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How to optimize the drop plate method for enumerating bacteria

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Abstract

The drop plate (DP) method can be used to determine the number of viable suspended bacteria in a known beaker volume. The drop plate method has some advantages over the spread plate (SP) method. Less time and effort are required to dispense the drops onto an agar plate than to spread an equivalent total sample volume into the agar. By distributing the sample in drops, colony counting can be done faster and perhaps more accurately. Even though it has been present in the laboratory for many years, the drop plate method has not been standardized. Some technicians use 10-fold dilutions, others use twofold. Some technicians plate a total volume of 0.1 ml, others plate 0.2 ml. The optimal combination of such factors would be useful to know when performing the drop plate method.

This investigation was conducted to determine (i) the standard deviation of the bacterial density estimate, (ii) the cost of performing the drop plate procedure, (iii) the optimal drop plate design, and (iv) the advantages of the drop plate method in comparison to the standard spread plate method. The optimal design is the combination of factor settings that achieves the smallest standard deviation for a fixed cost. Computer simulation techniques and regression analysis were used to express the standard deviation as a function of the beaker volume, dilution factor, and volume plated. The standard deviation expression is also applicable to the spread plate method. © 2001 Published by Elsevier Science B.V.

Keywords: Colony-forming units; Drop plate; Experimental design; Spread plate; Statistics

1. Introduction

The drop plate (DP) method exhibits many positive characteristics. The plating and counting procedures require less labor than alternative methods. The plating and counting steps are very convenient and manageable. On appropriately dried plates, the drops will absorb quickly into the agar. By distributing the sample in drops, colony counting can be done faster and perhaps more accurately. The drop plate method expends relatively few supplies. A bibliographic-database search and a worldwide web search showed that the drop plate method is being used in numerous laboratories across the world. In spite of its widespread use, the DP method has not been standardized.

Early advancements in the development of the drop plate method are accredited to several authors. These authors described, in brief form, the adaptation of dropping pipettes to the technique of plate

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counts of bacteria, particularly Wilson (1922), Aitken et al. (1936), Kenny et al. (1937), von Haebler and Miles (1938), Miles et al. (1938), Snyder (1947), Reed and Reed (1948), and Badger and Pankhurst (1960). The Miles et al. and Snyder papers include a statistical analysis of the accuracy of the method. In particular, Miles et al. derived the variance of the plate counts using binomial and Poisson distributions where the plate counts were averaged over all drops and plates. Badger et al. tested the effects of the use of different pipettes, of variations between drops from the same pipette, of variations between successive fillings of a pipette from the same dilution, and of variations between plates. The results of these tests show that there is no significant difference between pipettes, drops, or plates.

The plating process distinguishes the drop plate (DP) method from alternative methods. The most popular alternative is the spread plate (SP) method. In the SP method, 0.1 ml of a liquid sample is inoculated onto an agar plate. The liquid sample is spread into the agar with a flame-sterilized hockey stick, immobilizing the cells on the surface of the agar. The colony-forming units (CFUs) are counted after an appropriate incubation period. For the DP method, however, the sample volume is dispensed on the agar plate in a fixed number of separated, small drops. After incubation of the plates, the colonies within the drops are counted and the counts are scaled up to estimate the total number of CFUs in the initial beaker volume. Because counting is confined to the drops, the DP method is not recommended for organisms that display a swarming type of motility; e.g., *Proteus mirabilis*, *P. vulgaris*, and *Vibrio parahaemolyticus*.

Accurate and precise measurement of the drop volume is absolutely necessary to the DP method. Donald (1915) was the first to describe a method for the precise measurement of fluid volume by means of drops. Fildes and Smart (1926) expanded the procedure and developed methods of preparing and calibrating the pipette. Today, an electronic pipetter, costing less than $400 US, possesses the qualities of high accuracy and precision.

