1} \right) + t\left(\frac{\zeta(\alpha+1)}{k_2} - \frac{\zeta(\alpha+1)\beta v}{k_1}\right) \right],$$

$$\frac{\partial g_4}{\partial \alpha} = \log(e) \times \frac{\beta u}{\beta u+1} \Psi'\left(\frac{\alpha\beta u}{\beta u+1}\right) - \left[\sum_{t=1}^N \log(t) p_2^*(t) \left[\left(\Psi\left(t + \frac{\alpha}{d}\right) - \Psi\left(\frac{\alpha}{d}\right) \right) + \ln\left(\frac{\phi}{k_1}\right) \times \left(\frac{1}{d} - \frac{\zeta^2\alpha}{dk_2} \right) - \frac{\zeta^2\alpha\beta v}{dk_1} + t\left(\frac{\zeta^2}{k_2} - \frac{\zeta^2\beta v}{k_1}\right) \right] \right], \quad \text{and}$$

$$\frac{\partial g_4}{\partial \beta} = \log(e) \times \left[\frac{\alpha u}{\beta u+1} \Psi'\left(\frac{\alpha\beta u}{\beta u+1}\right) + \frac{u}{\beta u+1} \right] - \left[\sum_{t=1}^N \log(t) p_2^*(t) \left[\frac{\phi^2\alpha v}{k_1} + t\left(\frac{1}{\beta} - \frac{k_2 v}{k_1}\right) \right] \right].$$

The multivariate delta method is applicable to determine the asymptotic distribution of $\hat{\Upsilon}_{2, \text{MLE}_{\text{OVER}}}$. Let $\Sigma^*_{[1,1]}$ denote the one-one element of Σ^* . Note that $\Sigma^*_{[1,1]}$ is the asymptotic variance of $\hat{\phi}$. Then the asymptotic distribution of $\hat{\Upsilon}_{2, \text{MLE}_{\text{OVER}}}$ is,

$$\sqrt{A} \left[\hat{\Upsilon}_{2, \text{MLE}_{\text{OVER}}} - \Upsilon_2 \right] \xrightarrow{L} \text{MVN} \left(0, \log(e)^2 \times \frac{A \Sigma^*_{[1,1]}}{\phi^2} \right).$$

Efficiency Calculations for the Model with Over-dispersion

$$\text{ARE} \left(\hat{\Upsilon}_{1, \text{MOM}} : \hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}} \right) = \begin{cases} \frac{\left(\frac{\partial g_3}{\partial \beta^*} \right)' A \Sigma^* \left(\frac{\partial g_3}{\partial \beta^*} \right)}{\sigma_{X^*}^2 + \sigma_{Y^*}^2 / R}, & \text{assuming (2.20)} \\ \frac{\left(\frac{\partial g_4}{\partial \beta^*} \right)' A \Sigma^* \left(\frac{\partial g_4}{\partial \beta^*} \right)}{\sigma_{X^*}^2 + \sigma_{Y^*}^2 / R}, & \text{assuming (2.21)}, \end{cases}$$

$$\text{ARE} \left(\hat{\Upsilon}_{2, \text{MOM}} : \hat{\Upsilon}_{2, \text{MLE}_{\text{OVER}}} \right) = \frac{\frac{A \Sigma^*_{[1,1]}}{\phi^2}}{\frac{R\phi v(\beta u+1) + u(\phi\beta v(1+\zeta^2(\alpha+1)/\phi^2)+1)}{R\phi\alpha\beta uv}},$$

$$\text{ARE} \left(\hat{\Upsilon}_{1, \text{MOM}} : \hat{\Upsilon}_{2, \text{MOM}} \right) = \frac{\frac{\sigma_{X^*}^2 + \sigma_{Y^*}^2 / R}{\Upsilon_2^2}}{[\log(e)]^2 \times \left(\frac{R\phi v(\beta u+1) + u(\phi\beta v(1+\zeta^2(\alpha+1)/\phi^2)+1)}{(R\phi\alpha\beta uv) \times \Upsilon_2^2} \right)}, \quad \text{and}$$

$$\text{ARE} \left(\hat{\Upsilon}_{1, \text{MLE}_{\text{OVER}}} : \hat{\Upsilon}_{2, \text{MLE}_{\text{OVER}}} \right) = \begin{cases} \frac{\left(\frac{\partial g_3}{\partial \beta^*} \right)' A \Sigma^* \left(\frac{\partial g_3}{\partial \beta^*} \right)}{\frac{\Upsilon_2^2}{\frac{A \Sigma^*_{[1,1]}}{\phi^2 \Upsilon_2^2}}}, & \text{assuming (2.20)} \\ \frac{\left(\frac{\partial g_4}{\partial \beta^*} \right)' A \Sigma^* \left(\frac{\partial g_4}{\partial \beta^*} \right)}{\frac{\Upsilon_2^2}{\frac{A \Sigma^*_{[1,1]}}{\phi^2 \Upsilon_2^2}}}, & \text{assuming (2.21)}. \end{cases}$$

It should be noted that σ_{X*}^2 , σ_{Y*}^2 , and Σ^* all had to be approximated for efficiency calculations. Guidance on approximating these quantities is given in the section on *Efficiency Calculations for the Model with No Over-dispersion* of this appendice.

APPENDIX B

Appendix Material for the Presence/Absence Assay

Derivation of the Marginal Distribution of $\vec{r}_i$

Suppose a serial-dilution experiment is to be performed. Let the distribution of the number of organisms originally in suspension be denoted by $g(\theta)$, where θ is a non-negative random variable with moment generating function $m_\theta(t)$. Let $\vec{r}$ and $\vec{u}$ denote vectors of the number of wells responding positive and the volumes sampled, respectively. Let the number of wells assayed at dilution j be denoted as n_j .

Theorem 1: The marginal distribution of $\vec{r}$, denoted by $f(\vec{r})$, is given as:

$$f(\vec{r}) = \left[\prod_j \binom{n_j}{r_j} \right] \left(\sum_{L=1}^M \gamma_L \times m_\theta(-\xi_L) \right)$$

where M , γ_L , and ξ_L are constants which are calculable.

Proof:

For convenience, assume θ is a continuous random variable, although the proof works equally well for θ discrete. The conditional distribution of $\vec{r}$ given θ is given in (3.11) and is repeated here for ease of reading.

$$f(\vec{r} | \theta) = \prod_{j=1} \binom{n_j}{r_j} (1 - e^{-\theta \cdot u_j})^{r_j} (e^{-\theta \cdot u_j})^{n_j - r_j}.$$

The marginal distribution of $\vec{r}$ is obtained by integrating out θ ; i.e.

$$f(\vec{r}) = \int_0^\infty f(\vec{r} | \theta) \times g(\theta) d\theta.$$

$$\text{Thus, } f(\vec{r}) = \left[\prod_j \binom{n_j}{r_j} \right] \int_0^\infty \prod_j (1 - e^{-\theta \cdot u_j})^{r_j} (e^{-\theta \cdot u_j})^{n_j - r_j} \times g(\theta) d\theta.$$

Note that $(1 - t)^p = \sum_{k=0}^p \binom{p}{k} (-1)^k (t)^k$ (Mood, Graybill, and Boes 1974), hence

$$(1 - e^{-\theta \cdot u_j})^{r_j} = \sum_{k=0}^{r_j} \binom{r_j}{k} (-1)^k (e^{-\theta \cdot u_j})^k.$$

$$\text{So, } f(\vec{r}) = \left[\prod_j \binom{n_j}{r_j} \right] \int_0^\infty \prod_j \left[\sum_{k=0}^{r_j} \binom{r_j}{k} (-1)^k (e^{-\theta \cdot u_j})^k \right] (e^{-\theta \cdot u_j})^{n_j - r_j} \times g(\theta) d\theta$$

$$= \left[\prod_j \binom{n_j}{r_j} \right] \int_0^\infty \prod_j \left[\sum_{k=0}^{r_j} \binom{r_j}{k} (-1)^k e^{-\theta \cdot [u_j \cdot (k + n_j - r_j)]} \right] \times g(\theta) d\theta.$$

Let $M = \prod_j (r_j + 1)$. Notice there are M terms involved in $f(\vec{r})$. Realizing that $(a \cdot e^c) \times (b \cdot e^d) = ab \times e^{c+d}$ for all constants a, b, c , and d , $f(\vec{r})$ can be written as:

$$f(\vec{r}) = \left[\prod_j \binom{n_j}{r_j} \right] \int_0^\infty \left[\sum_{L=1}^M \gamma_L \times e^{-\theta \xi_L} \right] g(\theta) d\theta$$

where γ_L and ξ_L are appropriate constants. Notice that the γ_L terms are formed from the products of the coefficients from the binomial expansions of the $(1 - e^{-\theta \cdot u_j})^{r_j}$ terms. The ξ_L terms are formed from the products of the appropriate exponential terms. Thus,

$$f(\vec{r}) = \left[\prod_j \binom{n_j}{r_j} \right] \sum_{L=1}^M \gamma_L \int_0^\infty e^{-\theta \xi_L} \times g(\theta) d\theta$$

because integration is a linear operator. Notice that $\int_0^\infty e^{-\theta \xi_L} \times g(\theta) d\theta$ is the moment generating function of θ . Therefore,

$$f(\vec{r}) = \left[\prod_j \binom{n_j}{r_j} \right] \left(\sum_{L=1}^M \gamma_L \times m_\theta(-\xi_L) \right)$$

as claimed.

