Machine for Automatic Bacteriological Pour Plate Preparation
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A fully automatic system for preparing poured plates for bacteriological analyses has been constructed and tested. The machine can make decimal dilutions of bacterial suspensions, dispense measured amounts into petri dishes, add molten agar, mix the dish contents, and label the dishes with sample and dilution numbers at the rate of 2,000 dishes per 8-hr day. In addition, the machine can be programmed to select different media so that plates for different types of bacteriological analysis may be made automatically from the same sample. The machine uses only the components of the media and sterile polystyrene petri dishes; requirements for all other materials, such as sterile pipettes and capped bottles of diluents and agar, are eliminated.

Development of instrumentation for bacteriological analysis has been slow compared with progress in many other branches of science. The plate count, particularly in view of its wide use, has received very little attention, and demands almost the same repetitive manual work that it did at the beginning of the century. Success has been achieved in automation of various bacteriological processes (1, 3-6, 10) but only recently have there been signs that whole, or even usefully large, portions of plate counting can be satisfactorily automated (2, 3, 7-13). Several aids of undoubted value recently have been introduced, for example, the Petri-Scan automatic colony counter (American Instrument Co. Inc., 8030 Georgia Ave., Silver Spring, Md. 20910), the Autofill 600 petri dish filler (Buchler Instruments Div., Nuclear-Chicago Corp., 1327 16th St., Fort Lee, N.J. 07024) and the Petrimat petri dish filler (Belco Glass Inc., Vineland, N.J. 08360). The last two instruments are intended for use in laboratories where surface-inoculated plates are used routinely and would be of little value where counts are made routinely with poured plates.

The problems encountered in the construction of automatic "classic" bacteriological process equipment are probably unique among the sciences. The most important requirements are for constant provision of sterile containers and media, minimization of hazards and errors caused by carry-over of contamination, dealing with the tremendous range of bacterial concentrations encountered (10^8 or more), and the difficulties of detecting instrumentally the cues commonly used in recognition or counting (such as colonial morphology or color, or even just distinguishing bacterial colonies from sample debris). In addition, the variety of tests which may be necessary on different samples demand considerable versatility from an instrument system.

We have developed an instrument which, we believe, satisfactorily carries out the most time-consuming parts of the preparative and technical work involved in a bacteriological pour-plate count and which can dramatically increase the output of one or more technicians. The instrument, for which patent application has been made (British patent application no. 51377/70), was produced in response to requests from quality control bacteriologists in the food industry and represents one of our approaches to the problems in this area.

MATERIALS AND METHODS
General description of the bacteriological plating machine. The prototype is shown in Fig. 1-6. From a sample suspension it automatically prepares a stack of inoculated, agar-filled petri dishes, labeled with sample and dilution number, ready for incubation. According to the setting of its controls it will select and prepare up to two dilutions in one agar (e.g., violet red and bile (VRB) for coliform counts) and up to eight dilutions in a second agar (e.g., plate count agar (PCA) for total aerobic counts). The various operations are carried out sequentially and simultaneously. All materials are handled in
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FIG. 1. Layout (plan) of the main working areas in the pour plate machine.

FIG. 2. Layout (elevation) of the main working areas in the pour plate machine.

FIG. 3. Overall view of the pour plate preparing machine. 1, Petri dish dispenser; 2, agar store; 3, diluents store.

bulk, and fresh, sterile pipettes for dilutions are produced within the machine as required.

Dilutions are made within the petri dish itself, the residual liquid after transferring a sample serving as the inoculum. In its present form, the machine adds 10 ml of diluent to each dish and transfers 1.1-ml samples through the dilution range, thus achieving a set of decimally diluted inocula of 10 ml each. To these are added either 10 ml of double-strength or 5 ml of triple-strength agar. The final content of each petri dish is, therefore, 20 or 15 ml of normal-strength agar. It is convenient to use suitably concentrated nutrient solutions as the diluent and to add blank agar to avoid keeping nutrients for long periods at elevated temperatures; the agar itself is not significantly affected by such holding.

Pipettes are produced automatically, as required, from the concentrated agar used to fill the dishes. They are extruded as cylinders from a water-cooled die and discarded after use. Cutting is carried out with a stainless-steel blade, sterilized by electrical heat to 250°C. A constant supply of sterile pipettes is thus assured and carry-over of contamination eliminated. A separate diaphragm pump, connected through the center of the die, controls the volume of liquid taken up and ejected by each pipette. The shape of the agar cylinder used to transfer liquid is unimportant, provided that it is long enough to make contact with the diluent in the petri dish. The machine uses the clover leaf form (Fig. 7) to obtain a large cross-sectional area without tendency to drip.

