A QUANTITATIVE DIFFERENTIAL METHOD FOR COUNTING MIXED CULTURES OF BACTERIA

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Studies relating to the association of bacteria in mixtures have demonstrated the need of a practical technique for obtaining accurate growth curves of each of several species which are growing together.

The standard method of plate counting gave unreliable quantitative differential counts when used with mixtures of *Streptococcus faecalis* and *Salmonella Schottmuelleri*, *Escherichia coli* and *Salmonella schottmuelleri*, *Streptococcus faecalis* and *Escherichia coli*. In many cases the subsurface colonies were easily confused, one species with the other. In other cases, since subsurface colonies require twenty-four hours longer incubation than do surface colonies, diffusion of acid formed by one member of a pair rendered the differential medium useless. In all cases, however, the surface colonies were readily distinguishable, due to differences in size, coloration, or medium change. The method to be described is the result of an attempt to find a procedure suitable for mixed culture work that would give counts of an accuracy comparable to those obtained by using the standard plate counting method with pure cultures.

For this method Petri dishes (100 x 15 mm.) having porous covers are used. The media employed should be poured while quite warm, sufficient amounts being used to give fairly thick plates. The plates are allowed to harden and are placed top down, five or six deep, in a dry incubator for fourteen to eighteen hours prior to use.

The dilutions of the culture to be counted are made in the usual manner. The plates are removed from the incubator just

before using, a warm plate being important in bringing about rapid drying of the flooded plate. Of the dilution, 1 ml. is deposited on the agar surface near the edge of the plate. In delivering, the pipette is allowed to drain and then carefully touched to a dry portion of the plate's surface to insure uniform delivery.

The flooding of the plate is accomplished by removing the cover and tilting the plate back and forth in such a manner that the liquid floods its entire surface in a thin even film. Should excess liquid remain at any edge it may be flooded to the center by further tilting of the plate. With a little practice this flooding takes but a few seconds for each plate. After the liquid is evenly distributed, the cover is replaced and the plates left on a level

TABLE 1

Statistical analysis of counts obtained by the flooded and standard methods of counting bacteria

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3	
	F	S	F	S	F	S
Arithmetic mean (colonies)	35.40	35.67	44.14	45.37	80.69	79.74
Median (colonies)	35.00	36.00	45.00	45.00	81.00	79.00
Quartile deviation (±)	4.50	4.00	4.00	4.50	6.00	6.00
Standard deviation (±)	6.48	5.71	7.00	6.77	9.56	9.15
Coefficient of variation (per cent)	18.31	15.01	15.68	14.92	11.85	11.48
Number of plates	150	150	195	195	193	199

bench in a warm room, or placed top up in the incubator until dry. This is usually accomplished within twenty minutes. When dry the plates are incubated in the usual manner until sufficient colony growth has occurred. The distribution of colonies on the flooded plate is very uniform, surpassing that usually obtained in the standard plate.

To compare the accuracy of the flooded plate method with that of the standard plate method three correlation experiments with pure cultures, involving a total of 1082 plates, were made. In these experiments an equal number of plates were seeded by each method, the work being done in a manner insuring uniform conditions for both methods. The counts obtained were analysed statistically with the results shown in table 1.

From table 1 it is seen that although some of the measures indicate the standard method to be slightly more accurate, the difference between the two methods is statistically insignificant. Since the coefficient of variation is independent of both mean and number of samples taken this measure may be averaged for the total number of plates. These averages, flooded 15.34, standard 14.14, probably do not differ by a significant amount. The moving averages of the frequency distributions of the counts obtained by both methods give very similar curves which conform to those of normal distributions. From the results of these correlation experiments it is apparent that the flooded plate method gives counts of an accuracy equal to that of the standard method.

The flooded plate method has been successfully used in this laboratory in obtaining growth curves of both pure and mixed cultures and in the isolation of pure cultures from fecal samples.