Applications of Theorem 1

Example 1: Let θ be distributed as gamma (mean = $\alpha\beta$, variance = $\alpha\beta^2$). The moment generating function for the gamma distribution parameterized in this manner is $m_\theta(t) = \left(\frac{1}{1-\beta t} \right)^\alpha$. Then for appropriate constants M , γ_L , and ξ_L , the marginal distribution of $\vec{r}$ is,

$$f(\vec{r}) = \left[\prod_j \binom{n_j}{r_j} \right] \left(\sum_{L=1}^M \gamma_L \times \left(\frac{1}{1 + \beta \xi_L} \right)^\alpha \right). \quad (\text{B.1})$$

Example 2: Let θ be distributed as negative binomial (mean = $\frac{\rho q}{p}$, variance = $\frac{\rho q}{p^2}$).

The moment generating function for the negative binomial distribution

parameterized in this manner is $m_\theta(t) = \left(\frac{p}{1-qe^t}\right)^\rho$. Then for appropriate constants M , γ_L , and ξ_L , the marginal distribution of $\vec{r}$ is,

$$f(\vec{r}) = \left[\prod_j \binom{n_j}{r_j} \right] \left(\sum_{L=1}^M \gamma_L \times \left(\frac{p}{1-qe^{-\xi_L}} \right)^\rho \right). \quad (B.2)$$

It should be pointed out that (3.9) was derived using result (B.2) with $\rho = \frac{\alpha}{d}$, $p = \frac{1}{1+\beta\phi d}$, $q = 1 - p$.

Computing the MLE of $\vec{\tau}^*$

The MLE of $\vec{\tau}^*$ is the value $\hat{\vec{\tau}}^*$ which maximizes the likelihood function of the observable data, as given in (3.10). Because the components of $\vec{\tau}^*$ are at times on vastly different scales, I decided to transform the parameters as follows:

$$\text{Let } \tilde{\phi} = -\log(\phi), \quad \tilde{\zeta} = -\log(\zeta), \quad \tilde{\alpha} = \log(\alpha), \quad \tilde{\beta} = \log(\beta).$$

Let $\tilde{\tau}^* = (\tilde{\phi}, \tilde{\zeta}, \tilde{\alpha}, \tilde{\beta})'$. Denote as $l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{r}_A)$ and $l(\tilde{\tau}^* | \vec{g}_1, \dots, \vec{g}_B)$ the log likelihood functions for the observable check and test carrier data, respectively, under the transformed parameter space. Denote as $l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{g}_S)$ the complete log likelihood function under the transformed parameter space. Then $l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{r}_A)$ is given by (B.3), $l(\tilde{\tau}^* | \vec{g}_1, \dots, \vec{g}_B)$ is given by (B.4), and $l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{g}_B)$ is given by (B.5).

$$l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{r}_A) = \sum_{i=1}^A \ln \left[\left(\prod_{j=1}^{D_i} \binom{n_j}{r_j} \right) \left(\sum_{L_i=1}^{M_i} \gamma_{L_i} \times \left(\frac{1}{1 + 10^{\tilde{\beta} \tilde{\xi}_{L_i}}} \right)^{10^{\tilde{\alpha}}} \right) \right]. \quad (B.3)$$

$$l(\tilde{\tau}^* | \vec{g}_1, \dots, \vec{g}_B) = \sum_{s=1}^B \ln \left[\left(\prod_{m=1}^{E_s} \binom{w_{sm}}{g_{sm}} \right) \left(\sum_{L_s=1}^{M_s} \gamma_{L_s} \times \left(\frac{1}{1 + (10^{\tilde{\beta} - \tilde{\phi}}) d (1 - e^{-\xi_{L_s}})} \right)^{\frac{10^{\tilde{\alpha}}}{d}} \right) \right]. \quad (B.4)$$

$$l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{g}_B) = l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{r}_A) + l(\tilde{\tau}^* | \vec{g}_1, \dots, \vec{g}_B). \quad (B.5)$$

It should be noted that the value, d , in equation (B.4) can be written down as

$$d = 1 + \frac{(10^{-2\hat{\zeta}})(10^{\hat{\alpha}}+1)}{10^{-2\hat{\phi}}}.$$

Let $\hat{\tau}^*$ denote the value of $\tilde{\tau}^*$ which maximizes (B.5). Using the invariance property of mle (Mood, Graybill, and Boes 1974), the mle of $\tilde{\tau}^*$ is then

$$(\hat{\phi} = \frac{1}{10^{\hat{\phi}}}, \quad \hat{\zeta} = \frac{1}{10^{\hat{\zeta}}}, \quad \hat{\alpha} = 10^{\hat{\alpha}}, \quad \hat{\beta} = 10^{\hat{\beta}})'$$

A modified Newton-Raphson procedure was used to compute $\tilde{\tau}^*$. The

Newton-Raphson method requires knowledge of $\frac{\partial l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{g}_B)}{\partial \tilde{\tau}^*}$ and $\frac{\partial^2 l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{g}_B)}{(\partial \tilde{\tau}^*)(\partial \tilde{\tau}^*)'}$. By independence,

$$\frac{\partial l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{g}_B)}{\partial \tilde{\tau}^*} = \sum_{i=1}^A \frac{\partial l(\tilde{\tau}^* | \vec{r}_i)}{\partial \tilde{\tau}^*} + \sum_{s=1}^B \frac{\partial l(\tilde{\tau}^* | \vec{g}_s)}{\partial \tilde{\tau}^*} \quad \text{and,}$$

$$\frac{\partial^2 l(\tilde{\tau}^* | \vec{r}_1, \dots, \vec{g}_B)}{(\partial \tilde{\tau}^*)(\partial \tilde{\tau}^*)'} = \sum_{i=1}^A \frac{\partial^2 l(\tilde{\tau}^* | \vec{r}_i)}{(\partial \tilde{\tau}^*)(\partial \tilde{\tau}^*)'} + \sum_{s=1}^B \frac{\partial^2 l(\tilde{\tau}^* | \vec{g}_s)}{(\partial \tilde{\tau}^*)(\partial \tilde{\tau}^*)'}.$$

The functional forms of the first and second partial derivatives are rather messy, and for this reason some notation will be introduced to shorten the equations. Let $\vec{u}$ and $\vec{v}$ be vectors of the length n . Let $\odot$ represent the Hadamard product (the element-by-element direct product of two matrices) and let $\odot^{-1}$ represent the element-by-element direct division of two matrices. For example, consider vectors $\vec{u}$ and $\vec{v}$ of equal length. Then the i th elements of $\vec{u} \odot \vec{v}$ and $\vec{u} \odot^{-1} \vec{v}$ are $u_i \times v_i$ and $u_i \div v_i$, respectively. Define the operator, $S(\cdot)$, as the summation of the individual components of the argument in parentheses; i.e. $S(\vec{u}) = \sum_{i=1}^n u_i$. Also, for the remainder of this appendix, denote a mathematical operation on a vector to be the mathematical operation on each element of the vector. For example, the i th element of $\ln(\vec{u})$ is $\ln(u_i)$. Let,

$\gamma_i = (\gamma_1, \dots, \gamma_{M_i})'$, the vector of γ constants for check carrier i ,

$\gamma_s = (\gamma_1, \dots, \gamma_{M_s})'$, the vector of γ constants for test carrier s ,

$\xi_i = (\xi_1, \dots, \xi_{M_i})'$, the vector of ξ constants for check carrier i , and

$\xi_s = (\xi_1, \dots, \xi_{M_s})'$, the vector of ξ constants for test carrier s .

The ten variables defined below are introduced for notational convenience.

$$a_1 = \left(\frac{1}{\vec{1} + (10^{\bar{\beta}}) \times \xi_i} \right)^{10^{\bar{\alpha}}},$$

$$a_2 = S(\gamma_i \odot a_1),$$

$$a_3 = \vec{1} + (10^{\bar{\beta}}) \times \xi_i,$$

$$k_1 = 1 + \frac{(10^{-2\bar{\zeta}})(10^{\bar{\alpha}} + 1)}{10^{-2\bar{\phi}}},$$

$$k_2 = \left(\frac{1}{\vec{1} + (10^{\bar{\beta} - \bar{\phi}}) \times k_1 \times \xi_s} \right)^{\frac{10^{\bar{\alpha}}}{k_1}},$$

$$k_3 = S(\gamma_s \odot k_2),$$

$$k_4 = \vec{1} + (10^{\bar{\beta} - \bar{\phi}}) \times k_1 \times \xi_s,$$

$$k_5 = -(10^{\bar{\beta} - \bar{\phi}}) \times \ln(10) \times k_1 \times \xi_s + 2 \times (10^{\bar{\beta} + \bar{\phi} - 2\bar{\zeta}}) \times \ln(10) \times (10^{\bar{\alpha}} + 1) \odot \xi_s,$$

$$k_6 = \frac{10^{\bar{\alpha}} \times \ln(10)}{k_1} - \frac{(10^{2 \times (\bar{\beta} + \bar{\phi} - 2\bar{\zeta})}) \times \ln(10)}{k_1^2}, \text{ and}$$

$$k_7 = \frac{1}{k_1^2} \left((10^{\bar{\alpha} + 2\bar{\phi} - 2\bar{\zeta}}) \times 10^{\bar{\alpha} + 1} \times \ln(10) \times -\ln(k_4) \right).$$

