A METHOD FOR THE QUANTITATIVE ESTIMATION OF BACTERIA IN SUSPENSIONS

By ALBERT P. KRUEGER

(From the Laboratories of The Rockefeller Institute for Medical Research, Princeton, N. J.)

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Determinations of the number of bacterial cells present in a given suspension may be accomplished by several different means. Besides the classical plate and dilution methods which have contributed so much to the development of bacteriology, there are suitable procedures for direct microscopical counts, using either a counting chamber or a stained mount. Further, in certain instances, the total cell concentration of a suspension may be estimated by nephelometric comparison, by weighing the washed and dried sediments obtained from centrifuged aliquots, or by measuring a product of metabolism.

For some types of work the volume occupied by organisms has proven a satisfactory measure of the number of cells. Total cell volume is determined by centrifuging, the sediment being deposited in a calibrated cup from which the volume of packed cells is read directly. Having already established the volume-cell number ratio, preferably by direct count, volume readings are readily convertible into total cells per cubic centimeter.

Paine (1), Carlson (2), and Slator (3) have reported favorable experiences with such a technic used by each of them to determine the cell content of yeast cultures, while Hopkins (4) has successfully utilized the method for standardizing bacterial vaccines.

During the course of a study of bacteriophage action on staphylococci there arose a two-fold necessity: first, for a method by which estimates of cell numbers in bacteriophage-containing suspensions might be obtained; and second, for a technic giving rapid and accurate cell number determinations in dense preparations to be used for making up dilutions of known bacterial concentrations. Obviously, the plate
and dilution methods could not be employed in the presence of bacteriophage, and neither procedure would furnish immediate information for preparing cell dilutions. It was finally found possible to adapt the centrifuged-sediment method to these problems with satisfactory results.

The centrifuge tubes (Fig. 1) constitute the essential feature of the method. They are calibrated individually with dense cell suspensions which are made up in buffer solution, and standardized by direct count. Five consecutive 1 ml. aliquots are centrifuged at known speed for a period of time sufficient to insure maximal packing. The column length of the sediment is measured in each instance with a
cathetometer or vernier calipers and the average bacterial content per millimeter of tube length calculated. Both menisci are level and sharp and permit accurate measurement.

 Tubes calibrated in this way were employed in actual routine as follows: 16 hour cultures of a single strain of Staphylococcus aureus grown in Blake flasks were washed from the agar with $\frac{M}{200}$ phosphate buffer of pH 7.6 using 30 ml. to each flask. The emulsion was filtered through a sterile Schleicher and Schüll Faltenfilter No. 588 and 1 ml. aliquots centrifuged in the small tubes (Fig. 1) at 2600 r.p.m. for 15 minutes. The lengths of the columns of packed bacterial cells were then measured and the figures converted into cells per milliliter of original suspension. From such suspensions dilutions of any required cell content were of course readily prepared.

 The difference between consecutive measurements of the same suspension was found to be <1.0 per cent (triplicate determinations on 65 emulsions), while the total error based on repeated checks with careful direct counts was consistently <2 per cent. These figures apply to the measurement of very dense suspensions containing approximately $14 \times 10^9$ cells/ml. and giving a column length of about 20 mm.

 In the case of light suspensions of staphylococci in nutrient broth, 10 ml. aliquots added to 2 ml. of a mixture of acetone 10 ml. $\frac{M}{1}$ NaCl 3.0 ml., neutral formalin 2.0 ml., were centrifuged at 2600 r.p.m. for 15 minutes in large cups (Fig. 2). These cups were calibrated with light suspensions of known cell content (direct count), which were mixed with the acetone-salt-formalin reagent before centrifuging. Here a larger percentage of the organisms remained in the supernatant fluid and the difference between successive determinations on the same suspension was greater than in the case of the dense preparations, averaging approximately 4 per cent. The total error based on direct count figures averaged 5 per cent.

**SUMMARY**

 A method is described for estimating the cell concentration of bacterial suspensions by measurement of the column lengths of packed
cells centrifuged into a capillary tube of fine bore. The total error, under the experimental conditions employed, checked by direct count, was <2 per cent for dense suspensions and about 5 per cent for light suspensions. The method is suitable for work requiring rapid and accurate routine preparation of bacterial suspensions of known cell concentrations.

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BIBLIOGRAPHY
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