The DP method is a mixture of microbiological components and design components. The microbiological factors are fixed by the purpose of the experiment. They include the bacterial species, strain, and growth conditions (e.g., media, agar, temperature, time). This paper will focus only on the design factors (i) beaker volume, (ii) dilution factor, and (iii) volume plated. Throughout this paper, any specific combination of levels of these three factors will be called a design case. Beaker volume is synonymous with initial culture volume. The bacteria in the beaker may have originated in a sample from the environment or experimental apparatus. The sample could be a volume of liquid from a laboratory chemostat, recreational water, or drinking water. If the sample were a semi-solid, such as sediment, soil, or food, it would be blended in with a liquid, thereby creating the beaker volume suspension of disaggregated bacteria. In biofilm studies, it is common practice to remove the biofilm from a known surface area and disaggregate the bacteria in the beaker. Note that disaggregation methods are outside the scope of this paper. Our analyses assume that the bacteria have been properly disaggregated and are randomly mixed in the beaker.

To obtain distinct, non-overlapping colonies on the agar plate, the sample to be counted must almost always be diluted. Since the technician has only a rough guess of the viable count ahead of time, it is usually necessary to make more than one dilution. The dilution factor is a number defining the level of dilution. A larger dilution factor indicates a higher multiplicative fold dilution. For example, a dilution factor of 10 specifies 10-fold dilutions of the sample and a dilution factor of two specifies twofold dilutions. This factor partially governs the length of the dilution series. Density estimates based on 10-fold dilutions will usually involve fewer total dilutions than if based on twofold dilutions. Twofold dilutions, however, usually improve the precision of the density estimate.

Many different designs exist and have been implemented in laboratories. For example, the volume plated has varied from 0.1 ml (10 drops of 10-μl volume; Zelver et al., 1999) to 0.12 ml (six drops of 20-μl volume; Miles et al., 1938) to 0.15 ml (six drops of 25-μl volume; Reed and Reed, 1948). To apply the DP method, laboratory technicians must choose the number of drops that make up the volume plated. However, the number of drops does not directly affect the standard deviation of the density estimate. The number of drops is incorporated into

the calculation only because the volume plated equals the number of drops times the drop volume. The calculations are the same for 10 drops of 20 μl as for 20 drops of 10 μl, which are the same as for any combination of number of drops and drop size, which when multiplied together equals a volume plated of 0.2 ml.

The outcome (or result) of the DP method is an estimate of the density of microorganisms with units CFUs per beaker volume. The precision of the density estimate is indicated by its standard deviation (SD). The plating of a larger volume leads to more information about the true density of microorganisms. In turn, more prior information about the true density leads to less variability in the density estimate; i.e. a smaller SD. In theory, if the entire beaker volume is plated and counted, the SD would be zero. Of course, plating and counting the entire beaker volume is not realistic in terms of cost. Therefore, a compromise between precision and cost must be made.

The goals of this paper are to determine (i) the SD of the bacterial density estimate, (ii) the cost of performing the DP procedure, (iii) the optimal DP design, and (iv) the advantages of the DP method in comparison to the SP method.

2. Methods

2.1. Drop plate method

In this study, the laboratory experiments were run according to the following protocol. *Pseudomonas aeruginosa* (ERC-1) was used for all experiments. Using proper aseptic technique, an isolated colony was inoculated into a flask containing 100 ml of sterile Tryptic Soy Broth (Difco) at 300 mg/l. The flask was allowed to incubate in an orbital incubator at 35°C for a maximum of 24 h. The viable cell density was approximately 10^8 CFU/ml.

All serial dilutions were performed using sterile buffered water (AHPA, Standard Methods for the Examination of Water and Waste Water, 19th ed.). All test tubes used contained 9 ml of sterile buffered water. R2A agar (Difco) was used for all plate media. Drop plating was performed using an electronic pipetter (Rainin electronic EDP2 pipetter).

One milliliter of the bacterial suspension was pipetted into a dilution tube containing 9 ml of sterile buffered water. This tube was vortexed for approximately 8 s. After vortexing, 1 ml of this volume was removed and placed into a second dilution tube containing 9 ml of sterile buffered water. This process was repeated exactly until there was sufficient diluting of the sample.

