Technique and Apparatus for Rapid and Inexpensive Enumeration of Bacteria

A. N. SHARPE, E. J. DYETT, A. K. JACKSON, AND D. C. KILSBY
Unilever Research Laboratory, Colworth/Weluyn, Colworth House, Sharnbrook, Bedford, England

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A foot-operated diluter/dispenser and a projection viewer were developed for use with a rapid bacterial counting technique employing agar droplets. The equipment allows the advantages of the technique to be properly realized and assists many conventional bacteriological tests which may be made at the same time. Significant cost and labor savings are made, with reductions in incubation time, incubator space, and preparative work.

A rapid and inexpensive technique for enumerating bacteria, in which a sample suspension is diluted in molten agar and plated out in standard petri dishes as a series of 0.1-ml agar droplets, has recently been described (3). The unassisted manual method discussed in the first publication has several advantages over conventional pour-plate counting methods in, for example, quality control work in food factories, but its attractions have been greatly increased by the development of two simple aids. One, a foot-operated diluter/dispenser, allows decimal or centimal dilutions to be made and the droplets to be plated out rapidly with a minimum of effort. The other, a projection viewer, allows easy enumeration of colonies by throwing a bright image of a droplet, magnified to the size of a standard petri dish, toward the technician as he sits at the bench.

The droplet technique and the two aids are in use in a number of laboratories where the method has been met with remarkable enthusiasm. The present paper describes the devices and discusses the application of the technique in quality control and research laboratories.

MATERIALS AND METHODS

Diluter/dispenser. The two pedals (Fig. 1) act on pistons which are connected to each other and also to a small handpiece by flexible tubing. One piston can be adjusted to suck or blow 0.5 to 2.0 ml of liquid; in normal use it is set at 1.0 or 1.5 ml. By operating the pedal, liquid can be drawn into and ejected from a pipette or straw. The other piston is valved and blows 0.1 ml when its pedal is released.

Liquid enters only the pipette, which is discarded after use. The handpiece accepts either glass pipettes or polypropylene drinking straws. When using the diluter/dispenser (during or after pumping), it is important for the tip of the pipette to be in contact with the liquid in the droplet or agar diluent.

Otherwise, inaccuracies may occur from the effects of surface tension in the liquid and a variable orifice diameter in the pipette.

Projection viewer. The base (Fig. 1) contains a 12-v, 50-w tungsten/halogen lamp and transformer. Petri dishes are moved by hand over the top of the base until a droplet is over the illumination hole. The droplet acts as its own condenser. A reflex system throws an image (×10) of the droplet onto a ruled screen. The image is approximately equal in size to a standard 9-cm petri dish. The screen is angled so that it faces a seated technician, and the image is bright enough to be seen easily in a sunlit room. No focusing is necessary. Both the diluter/dispenser and the viewer were obtained from A. J. Seward and Co. Ltd. (P.O. Box 1, 6 Stamford St., London S.E.1, England).

Bacterial suspensions. All suspensions were prepared by homogenization, vortex stirring (2), shaking, or swabbing, for routine pour plate counting in several quality control or research laboratories. After samples had been taken for routine counts, the suspensions were analyzed by the droplet technique. Therefore, each droplet count was in direct comparison with a standard plating procedure.

Counting procedures. Four-plate counting followed a fairly standard procedure in which decimal dilutions of the sample were made in 0.1% peptone solution, 1.0 ml of each dilution being inoculated singly or in duplicate into petri dishes and mixed with 15 to 20 ml molten agar at 45 C. Incubation times were generally 48 hr at 30 or 37 C. Viable Staphylococcus aureus in pure cultures were enumerated on surface-inoculated plates of Baird-Parker's medium (1) at 37 C. For droplet counting, the same agar and incubation temperatures were used.

1 Present address: T. Wall and Sons (Meat and Handy Foods) Ltd., Willesden, London, England.
but colonies were counted after 24 hr.

Droplet counts were made using the two aids and following the method of Sharpe and Kilby (3). In a typical case, one centimal followed by one decimal dilution might be made. For this, two bottles containing 9 to 10 ml of melted, cooled agar would be used. The first pipette, either a 5.75-inch glass capillary pipette (Harshaw Chemicals Ltd., Daventry, Northants) or polypropylene drinking straw (Hygienic Drinking Straw Co. Ltd., Fishponds, Bristol), was filled with sample suspension. 0.1 ml was added to the first bottle of agar (taking care to immerse the tip of the pipette in the agar), and the pipette was discarded. The bottle contents were mixed by shaking gently, or touching against a vortex stirrer. A fresh pipette was filled with this centimal dilution, and the first row of 0.1-ml droplets was plated out in a standard polystyrene petri dish. If the pump was set at 1.5 ml, the remaining contents of the pipette (1.0 ml) were transferred directly to the second agar bottle and the plating out process was repeated (Fig. 2). If the pump was set at 1.0 ml, the pipette was refilled before making this decimal dilution. Numerous permutations of dilution ratios were possible. If other analyses, e.g., coliform counts (most probable number [MPN] or plate) or S. aureus (spread plate), were to be made, the pump was set at 1.0 ml. The diluter/dispenser considerably facilitated these analyses as well.

For most droplet counts, two dilutions (two rows of droplets) were adequate. By turning the dish over and using the lid, eight rows could be accommodated easily; four samples were thus plated out in each petri dish. Sample numbers were written only once. Dilution numbers were unnecessary, since the order of concentrations was obvious.

The method of colony counting in droplets was left to personal choice. The viewer screen could be marked with a felt-tipped pen, but the rulings satisfactorily prevented overcounting when colony densities were high. It was generally preferred to count all five droplets at a low colony concentration rather than only one or two at a high concentration. A minimum of 20 colonies was counted whenever possible. With two complete counts contained in a petri dish in one hand, most of the dish handling associated with counting was avoided (Fig. 3).

