



Using a serial dilution experiment to estimate the density of organisms
by Milton Wayne Loyer

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Abstract:

The serial dilution assay is a standard microbiological method for determining the density of organisms in a solution. This paper presents alternatives to current standard serial dilution confidence interval, point estimate and design recommendations.

Original exact confidence intervals are given which are narrower than those available in standard tables. Point estimates are given which have smaller mean squared error than the standard most probable number (MPN) maximum likelihood estimator. An algorithm is given which, for the techniques discussed and within certain researcher-chosen constraints, identifies the optimal design and the most efficient estimator.

This paper also gives the solution to the general finite population serial dilution problem, discusses finite population analogs of the confidence interval and point estimate techniques discussed, and compares the finite and the infinite population models.

The computer programs which were used to obtain the confidence intervals, point estimates and tables presented in the text are given in the Appendix. These programs, including the one for identifying the optimal design, generalize to any number of dilutions, any number of samples per dilution and any dilution factor.

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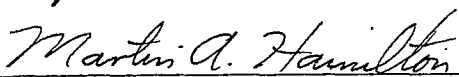
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Statistics

Approved:



Head, Major Department



Chairman, Examining Committee



Graduate Dean

MONTANA STATE UNIVERSITY
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ABSTRACT

The serial dilution assay is a standard microbiological method for determining the density of organisms in a solution. This paper presents alternatives to current standard serial dilution confidence interval, point estimate and design recommendations.

Original exact confidence intervals are given which are narrower than those available in standard tables. Point estimates are given which have smaller mean squared error than the standard most probable number (MPN) maximum likelihood estimator. An algorithm is given which, for the techniques discussed and within certain researcher-chosen constraints, identifies the optimal design and the most efficient estimator.

This paper also gives the solution to the general finite population serial dilution problem, discusses finite population analogs of the confidence interval and point estimate techniques discussed, and compares the finite and the infinite population models.

The computer programs which were used to obtain the confidence intervals, point estimates and tables presented in the text are given in the Appendix. These programs, including the one for identifying the optimal design, generalize to any number of dilutions, any number of samples per dilution and any dilution factor.

1. INTRODUCTION

Halvorson and Ziegler (1933a) state "the use of dilution methods ...dates back to the early days of science" and note that Pasteur, for example, was using serial dilution techniques about 1875. Typically, one seeks to estimate the number of organisms per unit volume of solution under the assumptions that (1) the organisms are randomly distributed throughout the solution and (2) each sample from the solution, when incubated in the culture medium, is certain to exhibit fertility whenever the sample contains one or more organisms. If the solution averages λ organisms per unit volume and z is the dilution (multiple of the unit volume selected for analysis), then, under the Poisson probability model, $P(\text{sterile sample}) = e^{-\lambda z}$. In practice, one guards against obtaining samples which are likely to be either all sterile or all fertile by using more than one dilution. Letting X_i equal the number of fertile samples in n_i trials at the i^{th} dilution, $P(X_i=r) = \binom{n_i}{r} (1-e^{-\lambda z_i})^r (e^{-\lambda z_i})^{n_i-r}$.

In the first definitive study of the problem of estimating λ using serial dilutions, McCrady (1915) described the estimate $\hat{\lambda}$, the value of λ that maximizes the probability of obtaining the specific arrangement of fertile and sterile samples observed. McCrady called $\hat{\lambda}$ the "most probable number" (MPN) and presented the procedure, which today is known as maximum likelihood (ML) estimation, as Bayes estimation with an improper uniform prior on λ .^a To justify the procedure, he cites, among others, distinguished late nineteenth

century mathematician Richard L. Edgeworth who stated, "The assumption that any probability constant about which we know nothing in particular is as likely to have one value as another, is grounded upon the rough but solid experience that such constants do, as a matter of fact, as often have one value as another."

For k dilutions, the likelihood function is given by

$$(1.1) \quad L(x_1, x_2, \dots, x_k; \lambda) = \prod_{i=1}^k \binom{n_i}{x_i} (1 - e^{-\lambda z_i})^{x_i} (e^{-\lambda z_i})^{n_i - x_i}$$

and the maximum likelihood estimate for λ , still most commonly

referred to as the MPN, is the λ which solves $\sum (x_i z_i e^{-\lambda z_i}) / (1 - e^{-\lambda z_i}) = \sum (n_i - x_i) z_i$, which simplifies (deMan 1977) to

$$(1.2) \quad \sum n_i z_i = \sum x_i z_i / (1 - e^{-\lambda z_i}).$$

For $k > 1$, the solution to (1.2) must be obtained by iterative methods. Several programs (e.g., Parnow 1972) to obtain the MPN for any k , any z_i and any n_i are readily available.

While the methods of all sections of this paper generalize to any k , any z_i and any n_i (except in Chapter 5 where it is required that $\sum n_i z_i < 1$), the numerical examples given are for the commonly encountered case of $k=3$ decimal dilutions $z_i = (.1)^i$ with $n_i = 3$ for $i=1, 2, 3$. The 64 possible (X_1, X_2, X_3) sample results will be referred to by the codes 000, 001, ..., 332, 333. For these k , z_i and n_i , the first three columns of Table 1 summarize the results presented and recommended by standard reference works.

TABLE 1

95% Confidence Intervals: $n=3$, $z_1=.1$ $z_2=.01$ $z_3=.001$

result	MPN ^a	Woodward ^b	deMan ^c	Combining ^d Independent Results	Minimum ^e Expected Width
000	0.0	0-9 ^f		0-12	0-13
001	3.0	0-9	<1-17	2-15	
002	6.0				
003	9.0				
010	3.0	.085-13	<1-17	<1-16	2-10
011	6.1			7-19	
012	9.2				
013	12				
020	6.2		2-22	4-17	
021	9.3			16-20	
022	12				
023	16				
030	9.4			17-17	
031	13				
032	16				
033	19				
100	3.6	.085-20	<1-21	<1-24	<1-25
101	7.2	.87-21	2-27	3-28	
102	11			26-28	
102	15				
110	7.3	.88-23	2-28	1-30	3-20
111	11	3-36	4-34	7-35	
112	15			35-35	
113	19				
120	11	2.7-36	4-35	5-32	
121	15		6-41	17-37	
122	20				
123	24				
130	16		6-42	18-32	
131	20				
132	24				
133	29				
200	9.1	1.0-36	2-38	1-42	<1-37
201	14	2.7-37	5-48	5-50	11-14
202	20			27-50	
203	26				
210	15	2.8-44	5-50	3-55	5-42
211	20	7-89	7-60	9-64	
212	27			36-65	
213	34				
220	21	3.5-47	8-62	7-61	10-32
221	28	10-150	11-74	18-71	
222	35			51-72	
223	42				
230	29		11-77	19-63	
231	36			40-74	
232	44				
233	53				
300	23	3.5-120	<10-130	3-137	4-120
301	39	6.9-130	10-180	10-175	14-69
302	64	15-380	20-230	42-183	
303	95				
310	43	7.1-210	10-210	5-257	7-200
311	75	14-230	20-280	15-320	21-180
312	120	30-380	40-350	51-340	
313	160			195-345	
320	93	15-380	30-380	11-456	12-360
321	150	30-440	50-500	26-594	38-400
322	210	35-470	80-640	69-659	120-260
323	290		110-790	208-687	
330	240	36-1300	<100-1400	27-1612	26-990
331	460	71-2400	100-2400	54-1800	70-2000
332	1100	150-4800	300-4800	115-1800	140-4070
333	∞	460- ∞ ^f		298- ∞	370- ∞

^aAmerican Public Health Association (1970, page 101)^bAmerican Public Health Association (1971, page 676); see section 2.1^cdeMan (1977); see section 2.2^dsee section 2.3^esee section 2.4^fone-sided 95% confidence interval

This paper examines presently recommended serial dilution interval estimation (Chapter 2), point estimation (Chapter 3) and design (Chapter 4) techniques. In each chapter, alternatives are developed and compared to the currently standard methods. In Chapter 5, the exact solution is given to the finite population serial dilution problem.

2. CONFIDENCE INTERVALS

Sections 2.1-2.4 present two commonly used and two new methods for constructing exact $100(1-\alpha)\%$ confidence intervals. Several approximate confidence interval techniques are discussed briefly in section 2.5. The 95% confidence intervals obtained by the methods in sections 2.1-2.4 are given in the final four columns of Table 1. As apparent from the discussion below, the methods of sections 2.1-2.3 can be used to construct one-sided confidence intervals, and for these methods the endpoints given in Table 1 may be used as the endpoints for appropriate 97.5% one-sided confidence intervals.

2.1 Woodward's Method

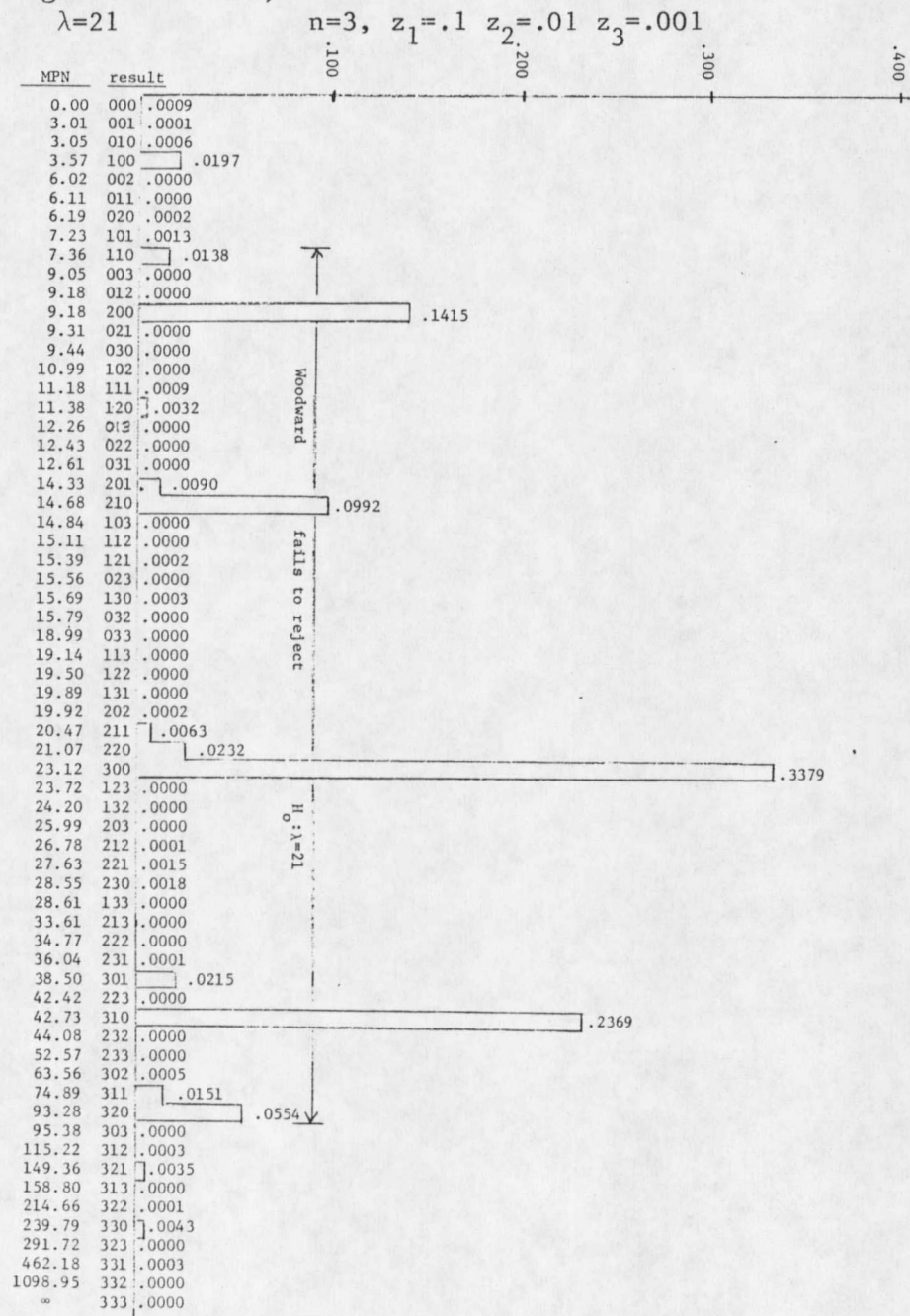
Perhaps the most commonly used 95% confidence intervals are those given by the American Public Health Association (1971, page 676). Prepared by Woodward (1957) and appearing in the "Woodward" column of Table 1, these intervals are the accepted norm by which other procedures are often judged (e.g., Martins and Selby 1980). Woodward ranked each of the 64 possible $X_1X_2X_3$ outcomes according to the magnitude of the MPN and then constructed 95% confidence intervals (i.e., approximate intervals, since the outcome space is discrete) by testing $H_0: \lambda = \lambda_0$ for selected λ_0 values in $[0, \infty)$. For a given $X_1X_2X_3$ outcome, Woodward rejected $H_0: \lambda = \lambda_0$ if and only if that $X_1X_2X_3$ outcome produced an MPN in the lower 2.5% or the upper 2.5% of the probability distribution of MPN's generated under H_0 . The set of all

λ_0 's not rejected for any $X_1X_2X_3$ outcome comprise the two-sided 95% confidence interval associated with that outcome.

Figure 1 illustrates the Woodward method. When testing $H_0: \lambda=21$ vs. $H_a: \lambda \neq 21$, one obtains the distribution of MPN's shown. Rejecting the .025 most extreme results in each tail, Woodward rejects $\lambda=21$ for $X_1X_2X_3=000, 001, 010, 100, 002, 011, 020, 101$ in the lower tail and for $X_1X_2X_3=333, 332, 331, 323, 330, 322, 313, 321, 312, 303$ in the upper tail. The Woodward 95% confidence intervals of Table 1 for these results (when given) should not include the value 21. This is true for all cases except $X_1X_2X_3=101$, which represents an error in Woodward's calculations. Nor is this the only error in Woodward's work, as deMan (1975) notes. "In the table presented by Woodward (1957)," he states, "a few mistakes were also found, but they were minor. Undoubtedly, this table should have been given more attention than it apparently received."^b The program used to generate the probabilities for Figure 1 is given in Appendix I. The MPN's in Figure 1 were obtained by Newton's method within the program of Appendix III.

Two additional comments regarding Woodward's confidence intervals need to be made. First, a caveat given by Woodward but often omitted by those reproducing his tables should be repeated. For the 000 (333) result, Woodward rejects $H_0: \lambda=\lambda_0$ if and only if the MPN is in the upper (lower) 5% of the sampling distribution and presents only upper (lower) 95% confidence intervals.

FIGURE 1
Distribution of Possible Sample Results (arranged by
magnitude of MPN)



Secondly, while Woodward's method provides a 95% confidence interval for each of the $X_1X_2X_3$ possible outcomes, his 1957 table includes confidence intervals only for what he determines to be the 22 most likely $X_1X_2X_3$ outcomes. The remaining 42 $X_1X_2X_3$ outcomes he calls "improbable" and recommends that they not be used for making inferences. In other words, there are some $X_1X_2X_3$ outcomes (e.g., the result 003 -- no organisms present in the more concentrated .1 or .01 dilutions, but organisms present in all three samples at the weakest .001 dilution) for which Woodward's method gives a 95% confidence interval in which he apparently does not have 95% confidence. The last two methods of Table 1 eliminate this subjectivity by inherently failing to give confidence intervals (i.e., by giving empty confidence intervals) for improbable results.

A further inspection of Woodward's method reveals some serious practical flaws. Note from Figure 1 that ordering the $X_1X_2X_3$ results by the magnitude of the MPN does not yield a unimodal sampling distribution. According to most statistical inference texts (e.g., Cox and Hinkley 1974, page 66), this means that the MPN is not an acceptable test statistic since more extreme values of the MPN do not necessarily give stronger evidence of departure from H_0 . Fisher (1956, page 98) objected to a procedure of Bartlett for similar reasons since his statistic "does not increase or decrease monotonically for changes in the weight of the evidence."

The difficulty caused by a multi-modal sampling distribution can be seen from Figure 1. Woodward's rejection region includes the result 100 for which $P(X_1X_2X_3=100) = .0197$ but fails to include the less likely result 110 for which $P(X_1X_2X_3=110) = .0138$. In fact, Woodward cannot place in his rejection region any result, no matter how unlikely, which gives an MPN larger than 3.57 unless the result 100 were already in the rejection region. Woodward's intervals, then, form a "staircase" based on the magnitude of the MPN so that the lower (upper) confidence limit associated with one sample result cannot be lower (higher) than the limit associated with another sample result yielding a lower (higher) MPN.^c Consequently, the width of an interval is not determined by the precision associated with the sample result, and preliminary calculations verify that the actual level of Woodward's intervals is greater than 95%.

2.2 DeMan's Method

Another set of commonly used confidence intervals is given by deMan (1975) and appears in the "deMan" column of Table 1. Even though deMan uses the term "confidence interval," his procedure does not meet the necessary and sufficient conditions given by Neyman (1941), the originator of the concept of confidence intervals as presently employed. In the opinion of many authors (e.g., von Mises 1942), however, this is not necessarily to deMan's detriment. DeMan does, in fact, provide the limits of the middle 95% of the likelihood

distribution for each $X_1X_2X_3$ result or, equivalently, the Bayesian interval for λ under an improper uniform prior. While the posterior function $f(\lambda; x_1, x_2, x_3)$ is defined continuously for $\lambda \in [0, \infty)$, deMan used discrete approximations in both directions from the MPN and truncated the posterior distribution whenever an additional tail histogram area contributed less than .000005 of the cumulative total.

DeMan's method, like Woodward's, provides a 95% confidence interval for each of the 64 possible $X_1X_2X_3$ results. Also like Woodward, deMan states that "MPN tables should be restricted to results having a defined minimal probability" and gives no confidence intervals for "improbable" $X_1X_2X_3$ results. In their original articles, deMan and Woodward agree in all but two cases on what is improbable (deMan considers the result 312 improbable, but not the result 211). It is clear that each finds himself deciding which of his 95% intervals he chooses not to accept with nominal level 95 per cent.

If one desires to use a Bayesian procedure, of course, he is not limited to an improper uniform prior. In general, the more specific prior information the researcher has (or is willing to assume) about λ , the narrower he can make his "confidence interval." Even if the researcher begins with complete ignorance about λ , however, the uniform prior may not be the appropriate prior. Box and Tiao (1973) discuss Bayesian interval estimation in general and define a

"noninformative prior," based on Fisher's information, that they recommend for the researcher with little or no prior information.

2.3 Methods of Combining Independent Results

Since each of the three dilutions gives results independent of those of the other dilutions, the total serial dilution experiment yields three independent point estimates and three independent confidence intervals for λ . First impressions might suggest constructing $\sqrt[3]{.95}$ confidence intervals C_1 , C_2 and C_3 for each of the three dilutions and using the intersection $C_1 \cap C_2 \cap C_3$ as an experiment-wide 95% confidence interval. This is certainly statistically acceptable and has the advantage of permitting certain unlikely results to produce empty confidence intervals whenever $C_1 \cap C_2 \cap C_3 = \emptyset$. There are, however, at least two disadvantages major enough to discourage the use of this procedure.

First, the three independent confidence intervals have at most three distinct lower endpoints and three distinct upper endpoints. This means that the 64 possible $X_1X_2X_3$ results generate a maximum of $3^2=9$ distinct non-empty confidence intervals. Certainly, there exists the possibility of different $X_1X_2X_3$ results yielding identical confidence intervals. This would not be undesirable if the minimal sufficient statistic were some function of the X_i (e.g., $Y=\sum X_i$) that could assume only some number of values considerably less than 64.

Here, unfortunately, the minimal sufficient statistic is the ordered triple (X_1, X_2, X_3) and none of the 64 possible $X_1 X_2 X_3$ outcomes gives the same information as any of the other outcomes.

Secondly, one intuitively has more confidence in the interval associated with the strongest concentration z_1 since it represents, in some sense, the largest sample size. Or should one have more confidence in the interval associated with the weakest concentration z_3 since it represents, in some sense, the finest scrutiny of the solution? What if two of the three intervals agree and the third appears to be an outlier? While the weighting of estimates is usually associated with point estimation, one can't help but feel that merely intersecting three intervals obtained at three different levels of examination would be naive and inefficient.

Fisher (1932) introduced a method of combining the results of independent tests using p-values, and Lancaster (1976) gives an updated review of the procedure. Here, one combines the p-values associated with $H_0: \lambda = \lambda_0$ for each of the three dilutions to obtain an experiment-wide p-value. The method uses the well-known (e.g., Hogg and Craig 1970, pages 349, 104 and 159) facts

- (i) For any continuous random variable Y with distribution function $F(Y)$, the random variable $W = F(Y)$ has a uniform $[0,1]$ distribution.
- (ii) If Y is a random variable having a uniform $[0,1]$

distribution, then $W = -2[\ln(Y)]$ has a chi-square distribution with 2 degrees of freedom.

(iii) If Y_1, Y_2, \dots, Y_k are independent random variables each having a chi-square distribution with r_i degrees of freedom, then $W = Y_1 + Y_2 + \dots + Y_k$ has a chi-square distribution with $r = r_1 + r_2 + \dots + r_k$ degrees of freedom.

