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Direct Counts of Bacterial Spores on Membrane Filters Under Phase Optics

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Direct microscopic counts of bacterial spores are useful, and often essential. A rapid and convenient method in routine use in our laboratory apparently has not been reported previously.

Membrane filters, with subsequent colony growth, are used frequently for bacterial counts. Filters which become transparent when immersed in oil of matching refractive index also are used for direct microscopic counting of various biological materials, with or without staining. (Methods and bibliography are available from the Millipore Corp., Bedford, Mass.) The latter procedure may be used for the direct counting of unstained bacterial spores with many advantages over conventional methods. Dormant bacterial spores, which would appear bright by negative-contrast phase microscopy when mounted in water, appear dark and are counted easily when on a filter surface impregnated with immersion oil.

Spores are dispersed uniformly with a motor-driven Potter-Elvehjem Teflon homogenizer in a small volume of water. A sample containing an estimated 0.5×10^7 to 2×10^7 spores is suspended in approximately 100 ml of water or broth from which extraneous particles have been removed by membrane prefiltration. Glassware is rinsed with prefiltered water. Suspensions are filtered through Millipore membranes 47 mm in diameter, with pore size of 0.22 or 0.45 μ. The smaller pore size gives a less granular surface. Uniform distribution of the spores on the filter surface depends on cleanliness of the sintered glass under the filter membrane. Uniformity also is aided by prewetting the filter and by using a substantial volume of filter liquid, all of which should be in the filter cup before vacuum is applied. No rinse should be used. A small portion of the air-dried membrane is mounted, under a cover slip, in Cargille's non-drying immersion oil, type A. Inadequately dried membranes may fail to accept the oil. Counting is done under oil immersion with an American Optical Co. dark M 97× objective. A combination of calibrated eyepiece grid and microscope-stage scale is used to measure the area counted. The number of spores on the total membrane is calculated from the count for a small measured portion.

Counts for standard spore preparations agree with counts made in a Petroff-Hausser bacteria counting chamber. Counts from different filterings of the same spore suspension and from various regions of the same filter membrane show uniformity within the Poisson variation for 500 spores.

Filter membrane counting has several advantages over conventional chamber counting. (i) Ease of loading and a nearly uniform plane of focus for spores on the membrane enable counts to be obtained much more quickly than with counting chambers. (ii) Counts from membranes stored for up to 3 years (the longest period tested) agreed with counts done promptly after preparation. Since only a small portion of a membrane is mounted for a count, the remainder is available for statistical replication at a later date. For a small investment of time, labile samples can be converted to a stable form, and the counting or even the decision to count can be made at a later, more convenient time, pending the outcome of outgrowth or other tests. (iii) Abnormal appearance or uneven dispersion of spores can be evaluated quantitatively.

The speed and convenience of this procedure make spore counts practical for routine use in many studies. Studies of dormancy and activation are difficult to interpret if based only on maximal outgrowth rather than on the number of spores actually present. Direct counts are useful whenever procedures such as centrifuging or drying may cause physical losses of spores, or where circumstances require the use of amounts of spores not adequate for weighing. A quick preliminary survey of spore numbers in samples from fractionations, etc., facilitates planning. More accurate counts, if desired, can follow at a convenient time. Effectiveness of spore production conditions can be evaluated easily. Suspensions from crude cultures may be filtered for spore counts, as mounting in immersion oil causes vegetative cells or debris to be nearly or totally transparent.