Functional Form of the First Partial Derivatives

$$\frac{\partial l(\tau^* | \vec{r}_i)}{\partial \phi} = 0;$$

$$\frac{\partial l(\tau^* | \vec{r}_i)}{\partial \zeta} = 0;$$

$$\frac{\partial l(\tau^* | \vec{r}_i)}{\partial \bar{\alpha}} = \left(\frac{1}{a_2} \right) \cdot \left[S \left((10^{\bar{\alpha}}) \cdot \ln(10) \cdot \gamma_i \odot a_1 \odot (-\ln(a_3)) \right) \right];$$

$$\frac{\partial l(\tau^* | \vec{r}_i)}{\partial \bar{\beta}} = \left(\frac{1}{a_2} \right) \cdot \left[S \left(\left\{ -(10^{\bar{\alpha} + \bar{\beta}}) \cdot \ln(10) \cdot \gamma_i \odot a_1 \odot \xi_i \right\} \odot^{-1} \{a_3\} \right) \right];$$

$$\begin{aligned} \frac{\partial l(\tau^* | \vec{g}_s)}{\partial \phi} = & \left(\frac{1}{k_3} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[-(2 \cdot k_7) - \left\{ -(10^{\bar{\alpha} + \bar{\beta} - \bar{\phi}}) \cdot \ln(10) \cdot k_1 \cdot \xi_s + \right. \right. \right. \right. \\ & \left. \left. \left. 2 \cdot (10^{\bar{\alpha} + \bar{\beta} + \bar{\phi} - 2\bar{\zeta}}) \cdot (10^{\bar{\alpha} + 1}) \cdot \ln(10) \cdot \xi_s \right\} \odot^{-1} \{k_1 \cdot k_4\} \right] \right) \right]; \end{aligned}$$

$$\begin{aligned} \frac{\partial l(\tau^* | \vec{g}_s)}{\partial \zeta} = & \left(\frac{1}{k_3} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[(2 \cdot k_7) + \right. \right. \right. \\ & \left. \left. \left. \left\{ (2 \cdot 10^{\bar{\alpha} + \bar{\beta} + \bar{\phi} - 2\bar{\zeta}}) \cdot (10^{\bar{\alpha} + 1}) \cdot \ln(10) \cdot \xi_s \right\} \odot^{-1} \{k_1 \cdot k_4\} \right] \right) \right]; \end{aligned}$$

$$\frac{\partial l(\tau^* | \vec{g}_s)}{\partial \bar{\alpha}} = \left(\frac{1}{k_3} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[(k_6 \cdot -\ln(k_4)) - \right. \right. \right.$$

$$\left\{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot \xi_s \} \odot^{-1} \{k_1 \cdot k_4\} \right\} \Bigg]; \quad \text{and}$$

$$\frac{\partial l(\tilde{\tau}^*|\tilde{g}_s)}{\partial \tilde{\beta}} = \left(\frac{1}{k_3}\right) \cdot \left[S \left(\{ -\ln(10) \cdot (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot \gamma_s \odot k_2 \odot \xi_s \} \odot^{-1} \{k_4\} \right) \right]$$

Functional Form of the Second Partial Derivatives

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\phi}^2} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\phi} \partial \tilde{\zeta}} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\phi} \partial \tilde{\alpha}} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\phi} \partial \tilde{\beta}} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\zeta} \partial \tilde{\phi}} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\zeta}^2} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\zeta} \partial \tilde{\alpha}} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\zeta} \partial \tilde{\beta}} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\alpha} \partial \tilde{\phi}} = 0;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\alpha} \partial \tilde{\zeta}} = 0;$$

$$\begin{aligned} \frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\alpha}^2} = & \left(\frac{1}{a_2}\right) \cdot \left[S \left((10^{\tilde{\alpha}}) \cdot (\ln(10))^2 \cdot \gamma_i \odot a_1 \odot (-\ln(a_3)) \odot \right. \right. \\ & \left. \left. [\tilde{I} + 10^{\tilde{\alpha}} \cdot (-\ln(a_3))] \right) \right] - \\ & \left(\frac{1}{(a_2)^2}\right) \cdot \left[S \left((10^{\tilde{\alpha}}) \cdot \ln(10) \cdot \gamma_i \odot a_1 \odot (-\ln(a_3)) \right) \right]^2; \end{aligned}$$

$$\begin{aligned} \frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\alpha} \partial \tilde{\beta}} = & \left(\frac{1}{a_2}\right) \cdot \left[S \left(- (10^{\tilde{\alpha}+\tilde{\beta}}) \cdot (\ln(10))^2 \cdot \gamma_i \odot a_1 \odot \xi_i \odot \right. \right. \\ & \left. \left. [\{\tilde{I} + 10^{\tilde{\alpha}} \cdot (-\ln(a_3))\} \odot^{-1} \{a_3\}] \right) \right] - \\ & \left(\frac{1}{(a_2)^2}\right) \cdot \left[S \left((10^{\tilde{\alpha}}) \cdot \ln(10) \cdot \gamma_i \odot a_1 \odot (-\ln(a_3)) \right) \right] \times \\ & \left[S \left(\{ - (10^{\tilde{\alpha}+\tilde{\beta}}) \cdot \ln(10) \cdot \gamma_i \odot a_1 \odot \xi_i \} \odot^{-1} \{a_3\} \right) \right]; \end{aligned}$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\alpha} \partial \tilde{\beta}} = \frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\beta} \partial \tilde{\alpha}};$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{r}_1)}{\partial \tilde{\beta}^2} = \left(\frac{1}{a_2}\right) \cdot \left[S \left((10^{\tilde{\alpha}}) \cdot (10^{\tilde{\alpha}} + 1) \cdot \gamma_i \odot a_1 \odot \left(\{ \ln(10) \cdot (10^{\tilde{\beta}}) \cdot \xi_i \} \odot^{-1} \{a_3\} \right)^2 \right) \right] -$$

$$\left\{ (\ln(10))^2 \cdot (10^{\tilde{\alpha}+\tilde{\beta}}) \cdot \gamma_i \odot a_1 \odot \xi_i \odot \odot^{-1}\{a_3\} \right\} \Bigg] -$$

$$\left(\frac{1}{(a_2)^2} \right) \cdot \left[S \left(\left\{ - (10^{\tilde{\alpha}+\tilde{\beta}}) \cdot \ln(10) \cdot \gamma_i \odot a_1 \odot \xi_i \odot \odot^{-1}\{a_3\} \right\} \right) \right]^2;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{g}_s)}{\partial \phi^2} = \left(\frac{1}{k_3} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[(-2 \cdot k_7 - \{(10^{\tilde{\alpha}}) \cdot k_5\} \odot \odot^{-1}\{k_1 \cdot k_4\})^2 + \right. \right. \right.$$

$$(8 \cdot 10^{\tilde{\alpha}+4\tilde{\phi}-4\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1})^2 \cdot (\ln(10))^2 \cdot -\ln(k_4) \div k_1^3 - 4 \cdot \ln(10) \cdot k_7 +$$

$$\{(4 \cdot 10^{\tilde{\alpha}+2\tilde{\phi}-2\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot \ln(10) \cdot k_5\} \odot \odot^{-1}\{k_1^2 \cdot k_4\} +$$

$$\left. \left. \{(10^{\tilde{\alpha}}) \cdot k_5^2\} \odot \odot^{-1}\{k_1 \cdot k_4^2\} - \{(10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot (\ln(10))^2 \cdot \xi_s\} \odot \odot^{-1}\{k_4\} \right] \right) \Bigg] -$$

$$\left(\frac{1}{(k_3)^2} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[(-2 \cdot k_7) - \{(10^{\tilde{\alpha}}) \cdot k_5\} \odot \odot^{-1}\{k_1 \cdot k_4\} \right] \right) \right]^2;$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{g}_s)}{\partial \phi \partial \zeta} = \left(\frac{1}{k_3} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[(2 \cdot k_7 + \{(2 \cdot 10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot \ln(10) \cdot \xi_s\} \right. \right. \right.$$

$$\odot \odot^{-1}\{k_1 \cdot k_4\}) \odot (-2 \cdot k_7 - \{(10^{\tilde{\alpha}}) \cdot k_5\} \odot \odot^{-1}\{k_1 \cdot k_4\}) +$$

$$4 \cdot \ln(10) \cdot k_7 - (8 \cdot 10^{\tilde{\alpha}+4\tilde{\phi}-4\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1})^2 \cdot (\ln(10))^2 \cdot -\ln(k_4) \div k_1^3 -$$

$$\{(4 \cdot 10^{\tilde{\alpha}+\tilde{\beta}+3\tilde{\phi}-4\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1})^2 \cdot (\ln(10))^2 \cdot \xi_s\} \odot \odot^{-1}\{k_1^2 \cdot k_4\} -$$