When the test $H_0: \lambda = \lambda_0$ vs. $H_a: \lambda > \lambda_0$ at the single dilution z yields m fertile samples in n trials, the associated p-value is given by

$$\begin{aligned}
 (2.1) \quad p &= \sum_{x=m}^n \binom{n}{x} (1 - e^{-\lambda_0 z})^x (e^{-\lambda_0 z})^{n-x} \\
 &= P(X \geq m \mid \lambda = \lambda_0) \\
 &= 1 - P(X < m \mid \lambda = \lambda_0) \\
 &= 1 - F(X)
 \end{aligned}$$

(ignoring the lack of continuity, a correction for which will be given later). Since $p = 1 - F(X)$ has a uniform $[0, 1]$ distribution whenever $W = F(X)$ has a uniform $[0, 1]$ distribution, (i), (ii) and (iii) above imply that an experiment-wide p-value for three independent dilutions can be obtained by calculating the probability that $-2\sum[\ln(p_i)]$ is greater than a random variable having a chi-square distribution with 6 degrees of freedom. Collecting for each $X_1 X_2 X_3$ result the λ_0 's for which one fails to reject $H_0: \lambda = \lambda_0$ vs. $H_a: \lambda > \lambda_0$ at the $\alpha = .025$ level (i.e., for which the p-value is greater than .025) and for which one fails to reject $H_0: \lambda = \lambda_0$ vs. $H_a: \lambda < \lambda_0$ at the $\alpha = .025$ level, one constructs two-sided 95% confidence intervals.

Rosenthal (1978) reviews several methods of combining the results of independent studies and for $k=3$ independent results such as those in the serial dilution problem, he recommends Fisher's method.

Rosenthal does, however, remind his readers of two disadvantages inherent in the method. First, for several independent p -values just slightly lower than .50, Fisher's method might not yield an overall significant p -value when such simple tests as the sign test would. Secondly, independent trials with strongly significant results in opposite directions could cause Fisher's method to support the significance of either outcome. While it is precisely this second phenomenon that proves to be the desired advantage in the serial dilution problem by causing empty confidence intervals for improbable results, not all authors would see this as an asset. Cox and Hinkley (1974, page 225), for example, discuss procedures that give empty confidence intervals and say, "the assertion that [the parameter] lies in a null region is certainly false... What is the point of making assertions known to be false?"

Lancaster (1949) gives a continuity correction for Fisher's method which, according to recent surveys (e.g., Rosenthal 1978), others have not been able to improve upon since. Moses (1956) discusses the Lancaster-corrected Fisher technique in detail. Confidence intervals obtained using Fisher's technique and Lancaster's correction for continuity are given in the "Combining Independent

Results" column of Table 1. While Woodward's method and deMan's method yield confidence intervals for all possible $X_1X_2X_3$ results (recall that those authors give intervals only for what they consider "probable" and acceptable results), the method of combining independent results described above yields only the confidence intervals given in Table 1. $X_1X_2X_3$ results for which no confidence interval is given are results inconsistent with any value of λ_0 . The program used to obtain the "Combining Independent Results" entries in Table 1 is given, along with an example, in Appendix II.

2.4 The Method of Minimum Expected Width

It is axiomatic that methods giving narrower confidence intervals are to be preferred over competing methods (all other considerations being equal). While the combination method of section 2.3 certainly gives narrower confidence intervals than Woodward's or deMan's method whenever it yields an empty interval, Table 1 indicates other $X_1X_2X_3$ results (e.g., 120) for which the combination method gives the narrowest non-empty confidence interval of the three methods. This prompts a search for yet another method which deliberately seeks to minimize the widths of the 95% confidence intervals. It will be apparent, however, that such a method produces only two-sided intervals and does not have the ability of the Woodward, deMan or combination method to use upper or lower 95% confidence interval

endpoints as endpoints of corresponding 97.5% one-sided confidence intervals.

The method of minimum expected width, like Woodward's method, starts by testing $H_0: \lambda = \lambda_0$ vs. $H_a: \lambda \neq \lambda_0$ at the $\alpha = .05$ level, considering the probability distribution of the 64 possible $X_1X_2X_3$ results under H_0 and rejecting H_0 if the observed result falls within the extreme 5% of the sampling distribution. The two methods differ according to how each determines whether a particular $X_1X_2X_3$ result lies within the extreme 5% of all results possible under H_0 .

Most textbook presentations of the minimum expected width technique are confined to continuous unimodal problems, for which the technique gives the shortest confidence interval (e.g., Guenther 1969). Mood, Graybill and Boes (1974, page 383) describe the technique for this case and contrast it with the usual equal tails method of constructing confidence intervals. Larson and Marx (1981, page 290) note that the technique, unlike the equal tails method, preserves the likelihood ratio criterion. Sterne (1954) first applied the technique in a discrete problem when he constructed $1 - \alpha_0$ confidence intervals for the binomial parameter p by forming rejection regions of size $\alpha \leq \alpha_0$ that contained as many points as possible under $H_0: p = p_0$. Crow (1956) indicates that this technique does achieve the minimum possible expected width among all non-randomized techniques meeting the usual Neyman confidence interval definition.

In general, a 95% minimum expected width confidence interval may be formed as follows. For the parameter λ and the statistic T having the sampling distribution $f(t;\lambda)$, increase r until $\int_R f(t;\lambda_0) dt = .05$, where $R = \{T | f(t;\lambda_0) \leq r\}$. R then forms the rejection region for testing $H_0: \lambda = \lambda_0$ vs. $H_a: \lambda \neq \lambda_0$ and the collection of λ_0 's for which a particular value of T is not in R forms the confidence interval associated with that particular T . In Figure 1, $\int_R f(t;\lambda_0) dt = \sum_P (X_1 X_2 X_3; \lambda_0 = 21) = .0496$ when $r = .0138$ and the rejection region R is the set of all 64 $X_1 X_2 X_3$ outcomes except 100, 200, 210, 220, 300, 301, 310, 311 and 320. Accordingly, the "Minimum Expected Width" column of Table 1 includes $\lambda = 21$ in precisely the confidence intervals associated with 100, 200, 210, 220, 300, 301, 310, 311 and 320.

The confidence intervals appearing in the "Minimum Expected Width" column of Table 1 were obtained following the procedure described above and using the program employed to construct Figure 1 (and given in Appendix I) to produce the sampling distributions for various λ 's. $X_1 X_2 X_3$ results for which no confidence interval is given are those results which, as with the combination method in section 2.3, were inconsistent with all values of λ . In this author's opinion, the combination method and minimum expected width method are significant improvements over currently used techniques and should be the recommended serial dilution analyses for one-sided and two-sided confidence intervals respectively. There are, however, some general

cautions and areas for further work associated with the method of minimum expected width of which the reader should be made aware.

First, while the confidence intervals given in the "Minimum Expected Width" column of Table 1 are generally narrower than those in the other columns (recall that the deMan intervals, while generally wider than those of this section anyway, are not true Neyman confidence intervals at all), there are a few specific exceptions (e.g., for $X_1X_2X_3=100$). This reminds one that the method guarantees minimum expected width over all 64 possible $X_1X_2X_3$ results and not necessarily the minimum width confidence interval for each $X_1X_2X_3$ result individually.

Secondly, the failure of the sampling distribution to be unimodal can cause the minimum expected width confidence interval to be the union of disjoint intervals. For the k , z_{α} , n_i and α of this chapter, this occurred only once, and to two significant digits the confidence interval properly associated with the result $X_1X_2X_3=330$ is $26 \cup [32,990]$. Since this would not be an acceptable result to most researchers, this author recommends the procedure used in Table 1 of extending the confidence intervals across any such gaps from the lowest included λ value to the highest included λ value. Santner and Snell (1980), in the first paper applying the minimum expected width technique to a multi-modal distribution and, hence, the first paper addressing this problem, describe an algorithm that makes adjustments

that guarantee continuous minimum expected width intervals.

Finally, as discussed in Chapter 3, the MPN is a positively biased point estimate for λ . Blyth and Hutchinson (1960) note that the minimum expected width confidence interval technique presented here is also biased and that minimum expected width unbiased confidence intervals for discrete distributions require randomization, a technique unacceptable to most researchers. After examining the endpoints of the confidence intervals in Table 1 and considering the positive bias of the MPN discussed in Chapter 3, this author conjectures that the minimum expected width confidence intervals are not only generally narrower but also less biased than the other competing intervals considered. Since, however, there may exist a non-randomized confidence interval procedure with greater expected width but less bias than the minimum expected width technique, bias investigations of these and other methods might be appropriate.

2.5 Approximate Methods

The methods of sections 2.1-2.4 use the true probabilities generated under the Poisson assumptions to provide exact confidence intervals which, by definition, are to be preferred over approximate methods advocated before the availability of advanced computing techniques. Various approximate methods proposed by such authorities as Neyman (Matuszewski, Neyman and Supinska 1935), Haldane (1939),

Fisher and Yates (1943), Cochran (1950), Ferguson (1958) and Finney (1978), however, continue to enjoy widespread use and do offer useful insights into the problem. Eisenhart and Wilson (1943) adequately review the pre-computer history of the MPN $\hat{\lambda}$, the search for estimates of the variance of $\hat{\lambda}$ and $\ln(\hat{\lambda})$, and approximate confidence intervals. They conclude, "With regard to further research, it seems highly desirable to construct mathematically exact charts giving .95 and .99 confidence intervals for the bacterial density for the case of several tubes at a single dilution, and for the case of one or more tubes at each of the successive dilutions." Woodward's (1957) exact tables, a direct response to the above challenge, are preceded by further warnings about the inadequacies of continuous approximations when $n=3$ and $n=5$ samples are used at each dilution. Inspection of Figure 1 indicates the degree of departure of the true sampling distribution of $\hat{\lambda}$ and $\ln(\hat{\lambda})$ from any normal approximation.

As a final note, one needs to be on guard against computer generated confidence intervals which, although sometimes even narrower than those of sections 2.1-2.4, are based on only approximate methods. Parnow (1972), for example, gives a program to determine confidence intervals based on the normal approximation to $\ln(\hat{\lambda})$. Not only is his estimate $\sigma_{\ln(\hat{\lambda})} = (1/\lambda)\sigma_{\hat{\lambda}}$ based on asymptotic theory, but his starting estimate of $\sigma_{\hat{\lambda}}$, taken from Haldane (1939),

uses the very crude approximations $\hat{\sigma}_\lambda = 1/\sqrt{I(\hat{\lambda})}$ and $I(\lambda) = -\partial^2 \ln(L)/\partial \lambda^2$, where L is the likelihood function defined by equation (1.1) evaluated at the observed (X_1, X_2, X_3) with $\hat{\lambda}$ estimating λ . Even for large values of \hat{k} and $n_{\hat{i}}$, Parnow's method involves at least five distinct approximations. For the $\hat{k} \leq 5$ and $n_{\hat{i}} \leq 10$ typically encountered in practice, one should certainly prefer exact confidence intervals.

3. POINT ESTIMATES

The MPN is precisely defined as the solution to equation (1.2). Being the ML estimate for λ , the MPN is asymptotically unbiased and asymptotically fully efficient. In fact, Cochran (1950) states, "The limiting distribution of the MPN has the smallest standard deviation that can be achieved by any method of estimation... There is no point in seeking further for a more precise estimate." Because, however, the MPN is tedious to compute, there has been a steady stream of alternatives and adjustments starting with Wolman and Weaver (1917) ever since McCrady (1915) introduced the concept. In addition, modern computing techniques are allowing authors to discover just how biased the MPN really is for the small n encountered in practice.

Section 3.1 examines the small sample behavior of the MPN, section 3.2 discusses alternatives and adjustments proposed in the literature as well as some original estimates, and section 3.3 compares the bias and the mean squared error (MSE) of the estimates presented. The numerical examples are, as in Chapter 2, for the commonly encountered and tabled $k=3$ decimal dilutions $z_1=.1$, $z_2=.01$ and $z_3=.001$ with $n_1=n_2=n_3=3$.

3.1 The MPN

Most review articles and textbooks on statistical methodology in the biological sciences (e.g., Eisenhart and Wilson 1943 and Finney 1978) begin the serial dilution problem by examining the single

dilution experiment for which $f(X;\lambda) = \binom{n}{x} (1-e^{-\lambda z})^x (e^{-\lambda z})^{n-x}$. As first noted by Finney (1952), Fisher's information $I(\lambda) = -E[\partial^2 \ln(f)/\partial \lambda^2] = nz^2/(e^{\lambda z}-1)$ is maximized for $\lambda z = 1.59$. This suggests selecting $z = 1.59/\hat{\lambda}$, where $\hat{\lambda}$ represents the researcher's best *a priori* guess for λ , would provide the most efficient single solution design and selecting $z = 1.59/\hat{\lambda}$ as the middle dilution would provide the most efficient $k=3$ serial dilution design. Indeed, this is the current recommendation of most authorities (e.g., Finney 1978).

The design of Chapter 1, then, with $z_1 = .1$, $z_2 = .01$ and $z_3 = .001$ should be appropriate (if not optimal in some sense) for λ 's near 160. Table 2 gives the expected value, variance and MSE of the MPN for $\lambda = 10(10)300$. As the MPN is infinite for $X_1 X_2 X_3 = 333$, only the reduced sample space consisting of the remaining 63 possible $X_1 X_2 X_3$ results was used for all calculations. The "T" (for "Total") column gives, for each λ , the sum of the probabilities over this restricted space. Even though some of the estimators to be considered give finite point estimates when $X_1 X_2 X_3 = 333$, this will be the procedure throughout all of Chapter 3. Table 2 was constructed from the output of the program given in Appendix III.

Inspection of Table 2 reveals several disturbing facts. First, for the k , z_i and n_i of the example, the MPN has a positive bias of about 45%. While the positive bias of the MPN is well known (e.g., Eisenhart and Wilson 1943 and Thomas and Woodward 1955), there has

TABLE 2

MPN Results: $n=3$, $z_1=.1$ $z_2=.01$ $z_3=.001$

λ	T	E(MPN)	VAR(MPN)	MSE(MPN)
10	1.000	13.73	165	179
20	1.000	29.17	656	740
30	1.000	43.68	1504	1691
40	1.000	57.26	2720	3018
50	1.000	70.56	4304	4727
60	1.000	84.06	6248	6827
70	1.000	97.95	8538	9318
80	1.000	112.28	11208	12186
90	1.000	127.01	14038	15408
100	1.000	142.06	17179	18948
110	1.000	157.34	20526	22767
120	1.000	172.77	24034	26819
130	.999	188.26	27665	31059
140	.999	203.74	31376	35439
150	.999	219.14	35136	39916
160	.998	234.40	38912	44447
170	.998	249.49	42673	48992
180	.997	264.35	46401	53516
190	.997	278.96	50073	57987
200	.996	293.28	53677	62378
210	.995	307.32	57194	66665
220	.995	321.04	60618	70827
230	.994	334.43	63943	74849
240	.993	347.50	67161	78717
250	.992	360.25	70267	82422
260	.990	372.66	73263	85955
270	.989	384.74	76621	89313
280	.988	396.50	78919	92491
290	.987	407.95	81578	95490
300	.985	419.08	84129	98309

been very little investigation concerning the exact magnitude of the bias for small n . For $k=3$ decimal dilutions, however, McCarthy, Thomas and Delaney (1958) empirically estimate the bias to be 30% for $n=5$ samples per dilution and Salama, Koch and Tolley (1978) calculate exact biases of about 10% for $n=10$. In addition, Thomas (1942) and Johnson and Brown (1961) give mathematical approximations expressing the bias in the serial dilution problem as being inversely related to n . Halvorson and Ziegler (1933c) state, "Evidence has been presented in support of the thesis that when three effective dilutions are used to determine the bacterial population, the accuracy is... dependent only on the number of tubes used in each dilution." Unfortunately, neither the "Standard Methods" volumes (American Public Health Association 1966, 1967, 1970, 1971) nor current literature providing MPN tables (e.g., deMan 1975, 1977) warn the researcher of the magnitude of the bias in their tabled MPN values for small n .

Secondly, note that even the large bias discussed above does not significantly increase $MSE(MPN)$ over $VAR(MPN)$. This suggests that the MPN suffers extremely large variability, a fact long known to researchers in the field. Olson, Turbak and McFeters (1979), for example, bemoan "the large confidence intervals inherent in the MPN procedure" and Georgia (1942) even proposed that this variability be acknowledged by abandoning the MPN in favor of the "most probable range" (MPR). In addition, one standard reference (American Public

Health Association 1966, page 139) cautions, "It is desirable to remember that, unless a large number of portions of samples are examined, the precision of the [MPN] test is rather low." Indeed, the Rao-Cramer lower variance bound for unbiased estimators, $[I(\lambda)]^{-1} = [\sum n_i z_i / (e^{\lambda z_i} - 1)]^{-1}$, is 10731 for $\lambda=160$ and suggests that considerable improvement over the MPN is possible.

Finally, notice that without exception $MSE(MPN) > \lambda^2$. This means that an estimator which completely ignores the experimental data and constantly guesses $\lambda=0$ (even when some of the samples are fertile) would, for this example, dominate the MPN! Clearly, while the MPN might possess desirable asymptotic properties, its behavior for small n calls for consideration of alternative techniques.

3.2 Alternative Procedures

Table 3 gives the point estimates associated with each of the 64 possible $X_1 X_2 X_3$ results and Table 4 gives the expected value and MSE for $\lambda=10(10)300$ for each procedure discussed in this section. The program used to generate the point estimates, expected values and MSE values for the procedures in sections 3.2.1 to 3.2.4 is given in Appendix III. While the program is given for the k , n_i and z_i of section 3.1, it readily generalizes. The estimates for the procedures of section 3.2.5 were obtained as indicated in the text and their expected values and MSE values were calculated in the usual way.

TABLE 3

Point Estimates: $n=3$, $z_1=.1$ $z_2=.01$ $z_3=.001$

result	MPN ^a	$\hat{\lambda}_C^b$	$\hat{\lambda}_F^c$	$\hat{\lambda}_T^d$	$\hat{\lambda}_S^e$	$\hat{\lambda}_1^f$	$\hat{\lambda}_2^g$
000	0.000	0.00	0.00	0.000	1.40	0.00	0
001	3.008	2.30	3.50	3.008	3.02	2.09	9
002	6.024	4.61	8.58	6.024	6.51	5.97	18
003	9.050	6.92	17.35	9.050	14.02	6.85	30
010	3.049	2.33	3.50	3.049	3.02	2.20	5
011	6.108	4.67	8.58	6.108	6.51	5.98	14
012	9.177	7.02	17.35	9.176	14.02	6.85	23
013	12.255	9.37	38.23	12.254	30.21	12.3	35
020	6.194	4.74	8.58	6.195	6.51	5.99	10
021	9.307	7.12	17.35	9.307	14.02	12.1	19
022	12.430	9.50	38.23	12.430	30.21	12.3	28
023	15.565	11.90	87.48	15.562	65.10	15.1	39
030	9.441	7.22	17.35	9.444	14.02	12.1	17
031	12.611	9.64	38.23	12.613	30.21	12.3	26
032	15.793	12.08	87.48	15.793	65.10	15.1	34
033	18.986	14.52	180.23	18.983	140.24	15.1	45
100	3.571	2.73	3.50	3.590	3.02	3.94	2
101	7.233	5.53	8.58	7.196	6.51	6.03	15
102	10.988	8.40	17.35	10.817	14.02	12.1	28
103	14.839	11.35	38.23	14.454	30.21	15.1	44
110	7.357	5.63	8.58	7.339	6.51	6.44	10
111	11.183	8.55	17.35	11.034	14.02	12.1	23
112	15.109	11.55	38.23	14.745	30.21	15.1	35
113	19.136	14.63	87.48	18.473	65.10	15.1	51
120	11.384	8.70	17.35	11.264	14.02	12.2	17
121	15.391	11.77	38.23	15.055	30.21	15.1	29
122	19.504	14.91	87.48	18.863	65.10	15.1	41
123	23.718	18.14	180.23	22.689	140.24	27.8	56
130	15.684	11.99	38.23	15.385	30.21	15.1	26
131	19.886	15.21	87.48	19.278	65.10	15.1	38
132	24.198	18.50	180.23	23.192	140.24	27.8	49
133	28.611	21.88	426.72	27.124	302.14	28.0	65
200	9.178	7.02	8.58	9.503	6.51	9.22	6
201	14.327	10.96	17.35	14.309	14.02	12.4	24
202	19.920	15.23	38.23	19.151	30.21	15.1	43
203	25.990	19.87	87.48	24.031	65.10	27.8	67
210	14.689	11.23	17.35	14.823	14.02	13.7	16
211	20.474	15.66	38.23	19.845	30.21	15.1	36
212	26.781	20.48	87.48	24.909	65.10	27.8	55
213	33.608	25.70	180.23	30.015	140.24	28.0	80
220	21.065	16.11	38.23	20.620	30.21	15.5	26
221	27.632	21.13	87.48	25.890	65.10	27.9	46
222	34.771	26.59	180.23	31.208	140.24	28.0	65
223	42.421	32.44	426.72	36.575	302.14	29.5	90
230	28.551	21.83	87.48	26.998	65.10	28.0	41
231	36.036	27.56	180.23	32.556	140.24	28.0	61
232	44.081	33.71	426.72	38.169	302.14	60.9	79
233	52.571	40.20	1098.66	43.840	650.95	60.9	105
300	23.116	17.68	17.35	28.618	14.02	20.6	15
301	38.500	29.44	38.23	38.749	30.21	28.7	59
302	63.558	48.60	87.48	49.212	65.10	61.0	113
303	95.376	72.93	180.23	60.030	140.24	124	187
310	42.729	32.67	38.23	45.706	30.21	42.1	40
311	74.885	57.26	87.48	58.417	65.10	64.8	118
312	115.215	88.10	180.23	71.750	140.24	124	190
313	158.797	121.42	426.72	85.775	302.14	152	291
320	93.280	71.33	87.48	75.993	65.10	93.4	84
321	149.357	114.21	180.23	94.916	140.24	138	196
322	214.657	164.14	426.72	115.659	302.14	155	305
323	291.724	223.07	1098.66	138.633	650.95	294	466
330	239.790	183.36	180.23	189.832	140.24	211	207
331	462.183	353.41	426.72	271.244	302.14	447	632
332	1098.950	840.32	1098.66	438.397	650.95	1058	1433
333	"	"	"	"	1402.43	>1800	>1800