**Statistical evaluation.** Droplet and plate counts were compared using a paired "t" test, and results are quoted at the 95% confidence level.

**RESULTS**

Results from 525 comparisons of standard pour- or spread-plate counts with droplet counts using the diluter/dispenser and the projection viewer showed that, for occasional samples, particularly of raw beef, droplet counts were significantly greater than the pour-plate counts. This had been noticed previously, using this technique, and is probably due to the effect of better aeration in droplets, compared with pour plates, on some meat flora (3). At the $P = 0.95$ confidence level, no significant difference was found for most of the other beef samples tested, or for samples of dried beef, kidney, brisket, mutton, rolled mutton, chicken, dried chicken, beefburger, sausage, raw ham, prawns, cod, peas, cooling water, and skin bacteria, or with disinfectant.
lethality tests or *Staphylococcus aureus* pure cultures. Evaluation of colony numbers was difficult in some of the droplets made from mutton because of a high proportion of fatty debris, but in most other samples colonies were easily distinguished from debris, even in the highest concentrations. For most of the samples, in fact, the visibility of colonies in droplets was better at 24 hr than on pour plates at 48 hr, owing to their relatively larger size after magnification. Colonies normally had a characteristic straw to dark brown elliptical appearance which was easy to distinguish against any ragged background of debris.

Few problems from swarming have been found, compared with conventional pour-plate methods. Occasionally, the droplet surface may be covered by a film of organisms, but this is almost invariably very thin compared with the plaques found on plates and does not interfere significantly with the visibility of subsurface colonies.

**DISCUSSION**

A rapid acceptance of the droplet technique by many of the laboratories to which it has been shown indicates growing impatience with costly and labor-intensive plating methods. It is by no means the first miniature method to be described. However, it would seem that the simple technique, combined with the two aids, possesses advantages and few of the disadvantages that, in our experience, are frequently leveled against miniature methods (e.g., in requiring special manipulative skills and fussy procedural steps, or causing eyestrain). Its accuracy is unquestionable, and its sensitivity good (down to 100 organisms/g for products not requiring dispersal or that are sampled by vortex stirring). Although originally developed for estimating total viable aerobic bacteria, the technique can be used for a variety of other types of count.

The technique is very easily learned when the two aids are used, and we find that technicians prefer its simplicity. Because familiar materials are used, it tends not to be regarded as a miniature method. In this respect also, the appearance of magnified droplets on the screen at approximately the same size as a standard petri dish has provided an excellent psychological stimulus to its acceptance. Equally important, although essentially a total viable counting technique, it integrates easily with, or even facilitates, other analyses which may be done. Diluter/dispensers are used, for example, to dilute and inoculate bottles for MPN counts of coliform organisms or *Escherichia coli*, to inoculate plates for *S. aureus* enumeration, and for dispensing broth or serum for coagulase tests on these organisms. The diluter/dispenser and viewer virtually eliminate fatigue in diluting, plating, and particularly counting in comparison to methods requiring the use of a microscope.

Labor savings are made on both technical and preparative work. Technical savings result from the reduced number of liquid transfers made during each analysis, from reduced manipulation of petri dishes, elimination of much writing on dishes, and the decrease in volume of all apparatus at the bench, resulting in, for example, less reaching and fewer trips to stores. Diluting and plating on most samples take less than 40 sec, and, if only the droplet technique is being used, output can be increased threefold without extra strain on technicians. At the same time, reduced consumption of media or diluent bottles, elimination of labor for recycling pipettes (if reusable), and a reduced disposal problem lightens the load of preparative staff.

Direct cost savings result mainly from reduced consumption of petri dishes (one quarter, instead of four or more per count), pipettes, if disposable graduated ones were used, and, to a lesser extent, from the smaller quantities of media consumed. In an average laboratory, the cost of diluter/dispensers and viewers is recovered in a few weeks from materials savings alone.

Benefit obtained from reduced incubation time is variable. Where products are held pending results for total viable counts only, the speed of the droplet technique could be of very real value. When other tests are carried out, however, a shorter incubation is less valuable, and it may be better to allow the same incubation time for droplets, to avoid the risk of increasing paperwork. The extra incubation does not introduce error. Incubation times greater than 48 hr should be avoided, particularly if dishes having raised lids are employed, unless dishes are placed in a sealed incubation vessel. Otherwise, excessive drying of droplets may occur. A slightly greater tendency to surface drying, caused by the greater surface to volume ratio of droplets, is of benefit in reducing the tendency of some organisms to swarm.

For much routine work, large-diameter (4 to 5 mm), stiff-walled, polypropylene drinking straws may be preferable to glass capillary pipettes, since they are both less expensive and less easily blocked by food particles. If
straws are used, it is advisable to check batches for contamination. Our experience is that, although standard straws may not be sterile, the level of contamination is so low as to be unnoticeable, considering the type of analysis for which the droplet technique is normally used. Much depends on the degree of handling that straws receive in packing and redistribution and it is certainly preferable to purchase direct from the manufacturer.

Although little study has been made of the applicability of the droplet technique to other types of bacterial count, there is no obvious reason why a variety of agars or atmospheres could not be used. The technique could be particularly valuable in thermal inactivation studies on pathogenic anaerobic bacteria or their spores, in which great savings on anaerobic jar space could be made. A little caution may be necessary if organisms sensitive to oxygen are used. The time required to fill an ordinary anaerobic jar is small if one petri dish is used per dilution; the exposure time for organisms will be longer if the same jar is filled with a large number of sets of droplet counts, and it would be necessary to check that oxygen was not rapidly lethal to the organisms. The application of the droplet technique to the counting of anaerobic organisms is currently being studied in detail.

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