$$\{(2 \cdot 10^{\tilde{\alpha}+2\tilde{\phi}-2\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot \ln(10) \cdot k_5\} \odot \odot^{-1}\{k_1^2 \cdot k_4\} -$$

$$\{(2 \cdot 10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot \ln(10) \cdot k_5 \odot \xi_s\} \odot \odot^{-1}\{k_1 \cdot k_4^2\} +$$

$$\left. \left. \{(2 \cdot 10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot (\ln(10))^2 \cdot \xi_s\} \odot \odot^{-1}\{k_1 \cdot k_4\} \right] \right) \Bigg] -$$

$$\left(\frac{1}{(k_3)^2} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left(- (2 \cdot k_7) - \{(10^{\tilde{\alpha}}) \cdot k_5\} \odot \odot^{-1}\{k_1 \cdot k_4\} \right) \right) \right] \times$$

$$S \left(\gamma_s \odot k_2 \odot \left((2 \cdot k_7) + \{(2 \cdot 10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot \ln(10) \cdot \xi_s\} \odot \odot^{-1}\{k_1 \cdot k_4\} \right) \right);$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{g}_s)}{\partial \phi \partial \tilde{\alpha}} =$$

$$\left(\frac{1}{k_3} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[((k_6 \cdot -\ln(k_4)) - \{(10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \ln(10) \xi_s\} \odot \odot^{-1}\{k_1 \cdot k_4\}) \odot \right. \right. \right.$$

$$\left. \left. (- (2 \cdot k_7) - \{(10^{\tilde{\alpha}}) \cdot k_5\} \odot \odot^{-1}\{k_1 \cdot k_4\}) - 2 \cdot \ln(10) \cdot k_7 + \right. \right.$$

$$(4 \cdot 10^{2\tilde{\alpha}+4\tilde{\phi}-4\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot (\ln(10))^2 \cdot (-\ln(k_4)) \div k_1^3 -$$

$$(2 \cdot 10^{2\tilde{\alpha}+2\tilde{\phi}-2\tilde{\zeta}}) \cdot (\ln(10))^2 \cdot (-\ln(k_4)) \div k_1^2 +$$

$$\left. \left. \{(2 \cdot 10^{2\tilde{\alpha}+\tilde{\beta}+3\tilde{\phi}-4\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot ((\ln(10))^2) \cdot \xi_s\} \odot \odot^{-1}\{k_1^2 \cdot k_4\} + \right. \right.$$

$$\left. \left. \{(10^{2\tilde{\alpha}+2\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot k_5\} \odot \odot^{-1}\{k_1^2 \cdot k_4\} - \right. \right]$$

$$\begin{aligned}
& \left\{ (10^{\tilde{\alpha}}) \cdot \ln(10) \cdot k_5 \right\} \odot^{-1} \{k_1 \cdot k_4\} - \\
& \left\{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot ((\ln(10))^2) \cdot \xi_s \right\} \odot^{-1} \{k_1 \cdot k_4\} + \\
& \left\{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot k_5 \cdot \xi_s \right\} \odot^{-1} \{k_1 \cdot k_4^2\} \Big] \Big] - \\
& \left(\frac{1}{(k_3)^2} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left(- (2 \cdot k_7) - \{ (10^{\tilde{\alpha}}) \cdot k_5 \} \odot^{-1} \{k_1 \cdot k_4\} \right) \right) \right] \times \\
& S \left(\gamma_s \odot k_2 \odot \left(k_6 \cdot (-\ln(k_4)) - \{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot \xi_s \} \odot^{-1} \{k_1 \cdot k_4\} \right) \right);
\end{aligned}$$

$$\begin{aligned}
\frac{\partial^2 l(\tilde{\tau}|\tilde{g}_s)}{\partial \tilde{\phi} \partial \tilde{\beta}} &= \left(\frac{1}{k_3} \right) \cdot \left[S \left(-\gamma_s \odot k_2 \odot \left[\{ (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot \ln(10) \cdot \xi_s \odot \right. \right. \right. \\
& \left. \left. \left(- (2 \cdot k_7) - \{ (10^{\tilde{\alpha}}) \cdot k_5 \} \odot^{-1} \{k_1 \cdot k_4\} \right) \} \odot^{-1} \{k_4\} + \right. \right. \\
& \left. \left\{ (2 \cdot 10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot (\ln(10))^2 \cdot \xi_s \right\} \odot^{-1} \{k_1 \cdot k_4\} - \right. \\
& \left. \left\{ (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot \ln(10) \cdot k_5 \cdot \xi_s \right\} \odot^{-1} \{k_4^2\} - \right. \\
& \left. \left. \left. (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot (\ln(10))^2 \cdot k_1 \cdot \xi_s + \right. \right. \right. \\
& \left. \left. \left. \left\{ (2 \cdot 10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot ((\ln(10))^2) \cdot \xi_s \right\} \odot^{-1} \{k_1 \cdot k_4\} \right] \right) \right] - \\
& \left(\frac{1}{(k_3)^2} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left(- (2 \cdot k_7) - \{ (10^{\tilde{\alpha}}) \cdot k_5 \} \odot^{-1} \{k_1 \cdot k_4\} \right) \right) \right] \times \\
& S \left(-\gamma_s \odot k_2 \odot \left(\{ (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot \ln(10) \cdot \xi_s \} \odot^{-1} \{k_4\} \right) \right);
\end{aligned}$$

$$\frac{\partial^2 l(\tilde{\tau}|\tilde{g}_s)}{\partial \tilde{\zeta} \partial \tilde{\phi}} = \frac{\partial^2 l(\tilde{\tau}|\tilde{g}_s)}{\partial \tilde{\phi} \partial \tilde{\zeta}};$$

$$\begin{aligned}
\frac{\partial^2 l(\tilde{\tau}|\tilde{g}_s)}{\partial \tilde{\zeta}^2} &= \left(\frac{1}{k_3} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[\right. \right. \\
& \left. \left((2 \cdot k_7) + \left\{ 2 \cdot (10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot (10^{\tilde{\alpha}}+1) \cdot \xi_s \right\} \odot^{-1} \{k_1 \cdot k_4\} \right)^2 + \right. \\
& \left. (8 \cdot 10^{\tilde{\alpha}+4\tilde{\phi}-4\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1})^2 \cdot (\ln(10))^2 \cdot (-\ln(k_4)) \div k_1^3 - \right. \\
& \left. (4 \cdot 10^{\tilde{\alpha}+2\tilde{\phi}-2\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1}) \cdot (\ln(10))^2 \cdot (-\ln(k_4)) \right) \div k_1^2 + \\
& \left\{ 8 \cdot (10^{\tilde{\alpha}+\tilde{\beta}+3\tilde{\phi}-4\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1})^2 \cdot (\ln(10))^2 \cdot \xi_s \right\} \odot^{-1} \{k_1^2 \cdot k_4\} + \\
& \left\{ 4 \cdot (10^{\tilde{\alpha}+2\tilde{\beta}+2\tilde{\phi}-4\tilde{\zeta}}) \cdot (10^{\tilde{\alpha}+1})^2 \cdot (\ln(10))^2 \cdot \xi_s^2 \right\} \odot^{-1} \{k_1 \cdot k_4^2\} - \\
& \left. \left. \left. \left\{ 4 \cdot (10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot (\ln(10))^2 \cdot (10^{\tilde{\alpha}+1}) \cdot \xi_s \right\} \odot^{-1} \{k_1 \cdot k_4\} \right] \right) \right] - \\
& \left(\frac{1}{(k_3)^2} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[(2 \cdot k_7) + \left\{ 2 \cdot (10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot (10^{\tilde{\alpha}}+1) \cdot \xi_s \right\} \odot^{-1} \right. \right. \right.
\end{aligned}$$

$$\begin{aligned}
\frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\alpha}^2} = & \left(\frac{1}{k_3}\right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[\right. \right. \right. \\
& \left(k_6 \cdot (-\ln(k_4)) - \{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot \xi_s \} \odot^{-1} \{ k_1 \cdot k_4 \} \}^2 + \right. \\
& \frac{(\ln(10))^2 \cdot (-\ln(k_4)) \cdot (10^{\tilde{\alpha}})}{k_1} \cdot \left(- (3 \cdot 10^{\tilde{\alpha}+2\tilde{\phi}-2\tilde{\zeta}}) \div k_1 + (2 \cdot 10^{2\tilde{\alpha}+4\tilde{\phi}-4\tilde{\zeta}}) \div k_1^2 \right) - \\
& \{ (10^{\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot k_6 \cdot \xi_s \} \odot^{-1} \{ k_4 \} - \\
& \{ 2 \cdot (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot (\ln(10))^2 \cdot \xi_s \} \odot^{-1} \{ k_1 \cdot k_4 \} + \\
& \{ (10^{3\tilde{\alpha}+\tilde{\beta}+3\tilde{\phi}-4\tilde{\zeta}}) \cdot (\ln(10))^2 \cdot \xi_s \} \odot^{-1} \{ k_1^2 \cdot k_4 \} + \\
& \left. \left. \left. \{ (10^{3\tilde{\alpha}+2\tilde{\beta}+2\tilde{\phi}-4\tilde{\zeta}}) \cdot (\ln(10))^2 \cdot \xi_s^2 \} \odot^{-1} \{ k_1 \cdot k_4^2 \} \right] \right] \right] - \\
& \left(\frac{1}{(k_3)^2} \right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left[k_6 \cdot (-\ln(k_4)) - \{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot \xi_s \} \odot^{-1} \{ k_1 \cdot k_4 \} \} \right] \right)^2 \right];
\end{aligned}$$