^athe MPN; see section 3.1^bthe MPN with Thomas' correction for bias; see section 3.2.1^cFisher's estimate; see section 3.2.2^dThomas' estimate; see section 3.2.3^ethe Johnson-Brown Spearman estimate; see section 3.2.4^fthe estimate from Woodward's C.I. method; see section 3.2.5^gthe estimate from the combination C.I. method; see section 3.2.5

TABLE 4

Expected Values and MSE Values: $n=3$, $z_1=.1$ $z_2=.01$ $z_3=.001$

λ	T	$E(\hat{\lambda})^a$	$E(\hat{\lambda}_C)^b$	$E(\hat{\lambda}_F)^c$	$E(\hat{\lambda}_T)^d$	$E(\hat{\lambda}_S)^e$	$E(\hat{\lambda}_1)^f$	$E(\hat{\lambda}_2)^g$	MSE($\hat{\lambda}$)	MSE($\hat{\lambda}_C$)	MSE($\hat{\lambda}_F$)	MSE($\hat{\lambda}_T$)	MSE($\hat{\lambda}_S$)	MSE($\hat{\lambda}_1$)	MSE($\hat{\lambda}_2$)
10	1.000	13.7	10.5	12.9	14.9	10.3	13.1	11.6	179	97	155	196	86	157	173
20	1.000	29.2	22.3	27.0	30.2	21.2	27.6	26.6	740	389	658	521	344	656	832
30	1.000	43.7	33.4	40.5	42.3	31.4	41.4	41.6	1691	891	1490	875	759	1465	2057
40	1.000	57.3	43.8	53.3	52.1	40.9	54.3	56.3	3018	1605	2633	1302	1317	2565	3917
50	1.000	70.6	54.0	65.9	61.0	50.3	66.8	70.9	4727	2532	4109	1819	2010	3983	6514
60	1.000	84.1	64.3	78.6	69.6	59.7	79.4	86.0	6827	3671	5947	2413	2828	5746	9939
70	1.000	98.0	74.9	91.7	78.2	69.3	92.2	101.8	9318	5015	8164	3058	3758	7870	14248
80	1.000	112.3	85.9	105.2	86.9	79.1	105.4	118.3	12186	6550	10763	3728	4787	10354	19459
90	1.000	127.0	97.1	118.9	95.6	89.0	118.9	135.5	15408	8259	13732	4399	5897	13185	25552
100	1.000	142.1	108.6	132.9	104.4	98.9	132.8	153.4	18948	10119	17047	5051	7073	16334	32477
110	1.000	157.3	120.3	147.1	113.2	108.9	146.8	171.9	22767	12107	20676	5671	8299	19769	40159
120	1.000	172.8	132.1	161.4	121.8	118.9	161.1	190.8	26819	14199	24579	6247	9560	23447	48508
130	.999	188.3	144.0	175.7	130.4	128.8	175.5	210.1	31059	16369	28713	6775	10842	27325	57425
140	.999	203.7	155.8	190.0	138.7	138.5	189.9	229.6	35439	18595	33032	7252	12134	31359	66804
150	.999	219.1	167.6	204.3	146.8	148.1	204.2	249.2	39916	20852	37492	7681	13424	35504	76542
160	.998	234.4	179.2	218.4	154.7	157.6	218.6	268.9	44447	23121	42049	8065	14704	39716	86538
170	.998	249.5	190.8	232.4	162.4	166.8	232.8	288.5	48992	25382	46662	8412	15968	43957	96695
180	.997	264.4	202.1	246.2	169.8	175.8	246.8	308.0	53516	27620	51294	8727	17211	48190	106927
190	.997	279.0	213.3	259.8	176.9	184.6	260.7	327.4	57987	29821	55910	9020	18430	52383	117152
200	.996	293.3	224.3	273.2	183.7	193.2	274.4	346.5	62378	31972	60481	9301	19621	56507	127299
210	.995	307.3	235.0	286.4	190.2	201.6	287.8	365.3	66665	34065	64979	9579	20785	60538	137306
220	.995	321.0	245.5	299.3	196.5	209.7	300.9	383.8	70827	36093	69382	9864	21922	64454	147118
230	.994	334.4	255.7	311.9	202.5	217.6	313.8	402.0	74849	38049	73671	10167	23033	68240	156691
240	.993	347.5	265.7	324.2	208.3	225.2	326.4	419.9	78717	39930	77831	10496	24120	71882	165984
250	.992	360.3	275.5	336.3	213.8	232.6	338.8	437.3	82422	41733	81849	10862	25186	75369	174968
260	.990	372.7	285.0	348.1	219.1	239.9	350.8	454.4	85955	43459	85716	11273	26234	78693	183618
270	.989	384.7	294.2	359.7	224.1	246.8	362.5	471.1	89313	45108	89423	11739	27269	81851	191913
280	.988	396.5	303.2	370.9	228.9	253.6	374.0	487.3	92491	46681	92967	12267	28293	84839	199842
290	.987	408.0	311.9	381.9	233.5	260.2	385.1	503.2	95490	48179	96344	12864	29311	87656	207392
300	.985	419.1	320.5	392.6	237.9	266.6	396.0	518.7	98309	49607	99553	13539	30328	90302	214560

^athe MPN; see section 3.1^bthe MPN with Thomas' correction for bias; see section 3.2.1^cFisher's estimate; see section 3.2.2^dThomas' estimate; see section 3.2.3^ethe Johnson-Brown Spearman estimate; see section 3.2.4^fthe estimate from Woodward's C.I. method; see section 3.2.5^gthe estimate from the combination C.I. method; see section 3.2.5

3.2.1 Bias Corrections

In general, the bias of an estimator $\hat{\lambda}$ is a function of the true parameter value and given by $b(\lambda) = E(\hat{\lambda}) - \lambda$. The positive bias of the MPN is well known and was noted for the numerical example of this paper in section 3.1. Thomas and Woodward (1955) compared MPN estimates to "exact" plate counts obtained by collecting organisms as the samples passed through a membrane filter (MF). "A considerable part of the disparity between MF and MPN values," they conclude, "may be attributed to the fact that mathematically considered, the MPN tends to overestimate the true density; on the average, MPN values are greater than the true density." McCarthy et al. (1958) made ten replicate $n=5$ MPN determinations and also used "exact" plate counts for their true λ 's.^d Their empirical estimate was that $E(\text{MPN}) = 1.29\lambda$.

Thomas (1955) uses a log-normal approximation to estimate $E(\text{MPN}) = e^{.805/n} \lambda$ and recommends the multiplicative bias correction $e^{-.805/n}$. Not being able to discover a better correction factor, this author recommends the Thomas adjustment for any k and z_i . Such bias corrected values for the example of this paper are given in the $\hat{\lambda}_C$ columns of Tables 3 and 4.

In a deliberate attempt to develop an estimator with less bias than the MPN, Salama et al. (1978) use expansions to obtain a $\hat{\lambda}$ for which $E(\hat{\lambda}) = \lambda + O(1/n^2)$. Unfortunately, their $k=3$ dilution numerical

example uses middle dilution $z_2 = .001$, which suggests their design would be appropriate for λ 's near 1600, while their bias and MSE calculations cover $\lambda = 25(25)100(50)200(100)1200$. Chapter 4 discusses the problems associated with λ 's outside the optimal range of the experimental design. Limited comparisons this author made for the Salama et al. design, however, indicate that their rather complicated estimator does not perform significantly better than the much simpler MPN with Thomas' bias correction. Further, subsequent sections will indicate that other non-MPN alternatives achieve even better results than does the Thomas bias-corrected MPN.

3.2.2 The Fisher Estimate

Fisher (1922) proposes an estimator based on $W = \sum (n_i - X_i)$, the total number of sterile responses. Since each dilution represents an independent experiment, $E(W) = \sum n_i P(X_i = 0) = \sum n_i e^{-\lambda z_i}$ and the λ that solves $W = \sum n_i e^{-\lambda z_i}$ would be an estimator both reasonable and relatively simple to compute. Fisher showed his estimator to be 87.71% efficient in the sense that for large n , the variance of the MPN (which is asymptotically fully efficient) is 87.71% that of his estimator. Values of his estimator, designated $\hat{\lambda}_F$ in Tables 3 and 4, for the example of this paper were obtained by Newton's method using the program of Appendix III. Fisher and Yates (1943) provide additional discussion and tables for obtaining $\hat{\lambda}_F$ for n trials at each

of $k \geq 4$ two-fold, four-fold and decimal dilutions.

3.2.3 The Thomas Estimate

Thomas (1942) gives the estimate $\hat{\lambda}_T = \Sigma X_i / \sqrt{[\Sigma(n_i - X_i)z_i][\Sigma n_i z_i]}$, which is the geometric mean of the estimates obtained using $e^{-\lambda z} \doteq 1 - \lambda z$ and $e^{-\lambda z} = 1/e^{\lambda z} \doteq 1/(1 + \lambda z)$ in equation (1.2). For $X_1 X_2 X_3 = 110$ and the n and z_i of Table 3, for example, Thomas' estimate is $\hat{\lambda}_T = 2/\sqrt{(.233)(.333)} = 7.339$. Thomas recommends his pre-computer estimator for its remarkable ability to approximate the MPN (which must be found iteratively), as seen by comparing the MPN and $\hat{\lambda}_T$ columns of Table 3. Apparently unknown to Thomas, however, his $\hat{\lambda}_T$ also enjoys considerably less variance than the MPN and deviates from the MPN so as to effect a significant reduction in bias.

The net result, as shown in Table 4, is that $\hat{\lambda}_T$ is the estimator, for the example of this paper, with by far the smallest MSE for values between 40 and 300. In fact, for $\lambda = 160$, $\hat{\lambda}_T$ approaches the Rao-Cramer lower variance bound $[1 + b'(\lambda)]^2 / I(\lambda)$ for estimators of its bias. Empirically estimating $b'(\lambda = 160)$ from Table 4 to be $[-7.6 - (-3.2)] / [170 - 150] = -.22$, one calculates the Rao-Cramer lower bound to be 6479, to which $\text{VAR}(\hat{\lambda}_T) = 8037$ favorably compares.

Finally, while Thomas gives $\hat{\lambda}_T / \sqrt{\Sigma X_i}$ as an approximate standard error for his estimator, he recognizes that the distribution of the estimator follows no tabled distribution and suggests no confidence

interval procedure.

3.2.4 The Johnson-Brown Estimate

Johnson and Brown (1961) develop an estimator based on the Spearman (1908) technique which, like Fisher's estimator, uses only the total number of fertile responses and is also 87.71% efficient. Their analysis, which requires $n_i = n$ and $z_i = z_1 d^{-(i-1)}$ for $i=1,2,\dots,k$, produces the estimate

$$(3.1) \quad \hat{\lambda}_S = \{2n/[2n+\ln(d)\ln(2)]\} e^{-\gamma - \ln(z_1) + [\ln(d)][(\sum X_i/n) - .5]},$$

where $2n/[2n+\ln(d)\ln(2)]$ is a multiplicative bias correction, $\gamma = .57722$ is Euler's constant and d is commonly called the dilution factor.

The Johnson-Brown Spearman estimator enjoys both practical use (e.g., Masover, Benson and Hayflick 1974) and further discussion (e.g., Church and Cobb 1975) in the literature. In fact, Cornell (1965) states; "The work of Johnson and Brown prompted several investigations which are summarized here." Cornell and Speckman (1967) discuss and compare by simulation several procedures, including Johnson and Brown's, for estimating (for different values of z) the parameter λ in the model with expectation $1 - e^{-\lambda z}$. Finally, Mantel (1967) discusses arithmetic (as opposed to the usual logarithmic) and arbitrary spacing of the z_i 's.

Identified as $\hat{\lambda}_S$, the Johnson-Brown Spearman estimator performs quite well for the example of Tables 3 and 4. Despite the fact that

it ignores the pattern of the X_i 's and, consequently, is not based on the minimal sufficient statistic, it is second in MSE only to Thomas' $\hat{\lambda}_T$ of section 3.2.3.

3.2.5 Estimates derived from Confidence Intervals

Typical mathematical statistics texts (e.g., Bickel and Doksum 1977, page 155) note that $1-\alpha$ confidence intervals must include the entire parameter space when $\alpha=0$ and, in general, decrease their coverage of the parameter space as α , the probability of error, increases. As α increases toward 1.00, the confidence interval decreases toward the empty set and, as illustrated by the combination method and the minimum expected width method of Chapter 2, may achieve the empty set even before α increases to .05. This suggests that increasing α until the $1-\alpha$ confidence interval decreases to a single point in the parameter space (i.e., finding the smallest non-empty confidence interval associated with a particular sample result) would be a reasonable point estimation procedure. This is equivalent to finding the point contained in every non-empty confidence interval. Applying this procedure to each of the true confidence interval techniques of Chapter 2 (i.e., the Woodward method, the combination method and the minimum expected width method) yields three additional competitive point estimates for λ .^e

Figure 1 gave the distribution of possible sample results

arranged by magnitude of the MPN and illustrated Woodward's technique for obtaining confidence intervals. As α increases toward 1.00 in the test of hypothesis, the only sample result for which one fails to reject $H_0: \lambda = \lambda_0$ is the median sample result. Although the discrete nature of the distribution leads to a range of λ values, a unique $\hat{\lambda}$ can be obtained for each $X_1X_2X_3$ sample result by finding the λ_0 associated with the distribution for which that $X_1X_2X_3$ is the exact median in the sense that the sum of all $P(X_1^*X_2^*X_3^* | \lambda = \lambda_0)$ for $MPN(X_1^*X_2^*X_3^*) < MPN(X_1X_2X_3)$ plus one half $P(X_1X_2X_3 | \lambda = \lambda_0)$ is exactly .5000. These values were calculated for each $X_1X_2X_3$ sample result and are designated $\hat{\lambda}_1$ in Tables 3 and 4.

The point estimate associated with confidence intervals obtained by the combination method is the λ_0 for which the p-value for the alternative $H_a: \lambda < \lambda_0$ equals the p-value for the alternative $H_a: \lambda > \lambda_0$. The smallest non-empty confidence interval occurs for an α equal to twice that common p-value. The sample output accompanying Appendix II indicates that for $X_1X_2X_3 = 301$ this occurs at $\lambda = 59$. These values were calculated for each $X_1X_2X_3$ sample result and are designated $\hat{\lambda}_2$ in Tables 3 and 4.

The procedure for determining the point estimate associated with the minimum expected width method for obtaining confidence intervals is well-defined but extremely tedious. For a given $X_1X_2X_3$ result, one must find the largest p-value for testing $H_0: \lambda = \lambda_0$, and the λ_0 at which

that p-value occurs is the desired point estimate. It is conjectured that this procedure leads to the MPN, as this author has found such to be the case for every $X_1X_2X_3$ examined. For the result $X_1X_2X_3=220$, for example, Figure 1 indicates that the p-value for testing $H_0:\lambda=21$ is .1290. A check of similar figures gives a p-value of .1091 for testing $H_0:\lambda=20$ and a p-value of .1257 for testing $H_0:\lambda=22$. The MPN for $X_1X_2X_3=220$ is 21.

3.3 Bias and MSE Comparisons

Recall that both Woodward and deMan cautioned against certain $X_1X_2X_3$ results that "are not mentioned in the table and are always unacceptable" (deMan 1975). While Table 4 was constructed under the obvious restriction of excluding $X_1X_2X_3=333$, one might be more interested in a table constructed excluding all "unacceptable" results. Table 5 gives selected expected values and MSE values over the severely truncated sample space consisting of only those 18 $X_1X_2X_3$ results having non-empty minimum expected width confidence intervals. Note that the "T" values are naturally somewhat (but, perhaps surprisingly, not significantly) smaller than those of Table 4 and that the expected values and MSE values are essentially unchanged. Since additional truncation of the sample space appears not to affect the "rankings" of the estimators, subsequent comparisons will continue to use the sample space obtained by eliminating only the $X_1X_2X_3=n_1n_2n_3$

TABLE 5

Selected Expected Values and MSE Values: $n=3$, $z_1=.1$ $z_2=.01$ $z_3=.001$

Truncated Sample Space: only those 18 $X_1X_2X_3$ results with non-empty minimum expected width confidence intervals

λ	T	$E(\hat{\lambda})^a$	$E(\hat{\lambda}_C)^b$	$E(\hat{\lambda}_S)^c$	$E(\hat{\lambda}_1)^d$	$E(\hat{\lambda}_2)^e$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_S)$	$MSE(\hat{\lambda}_1)$	$MSE(\hat{\lambda}_2)$
10	.979	13.8	10.5	10.1	13.1	11.4	182	98	84	159	172
50	.992	70.6	54.0	49.9	66.8	70.6	4733	2535	1984	3993	6493
100	.992	142.2	108.7	98.5	132.8	153.1	19008	10150	6995	16449	32635
150	.991	219.5	167.8	147.6	204.7	249.4	40048	20910	13241	35752	77019
200	.990	293.6	224.5	192.6	275.0	346.9	62549	32050	19351	56814	127955
250	.987	360.4	275.6	231.9	339.4	437.8	82635	41845	24910	75680	175675
300	.982	419.1	320.5	265.8	396.6	519.2	98583	49768	30114	90591	215255

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^a the MPN; see section 3.1

^b the MPN with Thomas' correction for bias; see section 3.2.1

^c the Johnson-Brown Spearman estimate; see section 3.2.4

^d the estimate from Woodward's C.I. method; see section 3.2.5

^e the estimate from the combination C.I. method; see section 3.2.5

result. Furthermore, since the estimators $\hat{\lambda}_1$ and $\hat{\lambda}_2$ are tedious to compute and did not perform well in the example of this paper, they will not be included in further comparisons.

As noted in section 3.2, Thomas' $\hat{\lambda}_T$ is without question the preferred estimator for the example of Table 4. There is no guarantee that some other estimator, however, would not perform better than $\hat{\lambda}_T$ for other z_i or n_i values. Table 6 compares the expected values and the MSE values for the estimators of the program in Appendix III (i.e., the MPN, $\hat{\lambda}_C$, $\hat{\lambda}_F$, $\hat{\lambda}_T$ and $\hat{\lambda}_S$) for $n=3,5,10$ and for $k=3$ two-fold, four-fold and decimal dilutions (i.e., for dilution factors $d=2,4,10$) centered at $z_2=.01$. Each of these nine experimental designs, which cover virtually all $k=3$ serial dilution settings found in the literature, should be appropriate for $\lambda=160$ and the comparisons will be made, as in Table 4, for $\lambda=10(10)300$.

The MSE values of Table 6 follow a definite pattern illustrated by Tables 6.1-6.3, which vary the dilution factor while maintaining $n=3$ samples per dilution. For dilution factor $d=10$ (Table 6.1), $\hat{\lambda}_T$ performs best as measured by MSE. For $d=4$ (Table 6.2), however, $\hat{\lambda}_S$ enjoys the smallest MSE. And for $d=2$ (Table 6.3), $\hat{\lambda}_C$ appears to be the superior estimator. As n increases, each estimator, as expected, performs better than it did for the previous n . For any single dilution factor, however, the "rankings" of the estimators do not change as n increases.