In its present form the machine can prepare one filled, labeled dish every 15 sec, or 2,000 per 8-hr day. Its speed can be increased by up to 30% if necessary. The number of samples for processing is determined by the number of dilutions required. At its lowest value, i.e., if two dilutions in VRB agar and eight in PCA are repeatedly called for, slightly less than 200 samples per day can be processed, allowing for start-up and run-down time.

Layout. The agar preparation unit, bulk diluent stores, and metering pumps are located in the lower part of the machine (Fig. 1 and 2) to avoid the need to lift heavy weights. Diluent bottles of up to 10 liters can be accommodated, allowing up to 4 hr of uninterrupted operation. Agar stores are replenished while the machine is running.

All bacteriological operations, i.e., dispensing
Agar. For routine work, agar can be prepared in the electrically heated, steam-jacketed lower tank. Agar powder and cold water are added to the tank about 35 min before agar is required. A centrifugal pump circulates the agar suspension during boil-up and is later used to pump the solution up to the storage tank, where it is held at 60 to 80 C until used. An alarm circuit in this tank warns of a low level in time for a fresh mix to be made.

Petri dishes. The rotating holder can take up to 200 disposable, plastic petri dishes in eight stacks. As one stack empties, the holder rotates to the next position to bring a fresh stack above the dispenser. Fresh dishes can be added at any time.

Electricity and water. A normal-main electric supply is required. The machine consumes about 3.5 kv with all units working, mostly by the agar preparation unit.

Cooling water is required for pipette extrusion and for a condenser on the agar steam jacket. Coolant temperatures up to 30 C can be tolerated, but pipette quality decreases rapidly above 25 C.

Operation. Start-up operations are completed quickly. These include switching on services and adding agar powder (conveniently by volume) and water to the preparation tank. Next, and before beginning work, occur the following procedures: (i) renewal of diluent bottles and supply tubes; (ii) filling of the petri dish store; (iii) removal of a disinfectant cup from the base of the pipette dispenser (if

petri dishes, removing their lids, performing dilutions, adding agar, mixing, replacing lids, and labeling dishes are carried out in the covered portion of the machine below the main working surface. Dishes are held in a rotating cross-shaped carrier which rotates from station to station to allow the various operations to be carried out at different positions.

Petri dishes and prepared agar are stored above the main working surface (Fig. 1 and 2). Dishes are added to the rotating store (200-dish capacity) whenever convenient. Agar (10 liters) can be pumped into the storage tank as soon as it is ready, leaving the preparation tank free for rinsing or further preparation.

Diluents. These are prepared in bulk, away from the machine, in bottles fitted with supply tubing and delivery nozzles sterilized by autoclaving at the same time as the diluent. Bottles of up to a 10-liter capacity can be accommodated. A length of silicone rubber tubing, included in each supply line, is stretched over the appropriate peristaltic pump head when a new bottle is fitted into the machine.

Diluents are prepared as solutions of the media nutrients at 1.5 or 2 times the intended final concentration. Diluents do not, therefore, deteriorate by being maintained at high temperatures.
Fig. 6. Stacked dishes containing dilutions in violet red and bile agar and plate count agar awaiting removal.

The instrument had been left overnight; (iv) pumping of boiled agar solution to the storage tank (if much work is to be done, the preparation tank is refilled immediately, but not boiled. Otherwise, it is rinsed and emptied; and (v) running of one complete set of dilutions in order to fill and clear the various supply lines.

During this time the first sample suspension will have been made ready. Preparing a set of dishes requires only: (i) placing a container of the sample suspension in the machine; (ii) dialing the required number of dilutions (up to 2 for VRB agar and up to 8 for PCA for example); and (iii) Pressing the START button.

The machine will then complete its bacteriological operations while the next sample is prepared. Before, or soon after dialing the next sample, the operator removes the stack of prepared dishes. Only one sample is accepted at a time.

Sequence of events after pressing the START button. All operations are carried out with the dishes seated in rings at the ends of a fairly complex cross-shaped carrier mechanism. The carrier indexes around one quarter of a revolution for each operation. The following sequence results when the START button is pressed.

(i) A petri dish (dish 1) is dispensed (station 1, Fig. 1) and carried around to the dilution position (station 2). On the way its lid is removed, and the dish is brought into an inclined position. At station 2 the dish engages with a rotating disc and, itself, begins to rotate.

(ii) An agar pipette is dispensed. The dispenser moves to the sample container, withdrawing 1.1 ml, and then to the first petri dish where the pipette is emptied. A 10-ml diluent (diluent 1) is pumped into the dish. The contents of the dish mix rapidly as a result of its rotation. The pipette is cut and falls into a waste receptacle. A new pipette removes 1.1 ml of the decimal dilution. A fresh dish (dish 2) is dispensed at station 1.

(iii) The carrier indexes around again, taking dish 1 to station 3 (agar addition) where it again rotates while in an inclined position. Agar is pumped into the dish and mixed. At the same time events (i) and (ii) are repeated with dishes 2 and 3, except that the pipette does not move to the sample container.