Samples plated were those resulting from serial dilutions. Each agar plate was divided into four quadrants, each quadrant reserved for one dilution in the series. These plates are prepared in duplicate. The electronic pipetter was programmed to pick up 100 μl and dispense 10-μl volumes. The first dilution tube was vortexed for approximately 6 s and 100 μl was picked up using the electronic pipetter. Fifty microliters was dispensed in five evenly spaced 10-μl drops onto the designated quadrant of the petri plate. The tip and the remaining 50 μl of sample were discarded. The sample was vortexed again for approximately 6 s and 100 μl was picked up using the electronic pipetter. Fifty microliters was dispensed in five evenly spaced 10-μl drops onto the designated quadrant of the duplicate plate. The tip and remaining 50 μl of the sample were discarded. This process was repeated for every tube in the dilution series. After the drops on the agar dried, the petri plates were inverted and incubated at 35°C for 17–20 h.

Colonies were counted using a Leica Darkfield Quebec Colony Counter. The appropriate magnification of the drops was used for ease in colony counting. The countable dilution is the dilution that gives 3 to 30 colonies per 10-μl drop of sample dispensed. This rule is consistent with the SP method for which one counts at the sample dilution containing 30 to 300 CFUs per plate (Koch, 1994). The technician records the total count of CFUs over all 10 drops at the countable dilution. Finally, the total count is scaled up and the viable cell counts are expressed as CFUs per beaker volume.

2.2. Computer simulation method

The optimal DP design achieves the minimum SD given a cost constraint. It is not feasible to derive mathematically the exact SD for each design case.

For this reason, the SD values were approximated using computer simulation methods. To elucidate a general formula for the SD, we found the SD for a wide range of cases and fit a SD polynomial response surface over the entire range using least squares multiple regression analysis.

The approximate SD was found at combinations of these levels of the factors: Beaker Volume (BV) = 1, 10, 100; Dilution Factor (DF) = 2, 10, 100; Volume Plated (VP) = 0.1, 0.2; and number of bacteria in the beaker (λ) = 10^4, 3.16×10^5, 10^6, 3.16×10^6, 10^8. A $3^2 \times 2$ factorial layout using these levels of BV, DF, and VP formed 18 design cases for each λ. Six of the design cases (listed in Table 2) were excluded because they were judged by experienced lab technicians to be either impossible or unreasonable. The computer simulation consisted of running 2000 experiments at each of the remaining 12 design cases for each of the five λ values.

The simulation study is based on these assumptions:

(i) the number of cells in a sample from a well-mixed suspension follows a Poisson distribution;
(ii) each plate offers the same conditions for the growth of each organism;
(iii) the growth of any organism is independent of other organisms present;
(iv) the growth of a single organism results only in one visible colony;
(v) each plate has an equal chance of receiving any organism;
(vi) the organisms are distributed independently;
(vii) the number of drops into which the VP is divided is irrelevant to the minimization of the SD;
(viii) the only relevant cost is the technician's labor (time) costs; and
(ix) the cost of supplies was considered unimportant relative to the cost of labor.

The computer simulation followed the steps illustrated in Fig. 1. Our mathematical notation is also illustrated in Fig. 1. Let X_i denote the number of bacteria in the ith dilution tube and V_i denotes the volume transferred from the $(i-1)$th dilution tube to the ith dilution tube. Let TV denote the dilution tube volume. The V_1 volume is a $(\mathrm{TV}/(\mathrm{DF} \cdot \mathrm{BV}))$ fraction of the beaker volume. The V_2 volume is a $(1/\mathrm{DF})$ fraction of the first TV. In this study, TV is fixed at 10 ml; consequently, V_i, for $i = 2, 3, 4, \ldots$, are all equal. Let XP_i denote the number of bacteria in a VP volume transferred from the ith dilution tube to the designated quadrant on the agar plate. For convenience of illustration in Fig. 1, the VP is partitioned into 10 drops. The first five drops of the first two dilutions are shown in the designated quadrants of the agar plate. Each dilution is plated in duplicate, totaling 10 drops across two agar plates.