TABLE 6

Expected Values and MSE Values

k=3 dilutions with middle dilution $z_2 = .01$

n = number of samples per dilution

d = the dilution factor

 $\hat{\lambda}$ = the MPN; see section 3.1 $\hat{\lambda}_C$ = the MPN with Thomas' correction for bias; see section 3.2.1 $\hat{\lambda}_F$ = Fisher's estimate; see section 3.2.2 $\hat{\lambda}_T$ = Thomas' estimate; see section 3.2.3 $\hat{\lambda}_S$ = the Johnson-Brown Spearman estimate; see section 3.2.4

Tables 6.1 - 6.9 vary n and d as indicated below.

	d			
	↑			
10	↑	6.1	6.4	6.7
	↑			
4	↑	6.2	6.5	6.8
	↑			
2	↑	6.3	6.6	6.9
				→ n
		3	5	10

TABLE 6.1

n = 3

d = 10

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λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	13.73	10.50	12.92	14.85	10.27	179	97	155	196	86
20	1.000	29.17	22.31	27.03	30.24	21.17	740	389	658	521	344
30	1.000	43.68	33.40	40.48	42.30	31.36	1691	891	1490	875	759
40	1.000	57.26	43.78	53.26	52.12	40.91	3018	1605	2633	1302	1317
50	1.000	70.56	53.96	65.87	60.98	50.28	4727	2532	4109	1819	2010
60	1.000	84.06	64.27	78.64	69.57	59.71	6827	3671	5947	2413	2828
70	1.000	97.95	74.90	91.74	78.18	69.30	9318	5015	8164	3058	3758
80	1.000	112.28	85.85	105.17	86.87	79.07	12186	6550	10763	3728	4787
90	1.000	127.01	97.12	118.91	95.63	88.97	15408	8259	13732	4399	5897
100	1.000	142.06	108.63	132.90	104.42	98.94	18948	10119	17047	5051	7073
110	1.000	157.34	120.31	147.07	113.17	108.92	22767	12107	20676	5671	8299
120	1.000	172.77	132.11	161.35	121.83	118.88	26819	14199	24579	6247	9560
130	.999	188.26	143.96	175.68	130.36	128.76	31059	16369	28713	6775	10842
140	.999	203.74	155.79	190.00	138.70	138.51	35439	18595	33032	7252	12134
150	.999	219.14	167.57	204.26	146.83	148.12	39916	20852	37492	7681	13424
160	.998	234.40	179.24	218.40	154.73	157.55	44447	23121	42049	8065	14704
170	.998	249.49	190.77	232.40	162.37	166.79	48992	25382	46662	8412	15968
180	.997	264.35	202.13	246.23	169.75	175.82	53516	27620	51294	8727	17211
190	.997	278.96	213.30	259.84	176.85	184.63	57987	29821	55910	9020	18430
200	.996	293.28	224.26	273.23	183.68	193.20	62378	31972	60481	9301	19621
210	.995	307.32	234.99	286.38	190.24	201.55	66665	34065	64979	9579	20785
220	.995	321.04	245.48	299.27	196.52	209.67	70827	36093	69382	9864	21922
230	.994	334.43	255.73	311.89	202.53	217.55	74849	38049	73671	10167	23033
240	.993	347.50	265.72	324.24	208.29	225.21	78717	39930	77831	10496	24120
250	.992	360.25	275.46	336.32	213.79	232.63	82422	41733	81849	10862	25186
260	.990	372.66	284.95	348.12	219.06	239.85	85955	43459	85716	11273	26234
270	.989	384.74	294.19	359.65	224.09	246.84	89313	45108	89423	11739	27269
280	.988	396.50	303.19	370.91	228.90	253.63	92491	46681	92967	12267	28293
290	.987	407.95	311.94	381.91	233.51	260.22	95490	48179	96344	12864	29311
300	.985	419.08	320.45	392.64	237.91	266.62	98309	49607	99553	13539	30328

TABLE 6.2

n = 3

d = 4

λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	11.54	8.82	11.37	11.75	12.65	116	68	109	127	54
20	1.000	23.70	18.12	23.13	24.42	20.68	337	192	311	376	156
30	1.000	36.40	27.83	35.26	37.67	29.42	708	395	654	763	322
40	1.000	49.46	37.82	47.64	51.10	38.37	1264	691	1172	1287	539
50	1.000	62.74	47.97	60.18	64.42	47.26	2019	1089	1878	1933	794
60	1.000	76.07	58.17	72.77	77.46	55.92	2963	1584	2764	2680	1077
70	1.000	89.37	68.34	85.34	90.13	64.28	4068	2162	3805	3500	1378
80	.999	102.55	78.41	97.84	102.41	72.32	5297	2802	4965	4362	1691
90	.998	115.56	88.36	110.20	114.30	80.04	6608	3484	6205	5239	2012
100	.997	128.36	98.15	122.41	125.80	87.43	7959	4186	7486	6103	2336
110	.996	140.92	107.76	134.42	136.93	94.50	9311	4890	8769	6934	2665
120	.994	153.23	117.17	146.19	147.70	101.27	10631	5578	10024	7712	2999
130	.992	165.25	126.36	157.71	158.11	107.74	11890	6239	11221	8424	3344
140	.989	176.97	135.32	168.94	168.17	113.92	13065	6862	12340	9059	3702
150	.986	188.37	144.04	179.85	177.89	119.81	14139	7441	13364	9610	4082
160	.982	199.43	152.50	190.44	187.26	125.42	15097	7974	14282	10075	4490
170	.977	210.15	160.69	200.67	196.28	130.76	15933	8460	15087	10454	4935
180	.972	220.50	168.61	210.55	204.95	135.84	16641	8900	15775	10750	5424
190	.967	230.49	176.25	220.06	213.28	140.66	17222	9300	16348	10967	5967
200	.961	240.11	183.60	229.20	221.27	145.24	17678	9664	16810	11112	6572
210	.954	249.36	190.67	237.97	228.91	149.57	18015	10000	17166	11194	7249
220	.947	258.24	197.46	246.37	236.23	153.68	18239	10317	17425	11222	8006
230	.939	266.75	203.97	254.42	243.22	157.57	18359	10622	17597	11207	8852
240	.931	274.90	210.21	262.11	249.90	161.26	18387	10926	17691	11159	9793
250	.922	282.71	216.17	269.46	256.27	164.75	18333	11238	17720	11090	10839
260	.913	290.17	221.88	276.47	262.34	168.05	18209	11568	17695	11012	11995
270	.904	297.29	227.33	283.17	268.13	171.17	18027	11926	17629	10935	13270
280	.894	304.10	232.53	289.56	273.64	174.14	17800	12321	17534	10872	14668
290	.884	310.60	237.50	295.65	278.89	176.94	17540	12764	17422	10832	16197
300	.874	316.80	242.24	301.46	283.89	179.60	17259	13262	17304	10826	17860

TABLE 6.3

n = 3

d = 2

λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	10.87	8.32	10.84	10.93	23.58	126	76	124	129	214
20	1.000	21.90	16.75	21.80	22.14	29.06	289	177	284	306	153
30	1.000	33.12	25.32	32.90	33.69	34.68	501	309	490	555	143
40	1.000	44.53	34.05	44.18	45.66	40.33	778	478	759	908	176
50	1.000	56.15	42.94	55.63	58.07	45.93	1129	688	1100	1392	249
60	.999	67.94	51.95	67.20	70.92	51.37	1556	937	1512	2022	360
70	.999	79.84	61.05	78.84	84.12	56.61	2044	1219	1980	2790	512
80	.997	91.72	70.14	90.43	97.57	61.59	2569	1519	2478	3666	712
90	.994	103.48	79.13	101.85	111.09	66.26	3099	1824	2973	4603	968
100	.990	114.98	87.92	112.97	124.52	70.61	3601	2120	3437	5548	1289
110	.984	126.12	96.44	123.69	137.70	74.62	4049	2399	3841	6451	1689
120	.976	136.80	104.61	133.92	150.49	78.30	4422	2657	4169	7268	2181
130	.967	146.96	112.37	143.61	162.79	81.66	4709	2895	4413	7967	2777
140	.955	156.55	119.71	152.71	174.52	84.70	4906	3120	4572	8528	3489
150	.941	165.56	126.59	161.21	185.62	87.47	5017	3340	4654	8940	4331
160	.925	173.98	133.03	169.12	196.09	89.96	5053	3567	4670	9206	5311
170	.907	181.82	139.03	176.46	205.91	92.22	5026	3816	4636	9331	6439
180	.888	189.11	144.61	183.23	215.10	94.26	4953	4100	4569	9330	7723
190	.867	195.88	149.78	189.49	223.68	96.10	4850	4433	4488	9217	9170
200	.846	202.14	154.57	195.25	231.66	97.77	4735	4830	4413	9013	10786
210	.823	207.94	159.00	200.56	239.10	99.28	4626	5303	4361	8737	12576
220	.799	213.31	163.11	205.44	246.01	100.64	4540	5865	4349	8409	14545
230	.775	218.27	166.90	209.94	252.43	101.88	4493	6527	4394	8047	16696
240	.750	222.87	170.42	214.08	258.41	103.01	4500	7301	4510	7673	19032
250	.725	227.13	173.68	217.89	263.96	104.03	4575	8194	4713	7303	21556
260	.700	231.08	176.69	221.40	269.13	104.97	4731	9216	5013	6954	24270
270	.675	234.74	179.49	224.64	273.94	105.82	4979	10375	5423	6642	27176
280	.650	238.13	182.09	227.63	278.42	106.60	5330	11678	5952	6381	30275
290	.625	241.29	184.50	230.39	282.60	107.31	5794	13130	6610	6185	33569
300	.601	244.22	186.74	232.94	286.49	107.97	6380	14738	7405	6066	37059

TABLE 6.4

n = 5
d = 10

λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	11.83	10.07	11.54	12.59	9.96	64	44	58	82	42
20	1.000	25.25	21.50	24.03	26.93	20.78	270	178	258	254	165
30	1.000	38.18	32.50	36.13	38.62	30.79	598	391	590	366	361
40	1.000	50.02	42.58	47.55	47.55	40.03	1031	681	1019	459	632
50	1.000	61.29	52.18	58.68	55.04	49.03	1587	1063	1546	598	980
60	1.000	72.52	61.73	69.84	62.02	58.12	2288	1547	2188	812	1404
70	1.000	83.99	71.50	81.23	68.95	67.44	3151	2144	2964	1106	1901
80	1.000	95.85	81.59	92.88	76.01	77.01	4191	2858	3894	1471	2465
90	1.000	108.10	92.03	104.80	83.26	86.81	5418	3693	4996	1893	3092
100	1.000	120.74	102.78	116.97	90.71	96.77	6840	4653	6287	2359	3776
110	1.000	133.72	113.83	129.36	98.31	106.85	8462	5739	7778	2853	4512
120	1.000	146.99	125.13	141.93	106.04	117.00	10283	6951	9478	3362	5296
130	1.000	160.50	136.63	154.66	113.84	127.16	12303	8286	11392	3877	6123
140	1.000	174.20	148.30	167.50	121.66	137.30	14518	9742	13520	4387	6989
150	1.000	188.05	160.08	180.43	129.47	147.37	16921	11315	15860	4887	7891
160	1.000	201.99	171.96	193.43	137.21	157.36	19503	12998	18406	5375	8825
170	1.000	216.00	183.88	206.46	144.86	167.23	22255	14787	21150	5850	9789
180	1.000	230.03	195.82	219.50	152.39	176.97	25165	16673	24082	6312	10780
190	1.000	244.04	207.75	232.54	159.76	186.57	28222	18651	27189	6766	11796
200	1.000	258.02	219.65	245.54	166.97	196.02	31412	20711	30460	7216	12834
210	1.000	271.93	231.49	258.50	174.00	205.30	34724	22847	33880	7668	13895
220	1.000	285.75	243.26	271.40	180.83	214.42	38145	25051	37435	8128	14975
230	1.000	299.46	254.93	284.22	187.46	223.37	41661	27316	41111	8603	16075
240	1.000	313.06	266.50	296.96	193.89	232.15	45261	29635	44895	9102	17192
250	1.000	326.51	277.96	309.61	200.12	240.77	48933	32000	48772	9631	18327
260	1.000	339.83	289.29	322.16	206.14	249.23	52664	34406	52729	10197	19478
270	.999	352.99	300.49	334.61	211.97	257.52	56446	36845	56754	10810	20645
280	.999	365.99	311.56	346.95	217.59	265.67	60266	39312	60834	11474	21828
290	.999	378.83	322.49	359.18	223.03	273.66	64116	41902	64957	12197	23026
300	.999	391.51	333.29	371.30	228.29	281.51	67986	44309	69113	12985	24240

TABLE 6.5

n = 5
d = 4

λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	10.82	9.21	10.76	10.92	12.76	56	41	55	59	33
20	1.000	21.94	18.68	21.73	22.40	20.61	150	108	146	167	84
30	1.000	33.39	28.42	32.90	34.35	29.26	299	211	291	335	176
40	1.000	45.09	38.38	44.23	46.53	38.20	515	357	506	559	299
50	1.000	56.95	48.48	55.67	58.65	47.13	810	554	800	831	448
60	1.000	68.88	58.64	67.14	70.53	55.87	1191	807	1181	1147	620
70	1.000	80.82	68.80	78.63	82.09	64.37	1662	1121	1653	1505	813
80	1.000	92.75	78.95	90.13	93.32	72.62	2230	1500	2220	1907	1027
90	1.000	104.65	89.08	101.65	104.27	80.63	2899	1946	2886	2358	1260
100	1.000	116.54	99.21	113.21	114.97	88.40	3670	2562	3652	2860	1512
110	1.000	128.44	109.34	124.81	125.50	95.97	4544	3047	4520	3413	1783
120	1.000	140.37	119.50	136.46	135.90	103.34	5519	3699	5487	4017	2072
130	1.000	152.34	129.68	148.17	146.21	110.51	6587	4412	6548	4666	2379
140	.999	164.35	139.91	159.92	156.44	117.51	7741	5180	7693	5355	2705
150	.999	176.38	150.16	171.69	166.62	124.32	8968	5994	8913	6075	3051
160	.999	188.44	160.42	183.48	176.75	130.95	10252	6844	10192	6817	3418
170	.998	200.50	170.68	195.26	186.81	137.39	11579	7717	11515	7570	3809
180	.997	212.53	180.93	206.99	196.81	143.64	12929	8604	12865	8325	4227
190	.997	224.51	191.12	218.66	206.71	149.70	14286	9491	14225	9071	4675
200	.995	236.40	201.25	230.22	216.50	155.56	15631	10369	15578	9798	5157
210	.994	248.19	211.28	241.67	226.17	161.23	16948	11227	16907	10498	5679
220	.992	259.83	221.19	252.97	235.70	166.70	18220	12055	18197	11164	6245
230	.991	271.31	230.97	264.10	245.06	171.97	19434	12847	19434	11789	6862
240	.988	282.61	240.58	275.03	254.24	177.04	20577	13597	20607	12369	7533
250	.986	293.69	250.02	285.76	263.24	181.93	21639	14298	21706	12902	8267
260	.983	304.55	259.26	296.26	272.02	186.62	22613	14950	22723	13384	9068
270	.980	315.17	268.30	306.53	280.60	191.13	23492	15549	23652	13818	9943
280	.976	325.53	277.12	316.55	288.95	195.45	24273	16096	24490	14203	10898
290	.973	335.63	285.72	326.32	297.07	199.60	24953	16592	25235	14542	11939
300	.968	345.46	294.09	335.83	304.96	203.58	25532	17040	25886	14838	13072

TABLE 6.6

n = 5
d = 2

λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	10.49	8.93	10.48	10.52	24.06	69	51	68	70	215
20	1.000	21.07	17.93	21.02	21.20	29.48	154	115	153	159	131
30	1.000	31.72	27.01	31.64	32.08	35.08	258	193	256	275	97
40	1.000	42.48	36.17	42.34	43.20	40.74	386	290	385	428	104
50	1.000	53.35	45.42	53.13	54.60	46.37	546	408	545	630	152
60	1.000	64.35	54.78	64.03	66.33	51.90	743	552	744	901	239
70	1.000	75.48	64.26	75.05	78.41	57.28	988	727	992	1260	369
80	1.000	86.76	73.86	86.20	90.87	62.47	1288	938	1294	1734	546
90	1.000	98.17	83.57	97.46	103.73	67.44	1648	1187	1657	2342	776
100	1.000	109.70	93.39	108.82	116.96	72.17	2068	1474	2079	3099	1066
110	.999	121.32	103.28	120.23	130.54	76.65	2541	1794	2551	4007	1425
120	.998	132.96	113.18	131.65	144.40	80.86	3055	2138	3060	5053	1860
130	.997	144.55	123.06	143.01	158.45	84.80	3589	2496	3585	6211	2381
140	.994	156.04	132.83	154.23	172.59	88.48	4123	2853	4105	7444	2999
150	.991	167.32	142.44	165.23	186.70	91.89	4636	3199	4599	8709	3723
160	.987	178.35	151.82	175.96	200.67	95.05	5106	3523	5047	9962	4564
170	.981	189.04	160.93	186.33	214.41	97.96	5518	3818	5434	11160	5530
180	.974	199.35	169.71	196.32	227.82	100.64	5860	4081	5749	12267	6631
190	.966	209.24	178.13	205.87	240.82	103.09	6126	4312	5989	13251	7875
200	.956	218.69	186.17	214.96	253.36	105.34	6314	4514	6153	14092	9271
210	.944	227.67	193.81	223.58	265.39	107.40	6427	4694	6246	14775	10824
220	.931	236.17	201.05	231.73	276.89	109.29	6471	4859	6275	15293	12542
230	.916	244.21	207.90	239.41	287.85	111.01	6456	5021	6251	15645	14430
240	.901	251.79	214.35	246.62	298.25	112.58	6392	5189	6187	15837	16493
250	.884	258.92	220.42	253.39	308.11	114.02	6292	5378	6098	15876	18734
260	.865	265.62	226.12	259.73	317.43	115.34	6171	5597	5997	15775	21158
270	.846	271.90	231.47	265.66	326.23	116.55	6042	5861	5899	15549	23767
280	.826	277.80	236.49	271.21	334.53	117.65	5920	6180	5820	15212	26564
290	.805	283.33	241.19	276.38	342.35	118.66	5818	6566	5774	14783	29552
300	.784	288.50	245.60	281.22	349.71	119.59	5750	7030	5774	14277	32731

TABLE 6.7

n =10
d =10

λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	10.75	9.91	10.68	11.17	9.80	20	16	20	26	18
20	1.000	22.40	20.66	21.93	24.09	20.64	89	71	89	111	72
30	1.000	34.24	31.59	33.10	35.85	30.65	213	168	221	174	154
40	1.000	45.36	41.85	43.77	44.88	39.74	368	292	392	187	269
50	1.000	55.82	51.50	54.16	52.01	48.51	552	444	591	199	421
60	1.000	66.03	60.93	64.51	58.23	57.36	771	626	817	251	612
70	1.000	76.30	70.40	74.99	64.14	66.47	1028	841	1073	356	838
80	1.000	86.75	80.04	85.61	70.01	75.88	1326	1090	1363	516	1097
90	1.000	97.42	89.89	96.39	75.97	85.57	1670	1374	1695	731	1386
100	1.000	108.32	99.95	107.31	82.07	95.49	2064	1698	2075	997	1702
110	1.000	119.44	110.20	118.35	88.33	105.57	2516	2066	2512	1310	2042
120	1.000	130.74	120.62	129.50	94.75	115.76	3031	2482	3013	1666	2405
130	1.000	142.21	131.21	140.74	101.32	126.01	3615	2952	3584	2058	2787
140	1.000	153.84	141.94	152.07	108.01	136.27	4275	3480	4232	2483	3188
150	1.000	165.61	152.80	163.48	114.82	146.50	5012	4067	4961	2933	3606
160	1.000	177.50	163.77	174.95	121.70	156.65	5829	4716	5773	3404	4038
170	1.000	189.50	174.85	186.47	128.62	166.72	6727	5427	6672	3892	4486
180	1.000	201.60	186.01	198.03	135.57	176.67	7706	6199	7657	4394	4947
190	1.000	213.77	197.23	209.61	142.50	186.48	8763	7031	8729	4907	5422
200	1.000	225.99	208.51	221.21	149.38	196.14	9897	7922	9886	5432	5911
210	1.000	238.26	219.83	232.82	156.21	205.65	11103	8869	11125	5968	6414
220	1.000	250.54	231.16	224.41	162.94	215.00	12379	9869	12444	6517	6931
230	1.000	262.83	242.50	255.98	169.55	224.18	13721	10919	13841	7084	7464
240	1.000	275.10	253.82	267.53	176.04	233.19	15124	12017	15312	7671	8012
250	1.000	287.34	265.12	279.04	182.39	242.05	16586	13161	16853	8284	8578
260	1.000	299.54	276.38	290.51	188.58	250.74	18102	14347	18461	8928	9163
270	1.000	311.69	287.58	301.94	194.61	259.28	19669	15573	20133	9610	9767
280	1.000	323.77	298.73	313.31	200.48	267.67	21285	16839	21866	10336	10393
290	1.000	335.78	309.81	324.63	206.18	275.92	22947	18142	23656	11112	11040
300	1.000	347.71	320.82	335.90	211.70	284.03	24652	19482	25503	11945	11712