$$\begin{aligned}
\frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\alpha} \partial \tilde{\beta}} = & \left(\frac{1}{k_3}\right) \cdot \left[S \left(-\gamma_s \odot k_2 \odot \left[\{ (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot \ln(10) \cdot \xi_s \odot \right. \right. \right. \\
& \left(k_6 \cdot (-\ln(k_4)) - \{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot \xi_s \} \odot^{-1} \{ k_1 \cdot k_4 \} \} \} \odot^{-1} \{ k_4 \} + \right. \\
& \{ (10^{\tilde{\beta}-\tilde{\phi}}) \cdot \ln(10) \cdot k_1 \cdot \xi_s \} \odot^{-1} \{ k_4 \} - \\
& \{ (10^{2\tilde{\alpha}+2\tilde{\beta}-2\tilde{\zeta}}) \cdot (\ln(10))^2 \cdot \xi_s^2 \} \odot^{-1} \{ k_4^2 \} + \\
& \left. \left. \left. \{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot ((\ln(10))^2) \cdot \xi_s \} \odot^{-1} \{ k_1 \cdot k_4 \} \right] \right] \right] - \\
& \left(\frac{1}{(k_3)^2} \right) \cdot \left[S \left(-\gamma_s \odot k_2 \odot \left(\{ (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot \ln(10) \cdot \xi_s \} \odot^{-1} \{ k_4 \} \} \right) \right) \right] \times \\
& \left[S \left(\gamma_s \odot k_2 \odot \left(k_6 \cdot (-\ln(k_4)) - \{ (10^{2\tilde{\alpha}+\tilde{\beta}+\tilde{\phi}-2\tilde{\zeta}}) \cdot \ln(10) \cdot \xi_s \} \odot^{-1} \{ k_1 \cdot k_4 \} \} \right) \right) \right];
\end{aligned}$$

$$\frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\beta} \partial \tilde{\phi}} = \frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\phi} \partial \tilde{\beta}};$$

$$\frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\beta} \partial \tilde{\zeta}} = \frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\zeta} \partial \tilde{\beta}};$$

$$\frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\beta} \partial \tilde{\alpha}} = \frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\alpha} \partial \tilde{\beta}}; \quad \text{and}$$

$$\begin{aligned}
\frac{\partial^2 l(\vec{\tau}|\vec{g}_s)}{\partial \tilde{\beta}^2} = & \left(\frac{1}{k_3}\right) \cdot \left[S \left(\gamma_s \odot k_2 \odot \left(\{ (10^{2\tilde{\alpha}+2\tilde{\beta}-2\tilde{\phi}}) \cdot (\ln(10))^2 \cdot \xi_s^2 \} \odot^{-1} \{ k_4^2 \} + \right. \right. \right. \\
& \{ (10^{\tilde{\alpha}+2\tilde{\beta}-2\tilde{\phi}}) \cdot (\ln(10))^2 \cdot k_1 \cdot \xi_s^2 \} \odot^{-1} \{ k_4^2 \} - \\
& \left. \left. \left. \{ (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot (\ln(10))^2 \cdot \xi_s \} \odot^{-1} \{ k_4 \} \} \right) \right] -
\end{aligned}$$

$$\left(\frac{1}{(k_3)^2}\right) \cdot \left[S \left(-\gamma_s \odot k_2 \odot \left[\left\{ (10^{\tilde{\alpha}+\tilde{\beta}-\tilde{\phi}}) \cdot \ln(10) \cdot \xi_s \right\} \odot^{-1} \{k_4\} \right] \right) \right]^2.$$

Expressions for $V^*(\hat{\tau}^*)$, $\frac{\partial g_5(\hat{\tau}^*)}{\partial \hat{\tau}^*}$ and $\frac{\partial g_6(\hat{\tau}^*)}{\partial \hat{\tau}^*}$

The formulas for the standard error of $\Upsilon_{1,\text{mle}}$ and $\Upsilon_{2,\text{mle}}$ are given by equations (3.32) - (3.34). Evaluating (3.32) - (3.34) requires the computation of the estimated variance-covariance matrix of $\hat{\tau}^*$, denoted as $V^*(\hat{\tau}^*)$. The computation of (3.32) and (3.33) requires that the derivatives with respect to $\hat{\tau}^*$ of the functions $g_5(\hat{\tau}^*)$ and $g_6(\hat{\tau}^*)$ be evaluated at the mle of $\hat{\tau}^*$.

Denote $V^*(\hat{\tau}^*)$ as the estimated variance-covariance matrix of $\hat{\tau}^*$. Assuming the usual regularity conditions hold, as given in (Lehmann 1991), then

$$V^*(\hat{\tau}^*) = \left[\frac{-\partial^2 l(\tilde{\tau})}{\partial \tilde{\tau} \partial \tilde{\tau}'} \mid \tilde{\tau}^* = \hat{\tau}^* \right]^{-1}.$$

$V^*(\hat{\tau}^*)$ can be obtained from $V^*(\hat{\tau}^*)$ as follows: $V^*(\hat{\tau}^*) =$

$$(\ln(10))^2 \times \begin{pmatrix} -10^{-\tilde{\phi}} & 0 & 0 & 0 \\ 0 & -10^{-\tilde{\zeta}} & 0 & 0 \\ 0 & 0 & 10^{\tilde{\alpha}} & 0 \\ 0 & 0 & 0 & 10^{\tilde{\beta}} \end{pmatrix} V^*(\hat{\tau}^*) \begin{pmatrix} -10^{-\tilde{\phi}} & 0 & 0 & 0 \\ 0 & -10^{-\tilde{\zeta}} & 0 & 0 \\ 0 & 0 & 10^{\tilde{\alpha}} & 0 \\ 0 & 0 & 0 & 10^{\tilde{\beta}} \end{pmatrix}.$$

Let,

$$g_5(\phi, \alpha, \beta, \zeta) = \frac{\Psi(\alpha) + \ln(\beta)}{\ln(10)} - \frac{\Psi\left(\frac{\phi\alpha\beta}{\phi\beta d+1}\right) + \ln(\phi\beta d+1)}{\ln(10)}, \quad \text{and,}$$

$$g_6(\phi, \alpha, \beta, \zeta) = \frac{\Psi(\alpha) + \ln(\beta)}{\ln(10)} - \sum_{t=1}^N \log(t) \left(\frac{\Gamma\left(t + \frac{\alpha}{d}\right)}{\Gamma\left(\frac{\alpha}{d}\right)} \right) \left(\frac{\phi\beta d}{\phi\beta d+1} \right)^t \left(\frac{1}{\phi\beta d+1} \right)^{\frac{\alpha}{d}}.$$

For notational convenience let,

$$p^*(t) = \left(\frac{\Gamma\left(t + \frac{\alpha}{d}\right)}{\Gamma\left(\frac{\alpha}{d}\right)} \right) \left(\frac{\phi\beta d}{\phi\beta d+1} \right)^t \left(\frac{1}{\phi\beta d+1} \right)^{\frac{\alpha}{d}},$$

$b_1 = \beta(\phi^2 + \zeta^2(\alpha + 1)) + \phi$, and

$b_2 = \phi^2 + \zeta^2(\alpha + 1)$. Then

$$\frac{\partial g_5}{\partial \phi} = \log(e) \times \left[-\Psi' \left(\frac{\phi^2 \alpha \beta}{b_1} \right) \left(\frac{2\phi \alpha \beta}{b_1} - \frac{\phi^2 \alpha \beta (2\phi \beta + 1)}{b_1^2} \right) - \frac{2\phi \beta + 1 - b_1/\phi}{b_1} \right],$$

$$\frac{\partial g_5}{\partial \zeta} = \log(e) \times \left[\frac{2\phi^2 \zeta \alpha(\alpha+1) \beta^2 \Psi' \left(\frac{\phi^2 \alpha \beta}{b_1} \right)}{b_1^2} - \frac{2\zeta \beta(\alpha+1)}{b_1} \right],$$

$$\frac{\partial g_5}{\partial \alpha} = \log(e) \times \left[\Psi'(\alpha) - \left(\frac{\phi^2 \beta}{b_1} - \frac{\phi^2 \zeta^2 \alpha \beta^2}{b_1^2} \right) \Psi' \left(\frac{\phi^2 \alpha \beta}{b_1} \right) - \frac{\zeta^2 \beta}{b_1} \right],$$

$$\frac{\partial g_5}{\partial \beta} = \log(e) \times \left[\frac{1}{\beta} - \left(\frac{\phi^2 \alpha}{b_1} - \frac{\phi^2 \alpha [b_1 - \phi]}{b_1^2} \right) \Psi' \left(\frac{\phi^2 \alpha \beta}{b_1} \right) - \frac{\phi^2 + \zeta^2 (\alpha+1)}{b_1} \right],$$