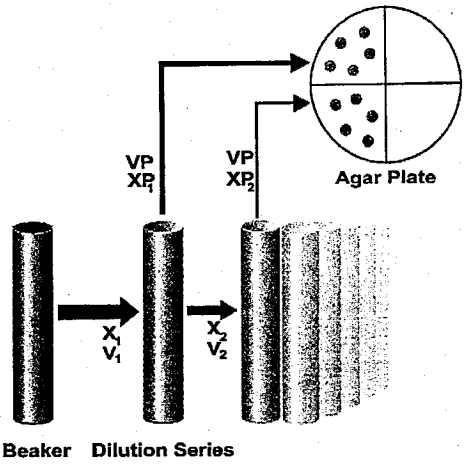

Fig. 1. Schematic showing the steps with associated mathematical symbols that make up the drop plate method. The shading of the tubes corresponds to the density of bacteria in the tubes, the lighter shading representing a higher dilution (more dilute sample). The width of the arrow corresponds to the number of bacteria in the volume, a thinner arrow representing fewer bacteria. A dilution is assigned a quadrant on an agar plate. When recording the time required for plating (see Section 2.3), each dilution was plated in duplicate with five drops per plate; however, the second plate is not shown here.

Theoretical considerations, initiated by Student (1907) and Fisher et al. (1922), and supported since by many others, show that the Poisson distribution is a useful model for the random number of particles in a sample from a well-mixed suspension (Miles et al., 1938). Based on this theory, our computer simulation generated Poisson distributed random variables sequentially, starting with the first dilution tube. The mean number of bacteria in a dilution tube is conditional on the number of bacteria in the previous

dilution tube. Eq. (1) gives the mean number of bacteria in the first dilution tube conditioned on the number of bacteria in the beaker.

$$E(X_1 \mid \lambda) = \frac{1}{\mathrm{DF}} \frac{\mathrm{TV}}{\mathrm{BV}}. \quad (1)$$

The conditional expectation of X_1 given λ has an additional correction factor for the situation in which the BV does not equal the TV. Throughout the remainder of the dilution series, this correction factor is not needed since the dilution tubes are of the same volume. Eq. (2) gives the mean number of bacteria in the $(i+1)$th dilution tube conditioned on the number of bacteria in the ith dilution tube.

$$E(X_{i+1} \mid X_i) = \frac{X_i}{\mathrm{DF}}, \quad \text{for } i = 1,2,\ldots,n-1. \quad (2)$$

Eq. (3) describes the mean number of bacteria plated from the ith dilution tube conditioned on the number of bacteria in the ith dilution tube.

$$E(\mathrm{XP}_i \mid X_i) = \left(1 - \frac{1}{\mathrm{DF}}\right) X_i \frac{\mathrm{VP}}{\left(1 - \frac{1}{\mathrm{DF}}\right)\mathrm{TV}}$$

$$= X_i \frac{\mathrm{VP}}{\mathrm{TV}}. \quad (3)$$

The fraction $(1-(1/\mathrm{DF}))$ is multiplied by X_i and TV because a $(1/\mathrm{DF})$ fraction of the dilution tube volume was already transferred to the next dilution tube in the dilution series. Correspondingly, a $1/\mathrm{DF}$ fraction of the bacteria was also transferred to the next dilution tube in the dilution series.

Typically, in the laboratory, the technician will count colonies at only one dilution in the dilution series. Let that dilution be called the "countable dilution". The intent is to count the dilution where there are the most colonies that are well spaced and non-overlapping in each drop. We created an objective rule for the computer to follow in selecting the countable dilution. For the spread plate method, one can usually count up to 300 CFUs in a 0.1-ml sample volume. It is our experience that the 300 CFUs limit applies also when the sample volume is distributed in drops. Our computer program therefore selected the first dilution for which the number of bacteria in the VP was less than 300 for the VP of 0.1 ml, and less the 600 for the VP of 0.2 ml. Let "Dilution" equal $(\mathrm{DF})^k$, where k is the number (integer) of DF-fold dilutions needed to obtain the countable dilution. Eq. (4) shows how the "Total count" is scaled up to estimate the density, which is the number of CFUs per beaker volume. In practice, such viable cell densities can be easily converted to viable cell densities per area, per volume, etc., depending on the original source of the bacteria.

$$\text{Density} = \frac{\text{Total count}}{\mathrm{VP}} \text{Dilution} \cdot \mathrm{BV}. \quad (4)$$

For each design case, the $\log_{10}$ SD (denoted by LSD) of the density estimates across 2000 independent simulated DP design experiments was computed and used as the response.