TABLE 6.8

n = 10
d = 4

λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	10.38	9.58	10.36	10.43	12.90	24	21	24	25	20
20	1.000	20.88	19.27	20.81	21.17	20.63	62	52	62	67	39
30	1.000	31.52	29.09	31.36	32.22	29.23	117	98	118	131	82
40	1.000	42.30	39.03	41.99	43.49	38.17	194	162	200	219	142
50	1.000	53.19	49.08	52.68	54.78	47.13	299	247	312	327	215
60	1.000	64.14	59.18	63.39	65.90	55.93	434	355	456	448	302
70	1.000	75.11	69.30	74.09	76.72	64.50	598	487	631	577	403
80	1.000	86.04	79.39	84.79	87.20	72.82	793	644	839	712	518
90	1.000	96.94	89.44	95.47	97.33	80.91	1018	826	1078	856	648
100	1.000	107.79	99.46	106.15	107.15	88.78	1275	1034	1350	1012	795
110	1.000	118.62	109.45	116.84	116.74	96.45	1566	1270	1656	1185	959
120	1.000	129.45	119.43	127.56	126.14	103.96	1893	1536	1997	1379	1143
130	1.000	140.28	129.43	138.32	135.41	111.29	2259	1833	2377	1596	1346
140	1.000	151.16	139.46	149.13	144.61	118.48	2665	2163	2798	1841	1570
150	1.000	162.08	149.54	159.99	153.76	125.51	3116	2529	3264	2114	1817
160	1.000	173.06	159.67	170.90	162.91	132.39	3615	2932	3778	2418	2089
170	1.000	184.11	169.87	181.87	172.06	139.13	4163	3374	4343	2753	2388
180	1.000	195.23	180.13	192.89	181.23	145.72	4764	3858	4963	3121	2717
190	1.000	206.43	190.47	203.97	190.44	152.17	5421	4385	5640	3521	3077
200	1.000	217.70	200.87	215.10	199.67	158.47	6135	4956	6376	3956	3472
210	1.000	229.04	211.33	226.28	208.94	164.62	6907	5573	7173	4424	3905
220	1.000	240.45	221.85	237.50	218.24	170.63	7739	6235	8031	4926	4378
230	1.000	251.91	232.42	248.76	227.57	176.49	8630	6944	8951	5464	4896
240	1.000	263.42	243.04	260.05	236.93	182.21	9579	7697	9932	6036	5460
250	1.000	274.97	253.70	271.37	246.30	187.78	10584	8493	10971	6642	6075
260	1.000	286.54	264.38	282.71	255.69	193.21	11643	9331	12065	7282	6744
270	1.000	298.14	275.08	294.06	265.08	198.50	12750	10206	13210	7955	7471
280	.999	309.75	285.79	305.42	274.48	203.65	13903	11115	14402	8658	8258
290	.999	321.35	296.50	316.77	283.86	208.66	15094	12055	15635	9391	9110
300	.999	332.94	307.19	328.11	293.24	213.54	16317	13019	16902	10151	10029

TABLE 6.9

n =10

d = 2

λ	T	$E(\hat{\lambda})$	$E(\hat{\lambda}_C)$	$E(\hat{\lambda}_F)$	$E(\hat{\lambda}_T)$	$E(\hat{\lambda}_S)$	$MSE(\hat{\lambda})$	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_F)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
10	1.000	10.24	9.45	10.23	10.25	24.45	32	28	32	32	217
20	1.000	20.51	18.92	20.49	20.59	29.84	71	61	71	72	117
30	1.000	30.82	28.43	30.78	31.05	35.41	117	101	117	122	64
40	1.000	41.17	37.98	41.11	41.67	41.07	172	149	173	185	52
50	1.000	51.56	47.58	51.48	52.47	46.72	236	205	239	263	79
60	1.000	62.01	57.22	61.89	63.46	52.29	314	271	319	363	146
70	1.000	72.51	66.91	72.35	74.68	57.73	405	349	414	489	255
80	1.000	83.08	76.65	82.86	86.13	62.99	513	440	527	647	410
90	1.000	93.70	86.46	93.43	97.83	68.05	641	546	661	847	618
100	1.000	104.40	96.33	104.06	109.79	72.88	791	671	819	1099	885
110	1.000	115.17	106.26	114.76	122.03	77.48	969	816	1006	1415	1220
120	1.000	126.02	116.27	125.52	134.55	81.84	1178	986	1226	1811	1629
130	1.000	136.95	126.36	136.36	147.39	85.97	1423	1183	1482	2308	2120
140	1.000	147.96	136.52	147.28	160.54	89.87	1708	1412	1780	2925	2702
150	1.000	159.06	146.76	158.27	174.04	93.55	2036	1674	2121	3688	3381
160	1.000	170.24	157.07	169.34	187.88	97.01	2410	1971	2508	4616	4166
170	1.000	181.49	167.45	180.46	202.07	100.27	2830	2303	2940	5729	5063
180	.999	192.79	177.88	191.64	216.59	103.33	3291	2666	3412	7037	6081
190	.999	204.11	188.33	202.84	231.44	106.20	3787	3057	3917	8544	7224
200	.998	215.44	198.77	214.02	246.56	108.89	4309	3466	4445	10241	8502
210	.997	226.72	209.19	225.16	261.91	111.41	4844	3886	4984	12109	9919
220	.995	237.93	219.52	236.22	277.43	113.76	5380	4307	5520	14120	11482
230	.993	249.00	229.75	247.14	293.05	115.95	5902	4717	6039	16239	13197
240	.990	259.92	239.81	257.89	308.69	117.99	6396	5107	6526	18423	15070
250	.986	270.62	249.69	268.42	324.28	119.89	6850	5469	6969	20628	17106
260	.982	281.07	259.33	278.70	339.74	121.66	7252	5796	7358	22809	19311
270	.976	291.24	268.72	288.69	354.99	123.29	7595	6083	7687	24923	21688
280	.970	301.10	277.81	298.36	369.98	124.81	7874	6329	7949	26930	24241
290	.962	310.63	286.60	307.69	384.64	126.22	8085	6532	8144	28795	26976
300	.953	319.80	295.06	316.66	398.93	127.52	8230	6697	8272	30490	29894

While it may be disappointing that no single estimator performs best across all dilutions, it should not be surprising. As the dilution factor decreases to 1 ($d=1$ is equivalent to using the single dilution $z=.01$ for all $3n=9$ samples), the Fisher estimator $\hat{\lambda}_F$ approaches the MPN and the Johnson-Brown Spearman estimator approaches the constant value $\hat{\lambda}_S = e^{-\gamma - \ln(z_1)} = e^{-\gamma - \ln(.01)} = 56.15$. As the dilution factor increases, it can be shown algebraically that the Thomas estimator $\hat{\lambda}_T$ shrinks and the Johnson-Brown estimator $\hat{\lambda}_S$ grows.

In short, the dilution factor affects each estimator in a particular manner and it seems, in general, that $\hat{\lambda}_T$ performs best at "high" dilution factors, $\hat{\lambda}_S$ performs best at "intermediate" dilution factors and $\hat{\lambda}_C$ performs best at "low" dilution factors. The choice of the dilution factor, however, moves one into the area of design considerations. Chapter 4 will examine current design recommendations, design suggestions in light of Table 6, and the final selection of an estimator.

4. DESIGN CONSIDERATIONS

Early serial dilution investigations (e.g., Fisher 1922) suggest that most researchers of the day simply used large numbers of dilutions over a range wide enough to be certain of obtaining at least one dilution for which some but not all of the samples were fertile. Matuszewski et al. (1935) noted that "the most accurate predictions are obtained" when between 59% and 66% of the total number of samples are fertile and recommended trying to select dilutions accordingly. Fisher and Yates (1943) advocated that the "two-fold dilution series should be used, with correspondingly fewer [samples] at each level, in preference to a four-fold or ten-fold series covering the same range." Stevens (1958) proposed a test to determine whether suspicious results (e.g., $X_1X_2X_3X_4X_5=30303$ for $n_i=3$ and $z_i=(.5)^i$) are suitable for further analysis.

Most current methods books recommend using $k=3$ decimal dilutions with $n=3$ or $n=5$ samples per dilution and that even when more than three dilutions are used to be certain of avoiding either all fertile or all sterile results, "the results from only three of these are used in computing the MPN" (American Public Health Association 1971). The more statistical works, however, echo Finney's design statements that (1) "If N , the total number of samples is fixed, the ideal allocation would be to use all [samples] at the dilution giving 1.59 organisms per sample" (Finney 1978, page 436) and (2) for serial dilutions, "The dilution factor should be as small as practicable; 2 and 4 are

definitely preferable to 10" (Finney 1978, page 437).

In this chapter, it is established that the above present recommendations are not necessarily correct. As in Chapter 3, the discussion commences with a consideration of the single dilution experiment (section 4.1). Finally, a workable algorithm is given for determining, under certain researcher-chosen constraints, an efficient serial dilution design (section 4.2).

4.1 The Single Dilution Experiment

Ever since Finney (1952) first noted that Fisher's information $I(\lambda)$ for the single dilution problem was maximized for $\lambda z = 1.59$, statements like (1) in section 4.1 have abounded in the literature (Mantel 1975). Unfortunately, $\lambda z = 1.59$ is not optimal for the small values of n encountered in practice.

Before Finney solved the single dilution problem asymptotically, Halvorson and Ziegler (1933b) set out "to show how $[\lambda]$, as well as the number of tubes, influences the accuracy of the results." Using the coefficient of variation as their measure of accuracy, they note that, "An examination of this table and graph shows that the point of maximum accuracy varies with the number of tubes. With 10 tubes, the maximum accuracy is obtained when 70% of the tubes show growth [i.e., for $\lambda z = 1.2$], but with 100 tubes, the maximum accuracy is obtained when 78% of the tubes show growth [i.e., for $\lambda z = 1.5$]." While their

conclusion, "Theoretically, the maximum accuracy is obtained with a bacterial population of approximately 1.2 to 1.5 organisms per [sample], this range shifting toward the higher values as the number of tubes is increased," fails to identify the exact asymptotic bound as 1.59, it is unfortunate that their small sample work has been largely ignored.

A further consideration discussed by neither Finney nor Halvorson and Ziegler is the effect of the MPN's sizable positive bias when MSE and not variance is used to judge precision.^f Table 7 gives expected values and MSE values for the single dilution design using all $n=9$ samples at either $z=.008$, $z=.010$ (Finney's optimal design for $\lambda=160$) or $z=.012$. While the varying degree of truncation caused by eliminating the result for which all samples were fertile makes comparisons across dilutions difficult, note that $MSE(\hat{\lambda})$ near $\lambda=160$ is actually smallest for $z=.012$ (i.e., for $\lambda z > 1.59$).

It should be noted that the figures in Table 7 indicate that $VAR(\hat{\lambda} | \lambda=160, z=.012) < VAR(\hat{\lambda} | \lambda=160, z=.010) < VAR(\hat{\lambda} | \lambda=160, z=.008)$, which seems to contradict the previously mentioned Halvorson and Ziegler result. The latter's work, however, uses binomial approximations without actually considering each of the $n+1$ possible sample results. Because Table 7 eliminates the result for which all samples were fertile, Halvorson and Ziegler's conclusions cannot be directly compared with those of this paper. At any rate, simply choosing

TABLE 7

MPN Expected Values and MSE Values for the Single Dilution: n=9

λ	$z=.008$			$z=.010$			$z=.012$		
	T	$E(\hat{\lambda})$	MSE(λ)	T	$E(\hat{\lambda})$	MSE(λ)	T	$E(\hat{\lambda})$	MSE(λ)
10	1.000	10.63	165	1.000	10.64	134	1.000	10.65	114
20	1.000	21.33	353	1.000	21.37	292	1.000	21.40	252
30	1.000	32.10	567	1.000	32.19	479	1.000	32.29	421
40	1.000	42.97	813	1.000	43.14	700	1.000	43.31	627
50	1.000	53.92	1094	1.000	54.19	958	.999	54.42	867
60	1.000	64.96	1412	.999	65.31	1248	.998	65.52	1123
70	1.000	76.07	1765	.998	76.42	1556	.994	76.45	1370
80	.999	87.20	2141	.995	87.41	1859	.987	87.01	1581
90	.998	98.28	2527	.991	98.14	2134	.976	97.02	1736
100	.995	109.26	2904	.984	108.50	2362	.960	106.37	1824
110	.992	120.03	3254	.974	118.36	2527	.939	114.95	1849
120	.987	130.51	3559	.960	127.64	2627	.912	122.75	1825
130	.980	140.63	3806	.943	136.29	2663	.880	129.75	1770
140	.971	150.33	3989	.922	144.28	2646	.844	136.01	1710
150	.960	159.55	4105	.897	151.62	2592	.803	141.57	1670
160	.947	168.27	4157	.869	158.30	2520	.760	146.48	1675
170	.931	176.46	4153	.837	164.38	2451	.715	150.82	1748
180	.912	184.12	4106	.803	169.88	2406	.668	154.64	1910
190	.892	191.26	4029	.767	174.85	2405	.621	158.01	2180
200	.869	197.88	3937	.730	179.32	2470	.575	160.97	2574
210	.844	204.02	3849	.691	183.35	2616	.530	163.57	3106
220	.817	209.68	3781	.652	186.98	2862	.486	165.86	3788
230	.789	214.91	3749	.614	190.23	3221	.445	167.88	4630
240	.760	219.72	3770	.575	193.16	3707	.405	169.66	5640
250	.730	224.15	3859	.537	195.79	4331	.368	171.22	6826
260	.699	228.22	4031	.501	198.16	5103	.334	172.60	8194
270	.688	231.96	4299	.465	200.29	6032	.302	173.82	9747
280	.637	235.40	4674	.431	202.20	7125	.273	174.89	11491
290	.606	238.56	5169	.399	203.92	8389	.245	175.84	13428
300	.575	241.45	5792	.368	205.47	9830	.221	176.67	15562

$\lambda z = 1.59$ clearly does not necessarily yield the optimal single dilution design.

Observing the "T" column in Table 7 for $z = .010$ reveals another difficulty with the $\lambda z = 1.59$ "ideal allocation." Even if the researcher's *a priori* guess of $\lambda = 160$ should happen to be exactly correct, he can expect to obtain usable experimental results only 86.9% of the time; 13.1% of the time he will observe all the samples fertile and be unable to calculate a meaningful point estimate for λ . If, moreover, the researcher guessed too low so that $\lambda = 300$ were the true density, he would obtain unusable results a full 63.2% of the time! This illustrates, of course, the wisdom of the serial dilution design, which protects against obtaining samples either all fertile or all sterile.

Finally, note from Table 7 just how large a change in the T and the MSE values occurs for such a small change of .002 in the dilution. It appears that the choice of the dilution(s) for the problem of estimating the density of organisms needs to be a carefully considered one.

4.2 A Design Algorithm for the Serial Dilution Experiment

The discussion of Table 7 in section 4.1 suggests that one could characterize the serial dilution problems as one of minimizing MSE while controlling $P(\text{all samples fertile}) = 1 - T$. It seems, then, that

the first design consideration of the researcher should be determining ρ , the risk he is willing to assume of obtaining all fertile samples. This being accomplished, one needs an estimate of λ_{\max} , the largest possible value the researcher believes that λ could reasonably assume.

The program in Appendix IV allows the user to input n (the number of samples per dilution), λ_{\max} and ρ (the maximum acceptable probability of obtaining all samples fertile, which will occur, of course, when $\lambda = \lambda_{\max}$). For various dilution factors, the program outputs z_2 , the middle dilution of a $k=3$ serial dilution experiment satisfying the input constraints. For any larger choice of z_2 , $P(X_1 X_2 X_3 = 333)$ will be greater than ρ . Any smaller choice of z_2 will, in general, increase the MSE values of the estimators. While the program is given for $k=3$, it readily generalizes to any k . Figure 2 gives the output of the program for $n=3$, $\lambda_{\max}=300$, $\rho=.01$ and $d=1(.5)10$.

Now the researcher must decide which of the (d, z) pairs meeting his n , λ_{\max} and ρ constraints gives the smallest MSE. Recall that the program in Appendix III generates expected values and MSE values for the estimators of sections 3.2.1 to 3.2.4 for designs with any dilution factor d and any middle dilution z_2 . Using the program in Appendix III, then, for each (d, z) pair and over the λ values of interest, the researcher may note which (d, z) pair achieves the

FIGURE 2

Output for the Program of Appendix IV

HOW MANY TUBES PER DILUTION?

?3

WHAT IS THE MAX EXPECTED L?

?300

WHAT IS THE RISK FOR HAVING ALL TUBES FERTILE?

?.01

INPUT THE STARTING D, ENDING D AND JUMPSIZE

?1,10,.5

D	Z
1.0	.00305
1.5	.00314
2.0	.00334
2.5	.00359
3.0	.00389
3.5	.00422
4.0	.00457
4.5	.00492
5.0	.00527
5.5	.00563
6.0	.00598
6.5	.00633
7.0	.00688
7.5	.00703
8.0	.00738
8.5	.00774
9.0	.00809
9.5	.00844
10.0	.00880

smallest MSE and for which estimator that smallest MSE occurs.

Appendix V gives the output obtained using the program in Appendix III over the (d,z) pairs of Figure 2 for $\lambda=10(10)300$. Note that in each case $T = .990 = 1-\rho$ for $\lambda = \lambda_{\max} = 300$. For the researcher's best *a priori* guess of $\lambda=30$, for example, he would construct from Appendix V a summary chart similar to Table 8 giving the MSE for each of the three competing estimators (the discussion of section 3.3 eliminated $\hat{\lambda}$ and $\hat{\lambda}_F$ from further consideration) at each (d,z) design. Note that the bias-corrected MPN $\hat{\lambda}_C$ achieves its minimum MSE of 506 for the design $(d,z)=(4.5,.00492)$; the Thomas estimator $\hat{\lambda}_T$ achieves its minimum MSE of 977 for the design $(d,z)=(4.0,.00457)$; the Johnson-Brown Spearman estimator $\hat{\lambda}_S$ achieves its minimum MSE of 400 also for the design $(d,z)=(4.0,.00457)$. The researcher should use the estimator $\hat{\lambda}_S$ and the design with dilution factor 4.0 centered at $z_2=.00457$.

Note that in both $\rho=P(\text{all samples fertile})$ and MSE this design is superior to the original example of Chapters 1-3 of $z_1=.1$, $z_2=.01$ and $z_3=.001$ which, according to Table 4 has $\rho = P(X_1X_2X_3=333) = .015$ and $MSE(\hat{\lambda}_S | \lambda=30) = 759$. Note also that the selected dilution factor of 4.0 contradicts the previously mentioned advice of Fisher and Yates (1943) and Finney (1978).

When constructing a summary chart similar to Table 8, however, the researcher may not wish to be so specific so as to design the

TABLE 8

MSE Comparisons: maximum $\lambda = 300$
 best guess $\lambda = 30$

d	z_2	$MSE(\hat{\lambda}_C)$	$MSE(\hat{\lambda}_T)$	$MSE(\hat{\lambda}_S)$
1.0	.00305	799	1332	23741
1.5	.00314	757	1268	5798
2.0	.00334	684	1161	1923
2.5	.00359	618	1071	848
3.0	.00389	566	1009	512
3.5	.00422	530	979	414
4.0	.00457	511	977	400
4.5	.00492	506	997	416
5.0	.00527	514	1027	444
5.5	.00563	532	1059	477
6.0	.00598	559	1087	511
6.5	.00633	591	1106	545
7.0	.00688	629	1113	580
7.5	.00703	670	1109	613
8.0	.00738	713	1093	646
8.5	.00774	756	1067	678
9.0	.00809	800	1034	708
9.5	.00844	844	997	737
10.0	.00880	886	957	765

experiment for precisely $\lambda=30$. Recall, for example, from the single dilution analysis the problems that can arise when the true λ value is far from the researcher's best *a priori* guess. In this case, the researcher could construct his summary chart by entering, for example, the maximum $\text{MSE}(\hat{\lambda} | 10 < \lambda < 100)$ instead of $\text{MSE}(\hat{\lambda} | \lambda=30)$ for each estimator.

While this author recommends the preceding algorithm and finds it to be both simple and reliable, a few additional comments and cautions need to be given. First, it should be noted that under every reasonable set of constraints imposed in connection with this paper, (1) the three estimators (i.e., $\hat{\lambda}_C$, $\hat{\lambda}_T$ and $\hat{\lambda}_S$) all achieved their minimum MSE values at approximately the same (d, z) pair and (2) the Johnson-Brown Spearman estimator $\hat{\lambda}_S$ proved consistently to be the preferred estimator. As there are no obvious analytical reasons why either of the above should be true without exception, however, they are presented as "observations" and not "conjectures."