$$\begin{aligned} \frac{\partial g_6}{\partial \phi} = & - \sum_{t=1}^N \log(t) p^*(t) \left[\left(\Psi(t + \frac{\alpha}{d}) - \Psi(\frac{\alpha}{d}) + \ln(\frac{\phi}{k_1}) \right) \times \left(\frac{2\phi \alpha}{k_2} - \frac{2\phi^3 \alpha}{k_2^2} \right) + \right. \\ & \left. \frac{\phi \alpha (1 - \phi(2\phi \beta v + 1)/k_1)}{k_2} + t \left(\frac{2\phi}{k_2} - \frac{2\phi \beta v + 1}{k_1} \right) \right], \end{aligned}$$

$$\begin{aligned} \frac{\partial g_6}{\partial \zeta} = & -2 \times \sum_{t=1}^N \log(t) p^*(t) \left[\left(\Psi(\frac{\alpha}{d}) - \Psi(t + \frac{\alpha}{d}) \right) \times \frac{\zeta \alpha(\alpha+1)}{dk_2} - \frac{2\zeta \alpha(\alpha+1)}{d} \times \left(\frac{\ln(\phi/b_1)}{b_2} + \frac{\beta}{b_1} \right) + \right. \\ & \left. t \left(\frac{\zeta(\alpha+1)}{b_2} - \frac{\zeta(\alpha+1)\beta b_2}{b_1} \right) \right], \end{aligned}$$

$$\begin{aligned} \frac{\partial g_6}{\partial \alpha} = & \log(e) \times \Psi'(\alpha) - \left[\sum_{t=1}^N \log(t) p^*(t) \left[\left(\Psi(t + \frac{\alpha}{d}) - \Psi(\frac{\alpha}{d}) + \ln(\frac{\phi}{b_1}) \right) \times \left(\frac{1}{d} - \frac{\zeta^2 \alpha}{db_2} \right) - \right. \right. \\ & \left. \left. \frac{\zeta^2 \alpha \beta}{db_1} + t \left(\frac{\zeta^2}{b_2} - \frac{\zeta^2 \beta}{b_1} \right) \right] \right], \quad \text{and} \end{aligned}$$

$$\frac{\partial g_6}{\partial \beta} = \log(e) \times \frac{1}{\beta} - \left[\sum_{t=1}^N \log(t) p^*(t) \left[\frac{\phi^2 \alpha}{b_1} + t \left(\frac{1}{\beta} - \frac{b_2}{b_1} \right) \right] \right].$$

Computation of $\hat{\mu}_C$, $\hat{\mu}_X$, and $V_C(\hat{\mu}_C, \hat{\eta}_C)$

Let μ_C and η_C denote the mean and coefficient of variation for the number of organisms surviving the simulated disinfection steps for check carriers. Theorem 1 was applied to derive the joint marginal distribution of $r_1^-, \dots, r_A^-$. The likelihood function, $l(\mu_C, \eta_C \mid r_1^-, \dots, r_A^-)$, is given in (3.13). Denote as $\hat{\mu}_C$ and $\hat{\mu}_X$ the values of μ_C and η_C which jointly maximize 3.13.

Because μ_C and η_C will typically differ by 6 or more orders of magnitude, I decided to compute first the mle of $\log(\mu_C)$, and then use the invariance property of mle to obtain $\hat{\mu}_C$. I used a modified Newton-Raphson method to perform the necessary maximizations. Let $U_C = \log(\mu_C)$. Note that $\mu_C = 10^{U_C}$. Thus (3.13) can be reparameterized in terms of U_C and η_C as follows:

$$l(U_C, \eta_C \mid r_1^-, \dots, r_A^-) = \sum_{i=1}^A \ln \left[\prod_{j=1}^A \binom{n_{ij}}{r_{ij}} \left(\sum_{L_i=1}^{M_i} \gamma_{L_i} \times \left(\frac{1}{1 + 10^{U_C} \eta_C^2 \xi_{L_i}} \right)^{\frac{1}{\eta_C^2}} \right) \right]. \quad (B.6)$$

The first and second partial derivatives of (B.6) with respect to U_C and η_C are required to use the modified Newton-Raphson algorithm. Let,

$\gamma_i = (\gamma_1, \dots, \gamma_{M_i})'$, the vector of γ constants for check carrier i , and

$\xi_i = (\xi_1, \dots, \xi_{M_i})'$, the vector of ξ constants for check carrier i .

The following four variables are defined for notational convenience.

$$k_{1i} = \vec{1} + 10^{U_C} \times \eta_C^2 \times \xi_i,$$

$$k_{2i} = k_{1i}^{\frac{-1}{\eta_C^2}},$$

$$k_{3i} = S(\gamma_i \odot k_{2i}), \text{ and}$$

$$l(\mu_C, \eta_C) = l(\mu_C, \eta_C \mid \vec{r}_1, \dots, \vec{r}_A).$$

First and Second Partial Derivatives

$$\frac{\partial l(\mu_C, \eta_C)}{\partial U_C} = \sum_{i=1}^A \frac{1}{k_{3i}} \times S\left(\left\{-\ln(10) \times 10^{U_C} \times \gamma_i \odot k_{2i} \odot \xi_i\right\} \odot^{-1}\{k_{1i}\}\right).$$

$$\frac{\partial l(\mu_C, \eta_C)}{\partial \eta_C} = \sum_{i=1}^A \frac{1}{k_{3i}} \times S\left(\gamma_i \odot k_{2i} \odot \left[\frac{2 \times \ln(k_{1i})}{\eta_C^3} - \{2 \times 10^{U_C} \times \xi_i\} \odot^{-1}\{\eta_C \times k_{1i}\}\right]\right).$$

$$\begin{aligned} \frac{\partial^2 l(\mu_C, \eta_C)}{\partial U_C^2} = \sum_{i=1}^A \left(\frac{1}{k_{3i}}\right) \times & \left[S\left(10^{U_C} \times (\ln(10))^2 \times \xi_i \odot \gamma_s \odot k_{2i} \odot \left[\right. \right. \right. \\ & \left. \left\{10^{U_C} \times \xi_i\right\} \odot^{-1}\{k_{1i}^2\} - \frac{1}{k_{1i}} + \left. \left\{10^{U_C} \times \eta_C^2 \times \xi_i\right\} \odot^{-1}\{k_{1i}^2\}\right] \right) \left. \right] - \\ & \sum_{i=1}^A \left(\frac{1}{(k_{3i})^2}\right) \times \left[S\left(\left\{-\ln(10) \times 10^{U_C} \times \xi_i \odot \gamma_s \odot k_{2i}\right\} \odot^{-1}\{k_{1i}^2\}\right) \right]^2. \end{aligned}$$

$$\begin{aligned} \frac{\partial^2 l(\mu_C, \eta_C)}{\partial U_C \partial \eta_C} = \sum_{i=1}^A \left(\frac{1}{k_{3i}}\right) \times & \left[S\left(-\gamma_s \odot k_{2i} \odot \left[\left(\frac{2 \times \ln(k_{1i})}{\eta_C^3} - \right. \right. \right. \right. \\ & \left. \left\{2 \times 10^{U_C} \times \xi_i\right\} \odot^{-1}\{\eta_C \times k_{1i}\} \odot \left(\left\{\ln(10) \times 10^{U_C} \times \xi_i\right\} \odot^{-1}\{k_{1i}\}\right) - \right. \\ & \left. \left. \left. \left\{2 \times 10^{2U_C} \times \eta_C \times \ln(10) \times \xi_i^2\right\} \odot^{-1}\{k_{1i}^2\}\right]\right) \right] - \\ & \sum_{i=1}^A \left(\frac{1}{(k_{3i})^2}\right) \times \left[S\left(\left\{-\ln(10) \times 10^{U_C} \times \xi_i \odot \gamma_s \odot k_{2i}\right\} \odot^{-1}\{k_{1i}\}\right) \right] \times \\ & \left[S\left(\gamma_s \odot k_{2i} \odot \left(\frac{2 \times \ln(k_{1i})}{\eta_C^3} - \{2 \times 10^{U_C} \times \xi_i\} \odot^{-1}\{\eta_C \times k_{1i}\}\right)\right) \right]. \end{aligned}$$

$$\frac{\partial^2 l(\mu_C, \eta_C)}{\partial \eta_C \partial U_C} = \frac{\partial^2 l(\mu_C, \eta_C)}{\partial U_C \partial \eta_C}.$$

$$\frac{\partial^2 l(\mu_C, \eta_C)}{\partial \eta_C^2} = \sum_{i=1}^A \left(\frac{1}{k_{3i}}\right) \times \left[S\left(\gamma_s \odot k_{2i} \odot \left[\left(\frac{2 \times \ln(k_{1i})}{\eta_C^3} - \{2 \times 10^{U_C} \times \xi_i\} \odot^{-1}\{\eta_C \times k_{1i}\}\right)^2 + \right. \right. \right.$$

$$\left\{ 6 \times 10^{U_C} \times \xi_i \right\} \odot^{-1} \left\{ \eta_C^2 \times k_{1i} \right\} - \frac{6 \times \ln(k_{1i})}{\eta_C^4} + \left\{ 4 \times 10^{2U_C} \times \xi_i^2 \right\} \odot^{-1} \left\{ k_{1i}^2 \right\} \Bigg] - \sum_{i=1}^A \left(\frac{1}{(k_{3i})^2} \right) \times \left[S \left(\gamma_s \odot k_{2i} \odot \left(\frac{2 \times \ln(k_{1i})}{\eta_C^3} - \left\{ 2 \times 10^{U_C} \xi_i \right\} \odot^{-1} \left\{ \eta_C \times k_{1i} \right\} \right) \right) \right]^2.$$

The Estimated Variance-covariance Matrix of $\hat{\mu}_C$ and $\hat{\eta}_C$

Denote as $V_C(\hat{\mu}_C, \hat{\eta}_C)$ the estimated variance-covariance matrix of $\hat{\mu}_C$ and $\hat{\eta}_C$.