2.3. Cost constraint

The technician's labor (time) is the only significant factor that varies among design cases and contributes to the cost. For many laboratory manipulations, the time required does not depend on the design case. The times required to perform the serial dilutions and the plating, however, do depend on the design case.

To estimate those times, experienced technicians timed the dilution series and drop plating processes on multiple occasions. These estimates were incorporated into the time analysis portion of this study. Eq. (5) was used to estimate k, the number of dilutions needed to reach the countable dilution. We are assuming that the technician has enough prior information about λ that the final dilution in the dilution series is the countable dilution or adjacent to the countable dilution.

$$k = \min([y]+1, 5),$$

$$\text{where } y = \frac{\log\left(\frac{10\lambda}{\mathrm{BV}\cdot\mathrm{DF}}\right) - 2}{\log(\mathrm{DF})}. \quad (5)$$

In Eq. (5), $[y]$ denotes the smallest integer greater than or equal to y. Completing k dilutions in a dilution series is sufficient to achieve the countable dilution.

When DF equals 2 and λ is large, $[y]+1$ in Eq. (5) is more dilutions than would ever be used in the laboratory. After consulting experienced laboratory

technicians, we decided to insert some bigger dilution steps into the series whenever k is too large; hence, the use of the minimum (min) function in Eq. (5). Following that strategy, the countable dilution can always be reached within $k = 5$ dilutions. The DF of 2 is therefore an index for a DF sequence of which the smallest DF is 2. For example, if the technician anticipated that λ is between 10^6 and 10^7, then the DF = 2 sequence is [2, 2, 2, 10, 100], starting the dilution series with twofold dilutions and ending the dilution series with a 100-fold dilution. One can think of DF as an index based on the smallest dilution factor in a sequence of five dilutions. Let A denote the estimated time involved in completing one dilution in the dilution series. Let B denote the estimated time involved in plating one dilution. In general, Eq. (6) describes time, T, as a function of k.

$$T(k) = \begin{cases} (A+B)k + B, & \text{for } k = 0,1,2 \\ Ak + 4B, & \text{for } k = 3,4,5 \end{cases}. \quad (6)$$

2.4. Minimization

For fixed λ, two optimization steps were implemented to find the BV, DF, and VP values that minimize LSD (Eq. (8)) when the minimization is constrained by Eq. (7) (utilizing Eq. (5) for k). First, the `fmincon` function in MATLAB was used. This function returns the minimum solution to Eq. (8) subject to the time constraint of Eq. (7). The lower and upper bounds (LB and UB, respectively) for the solution were used in the function `fmincon`. OPTIONS were set using `optimset`. The 'LargeScale' option was set to 'off' and the 'Levenberg-Marquardt' option was set to 'on'. All other options remained at the default settings. The function returns the values of BV, DF, and VP corresponding to the minimum LSD subject to the time constraint.

The second step was a brute force technique used to ensure the `fmincon` function was locating the global minimum, not a local minimum. A grid search, also programmed in MATLAB, was performed over the entire region circumscribed by the design cases. Fine partitions were used for each of the three predictors. The regression model was evaluated to find the LSD at each combination of the three predictors. The computer sorted all grid points by LSD and identified the grid point that produced the smallest LSD subject to the time constraint.