Secondly, Table 8 indicates that the Thomas estimator $\hat{\lambda}_T$ MSE value actually peaks for $d=7.0$ and begins to decrease as d increases. One wonders whether it will ever drop below the 400 value attained by $\hat{\lambda}_S$ for $d=4.0$. The answer, unfortunately, is yes, as $\text{MSE}(\hat{\lambda}_T | \lambda=30)$ drops to 376 for $(d, z)=(60, .04853)$ and keeps on dropping. In this author's opinion, however, the previously recommended $(4.0, .00457)$ design should still be preferred because (1) dilution factors much greater than 10 reflect too much uncertainty to be of practical

concern and indicate the need for a "preliminary investigation" and (2) the behavior of all the estimators, as mentioned in section 3.3, degenerates for both very large and very small (i.e., near $d=1.00$) dilution factors. The first statement is a matter of opinion, and Seligman and Mickey (1964), for example, state, "Not infrequently, however, the confidence in the pre-existing estimate is such that a 1,000-fold dilution interval is employed." The second statement recalls the fact that $\lim_{d \rightarrow \infty} (\hat{\lambda}_T) = 0$. As d increases, there will be some point at which $MSE(\hat{\lambda}_T)$ is artificially minimized before climbing back to $MSE(\hat{\lambda}_T) = (\text{best guess})^2$. A similar phenomenon occurs for the Johnson-Brown Spearman estimator which, as previously noted, approaches the constant $e^{-\gamma - \ln(z_1)}$ as d approaches 1. If the researcher's best guess happens to be close to $e^{-\gamma - \ln(z_1)}$, there will be some point at which $MSE(\hat{\lambda}_S)$ is artificially minimized before climbing back to $MSE(\hat{\lambda}_S) = ((\text{best guess}) - e^{-\gamma - \ln(z_1)})^2$.

5. THE FINITE POPULATION MODEL

Each technique described in Chapters 1-4 assumes that the X_i 's are independent responses or, equivalently, that the samples come from an infinite population. This is certainly acceptable when one is monitoring organism levels in, for example, water systems or dairy products. Suppose, however, the parameter λ is the number of organisms in a specific finite volume and not the average number per volume in some conceptually infinite population. In this case, the number of fertile samples cannot exceed λ and a point estimate or confidence interval value less than the total number of observed fertile responses is not appropriate. Furthermore, since the infinite model considers the given volume a random sample, it incorporates additional variation due to sampling into point estimates and confidence intervals.

Olson, Turbak and McFeters (1979), for example, employed membrane diffusion chambers to study the survival of organisms in mine waters. After using serial dilution techniques to estimate the number of organisms in a small volume, they placed the volume in mine water in a chamber that allowed the mine water but not the organisms to pass freely in and out of the chamber. Periodically, they used serial dilution techniques to estimate the number of organisms surviving in the chamber. While the authors were investigating a clearly finite population (i.e., they were trying to estimate the true count in a

particular chamber at a particular time), they were forced to turn to standard (i.e., infinite population) MPN tables for point estimates and confidence intervals and concluded "the large confidence intervals inherent in the MPN procedure make a more definite statement difficult to justify."

It is interesting to note that McCrady's (1915) original serial dilution paper dealt exclusively with finite populations. Acknowledging that his mathematical analysis represents only an approximation, he states

When more than one volume is to be drawn from the [population], these formulae demand that for each draw the initial conditions must be the same. That is, after the first volume has been drawn, this volume, together with its contained B. coli, must be replaced in the [population] before drawing the next volume. Such a procedure is obviously impossible in practice.

But perhaps, when the first volume has been drawn, it may be assumed that a proportionate number of the B. coli have also been drawn in this volume. If so... the value of the general factor... has remained practically unchanged.

But even if this assumption is not justified, calculation will show that the error due to non-replacement is, in general, negligible.

Although later in the paper he partially works out one specific example correctly accounting for this non-replacement, there is no evidence that he was aware of the exact solution to the general finite population problem.

Starting with Greenwood and Yule (1917), papers consider only the simpler and, in truth, more useful infinite model. Several

authors (e.g., Cochran 1950), however, use a finite population situation to motivate the serial dilution problem, state that "this is closely approximated by" the infinite model, and then proceed to discuss mathematically only the latter.

Section 5.1 develops the exact mathematical formulation of the general finite population problem. In section 5.2, point estimation and interval estimation results are given and compared with those obtained under the usual infinite model. Except where otherwise noted, the examples follow Chapters 1-4 in using the commonly employed and tabled $k=3$ serial dilution experiment with $n_i=3$ and $z_i=(.1)^{i-1}$ for $i=1,2,3$.

5.1 The General Formula

According to Johnson and Kotz (1977), Polya once maintained that "any problem of probability appears comparable to a suitable problem about bags containing balls." The finite population serial dilution problem is, in fact, a variation of the classical occupancy problem of urn modeling. Imagine that each of $Y \geq 1$ balls is placed at random into one of n equally likely urns and that X denotes the number of urns thus occupied. The probability that exactly r urns are occupied (see, for example, Johnson and Kotz 1977) is given by

$$(5.1) \quad P(X=r) = \binom{n}{r} \sum_{i=0}^r (-1)^{r-i} \binom{r}{i} (i/n)^Y \quad r=1,2,\dots,\min(n,Y).$$

Suppose there are n_i equally likely urns of type $i=1,2,3$ and

that the probability associated with each type i urn is z_i so that $P(\text{being placed in a type } i \text{ urn}) = n_i z_i$ and $P(\text{not being placed in any urn}) = 1 - \sum_i n_i z_i$. If Y_i represents the total number of balls placed in type i urns, then after λ balls have been placed (Y_1, Y_2, Y_3) has a multinomial distribution with parameters $p_i = n_i z_i$ and λ . In addition, letting $P(X_1, X_2, X_3 = r_1, r_2, r_3) = f(X_1 X_2 X_3)$,

$$\begin{aligned}
 (5.2) \quad f(X_1 X_2 X_3) &= E_{Y_1 Y_2 Y_3} f(X_1 X_2 X_3 | y_1 y_2 y_3) \\
 &= E[f(X_1 | y_1) f(X_2 | y_2) f(X_3 | y_3)] \\
 &= \binom{n_1}{X_1} \binom{n_2}{X_2} \binom{n_3}{X_3} \sum_{i=0}^{X_1} \sum_{j=0}^{X_2} \sum_{k=0}^{X_3} (-1)^{\sum X - i - j - k} \binom{X_1}{i} \binom{X_2}{j} \binom{X_3}{k} \\
 &\quad E[(i/n_1)^{Y_1} (j/n_2)^{Y_2} (k/n_3)^{Y_3}].
 \end{aligned}$$

The joint moment generating function for the multinomial distribution of (Y_1, Y_2, Y_3) with parameters p_1, p_2, p_3 and λ indicates that $E[A^{Y_1} B^{Y_2} C^{Y_3}] = [p_1 A + p_2 B + p_3 C + (1 - p_1 - p_2 - p_3)]^\lambda$ for any constants A, B and C . Here, $E[(i/n_1)^{Y_1} (j/n_2)^{Y_2} (k/n_3)^{Y_3}] = [1 - \sum n z + i z_1 + j z_2 + k z_3]^\lambda$. Substituting into equation (5.2) gives

$$\begin{aligned}
 (5.3) \quad f(X_1 X_2 X_3) &= \binom{n_1}{X_1} \binom{n_2}{X_2} \binom{n_3}{X_3} \sum_{i=0}^{X_1} \sum_{j=0}^{X_2} \sum_{k=0}^{X_3} (-1)^{\sum X - i - j - k} \binom{X_1}{i} \binom{X_2}{j} \binom{X_3}{k} \\
 &\quad (1 - \sum n z + i z_1 + j z_2 + k z_3)^\lambda,
 \end{aligned}$$

the exact probability function for the finite population serial dilution problem with λ total organisms and n_i samples at the z_i dilution for $i=1, 2, 3$.

5.2 Point and Interval Estimation

The method of combining independent results (section 2.3) and Fisher's $\hat{\lambda}_F$ estimate (section 3.2.2) require independent X_i 's and, consequently, cannot be used for finite populations. Each of the other techniques of Chapters 1-4, however, can be applied in the finite population situation by using equation (5.3) instead of equation (1.1) to obtain the sampling distribution of the $X_1X_2X_3$ values. The program used to generate these probabilities is given in Appendix VI and is the finite analog of the program in Appendix I.

Table 9 compares the MPN point estimates and the deMan and minimum expected width interval estimates of the infinite and finite models for the 18 $X_1X_2X_3$ results with non-empty minimum expected width confidence intervals. In both the infinite and the finite model, these 18 $X_1X_2X_3$ results include about 98% of the probability for $10 < \lambda < 300$. Note that for the finite model the MPN's are generally slightly smaller and the confidence intervals are generally slightly narrower. The fact that the differences are so minor is noteworthy in that this design involves sampling $\sum_{i=1}^3 n_i z_i = .333$ of the population. Even when the portion of the finite population sampled is quite significant, apparently, the difference between the infinite and the finite analyses is negligible. Since the Olson et al. (1979) mine water problem of this chapter, for example, involved sampling only very small portions of the population at each step, then, applying the

TABLE 9

Infinite and Finite Population Results: $n=3$, $z_1=.1$ $z_2=.01$ $z_3=.001$

result	MPN values		.95 deMan interval estimates		.95 minimum expected width intervals	
	infinite ^a	finite ^b	infinite ^c	finite ^b	infinite ^d	finite ^d
000	0.0	0		0-9	0-13	0-11
010	3.1	3	<1-17	1-14	2-10	2-9
100	3.6	3	<1-21	1-18	<1-25	1-21
110	7.4	7	2-28	2-24	3-20	3-19
200	9.2	9	2-38	2-35	<1-37	2-36
201	14.3	14	5-48	5-45	11-14	10-14
210	14.7	14	5-50	5-46	5-42	5-40
220	21.1	20	8-62	8-59	10-32	10-32
300	23.1	22	<10-130	8-126	4-120	4-120
301	38.5	37	10-180	15-174	14-69	15-69
310	42.7	42	10-210	16-210	7-200	8-200
311	74.9	74	20-280	27-278	21-180	20-170
320	93.3	93	30-380	33-383	12-360	12-360
321	149.4	149	50-500	55-503	38-400	37-400
322	214.7	214	80-640	86-637	130-260	130-250
330	239.8	239	<100-1400	91-1393	26-990	33-990
331	462.2	461	100-2400	178-2405	70-2000	70-1990
332	1099.0	1098	300-4800	381-4785	140-4070	150-4070

^a one decimal accuracy^b integer accuracy^c values for results 000-222 are given to the nearest integer
values for results 300-322 are given to the nearest 10
values for results 330-333 are given to the nearest 100^d values less than 100 are given to the nearest integer
values greater than 100 are given to the nearest 10

finite population analysis will not affect their conclusions.

The finite population deMan Bayesian intervals in Table 9 were obtained using the program in Appendix VII. Recall that deMan (1975) employed a truncated likelihood function to obtain his intervals for the infinite model. The sum of the finite model likelihood values across all possible λ 's (i.e., from $\lambda = \Sigma X$ to $\lambda = \infty$), however, converges analytically to

$$(5.4) \quad \Sigma f(\lambda; X_1 X_2 X_3) = \binom{n_1}{X_1} \binom{n_2}{X_2} \binom{n_3}{X_3} \sum_{i=0}^{X_1} \sum_{j=0}^{X_2} \sum_{k=0}^{X_3} \binom{X_1}{i} \binom{X_2}{j} \binom{X_3}{k} \\ [(1 - \Sigma nz + iz_1 + jz_2 + kz_3)^{\Sigma X}] / (\Sigma nz - iz_1 - jz_2 - kz_3)$$

so that the program in Appendix VII provides exact intervals. It can also be shown that the exact mean of the deMan Bayesian posterior distribution in the finite population case is given by

$$(5.5) \quad \frac{\Sigma \lambda f(\lambda; X_1 X_2 X_3)}{\Sigma f(\lambda; X_1 X_2 X_3)} = \Sigma X + [1 / \Sigma f(\lambda; X_1 X_2 X_3)] \binom{n_1}{X_1} \binom{n_2}{X_2} \binom{n_3}{X_3} \sum_{i=0}^{X_1} \sum_{j=0}^{X_2} \sum_{k=0}^{X_3} \\ \binom{X_1}{i} \binom{X_2}{j} \binom{X_3}{k} [1 - \Sigma nz + iz_1 + jz_2 + kz_3]^{\Sigma X - 1} / \\ (\Sigma nz - iz_1 - jz_2 - kz_3)^2.$$

Even though the positive skew of the likelihood distribution and the positive bias of the MPN (the mode of the likelihood distribution) prevent the mean and the median of the posterior distribution from being useful point estimates for λ , the program in Appendix VII gives these values for completeness.

The difference between the infinite and the finite population

models is greatest as $\sum_i z_i$ approaches 1, at which point all of the finite population is included in the sample. Table 10 considers such an extreme case of the finite population problem and makes the same comparisons as Table 9 but in the case for which $z_1=.3$, $z_2=.03$, $z_3=.003$. While the finite model intervals are, as in Table 9, narrower than their infinite counterparts, even when $\sum_i z_i=.999$ of the population is selected for observation, the finite analysis appears to serve mainly to prevent lower interval estimate endpoints from falling below the observed number of fertile tubes when ΣX (and, by inference, λ) is small.

Taken together, Tables 9 and 10 suggest that the finite population model results are essentially identical to those of the infinite model when $\sum_i z_i$ is small and differ from those of the infinite model as $\sum_i z_i$ approaches 1 only when λ is small. Chapters 1-4 suggested that the Johnson-Brown Spearman estimator $\hat{\lambda}_S$ was generally the "best" infinite population estimator, however, so that one should examine the finite analog of $\hat{\lambda}_S$ before reaching any tentative conclusions.

Using equation (5.3) instead of (1.1), this author mimicked the work of Johnson and Brown (1961) and obtained the following results.

(i) For large λ , the finite and infinite models yield the same non-bias-corrected point estimate $\hat{\lambda} =$

$$e^{-\gamma - \ln(z_1) + [\ln(d)] [(\Sigma X/n) - .5]}$$

TABLE 10

Infinite and Finite Population Results: $n=3$, $z_1=.1$ $z_2=.01$ $z_3=.001$

result	MPN values		.95 deMan interval estimates		.95 minimum expected width intervals	
	infinite ^a	finite ^b	infinite ^c	finite ^b	infinite ^d	finite ^d
000	0.0	0		0-0	0-4	0-0
010	1.0	1	<.3-5.7	1-2	<1-3	1-1
100	1.2	1	<.3-7.0	1-4	<1-8	1-4
110	2.5	2	.7-9.3	2-6	<1-6	2-5
200	3.1	2	.7-13	2-9	<1-12	2-10
201	4.8	4	1.7-16	3-13	4-4	4-5
210	4.9	4	1.7-20	3-13	2-14	3-12
220	7.0	6	2.7-31	4-17	4-10	5-11
300	7.7	7	<3.3-43	3-40	2-41	3-40
301	12.8	12	3.3-60	5-56	5-23	5-23
310	14.2	13	3.3-70	5-68	3-66	4-66
311	25.0	24	6.7-93	9-91	7-60	9-58
320	31.1	30	10-127	10-126	4-120	6-110
321	49.8	49	17-167	18-165	13-130	13-130
322	71.6	71	27-213	28-210	42-87	41-84
330	79.9	79	<33-467	30-463	11-330	10-330
331	154.1	153	33-800	59-800	24->600	24-660
332	366.3	366	100-1600	127-1593	47->600	49->1000

^aone decimal accuracy^binteger accuracy^cvalues from Table 9 divided by 3^dvalues less than 100 are given to the nearest integer
values greater than 100 are given to the nearest 10

(ii) When $\sum_i z_i$ is small, $\text{COV}(X_i, X_j)$ is negative but negligible and both the finite and the infinite models yield the multiplicative bias correction given in section 3.2.4 and produce the final estimator of equation (3.1).

These results, the development of which is sketched in Appendix VIII, support the suggestions of Tables 9 and 10 given above.

6. SUMMARY

Even though the serial dilution assay is a standard microbiological method for determining the density of organisms in a solution, there has been very little mathematical investigation into the statistical appropriateness of the decimal dilution design, the MPN point estimate and the Woodward (1957) confidence intervals that have been long accepted as standard procedure. This paper, the only recent review of the serial dilution problem, has been the first to compare exact MSE values of competing estimators, the first to provide an algorithm to determine efficient serial dilution designs, and the first to give the exact formulation of the finite population serial dilution problem. In addition, original serial dilution point estimates and exact confidence intervals were given and compared with the MPN and previously proposed alternatives.

It is the author's conclusion that the serial dilution design and estimation recommendations of standard textbooks (e.g., Finney 1978) and reference works (e.g., American Public Health Association 1971) are not adequate and, specifically,

(i) The combining independent results (section 2.3) and minimum expected width (section 2.4) confidence interval methods should be the recommended techniques for obtaining one-sided and two-sided confidence intervals respectively.

(ii) The algorithm of section 4.2 should be used to identify both the optimal serial dilution design and the most efficient

point estimate.

(iii) The finite population probabilities given by equation (5.3) should be used when applicable, especially when λ is small and many non-fertile samples are likely.

General computer programs that allow the researcher to put the recommendations of this paper into practice (or to compare completely new procedures to those in this paper) appear in the appendices. In addition, caveats and areas for further work have been identified throughout the paper.

FOOTNOTES

^a McCrady's paper spells out and applies ML estimation several years before Fisher popularized the concept during his "spectacular dispute" with Karl Pearson, who preferred the method of moments estimation (Owen 1976). Credit for the first clear and explicit formulation of ML estimation by differentiating the likelihood function, however, belongs to Daniel Bernoulli in 1777. Pearson and Kendall (1970) give a translation and discussion of Bernoulli's paper and of Euler's rebuttal (which was published in the same 1777 volume) of Bernoulli's ML ideas.

^b The MPN's given in Figure 1, calculated by the program given in Appendix III, also reveal two minor errors in the American Public Health Association (1970) MPN's given in Table 1. The MPN's corresponding to 110 and 200 should be 7.4 and 9.2 respectively.

^c Note that this is not the case for the other three 95% confidence intervals given in Table 1. The issue here is whether Woodward is really following the accepted testing procedure of rejecting the null hypothesis for the "most extreme" $\alpha=.05$ of the results when he chooses to reject for "likely" results and not for "unlikely" ones.

^d While it is not within the scope of this paper to discuss the biological appropriateness of either the serial dilution or the MF technique for various organisms, their frequent comparison in the literature deserves some comment. Most authors which consider this aspect of the problem (e.g., Middlebrooks, Middlebrooks, Johnson, Wight, Reynolds and Venosa 1978) stress that neither procedure should be regarded as absolute and note that "one technique does not appear to be more reliable than the other." DeMan (1977), however, opinions that "because microbiological standards for foods are becoming increasingly severe, in the future MPN [serial dilution] methods will probably often have to replace plate counts." Futhermore, one standard reference (American Public Health Association 1966, page 139) notes that "the limitations of the plate count method are well known" and "a more accurate method for the enumeration of small numbers of organisms is the MPN technique."

^eThis technique can certainly be applied to deMan's Bayesian intervals also, and the resulting point estimate would be the median of the posterior distribution. Since, however, deMan's posterior distributions are unimodal and positively skewed, the median of each posterior distribution would be larger than its mode (which is the MPN), and the resulting point estimate would be even more positively biased than the MPN. The same would be true for the estimate obtained by using the mean of the posterior distribution.

^fWhile Halvorson and Ziegler (1933c) were actually the first to note the positive bias of the MPN, they do not mention the bias as a design factor when selecting dilutions.