Denote as $V_{\tilde{C}}(\hat{U}_C, \hat{\eta}_C)$ the estimated variance-covariance matrix of $\hat{U}_C$ and $\hat{\eta}_C$.

Assuming the usual regularity conditions hold, as given in (Lehmann 1991), then

$$V_{\tilde{C}}(\hat{U}_C, \hat{\eta}_C) = \left[- \left(\begin{array}{cc} \frac{\partial^2 l(\mu_C, \eta_C)}{\partial U_C^2} & \frac{\partial^2 l(\mu_C, \eta_C)}{\partial U_C \partial \eta_C} \\ \frac{\partial^2 l(\mu_C, \eta_C)}{\partial \eta_C \partial U_C} & \frac{\partial^2 l(\mu_C, \eta_C)}{\partial \eta_C^2} \end{array} \right) \Big|_{(U_C = \hat{U}_C, \eta_C = \hat{\eta}_C)} \right]^{-1}.$$

$V_C(\hat{\mu}_C, \hat{\eta}_C)$ can be obtained from $V_{\tilde{C}}(\hat{U}_C, \hat{\eta}_C)$ as follows:

$$V_C(\hat{\mu}_C, \hat{\eta}_C) = \begin{pmatrix} \ln(10) \times 10^{\hat{U}_C} & 0 \\ 0 & 1 \end{pmatrix} V_{\tilde{C}}(\hat{U}_C, \hat{\eta}_C) \begin{pmatrix} \ln(10) \times 10^{\hat{U}_C} & 0 \\ 0 & 1 \end{pmatrix}.$$

Let $g_7(\mu, \eta) = \frac{\Psi(\frac{1}{\eta^2}) + \ln(\mu\eta^2)}{\ln(10)}$. The computation of the estimated variance of $\hat{\mu}_X$ requires knowledge of the partial derivatives of g_7 with respect to both μ and η .

$$\frac{\partial g_7(\mu, \eta)}{\partial \mu} = \frac{1}{\ln(10) \times \mu} \quad \text{and} \quad \frac{\partial g_7(\mu, \eta)}{\partial \eta} = \frac{1}{\ln(10)} \times \left(\frac{-2 \times \Psi'(\eta^{-2})}{\eta^3} + \frac{2}{\eta} \right).$$

Computation of $\hat{\mu}_T$, $\hat{\mu}_Y$, and $V_C(\hat{\mu}_T, \hat{\eta}_T)$

Let μ_T and η_T denote the mean and coefficient of variation of the number of organisms surviving the disinfection steps for test carriers. Theorem 1 was applied to derive the joint marginal distribution of $\vec{g}_1, \dots, \vec{g}_B$. The likelihood function, $l(\mu_T, \eta_T \mid \vec{g}_1, \dots, \vec{g}_B)$, is given in (3.14). Denote as $\hat{\mu}_T$ and $\hat{\eta}_T$ the values of μ_T and η_T which jointly maximize 3.14.

Because μ_T and η_T may be of differing orders of magnitude, I decided to compute first the mle of $\log(\mu_T)$, and then use the invariance property of mle to obtain $\hat{\mu}_T$. I used a modified Newton-Raphson method to perform the necessary maximizations. Let $U_T = \log(\mu_T)$. Note that $\mu_T = 10^{U_T}$. Thus (3.14) can be reparameterized in terms of U_T and η_T as follows: $l(U_T, \eta_T \mid \vec{g}_1, \dots, \vec{g}_B) =$

$$\sum_{s=1}^B \ln \left[\prod_{s=1}^B \left(\frac{w_{sm}}{g_{sm}} \right) \left(\sum_{L_s=1}^{M_s} \gamma_{L_s} \times \left[10^{U_T} \eta_T^2 (1 - e^{-\xi_{L_s}}) + e^{-\xi_{L_s}} \right]^{\left(\frac{-10^{U_T}}{10^{U_T} \times \eta_T^2 - 1} \right)} \right) \right]. \quad (B.7)$$

The first and second partial derivatives of (B.7) with respect to U_T and η_T are required to use the Newton-Raphson algorithm. Let,

$\gamma_s = (\gamma_1, \dots, \gamma_{M_i})'$, the vector of γ constants for test carrier s , and

$\xi_s = (\xi_1, \dots, \xi_{M_i})'$, the vector of ξ constants for test carrier s .

The following six variables are introduced for notational convenience.

$$k_{1s} = 10^{U_T} \times \eta_T^2 \times (1 - e^{\xi_s}) + e^{-\xi_s},$$

$$k_{2s} = k_{1s}^{\frac{-10^{U_T}}{10^{U_T} \times \eta_T^2 - 1}},$$

$$k_{3s} = S(\gamma_s \odot k_{2s}),$$

$$k_{4s} = 10^{U_T} \times \eta_T^2 - 1,$$

$$k_{5s} = \frac{-10^{U_T} \times \ln(10)}{k_{4s}} + \frac{10^{2U_T} \times \eta_T^2 \times \ln(10)}{k_{4s}^2}, \text{ and}$$

$$l(U_T, \eta_T) = l(U_T, \eta_T \mid \vec{g}_1, \dots, \vec{g}_B).$$

First and Second Partial Derivatives

$$\frac{\partial l(U_T, \eta_T)}{\partial U_T} = \sum_{s=1}^B \frac{1}{k_{3s}} \times S \left(\gamma_s \odot k_{2s} (k_{5s} \times \ln(k_{1s}) - \{10^{2U_T} \times \eta_T^2 \times \ln(10) \times (1 - e^{\xi_s})\} \odot^{-1} \{k_{1s} \times k_{4s}\}) \right).$$

$$\frac{\partial l(U_T, \eta_T)}{\partial \eta_T} = \sum_{s=1}^B \frac{1}{k_{3s}} \times S \left(\gamma_s \odot k_{2s} (\{2 \times 10^{2U_T} \times \eta_T \times \ln(k_{1s})\} \odot^{-1} \{k_{4s}^2\} - \{2 \times 10^{2U_T} \times \eta_T \times (1 - e^{\xi_s})\} \odot^{-1} \{k_{1s} \times k_{4s}\}) \right).$$

$$\frac{\partial^2 l(U_T, \eta_T)}{\partial U_T^2} = \sum_{s=1}^B \left(\frac{1}{k_{3s}} \right) \times \left[S \left((k_{5s} \times \ln(k_{1s}) - \{10^{2U_T} \times \eta_T^2 \times \ln(10) \times (1 - e^{\xi_s})\} \odot^{-1} \{k_{1s} \times k_{4s}\}) \right) \right]$$