(iv) The carrier indexes again, taking dish 1 to station 4. On the way its lid is replaced. At station 4 the dish is pushed from beneath by the printing mechanism and rides over a set of catches to form the first dish of a stack. Events (i) through (iii) are repeated.

(v) At a point determined by the operator when dialing, the pipette dispenser again moves to the sample container, picking up a fresh sample of the suspension. The sequence then continues and uses diluent 2 to obtain a second set of dilutions.

(vi) When the last dish is labeled and stacked, the control settings return to zero and the machine stops.

Fig. 7. Used agar pipettes.
RESULTS

**Speed.** The machine completes a full dilution-range count preparation from two media in 2.5 to 3 min and smaller count ranges in less time. The operator may use this time to prepare the next sample. With many types of material, sample preparation is then likely to be the rate-determining factor, and it is reasonable to expect that two or more operators can use the machine simultaneously. The potential output (2,000 plates per day or 200 sets of eight PCA and two VRB agar plates) exceeds the capabilities of most bacteriologists.

Labor saved on preparative work is marked. All operations concerned with pipettes and small bottles of agar and diluents (uncapping, washing, refilling, recapping, etc.) are eliminated. Agar preparation occupies very little time, equivalent to that spent steaming separate bottles for a normal manual count. Diluent (nutrient) preparation time is equivalent to that required to prepare the same volume prior to dispensation into bottles for the manual count technique. Some laboratories might have difficulty autoclaving the largest and most economical container sizes. Large diluent bottles may be kept in the machine overnight for reuse the next morning, provided the nozzles are removed from their brackets and immersed in Formalin. Total labor saving appears to be about 70% over the whole count in our laboratories when about 150 to 180 samples per day are processed. This represents a cost reduction of about 47% over each manual count. Against this saving, of course, must be weighed the cost of the machine.

**Accuracy.** The machine prepares excellent decimal dilutions, well mixed with agar, showing no sign of "carry-over" and with a maximum temperature of 40 to 42°C after mixing. There is no reason, therefore, to expect the accuracy of the machine to be inferior to that of a properly carried-out manual count. A comparison of approximately 800 sets of data from manual and machine counts, on a variety of foods, including results from the machine at all stages of its increasing operational reliability, showed a significantly lower count ($P = 0.01$) for the machine. A later set of 144 counts, however, obtained as quadruplicated analyses on 36 specimens (meats, vegetables and swabs) gave the following results ($\log_{10}$ counts/g): the machine count had a mean of 3.89 (estimate of variance was 0.0027); the manual count had a mean of 3.87 (estimate of variance was 0.082), indicating that the variability of counts made by the machine is significantly less ($P < 0.1\%$) than those done manually. Evaluation is continuing.

In the absence of nutrients, and at the high storage temperature, multiplication of bacteria in the boiled agar is most unlikely. However, when prepared by boiling, the agar has a very small but detectable level of residual bacterial contamination. For example, in the worst case during trials in which 250 plates were prepared at a time from boiled agar, 93% of the plates were clear and 6% had one contaminant colony. The corresponding figures for autoclaved agar were 98 and 2%. (This partially may, in fact, be due to aerial contamination, indicating that the addition of a filtered air supply might be desirable.) The convenience of preparing agar within the machine would make a small error in counting acceptable; in fact, for normal quality control work with foods etc., even a 6% chance of finding one contaminant colony on a plate leads to an undetectably small error, since it is unusual for plates containing fewer than 20 colonies to be counted. It is feasible, of course, to prepare agar outside the machine and to sterilize by autoclaving. In most instances, however, the extra labor would be unjustified.

Only one plate is prepared from each dilution. Many bacteriologists, however, like to inoculate petri dishes in duplicate from each dilution. The effect of such duplication on the accuracy of the count is questionable; it would be far better if the dilution sequence were also repeated and still better if the whole product were resampled. This is feasible with the machine described here. If the utmost in accuracy is required, therefore, it is more reasonable to take two samples from different parts of the product rather than to duplicate inocula from particular dilutions.

DISCUSSION

The labor involved in bacterial counting can be conveniently divided into "support" and "technical" work. In large laboratories support work may be carried out by low-level staff and includes preparation and dispensation of media into bottles, cleaning, sterilizing, and distributing homogenizers, bottles, and pipettes, etc., and disposal of contaminated wastes. Technical work includes everything pertaining to the actual count such as sample preparation, dilution and inoculation, counting or estimating numbers of colonies, calculation and reporting of results, and carrying out whatever subsequent special tests may be necessary.
Costs can be divided into "direct" costs which include, for example, media, petri dishes, and pipettes consumed during the test (either as disposables or breakages of reusable items) and "indirect" costs which include all labor, steam, gas or other services, and depreciation of equipment. The importance of each item naturally varies greatly among laboratories and according to the types of analyses made.