3. Results

The time estimates found in our laboratory were averaged and used to construct our time function. The estimated time involved in completing one dilution in the dilution series was $A = 22.8$ s. The estimated time involved in plating one dilution was $B = 44.4$ s. Eq. (7) specifies the function used as the time constraint in this study.

$$T(k) = \begin{cases} 67.2k + 44.4, & \text{for } k = 0,1,2 \\ 22.8k + 4 \cdot 44.4, & \text{for } k = 3,4,5 \end{cases}. \quad (7)$$

Eq. (8) is the multiple regression model for LSD, when the true density is λ_1, where $\lambda_1 = 10^4$, $\lambda_2 = 3.16 \times 10^5$, $\lambda_3 = 10^6$, $\lambda_4 = 3.16 \times 10^6$, and $\lambda_5 = 10^8$. For the five λ values, arranged from smallest to largest, $m_1 = 3.52190$, $m_2 = 4.60345$, $m_3 = 4.90655$, $m_4 = 5.50650$, and $m_5 = 6.88865$. The regression coefficients for BV, BV^2, DF, DF^2, and VP do not depend on λ. The R^2 value is 96.9%. Eq. (8) is also applicable to the spread plate method.

$$\begin{aligned} \mathrm{LSD}_i = m_i &- 6.66 \times 10^{-3}\mathrm{BV} + 8.12 \times 10^{-5}\mathrm{BV}^2 \\ &+ 2.48 \times 10^{-2}\mathrm{DF} - 1.95 \times 10^{-4}\mathrm{DF}^2 \\ &- 1.23\mathrm{VP}, \\ \text{for } i &= 1,\ldots,5. \end{aligned} \quad (8)$$

Table 1 shows the optimal design, along with the Center for Biofilm Engineering (CBE) design (Zelver et al., 1999) and our recommended design for the five λ values. Table 1 also shows the associated LSD and the coefficient of variation (CV), which equals $(100\mathrm{SD}/\lambda)\%$. The CBE design consists of a BV of 10 ml, a DF of 10, and a VP of 0.1 ml. Since the optimal designs are not easily applicable in a laboratory setting, the BV and DF values were rounded to more appropriate values, giving us the recommended designs shown in the table. The recommended design reduces the CV to half that of the CBE design previously used in our laboratories; that is, with no increase in cost, the assay's precision can be doubled. If the laboratory technician has sufficient

Table 1
For a given true number of bacteria in the beaker (density), the entries show the CBE, Optimal, and Recommended Designs (BV, DF, VP values for each design), with the associated log standard deviation (LSD) and coefficient of variation (CV) of the density estimate

Density (λ)	Design	BV (ml)	DF	VP (ml)	LSD	CV (%)
10^4	CBE	10	10	0.1	3.569	16.10
	Optimal	41	5	0.2	3.259	7.88
	Recommended	40	5	0.2	3.259	7.88
3.16×10^5	CBE	10	10	0.1	4.651	6.15
	Optimal	51	5	0.2	4.348	3.06
	Recommended	55	5	0.2	4.356	3.12
10^6	CBE	10	10	0.1	4.954	3.91
	Optimal	42	7	0.2	4.688	2.12
	Recommended	40	10	0.2	4.753	2.46
3.16×10^6	CBE	10	10	0.1	5.554	4.92
	Optimal	41	1.25	0.2	5.155	1.96
	Recommended	40	5	0.2	5.243	2.40
10^8	CBE	10	10	0.1	6.936	3.75
	Optimal	41	1.25	0.2	6.537	1.50
	Recommended	45	5	0.2	6.627	1.84

prior information about the λ value, then Table 1 shows the recommended design. Alternatively, if the laboratory technician cannot make a reliable prior guess of the λ value, then our recommended design across all λ values is a BV of 40 ml, a DF of 10, and a VP of 0.2 ml.

4. Discussion

The recommended designs for each λ (Table 1) may not be suitable for every technician in every laboratory. We suggest the following steps to determine an efficient DP design. First, estimate the time required to complete one dilution (A) and the time required to plate one dilution (B) (see Eq. (6)). Given the time estimates, construct a time function similar to Eq. (7) (utilizing Eq. (5) for k). Second, determine the DP design cases of interest by specifying the factor levels of BV, DF, and VP. Third, compute the LSD for each design case by substituting the values of the BV, DF, and VP into the regression model, Eq. (8). This suggestion assumes that the design cases of interest will fall within the range of cases simulated in this study. Extrapolation outside of the simulated range of cases will require more simulation and a newly calculated regression model. Once an LSD and time estimate are attached to each design case, the minimization can be carried out.