APPENDICES

APPENDIX I

```

C   PROGRAM TO GENERATE EXACT SAMPLING DISTRIBUTIONS FOR LAMBDA
C   FROM 1 TO 100 FOR N TUBES AT EACH OF 3 DILUTIONS
C   A IS THE BINOMIAL PROBABILITY W/O THE COEFFICIENT
C   D IS THE BINOMIAL COEFFICIENT
C   X IS THE NUMBER OF FERTILE TUBES
C   Z IS THE DILUTION
C   AMAT SAVES THE VALUES FOR OUTPUT A PAGE AT A TIME (10 LAMBDA
C   VALUES BY 64 TUBE COMBINATIONS)
C

```

```

DIMENSION A(3),D(0:10),X(3),Z(3),AMAT(66,40)
OUTPUT 'INPUT THE 3 Z VALUES'
INPUT Z(1),Z(2),Z(3)
OUTPUT 'INPUT N (# TUBES PER Z)'
INPUT N
D(0) = 1.0
DO 5 I=0,N-1
5  D(I+1)=D(I)*(N-I)/(I+1)
   L=1
9  DO 60 IM=1,40,4
   IF (L>100) GO TO 99
   AMAT(1,IM)=L
   C=0
   KOUNT=2
   DO 40 X1=0,N
   DO 40 X2=0,N
   DO 40 X3=0,N
   X(1)=X1
   X(2)=X2
   X(3)=X3
   CN = D(X1)*D(X2)*D(X3)
   DO 30 I=1,3
30  A(I)=(1-EXP(-Z(I)*L))**X(I)*(EXP(-Z(I)*L))**(N-X(I))
   F=CN*A(1)*A(2)*A(3)
   AMAT(KOUNT,IM)=X1
   AMAT(KOUNT,IM+1)=X2
   AMAT(KOUNT,IM+2)=X3
   AMAT(KOUNT,IM+3)=F
   KOUNT=KOUNT+1
   C=C+F
40  CONTINUE
   AMAT(66,IM)=C
   L=L+1
60  CONTINUE

```

APPENDIX I (CONTINUED)

```
      WRITE (108,200) AMAT(1,1),AMAT(1,5),AMAT(1,9),
C AMAT(1,13),AMAT(1,17),AMAT(1,21),AMAT(1,25),
C AMAT(1,29),AMAT(1,33),AMAT(1,37)
200  FORMAT (T7,I3,9(9X,I3))
      DO 70 I=2,65
70   WRITE (108,300) (AMAT(I,J),J=1,40)
300  FORMAT (X,10(2X,3I1,F7.4))
      WRITE (108,400) AMAT(66,1),AMAT(66,5),AMAT(66,9),
C AMAT(66,13),AMAT(66,17),AMAT(66,21),AMAT(66,25),
C AMAT(66,29),AMAT(66,33),AMAT(66,37)
400  FORMAT (T7,F6.4,9(6X,F6.4))
      GO TO 9
99   END
```

APPENDIX II

```

C      PROGRAM TO FIND .95 C.I. ENDPOINTS BY THE FISHER-LANCASTER
C      METHOD FOR 3 TUBES AT EACH OF THE DILUTIONS .1,.01,.001
C      THIS PROGRAM ALSO IDENTIFIES THE ASSOCIATED POINT ESTIMATE
C      D IS THE BINOMIAL COEFFICIENT
C      F IS THE BINOMIAL PROBABILITY
C      PU AND PL ARE THE UPPER AND LOWER P-VALUES
C      CU AND CL ARE THE UPPER AND LOWER CHI-SQUARE VALUES
C      14.45 IS THE CHI-SQUARE VALUE WITH 6 DF AND .025 BEYOND
C
      DIMENSION D(0:4),F(0:4,3),PU(3),PL(3),CU(3),CL(3),X(3),Z(3)
      REAL L
      D(0)=1
      D(1)=3
      D(2)=3
      D(3)=1
      Z(1)=.1
      Z(2)=.01
      Z(3)=.001
4     OUTPUT 'HOW MANY FERTILE TUBES PER DILUTION?'
      INPUT X(1),X(2),X(3)
      OUTPUT '          L  UPPER TAIL  LOWER TAIL'
      OUTPUT '          CHI**2      CHI**2'
      L=1.00
5     CONTINUE
      DO 10 I=0,3
      DO 10 J=1,3
C
C      I INDEXES THE # OF FERTILE TUBES PER DILUTION
C      J INDEXES THE DILUTIONS
C
      F(I,J)=D(I)*(1-EXP(-1*Z(J)))**I
C      *(EXP(-L*Z(J)))**(3-I)
10    CONTINUE
      DO 40 J=1,3
      S=0
      N=X(J)
      DO 11 I=0,N
11    S=S+F(I,J)
      IF (N.EQ.0) GO TO 19
      S1=0
      DO 15 I=0,N-1
15    S1=S1+F(I,J)
      PU(J)=(S+S1)/2

```

APPENDIX II (CONTINUED)

```

      CU(J)=-2*LOG(PU(J))
      GO TO 20
19  CU(J)=2-2*LOG(S)
20  CONTINUE
      T=0
      DO 21 I=N,3
21  T=T+F(I,J)
      T1=0
      IF (N.EQ.3) GO TO 29
      DO 25 I=N+1,3
25  T1=T1+F(I,J)
      PL(J)=(T+T1)/2
      CL(J)=-2*LOG(PL(J))
      GO TO 30
29  CL(J)=2-2*LOG(T)
30  CONTINUE
40  CONTINUE
      CHU=CU(1)+CU(2)+CU(3)
      CHL=CL(1)+CL(2)+CL(3)
C
C   THE FOLLOWING LINES ELIMINATE OUTPUT NOT NEAR THE POINT
C   ESTIMATE OR THE ENDPOINTS OF THE .95 C.I.
C
      IF ABS(CHU-14.45).LE.1.00) GO TO 50
      IF ABS(CHL-14.45).LE.1.11) GO TO 50
      IF ABS(CHU-CHL).LE.1.00) GO TO 50
      GO TO 60
50  WRITE (108,200) L,CHU,CHL
200 FORMAT (3X,I4,X,2F11.4)
60  IF ((CHU.GE.16).AND.(CHL.LE.13)) GO TO 4
      L=L+1
      GO TO 5
      END

```

APPENDIX II (CONTINUED)

SAMPLE OUTPUT AND EXPLANATION

HOW MANY FERTILE TUBES PER DILUTION?

?3,0,1

L	UPPER TAIL CHI**2	LOWER TAIL CHI**2
:	:	:
8	2.6784	15.4329
9	2.7878	14.7117
10	2.9001	14.0869
11	3.0145	13.5394
:	:	:
58	7.0247	7.1454
59	7.0895	7.1049
60	7.1541	7.0653
:	:	:
174	14.3686	4.8804
175	14.4319	4.8700
176	14.4951	4.8596
177	14.5583	4.8492
:	:	:

From the output above, one rejects $H_0: \lambda = \lambda_0$ in favor of $H_a: \lambda < \lambda_0$ at the .025 level for $\lambda_0 \geq 176$ and one rejects $H_0: \lambda = \lambda_0$ in favor of $H_a: \lambda > \lambda_0$ at the .025 level for $\lambda_0 < 9$. Hence the two-tailed 95% confidence interval associated with $X_1 X_2 X_3 = 301$ extends from 10 to 175 as indicated in the "Combining Independent Results" column of Table 1.

(As noted in the program, the χ^2 value with 6 degrees of freedom and .025 beyond it is 14.45. One rejects the null hypothesis when the calculated χ^2 value falls above 14.45.)

APPENDIX III

```

C      PROGRAM TO CALCULATE POINT ESTIMATES, EXPECTED VALUES AND
C      MSE VALUES FOR FIVE SERIAL DILUTION ESTIMATORS
C      MPN: CALCULATED IN LOOP 25, CALLED M IN PROGRAM AND OUTPUT
C      BIAS-CORRECTED MPN: CALCULATED BY ADJUSTING THE MPN, CALLED
C      C IN PROGRAM AND OUTPUT
C      FISHER ESTIMATE: CALCULATED IN LOOP 20, CALLED Y IN THE
C      PROGRAM AND F IN THE OUTPUT
C      JOHNSON-BROWN SPEARMAN ESTIMATE: CALCULATED IN LOOP 20, CALLED
C      B IN THE PROGRAM AND S IN THE OUTPUT
C      THOMAS ESTIMATE: CALCULATED IN LOOP 25, CALLED T IN THE
C      PROGRAM AND OUTPUT
C
C      C IS THE BINOMIAL COEFFICIENT
C      K IS A DUMMY VARIABLE FOR NEWTON ITERATIONS
C
C      THIS PROGRAM IS FOR 3 DILUTIONS, N TUBES PER DILUTION AND
C      DILUTION FACTOR D
C
C      THE PROGRAM DIMENSION STATEMENT MUST BE ADJUSTED FOR EACH N
C
DIMENSION B(0:3N-1),C(0:N),K(30),Y(0:3N-1)
REAL K,L,M
INTEGER X1,X2,X3
E1(W)=EXP(-Z1*W)
E2(W)=EXP(-Z2*W)
E3(W)=EXP(-Z3*W)
F1(W)=X1*Z1/(1-E1(W))+X2*Z2/(1-E2(W))+X3*Z3/(1-E3(W))
C  -N*(Z1+Z2+Z3)
G1(W)=-X1*Z1**2*E1(W)/(1-E1(W))**2
C  -X2*Z2**2*E2(W)/(1-E2(W))**2
C  -X3*Z3**2*E3(W)/(1-E3(W))**2
F2(W)=N*E1(W)+N*E2(W)+N*E3(W)-TN
G2(W)=-N*Z1*E1(W)-N*Z2*E2(W)-N*Z3*E3(W)
REWIND 1
OUTPUT 'WHAT IS N?'
INPUT N
OUTPUT 'WHAT IS THE MIDDLE DILUTION?'
INPUT Z2
OUTPUT 'WHAT IS THE DILUTION FACTOR?'
INPUT D
OUTPUT 'INPUT STARTING L, ENDING L, JUMPSIZE'
INPUT A1,A2,A3

```

APPENDIX III (CONTINUED)

```

      Z1=Z2*D
      Z3=Z2/D
      C(0)=1.0
      DO 5 I=0,N-1
5     C(I+1)=C(I)*(N-I)/(I+1)
      DO 20 I=0,3*N-1
      TN=3*N-I
      K(1)=20
      DO 10 J=1,19
      K(J+1)=K(J)-F2(K(J))/G2(K(J))
10    IF (K(J+1).LE.0) K(J+1)=.01
      IF (ABS(K(20)-K(19)).GE..01) GO TO 990
      Y(I)=K(20)
      B(I)=(1/Z1)*EXP(-.57722-.5*LOG(D)+((LOG(D))/N)*I)
      B(I)=B(I)*(2*N)/(2*N+LOG(D)*LOG(2))
20   CONTINUE
C
C     THE FISHER (Y) AND JOHNSON-BROWN (B) ESTIMATES ARE NOW STORED
C     IN MATRICES
C     THE MPN AND THOMAS ESTIMATES WILL NOW BE COMPUTED AND SENT
C     TO A FILE
C
      DO 25 X1=0,N
      DO 25 X2=0,N
      DO 25 X3=0,N
      IF (X1.EQ.N) IF (X2.EQ.N) IF (X3.EQ.N) GO TO 25
      S=X1+X2+X3
      S1=(N-X1)*Z1+(N-X2)*Z2+(N-X3)*Z3
      S2=N*(Z1+Z2+Z3)
      T=S/((S1*S2)**.5)
      IF (X1.EQ.0) IF (X2.EQ.0) IF (X3.EQ.0) GO TO 16
      K(1)=20
      DO 15 J=1,29
      K(J+1)=K(J)-F1(K(J))/G1(K(J))
15    IF (K(J+1).LE.0) K(J+1)=.01
      IF (ABS(K(30)-K(29)).GE..01) GO TO 991
      M=K(30)
      GO TO 17
16   M=0.00
17   WRITE (1,100) M,T
100  FORMAT (2F8.3)
25   CONTINUE

```

APPENDIX III (CONTINUED)

```

C
C   THE MPN (M) AND THOMAS (T) ESTIMATES HAVE NOW BEEN STORED
C   IN A FILE
C   THE PROGRAM WILL NOW CALCULATE EXACT PROBABILITIES, EXPECTED
C   VALUES, VARIANCES AND MSE VALUES FOR ALL THE ESTIMATORS
C
OUTPUT '  L      T    E(M)  E(C)  E(F)  E(S)  E(T)  MSE(M)
C MSE(C)  MSE(F)  MSE(S)  MSE(T)'
DO 80 L=A1,A2,A3
REWIND 1
TO=0
SM=0
SF=0
SB=0
ST=0
SSM=0
SSF=0
SSB=0
SST=0
DO 70 X1=0,N
DO 70 X2=0,N
DO 70 X3=0,N
S=X1+X2+X3
IF (X1.EQ.N) IF (X2.EQ.N) IF (X3.EQ.N) GO TO 70
P=C(X1)*C(X2)*C(X3)*(1-E1(L))**X1*(E1(L))**(N-X1)
C  *(1-E2(L))**X2*(E2(L))**(N-X2)
C  *(1-E3(L))**X3*(E3(L))**(N-X3)
READ (1,200) M,T
200 FORMAT (2F8.3)
TO=TO+P
PM=P*M
PF=P*Y(S)
PB=P*B(S)
PT=P*T
PPM=PM*M
PPF=PF*Y(S)
PPB=PB*B(S)
PPT=PT*T
SM=SM+PM
SF=SF+PF
SB=SB+PB
ST=ST+PT

```

APPENDIX III (CONTINUED)

```
SSM=SSM+PPM
SSF=SSF+PPF
SSB=SSB+PPB
SST=SST+PPT
70 CONTINUE
EM=SM/TO
EF=SF/TO
EB=SB/TO
ET=ST/TO
VM=SSM/TO-EM**2
VF=SSF/TO-EF**2
VB=SSB/TO-EB**2
VT=SST/TO-ET**2
SEM=VM+(EM-L)**2
SEF=VF+(EF-L)**2
SEB=VB+(EB-L)**2
SET=VT+(ET-L)**2
TH=EXP(-.805/N)
EC=EM*TH
VC=VM*TH**2
SEC=VC+(EC-L)**2
WRITE (108,300) L,TO,EM,EC,EF,EB,SEM,SEC,SEF,SEB,SET
300 FORMAT (I5,F6.3,5F7.2,5I10)
80 CONTINUE
GO TO 91
990 OUTPUT 'FISHER ESTIMATE DOES NOT CONVERGE'
GO TO 91
991 OUTPUT 'MPN ESTIMATE DOES NOT CONVERGE'
91 END
```

APPENDIX IV

```

C      PROGRAM TO FIND Z (THE MIDDLE DILUTION OF A K=3 DILUTION
C      SERIAL DILUTION EXPERIMENT) FOR A GIVEN DILUTION FACTOR
C      THAT WILL
C      -KEEP P(ALL FERTILE TUBES) TO SOME MAXIMUM INPUT VALUE
C      -ACCEPT L MAX (THE MAXIMUM REASONABLE VALUE LAMBDA ASSUMES)
C

```

```

DIMENSION Z(30)
REAL J,L,M,N
E1(W)=1-EXP(-L*D*W)
E2(W)=1-EXP(-L*W)
E3(W)=1-EXP(-1*W/D)
F1(W)=E1(W)*E2(W)*E3(W)-R**(1/N)
F2(W)=E1(W)*E2(W)*(L/D)*EXP(-L*W/D)
C +E1(W)*E3(W)*L*EXP(-1*W)
C +E2(W)*E3(W)*L*D*EXP(-L*D*W)
OUTPUT 'HOW MANY TUBES PER DILUTION?'
INPUT N
OUTPUT 'WHAT IS THE MAX EXPECTED L?'
INPUT L
OUTPUT 'WHAT IS THE RISK FOR HAVING ALL TUBES FERTILE?'
INPUT R
OUTPUT 'INPUT THE STARTING D, ENDING D AND JUMPSIZE'
INPUT A,B,C
OUTPUT '      D      Z'
ZD=-(1/L)*LOG(1-R**(1/3*N))
DO 20 D=A,B,C
Z(1)=ZD
DO 10 I=1,29
Z(I+1)=Z(I)-F1(Z(I))/F2(Z(I))
IF (Z(I+1).LE.0) Z(I+1)=.000001
10 CONTINUE
IF (ABS(Z(30)-Z(29)).GE..0001) GO TO 999
ZD=Z(30)
WRITE (108,100) D,ZD
100 FORMAT (F6.3,F8.5)
20 CONTINUE
GO TO 30
999 OUTPUT 'Z DOES NOT CONVERGE'
30 END

```

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

? .00305

WHAT IS THE DILUTION FACTOR?

?1.0

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

84

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	10.61	8.11	10.62	184.08	10.62	418	248	418	30305	420
20	1.000	21.25	16.25	21.25	184.08	21.29	857	514	857	26923	863
30	1.000	31.91	24.40	31.91	184.08	31.99	1316	799	1316	23741	1332
40	1.000	42.59	32.57	42.60	184.08	42.75	1798	1102	1798	20760	1830
50	1.000	53.31	40.76	53.31	184.08	53.56	2304	1426	2304	17978	2360
60	1.000	64.05	48.98	64.05	184.08	64.43	2835	1770	2835	15396	2926
70	1.000	74.82	57.21	74.83	184.08	75.37	3395	2135	3395	13015	3535
80	1.000	85.63	65.48	85.63	184.08	86.38	3984	2521	3984	10833	4190
90	1.000	96.47	73.76	96.47	184.08	97.47	4604	2931	4604	8851	4898
100	1.000	107.34	82.08	107.34	184.08	108.65	5258	3364	5258	7070	5666
110	1.000	118.25	90.42	118.25	184.08	119.92	5948	3821	5948	5488	6499
120	1.000	129.19	98.79	129.19	184.08	131.28	6674	4303	6674	4106	7404
130	1.000	140.17	107.18	140.17	184.08	142.75	7438	4809	7438	2925	8387
140	1.000	151.18	115.60	151.18	184.08	154.33	8239	5339	8239	1943	9454
150	1.000	162.22	124.05	162.22	184.08	166.02	9078	5894	9077	1161	10608
160	1.000	173.30	132.51	173.30	184.08	177.82	9952	6471	9952	580	11852
170	1.000	184.39	141.00	184.39	184.08	189.73	10860	7069	10860	198	13188
180	1.000	195.51	149.50	195.51	184.08	201.74	11798	7688	11798	16	14615
190	.999	206.64	158.01	206.64	184.08	213.87	12763	8324	12763	34	16131
200	.999	217.77	166.52	217.77	184.08	226.09	13749	8975	13749	253	17732
210	.999	228.89	175.02	228.89	184.08	238.39	14751	9639	14751	671	19412
220	.998	240.00	183.52	240.00	184.08	250.78	15762	10313	15762	1289	21164
230	.998	251.09	192.00	251.09	184.08	263.24	16776	10993	16776	2108	22979
240	.997	262.14	200.44	262.14	184.08	275.75	17786	11677	17786	3126	24845
250	.996	273.14	208.85	273.14	184.08	288.31	18784	12363	18784	4344	26751
260	.996	284.07	217.22	284.07	184.08	300.89	19763	13047	19763	5763	28685
270	.994	294.94	225.52	294.94	184.08	313.48	20716	13727	20716	7381	30634
280	.993	305.71	233.76	305.71	184.08	326.06	21636	14401	21636	9199	32582
290	.992	316.39	241.93	316.39	184.08	338.61	22516	15068	22516	11218	34517
300	.990	326.96	250.01	326.96	184.08	351.13	23350	15727	23350	13436	36425

STOP 0

APPENDIX V
TABLE A

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

? .00314

WHAT IS THE DILUTION FACTOR?

?1.5

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	10.69	8.17	10.69	96.99	10.71	393	232	391	7622	395
20	1.000	21.41	16.37	21.39	101.01	21.46	809	485	806	6674	816
30	1.000	32.16	24.59	32.12	105.01	32.27	1249	757	1244	5798	1268
40	1.000	42.94	32.84	42.89	109.01	43.14	1717	1050	1709	4994	1755
50	1.000	53.76	41.11	53.68	112.99	54.07	2213	1364	2201	4261	2280
60	1.000	64.61	49.41	64.51	116.96	65.08	2740	1702	2725	3599	2849
70	1.000	75.51	57.74	75.38	120.89	76.17	3302	2063	3282	3008	3469
80	1.000	86.44	66.10	86.28	124.81	87.35	3900	2449	3875	2487	4147
90	1.000	97.41	74.49	97.22	128.68	98.63	4538	2862	4508	2038	4891
100	1.000	108.43	82.91	108.20	132.53	110.00	5219	3302	5182	1661	5708
110	1.000	119.50	91.37	119.22	136.33	121.49	5945	3770	5900	1356	6607
120	1.000	130.61	99.87	130.29	140.10	133.09	6718	4267	6666	1125	7596
130	1.000	141.77	108.40	141.40	143.82	144.82	7540	4794	7478	969	8683
140	1.000	152.97	116.97	152.55	147.49	156.67	8412	5350	8340	890	9876
150	1.000	164.21	125.56	163.74	151.11	168.65	9333	5936	9250	888	11180
160	1.000	175.49	134.19	174.96	154.68	180.76	10304	6550	10206	966	12598
170	1.000	186.81	142.84	186.21	158.20	192.99	11321	7191	11207	1125	14134
180	1.000	198.16	151.52	197.48	161.65	205.36	12381	7857	12249	1368	15787
190	.999	209.52	160.21	208.77	165.05	217.84	13480	8546	13327	1696	17556
200	.999	220.90	168.91	220.07	168.39	230.43	14613	9255	14436	2113	19436
210	.999	232.29	177.62	231.37	171.66	243.13	15774	9981	15569	2619	21422
220	.998	243.66	186.32	242.65	174.86	255.92	16955	10720	16719	3219	23505
230	.998	255.02	195.00	253.91	178.00	268.79	18150	11470	17878	3915	25675
240	.997	266.35	203.67	265.13	181.06	281.73	19349	12227	19038	4710	27921
250	.996	277.64	212.30	276.30	184.06	294.72	20545	12987	20191	5607	30230
260	.995	288.87	220.89	287.41	186.98	307.75	21729	13747	21327	6609	32587
270	.994	300.03	229.42	298.44	189.84	320.79	22894	14505	22439	7720	34978
280	.993	311.12	237.90	309.38	192.61	333.83	24030	15257	23519	8942	37387
290	.992	322.11	246.30	320.22	195.31	346.86	25131	16000	24559	10279	39799
300	.990	332.99	254.62	330.95	197.94	359.85	26189	16735	25552	11735	42199

6TOP OY

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APPENDIX V
TABLE B

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

? .00334

WHAT IS THE DILUTION FACTOR?