$$\begin{aligned}
& \left\{ 10^{2U_T} \times \eta_T^2 \times \ln(10) \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} \Big)^2 + \\
& \frac{10^{U_T} \times \ln(k_{1s}) \times (\ln(10))^2}{k_{4s}} \times \left(-1 + 3 \times 10^{U_T} \times \eta_T^2 \div k_{4s} - 2 \times 10^{2U_T} \times \eta_T^4 \div k_{4s}^2 \right) + \\
& \left\{ 10^{U_T} \times \eta_T^2 \times \ln(10) \times k_{5s} \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \} - \\
& \left\{ 2 \times 10^{2U_T} \times \eta_T^2 \times (\ln(10))^2 \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} + \\
& \left\{ 10^{3U_T} \times \eta_T^4 \times (\ln(10))^2 \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s}^2 \} + \\
& \left\{ 10^{3U_T} \times \eta_T^4 \times (\ln(10))^2 \times (1 - e^{\xi_s})^2 \right\} \odot^{-1} \{ k_{1s}^2 \times k_{4s} \} - \\
& \sum_{s=1}^B \left(\frac{1}{(k_{3s})^2} \right) \times \left[S \left(\gamma_s \odot k_{2s} \odot (k_{5s} \times \ln(k_{1s}) - \right. \right. \\
& \left. \left. \left\{ 10^{2U_T} \times \eta_T^2 \times \ln(10) \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} \right) \right) \Big]^2. \\
\frac{\partial^2 l(U_T, \eta_T)}{\partial U_T \partial \eta_T} = & \sum_{s=1}^B \left(\frac{1}{k_{3s}} \right) \times \left[S \left(\gamma_s \odot k_{2s} \odot \left[\left\{ 2 \times 10^{2U_T} \times \eta_T \times \ln(k_{1s}) \right\} \odot^{-1} \{ k_{4s}^2 \} - \right. \right. \right. \\
& \left. \left\{ 2 \times 10^{2U_T} \times \eta_T \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} \right\} \odot \\
& \left\{ k_{5s} \times \ln(k_{1s}) - \left\{ 10^{2U_T} \times \eta_T^2 \times \ln(10) \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} \right\} + \\
& \frac{4 \times 10^{2U_T} \times \eta_T \times \ln(10) \times \ln(k_{1s})}{k_{4s}^2} \times \left(1 - \frac{10^{U_T} \times \eta_T^2}{k_{4s}} \right) + \\
& \left\{ 2 \times 10^{U_T} \times \eta_T \times k_{5s} \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \} + \\
& \left\{ 2 \times 10^{3U_T} \times \eta_T^3 \times \ln(10) \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s}^2 \} - \\
& \left\{ 2 \times 10^{2U_T} \times \eta_T \times \ln(10) \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} + \\
& \left. \left. \left. \left\{ 2 \times 10^{3U_T} \times \eta_T^3 \times \ln(10) \times (1 - e^{\xi_s})^2 \right\} \odot^{-1} \{ k_{1s}^2 \times k_{4s} \} \right] \right) \right] - \\
& \sum_{s=1}^B \left(\frac{1}{(k_{3s})^2} \right) \times \left[S \left(\gamma_s \odot k_{2s} \odot (k_{5s} \times \ln(k_{1s}) - \right. \right. \\
& \left. \left. \left\{ 10^{2U_T} \times \eta_T^2 \times \ln(10) \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} \right) \right) \Big] \times \\
& \left[S \left(\gamma_s \odot k_{2s} \odot \left(\left\{ 2 \times 10^{2U_T} \times \eta_T \times \ln(k_{1s}) \right\} \odot^{-1} \{ k_{4s}^2 \} - \right. \right. \right. \\
& \left. \left. \left. \left\{ 2 \times 10^{2U_T} \times \eta_T \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} \right) \right) \right].
\end{aligned}$$

$$\frac{\partial^2 l(U_T, \eta_T)}{\partial \eta_T \partial U_T} = \frac{\partial^2 l(U_T, \eta_T)}{\partial U_T \partial \eta_T}.$$

$$\begin{aligned}
\frac{\partial^2 l(U_T, \eta_C)}{\partial \eta_T^2} = & \sum_{s=1}^B \left(\frac{1}{k_{3s}} \right) \times \left[S \left(\gamma_s \odot k_{2s} \odot \left[\left(\left\{ 2 \times 10^{2U_T} \times \eta_T \times \ln(k_{1s}) \right\} \odot^{-1} \{ k_{4s}^2 \} - \right. \right. \right. \right. \\
& \left. \left. \left. \left\{ 2 \times 10^{2U_T} \times \eta_T \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{ k_{1s} \times k_{4s} \} \right)^2 - \frac{8 \times 10^{3U_T} \times \eta_T^2 \times \ln(k_{1s})}{k_{4s}^3} + \right. \right.
\end{aligned}$$

$$\begin{aligned}
& \frac{2 \times 10^{2U_T} \times \ln(k_{1s})}{k_{4s}^2} + \left\{ 8 \times 10^{3U_T} \times \eta_T^2 \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{k_{1s} \times k_{4s}^2\} - \\
& \left\{ 2 \times 10^{2U_T} \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{k_{1s} \times k_{4s}\} + \\
& \left\{ 4 \times 10^{3U_T} \times \eta_T^2 \times (1 - e^{\xi_s})^2 \right\} \odot^{-1} \{k_{1s}^2 \times k_{4s}\} \Big] - \\
& \sum_{s=1}^B \left(\frac{1}{(k_{3s})^2} \right) \times \left[S \left(\gamma_s \odot k_{2s} \odot \left(\left\{ 2 \times 10^{2U_T} \times \eta_T \times \ln(k_{1s}) \right\} \odot^{-1} \{k_{4s}^2\} - \right. \right. \right. \\
& \left. \left. \left. \left\{ 2 \times 10^{2U_T} \times \eta_T \times (1 - e^{\xi_s}) \right\} \odot^{-1} \{k_{1s} \times k_{4s}\} \right) \right)^2 \right].
\end{aligned}$$

The Estimated Variance-covariance Matrix of $\hat{\mu}_T$ and $\hat{\eta}_T$

Denote as $V_T(\hat{\mu}_T, \hat{\eta}_T)$ the estimated variance-covariance matrix of $\hat{\mu}_T$ and $\hat{\eta}_T$.

Denote as $V_{\hat{T}}(\hat{U}_T, \hat{\eta}_T)$ the estimated variance-covariance matrix of $\hat{U}_T$ and $\hat{\eta}_T$.

Assuming the usual regularity conditions hold, as given in (Lehmann 1991), then

$$V_{\hat{T}} = \left[- \begin{pmatrix} \frac{\partial^2 l(U_T, \eta_T)}{\partial U_T^2} & \frac{\partial^2 l(U_T, \eta_T)}{\partial U_T \partial \eta_T} \\ \frac{\partial^2 l(U_T, \eta_T)}{\partial \eta_T \partial U_T} & \frac{\partial^2 l(U_T, \eta_T)}{\partial \eta_T^2} \end{pmatrix} \Big|_{(U_T = \hat{U}_T, \eta_T = \hat{\eta}_T)} \right]^{-1}.$$

$V_T(\hat{\mu}_T, \hat{\eta}_T)$ can be obtained from $V_{\hat{T}}(\hat{U}_T, \hat{\eta}_T)$ as follows:

$$V_T(\hat{\mu}_T, \hat{\eta}_T) = \begin{pmatrix} \ln(10) \times 10^{\hat{U}_T} & 0 \\ 0 & 1 \end{pmatrix} V_{\hat{T}}(\hat{U}_T, \hat{\eta}_T) \begin{pmatrix} \ln(10) \times 10^{\hat{U}_T} & 0 \\ 0 & 1 \end{pmatrix}.$$

Let,

$$g_8(\mu, \eta) = \sum_{t=1}^N \log(t) \times \frac{\Gamma\left(t + \frac{\mu}{\mu\eta^2 - 1}\right)}{\Gamma\left(\frac{\mu}{\mu\eta^2 - 1}\right) t!} \left[\left(\frac{1}{\mu\eta^2} \right)^{\frac{\mu}{\mu\eta^2 - 1}} \right] \left[\left(\frac{\mu\eta^2 - 1}{\mu\eta^2} \right)^t \right].$$

The computation of the estimated variance of $\hat{\mu}_Y$, requires knowledge of the partial derivatives of either g_7 or g_8 with respect to both μ and η . The partial derivatives of g_7 have already been given in this appendix. The partial derivatives of g_8 are given below.

$$\begin{aligned}
\frac{\partial g_8(\mu, \eta)}{\partial \mu} = \sum_{t=1}^N \left\{ \log(t) \times \frac{\Gamma\left(t + \frac{\mu}{\mu\eta^2 - 1}\right)}{\Gamma\left(\frac{\mu}{\mu\eta^2 - 1}\right) t!} \left[\left(\frac{1}{\mu\eta^2} \right)^{\frac{\mu}{\mu\eta^2 - 1}} \right] \left[\left(\frac{\mu\eta^2 - 1}{\mu\eta^2} \right)^t \right] \times \left[\frac{-1}{(\mu\eta^2 - 1)^2} \times \right. \right. \\
\left. \left. \left(\Psi\left(t + \frac{\mu}{\mu\eta^2 - 1}\right) - \Psi\left(\frac{\mu}{\mu\eta^2 - 1}\right) \right) + \left(\frac{-1}{(\mu\eta^2 - 1)^2} \right) \times \ln\left(\frac{1}{\mu\eta^2}\right) - \frac{1}{(\mu\eta^2 - 1)} + \frac{t}{\mu \times (\mu\eta^2 - 1)} \right] \right\}.
\end{aligned}$$

$$\frac{\partial g_8(\mu, \eta)}{\partial \eta} = \sum_{t=1}^N \left\{ 2 \times \log(t) \times \frac{\Gamma\left(t + \frac{\mu}{\mu\eta^2 - 1}\right)}{\Gamma\left(\frac{\mu}{\mu\eta^2 - 1}\right) t!} \left[\left(\frac{1}{\mu\eta^2}\right)^{\frac{\mu}{\mu\eta^2 - 1}} \right] \left[\left(\frac{\mu\eta^2 - 1}{\mu\eta^2}\right)^t \right] \times \right. \\ \left. \left[\frac{\mu^2 \eta}{(\mu\eta^2 - 1)^2} \times \left(\Psi\left(\frac{\mu}{\mu\eta^2 - 1}\right) - \Psi\left(t + \frac{\mu}{\mu\eta^2 - 1}\right) \right) + \frac{\mu^2 \eta \ln(\mu\eta^2)}{(\mu\eta^2 - 1)^2} - \frac{\mu}{\eta \times (\mu\eta^2 - 1)} + \frac{t}{\eta \times (\mu\eta^2 - 1)} \right] \right\}.$$

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