Our main interests were the total viable aerobic and VRB agar coliform counts using poured plates. Average figures for technical time spent on various operations of the viable aerobic count in local laboratories are shown in Table 1. The largest single item was, surprisingly, accurate sample weighing, but diluting plating and (also surprisingly) labeling petri dishes and simply keeping materials at hand were notably time consuming. On the other hand, colony counting occupied a relatively small proportion of the total time.

Support labor was notably expensive (Table 2). A high proportion of this labor, associated with readying pipettes, bottles, etc. could obviously be eliminated if bulk handling were introduced. Pipettes alone accounted for 22.5% of total costs (replacement and labor) and were obviously a particularly important target for automation.

In an additional analysis of an individual count which was broken down into individual movements (such as pick up bottle, remove bottle cap, pick up pipette, place pipette in bottle, and so on) over 1,000 operations were noted. This did not include counting itself, for which an operational breakdown was open to many interpretations. The fundamental processes, however, formed less than 10% of these movements. (For example, dilution consists merely of transferring liquid from one container to another; all other operations such as opening bottles are wasted effort but are nevertheless essential to fit the technique to our manual abilities.) It was obviously inefficient, therefore, to design equipment to do single operations (e.g., diluting); at least two stages of the count would have to be automated and integrated (e.g., diluting and plating) to avoid the need for human handling before and after each operation. (This effect was excellently demonstrated by the electronic colony counter tested by Malligo [8]. Although scanning and counting plates in 1 sec, only 40% of counting time was saved, as it did not eliminate the need for human handling before and after operations.)

The following criteria were considered most important in the design of a bacteriological plate count machine.

(i) The operations carried out by the machine would deviate as little as possible from the accepted manual technique. (ii) Savings in support work were as important as savings in technical work. (iii) Automation of single stages only was not worth considering. (iv) The extent of automation would be related to the capital outlay involved for each stage and the savings resulting from it. (v) Elimination of pipettes and manual pipetting, or both, would be a major objective, partly because of associated costs and partly because of the central

| Table 2. Example of costs in pour plate count* |
|-----------------------------------------------|
| Cost                                          | Per cent |
| Direct                                        |          |
| Media                                         | 3.0      |
| Peptone                                       | 0.3      |
| Distilled water                               | 1.0      |
| Petri dishes                                  | 9.6      |
| Replacement pipettes                          | 7.5      |
| Replacement caps                              | 1.4      |
| Total                                         | 22.8     |
| Indirect                                      |          |
| Depreciation of all items (homogenizers,      | 7.4      |
| autoclave, incubators etc.)                   |          |
| Support labor                                 |          |
| Readying homogenizers                         | 7.8      |
| Readying pipettes                             | 15.0     |
| Filling and sterilizing bottles               | 11.0     |
| Total                                         | 33.8     |
| Technical labor                               | 36.0     |
| Total                                         | 100.0    |

* Services (gas, electricity etc.), management, and maintenance costs not included.

Table 1. Breakdown of technical time in pour plate count*

| Operation                                | Time (%) |
|------------------------------------------|----------|
| Attention to agar steaming/cooling       | 3        |
| Collection of petri dishes, bottles, etc.| 12       |
| Accurately weighing sample               | 24       |
| Homogenization                           | 13       |
| Opening packs and labeling petri dishes  | 14       |
| Diluting inoculating dishes and adding    | 22       |
| agar                                      |          |
| Inspecting and counting plates           | 12       |
| Total                                    | 100      |

*Generally five or six dilutions with duplicate plates from each dilution. All media etc. were taken from distributed stock.
position in the count. (vi) The machine would be sufficiently versatile to handle the major portion of routine counting requirements.

It was decided that an experimental machine should be made carrying out all parts of the count except sample preparation (on account of the variability encountered), colony counting (because of the likely expense associated with such a small item), and some parts of media preparation. These facilities could be added as peripheral units to the central system if the need later arose. In addition, the machine would have to be able to prepare plates for at least the total viable aerobic and coliform counts, since sequential dilutions were frequently required for these. The ability to prepare plates for counts of Staphylococcus aureus, for example, and yeasts or molds was considered less important, since the levels of these organisms encountered in foods do not normally require sequential dilutions to be made.

Work is now underway to redesign certain aspects of the machine to improve accessibility, versatility, and general smoothness of operation. We expect, for example, that increasing the number of media dispensed to at least four and the ability to make up to eight dilutions for each will allow the machine to satisfy any reasonable subsequent demands on performance. At the same time, evaluation work is being done on other types of counts besides those forming our initial interest (total and coliform) to ensure that the pour plate approach embodied in the machine can be used to its fullest in quantitative bacteriology.

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