?2.0

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	10.83	8.28	10.81	60.11	10.85	347	205	344	2589	350
20	1.000	21.70	16.59	21.64	65.32	21.78	724	433	716	2222	735
30	1.000	32.63	24.95	32.52	70.63	32.79	1134	684	1120	1923	1161
40	1.000	43.60	33.34	43.43	76.04	43.90	1580	961	1557	1685	1634
50	1.000	54.63	41.77	54.39	81.52	55.10	2067	1263	2033	1504	2162
60	1.000	65.72	50.25	65.39	87.06	66.41	2598	1595	2552	1377	2752
70	1.000	76.86	58.77	76.45	92.66	77.84	3179	1957	3118	1301	3415
80	1.000	88.08	67.35	87.56	98.28	89.40	3814	2352	3737	1272	4159
90	1.000	99.35	75.97	98.72	103.93	101.08	4509	2782	4413	1288	4995
100	1.000	110.70	84.65	109.94	109.59	112.90	5269	3249	5152	1346	5936
110	1.000	122.12	93.38	121.21	115.25	124.87	6097	3755	5958	1446	6990
120	1.000	133.60	102.16	132.55	120.89	136.99	6999	4302	6834	1586	8169
130	1.000	145.16	110.99	143.94	126.52	149.25	7976	4890	7782	1766	9481
140	1.000	156.78	119.88	155.39	132.11	161.67	9030	5520	8805	1984	10933
150	1.000	168.47	128.82	166.89	137.67	174.24	10163	6191	9902	2240	12531
160	1.000	180.21	137.80	178.43	143.17	186.95	11371	6902	11070	2535	14276
170	1.000	192.01	146.82	190.01	148.62	199.81	12652	7652	12306	2869	16168
180	.999	203.85	155.87	201.63	154.01	212.80	14002	8436	13606	3243	18205
190	.999	215.72	164.95	213.26	159.32	225.90	15414	9253	14963	3659	20382
200	.999	227.62	174.05	224.90	164.56	239.12	16881	10097	16368	4117	22690
210	.999	239.53	183.16	236.54	169.71	252.43	18394	10965	17813	4620	25119
220	.998	251.44	192.26	248.16	174.78	265.82	19944	11852	19288	5170	27655
230	.998	263.33	201.35	259.75	179.75	279.27	21520	12753	20782	5769	30286
240	.997	275.19	210.42	271.30	184.63	292.77	23111	13663	22285	6421	32995
250	.996	287.01	219.46	282.79	189.40	306.29	24707	14578	23786	7128	35764
260	.995	298.77	228.45	294.21	194.07	319.82	26296	15491	25273	7894	38578
270	.994	310.45	237.39	305.54	198.63	333.33	27867	16400	26737	8723	41416
280	.993	322.05	246.26	316.77	203.08	346.82	29411	17300	28166	9618	44262
290	.992	333.55	255.05	327.89	207.43	360.25	30916	18188	29553	10584	47098
300	.990	344.94	263.76	338.89	211.66	373.61	32374	19060	30888	11624	49906

STOP 0Y

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APPENDIX V
TABLE C

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

? .00359

WHAT IS THE DILUTION FACTOR?

?2.5

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	10.97	8.39	10.93	41.29	11.01	302	179	298	1059	306
20	1.000	22.02	16.84	21.91	47.09	22.14	644	384	632	918	660
30	1.000	33.15	25.35	32.94	53.12	33.41	1031	618	1006	848	1071
40	1.000	44.36	33.92	44.04	59.36	44.82	1467	884	1426	841	1547
50	1.000	55.67	42.56	55.20	65.77	56.39	1962	1183	1900	891	2099
60	1.000	67.06	51.28	66.43	72.33	68.12	2521	1521	2435	991	2739
70	1.000	78.55	60.07	77.73	79.01	80.01	3153	1899	3040	1136	3477
80	1.000	90.14	68.93	89.11	85.78	92.06	3866	2323	3721	1322	4327
90	1.000	101.83	77.87	100.56	92.62	104.28	4669	2795	4489	1545	5298
100	1.000	113.62	86.88	112.09	99.50	116.67	5568	3319	5349	1802	6403
110	1.000	125.51	95.97	123.70	106.40	129.23	6570	3897	6309	2088	7650
120	1.000	137.50	105.14	135.39	113.31	141.94	7680	4532	7372	2402	9047
130	1.000	149.58	114.38	147.14	120.20	154.80	8901	5224	8540	2741	10598
140	1.000	161.74	123.68	158.96	127.06	167.81	10234	5973	9815	3104	12306
150	1.000	173.99	133.04	170.84	133.87	180.95	11676	6778	11195	3490	14170
160	1.000	186.30	142.45	182.76	140.63	194.20	13224	7635	12674	3897	16186
170	1.000	198.66	151.91	194.72	147.31	207.56	14872	8543	14246	4324	18348
180	.999	211.07	161.39	206.71	153.92	220.99	16612	9495	15903	4773	20646
190	.999	223.50	170.90	218.71	160.43	234.50	18434	10487	17636	5242	23067
200	.999	235.95	180.42	230.70	166.84	248.05	20327	11513	19431	5734	25599
210	.998	248.39	189.93	242.67	173.14	261.63	22277	12566	21277	6249	28224
220	.998	260.81	199.43	254.62	179.33	275.22	24271	13640	23160	6789	30926
230	.997	273.20	208.90	266.51	185.41	288.79	26296	14729	25066	7356	33687
240	.997	285.53	218.33	278.34	191.35	302.33	28337	15825	26982	7953	36488
250	.996	297.80	227.71	290.09	197.17	315.82	30380	16924	28895	8581	39311
260	.995	309.98	237.03	301.75	202.86	329.24	32411	18017	30790	9245	42137
270	.994	322.06	246.27	313.30	208.41	342.58	34417	19101	32655	9948	44949
280	.993	334.04	255.42	324.74	213.84	355.80	36385	20171	34479	10692	47729
290	.992	345.88	264.48	336.05	219.12	368.91	38305	21222	36251	11483	50462
300	.990	357.60	273.44	347.22	224.27	381.89	40166	22250	37962	12325	53133

STOP DV

APPENDIX V
TABLE D

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

? .00389

WHAT IS THE DILUTION FACTOR?

?3.0

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	11.11	8.50	11.05	30.41	11.17	264	155	258	493	269
20	1.000	22.34	17.08	22.17	36.61	22.53	578	343	560	465	601
30	1.000	33.71	25.77	33.38	43.20	34.11	951	566	914	512	1009
40	1.000	45.20	34.57	44.68	50.12	45.91	1394	829	1332	626	1505
50	1.000	56.84	43.46	56.08	57.30	57.92	1918	1136	1824	799	2103
60	1.000	68.62	52.47	67.58	64.70	70.15	2533	1494	2403	1022	2814
70	1.000	80.54	61.58	79.18	72.26	82.58	3253	1908	3080	1292	3649
80	1.000	92.59	70.80	90.88	79.94	95.20	4089	2382	3868	1601	4618
90	1.000	104.78	80.12	102.68	87.70	108.00	5050	2922	4777	1946	5729
100	1.000	117.09	89.54	114.57	95.50	120.94	6146	3532	5815	2321	6988
110	1.000	129.52	99.04	126.55	103.32	134.02	7383	4214	6988	2724	8398
120	1.000	142.06	108.63	138.61	111.12	147.22	8765	4969	8299	3151	9961
130	1.000	154.69	118.29	150.73	118.89	160.51	10290	5797	9747	3599	11673
140	1.000	167.40	128.00	162.92	126.60	173.87	11957	6696	11330	4066	13529
150	1.000	180.17	137.77	175.15	134.23	187.28	13759	7662	13040	4549	15520
160	1.000	192.99	147.57	187.40	141.78	200.71	15688	8690	14870	5046	17636
170	.999	205.84	157.40	199.68	149.22	214.16	17732	9775	16809	5557	19863
180	.999	218.70	167.23	211.95	156.55	227.58	19878	10910	18842	6081	22186
190	.999	231.55	177.05	224.21	163.76	240.98	22111	12087	20955	6617	24590
200	.999	244.37	186.86	236.43	170.84	254.31	24416	13298	23134	7165	27057
210	.998	257.15	196.63	248.61	177.79	267.58	26776	14535	25361	7727	29569
220	.998	269.87	206.35	260.73	184.60	280.76	29175	15790	27621	8303	32111
230	.997	282.51	216.02	272.77	191.26	293.83	31594	17056	29898	8895	34663
240	.996	295.06	225.62	284.72	197.78	306.78	34020	18325	32176	9504	37211
250	.996	307.51	235.14	296.57	204.15	319.60	36435	19590	34440	10133	39738
260	.995	319.84	244.56	308.31	210.38	332.28	38825	20846	36676	10785	42229
270	.994	332.04	253.89	319.92	216.46	344.80	41177	22085	38872	11463	44670
280	.993	344.10	263.11	331.40	222.39	357.16	43478	23304	41015	12169	47050
290	.991	356.01	272.22	342.74	228.18	369.35	45717	24498	43095	12907	49357
300	.990	367.77	281.22	353.93	233.82	381.36	47883	25664	45102	13680	51581

STOP OY

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APPENDIX V
TABLE E

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

? .00422

WHAT IS THE DILUTION FACTOR?

?3.5

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	11.24	8.60	11.16	23.68	11.32	232	136	225	259	239
20	1.000	22.67	17.34	22.43	30.26	22.95	528	311	504	297	560
30	1.000	34.31	26.23	33.84	37.38	34.90	901	530	852	414	979
40	1.000	46.14	35.28	45.37	44.93	47.15	1368	800	1284	602	1511
50	1.000	58.18	44.48	57.05	52.81	59.69	1945	1128	1817	852	2167
60	1.000	70.40	53.83	68.85	60.94	72.46	2647	1522	2470	1157	2954
70	1.000	82.80	63.32	80.78	69.24	85.43	3490	1989	3256	1510	3881
80	1.000	95.37	72.92	92.83	77.65	98.56	4487	2535	4189	1906	4950
90	1.000	108.08	82.64	104.98	86.12	111.80	5648	3165	5280	2340	6163
100	1.000	120.91	92.45	117.23	94.60	125.11	6981	3883	6534	2807	7520
110	1.000	133.85	102.35	129.55	103.06	138.46	8488	4689	7954	3303	9014
120	1.000	146.87	112.30	141.93	111.45	151.82	10168	5582	9538	3823	10640
130	1.000	159.95	122.31	154.35	119.77	165.16	12016	6560	11282	4365	12388
140	1.000	173.07	132.34	166.81	127.99	178.45	14023	7618	13178	4924	14246
150	1.000	186.21	142.38	179.27	136.10	191.67	16177	8750	15213	5498	16201
160	1.000	199.34	152.43	191.73	144.08	204.81	18463	9948	17374	6085	18239
170	.999	212.46	162.46	204.18	151.93	217.83	20867	11203	19646	6683	20345
180	.999	225.54	172.46	216.59	159.63	230.74	23370	12508	22012	7290	22503
190	.999	238.56	182.42	228.95	167.20	243.52	25954	13854	24454	7906	24698
200	.998	251.51	192.32	241.26	174.61	256.15	28600	15230	26955	8529	26913
210	.998	264.38	202.16	253.49	181.88	268.64	31291	16628	29497	9160	29135
220	.998	277.16	211.93	265.65	188.99	280.97	34008	18039	32062	9800	31350
230	.997	289.82	221.62	277.71	195.96	293.13	36734	19456	34635	10448	33544
240	.996	302.38	231.21	289.67	202.77	305.14	39452	20870	37198	11107	35705
250	.995	314.80	240.72	301.51	209.44	316.97	42148	22274	39738	11778	37821
260	.995	327.10	250.12	313.24	215.95	328.62	44807	23663	42241	12463	39883
270	.994	339.25	259.41	324.84	222.32	340.11	47415	25031	44695	13165	41882
280	.993	351.26	268.59	336.31	228.55	351.41	49962	26373	47087	13886	43809
290	.991	363.12	277.66	347.64	234.63	362.54	52436	27685	49409	14628	45657
300	.990	374.83	286.61	358.82	240.57	373.49	54828	28963	51651	15396	47422

STOP OY

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APPENDIX V
TABLE F

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

?00457

WHAT IS THE DILUTION FACTOR?

?4.0

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	11.37	8.69	11.27	19.33	11.48	207	122	199	156	217
20	1.000	23.02	17.60	22.70	26.28	23.41	494	289	464	235	536
30	1.000	34.96	26.74	34.32	33.92	35.79	880	511	817	400	977
40	1.000	47.19	36.08	46.12	42.07	48.56	1389	797	1281	640	1552
50	1.000	59.67	45.63	58.10	50.59	61.62	2041	1157	1880	946	2264
60	1.000	72.38	55.34	70.23	59.35	74.89	2855	1601	2632	1313	3115
70	1.000	85.28	65.21	82.50	68.26	88.28	3848	2136	3554	1734	4105
80	1.000	98.33	75.19	94.88	77.22	101.71	5032	2769	4657	2202	5231
90	1.000	111.50	85.26	107.34	86.18	115.14	6415	3503	5948	2712	6485
100	1.000	124.75	95.39	119.87	95.10	128.49	7998	4339	7429	3259	7862
110	1.000	138.06	105.57	132.43	103.93	141.74	9779	5277	9097	3838	9350
120	1.000	151.40	115.77	145.02	112.66	154.86	11749	6311	10945	4445	10938
130	1.000	164.74	125.97	157.62	121.26	167.83	13898	7436	12963	5075	12614
140	1.000	178.06	136.16	170.20	129.73	180.63	16211	8646	15139	5723	14364
150	1.000	191.35	146.32	182.77	138.05	193.26	18672	9931	17457	6388	16175
160	.999	204.59	156.44	195.30	146.23	205.71	21263	11282	19901	7064	18033
170	.999	217.76	166.51	207.79	154.26	217.97	23965	12690	22451	7751	19924
180	.999	230.87	176.53	220.22	162.13	230.06	26758	14144	25092	8445	21836
190	.999	243.88	186.49	232.60	169.86	241.96	29623	15635	27802	9145	23756
200	.998	256.81	196.37	244.91	177.44	253.68	32541	17152	30566	9850	25672
210	.998	269.64	206.18	257.14	184.87	265.24	35493	18687	33363	10559	27572
220	.997	282.37	215.92	269.29	192.16	276.61	38461	20230	36178	11272	29446
230	.997	294.99	225.57	281.34	199.30	287.82	41428	21773	38994	11989	31285
240	.996	307.50	235.13	293.30	206.30	298.86	44379	23308	41796	12711	33079
250	.995	319.88	244.60	305.15	213.16	309.74	47298	24828	44568	13438	34821
260	.994	332.15	253.98	316.89	219.89	320.45	50172	26328	47299	14173	36504
270	.993	344.28	263.26	328.51	226.47	331.00	52988	27801	49976	14916	38122
280	.992	356.28	272.43	340.01	232.92	341.39	55733	29242	52588	15670	39669
290	.991	368.15	281.51	351.38	239.24	351.62	58399	30647	55125	16438	41141
300	.990	379.88	290.47	362.61	245.42	361.69	60976	32012	57578	17222	42534

STOP BY

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APPENDIX V
TABLE G

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

? .00492

WHAT IS THE DILUTION FACTOR?

?4.5

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	11.50	8.79	11.37	16.43	11.64	189	110	179	108	201
20	1.000	23.39	17.89	22.97	23.77	23.93	474	275	438	217	527
30	1.000	35.69	27.29	34.83	31.93	36.79	886	506	809	416	997
40	1.000	48.34	36.97	46.93	40.65	50.07	1452	817	1323	697	1611
50	1.000	61.29	46.87	59.22	49.72	63.59	2197	1219	2004	1051	2366
60	1.000	74.46	56.94	71.67	58.98	77.21	3141	1723	2874	1471	3254
70	1.000	87.80	67.14	84.24	68.32	90.79	4299	2336	3944	1951	4269
80	1.000	101.24	77.41	96.88	77.64	104.24	5679	3063	5222	2485	5400
90	1.000	114.73	87.73	109.56	86.90	117.51	7282	3905	6709	3066	6638
100	1.000	128.24	98.06	122.25	96.04	130.56	9105	4861	8402	3689	7971
110	1.000	141.74	108.38	134.94	105.05	143.36	11137	5925	10295	4347	9389
120	1.000	155.20	118.67	147.61	113.91	155.91	13367	7093	12375	5037	10878
130	1.000	168.61	128.93	160.26	122.62	168.21	15778	8355	14630	5752	12427
140	1.000	181.96	139.14	172.87	131.18	180.27	18354	9702	17044	6487	14024
150	1.000	195.25	149.30	185.44	139.58	192.11	21074	11125	19602	7240	15658
160	.999	208.46	159.40	197.98	147.84	203.73	23921	12614	22284	8005	17315
170	.999	221.60	169.44	210.46	155.96	215.14	26874	14157	25072	8779	18987
180	.999	234.66	179.43	222.90	163.93	226.37	29913	15743	27949	9560	20661
190	.999	247.64	189.36	235.29	171.77	237.42	33019	17363	30895	10345	22328
200	.998	260.55	199.23	247.61	179.47	248.31	36173	19007	33893	11132	23979
210	.998	273.37	209.03	259.87	187.05	259.03	39355	20663	36924	11920	25604
220	.997	286.11	218.77	272.06	194.49	269.60	42549	22324	39972	12708	27196
230	.997	298.75	228.44	284.17	201.80	280.02	45736	23980	43020	13495	28746
240	.996	311.30	238.04	296.19	208.98	290.29	48902	25624	46053	14282	30248
250	.995	323.76	247.56	308.12	216.04	300.42	52031	27247	49057	15068	31696
260	.994	336.10	257.00	319.95	222.96	310.41	55108	28844	52017	15855	33084
270	.993	348.34	266.36	331.68	229.76	320.26	58121	30408	54921	16645	34409
280	.992	360.45	275.62	343.29	236.44	329.97	61058	31934	57759	17438	35665
290	.991	372.45	284.79	354.77	242.98	339.53	63907	33418	60519	18238	36851
300	.990	384.32	293.87	366.13	249.40	348.95	66660	34856	63194	19045	37963

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STOP OY

APPENDIX V
TABLE H

WHAT IS N?

?3

WHAT IS THE MIDDLE DILUTION?

? .00527

WHAT IS THE DILUTION FACTOR?

?5.0

INPUT STARTING L, ENDING L, JUMP SIZE

?10,300,10

L	T	E(M)	E(C)	E(F)	E(S)	E(T)	MSE(M)	MSE(C)	MSE(F)	MSE(S)	MSE(T)
10	1.000	11.63	8.89	11.47	14.44	11.82	175	102	164	85	190
20	1.000	23.80	18.20	23.26	22.19	24.50	468	268	425	216	530
30	1.000	36.49	27.90	35.38	30.85	37.86	915	514	825	444	1027
40	1.000	49.58	37.91	47.78	40.07	51.60	1550	857	1400	760	1671
50	1.000	62.97	48.15	60.38	49.59	65.45	2399	1308	2176	1156	2447
60	1.000	76.54	58.53	73.10	59.21	79.19	3481	1877	3169	1626	3342
70	1.000	90.20	68.97	85.89	68.82	92.68	4806	2572	4386	2162	4345
80	1.000	103.88	79.43	98.69	78.32	105.85	6375	3394	5829	2757	5442
90	1.000	117.53	89.87	111.47	87.69	118.67	8184	4342	7494	3405	6624
100	1.000	131.12	100.26	124.22	96.91	131.14	10222	5410	9375	4099	7880
110	1.000	144.64	110.60	136.93	105.95	143.26	12476	6593	11461	4833	9199
120	1.000	158.09	120.88	149.60	114.84	155.07	14931	7883	13741	5602	10571
130	1.000	171.46	131.11	162.24	123.58	166.60	17570	9269	16199	6398	11985
140	1.000	184.76	141.28	174.84	132.17	177.87	20372	10741	18820	7217	13430
150	1.000	198.00	151.40	187.41	140.62	188.92	23321	12290	21588	8053	14897
160	.999	211.18	161.48	199.95	148.94	199.77	26395	13903	24483	8903	16377
170	.999	224.31	171.52	212.47	157.14	210.44	29574	15569	27489	9762	17859
180	.999	237.39	181.52	224.95	165.21	220.95	32839	17277	30587	10626	19334
190	.999	250.42	191.48	237.39	173.17	231.31	36170	19016	33758	11492	20793
200	.998	263.39	201.40	249.80	181.02	241.53	39546	20775	36984	12358	22228
210	.998	276.31	211.28	262.16	188.75	251.63	42950	22543	40246	13221	23632
220	.997	289.16	221.11	274.46	196.36	261.59	46362	24312	43528	14080	24998
230	.997	301.95	230.89	286.70	203.86	271.43	49765	26071	46811	14935	26318
240	.996	314.66	240.61	298.86	211.24	281.14	53141	27812	50080	15783	27587
250	.995	327.29	250.27	310.94	218.51	290.72	56474	29527	53320	16627	28801
260	.994	339.83	259.85	322.92	225.64	300.18	59750	31210	56516	17466	29956
270	.993	352.26	269.36	334.80	232.66	309.51	62955	32853	59655	18301	31047
280	.992	364.58	278.78	346.57	239.55	318.70	66076	34453	62725	19134	32074
290	.991	376.78	288.10	358.21	246.32	327.75	69102	36004	65715	19967	33033
300	.990	388.85	297.33	369.72	252.95	336.67	72023	37503	68614	20802	33924

STOP 0Y

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APPENDIX V
TABLE I