It is important to note that the cost, as described by Eq. (7), is not the total cost of the DP method. Rather, it focuses only on the time required to perform the serial dilutions and the plating, because these are the only components of the total cost that differ among the various DP design cases. We have not conducted a complete cost analysis as would be required for comparison to the total cost of the SP method, or any other popular method such as the pour plate method.

Statisticians have traditionally considered pipetting error to be a cause for concern when creating dilution series. For this reason, we considered the incorporation of pipetting error into the computer simulation at each pipetting step within the dilution series and the plating process. The precision for an electronic pipette was used as an estimate of the variability at each pipetting step (Anonymous, 1997—Rainin Pipetting Solutions, 1998). The computer simulation was run with and without the pipetting error for a few design cases. Pipetting error increased the SD less than 1%. Because the pipetting error was of no practical significance, we ignored pipetting error for our final analyses.

The volume plated can be partitioned into any number of drops and the countable number of CFUs depends on the volume of the drop. The appropriate volume of each drop depends on microbiological considerations such as the bacterial species and growth conditions. The DP method is primarily used for pure cultures because the small area of a single

Table 2
Design cases that were not included in the simulation study because they were judged to be either impossible or impractical

BV (ml)	DF	TV (ml)	VP (ml)
1	2	10	0.1
1	2	10	0.2
1	10	10	0.1
1	10	10	0.2
100	2	10	0.1
100	2	10	0.2

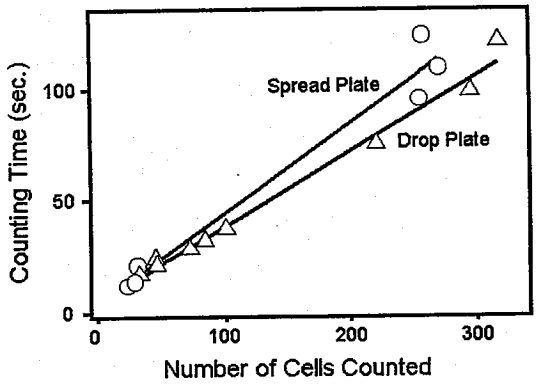

Fig. 2. For both the DP and SP methods, the observed technician's time required to count CFUs increased with the number of total CFUs being counted. When the total CFU count was large, the DP method was considerably faster.

drop may not enable the technician to make colony distinctions among the multiple species within a mixed sample.

The DP method possesses several practical advantages over the SP method. First, less time and effort are required to dispense the drops onto an agar plate than to spread an equivalent total sample volume into the agar. Second, less time is involved in counting drop plates compared to spread plates. The clear partitioning of the drops forms distinct groups of colonies allowing ease in counting. When counting a spread plate, the groups are all contiguous causing increased difficulty in counting. Fig. 2 shows the extent to which the DP method is less laborious than the SP method for larger plate counts. Third, the area covered by the CFUs can possibly influence the accuracy of the method. For the DP method, ten 10-μl drops typically cover a total area of 10 cm^2, whereas for the SP method, the equivalent total sample volume covers approximately 64 cm^2. We conjecture that, by distributing the sample in small drops, colony counting can be done more accurately. Fourth, the DP method expends relatively few supplies. For example, plating four dilutions using the DP method would make use of two petri plates. In contrast, plating four dilutions in duplicate using the SP method would make use of eight plates. Not only the cost of the plates need to be considered, but also the additional time required to handle four times more plates. Although a good pipetter is required for the DP method, the purchase price and maintenance are insignificant when prorated over many assays. It has been reported that the DP and SP methods provide insignificantly different density estimates when simultaneously applied to the same material (Donegan et al., 1991). Overall, considering time, accuracy, and resources, the DP method appears to be superior to the SP method. Similar reasoning indicates that the DP method is also superior to the pour plate method:

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