A Surface Plating Technic for Determining Bacterial Population of Milk

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The simplicity and practicality of this proposed new method—presented before the Coordinating Committee on Laboratory Methods last fall—suggests a wider acquaintance with it and so the paper is published here.

The agar pour plate technic for determining bacterial populations of milk has always been the accepted procedure for determining bacterial counts of pasteurized milk.[1] Burri [2] in 1928 presented a simple test, in which a standard loopful of milk is spread over the surface of an agar slant. It is used as a screening test and gives a rough evaluation of bacterial populations.

In 1952 Tomales-Lebron and Fernandos [3] presented a method of determining bacterial numbers in liquids. This method consists of depositing on the surface of an agar plate known small portions of the solution to be tested, incubating and counting the colonies that develop. The senior author became interested in this procedure in 1953 when it was used for checking bactericidal activity of hypochlorites.[4] After observing the simplicity of the test and the ease of counting, application of the procedure was attempted in areas where dilution pour plates are used. The determination of bacterial populations in milk by this procedure appeared feasible.

Tomales-Lebron and Fernandos [3] recommend that approximately 12 ml of nutrient agar be placed in each Petri dish. The plates are incubated for at least 24 hours and then stored in the refrigerator until used. Prior to use the Petri dishes are inverted in a 35° C incubator for two hours, while the bottoms are raised slightly to allow the evaporation of excess moisture. The test liquids are pipetted to the agar surfaces by means of 0.2 ml pipettes. Plantings of 0.01–0.1 ml can be made. The plates are not disturbed for one hour before incubation.

In a study by Mallmann and Peabody,[5] where an examination of sewage effluent was made, the colonies were evenly spaced over the area covered by the planting when plantings of 0.1–0.4 ml were used. When 1–10 dilutions of milk were placed on the agar surface there was a tendency for the suspended solids to collect at the periphery of the drop. The solids appeared to hold moisture so a confluent growth developed forming a distinct ring around an area containing discreet colonies. Obviously, counts of such a plating would be inaccurate.

A maximum amount of spread of the plantings would be desirable, both to eliminate periphery growths and to obtain a wider spacing of the colonies. A nonionic wetting agent, Triton X–100, was added to the dilution water to decrease surface tension. Varying concentrations of Triton X–100 were added to the buffered dilution water to yield final concentrations of 1–40,000, 1–20,000, 1–10,000, and 1–5,000. Six samples of milk, including pasteurized, raw and pasteurized (that had been incubated at room temperature for 24

hours) were added to the dilution blanks containing Triton X–100 in a series of 10-fold dilutions. One ml quantities, pipetted with a 0.2 ml pipette, were placed on tryptone glucose extract agar drop plates, prepared according to Tomales-Lebron and Fernandos. The plates were examined in 48 hours. The approximate area of each drop was found by determining the average diameter. The maximum increase in area of a 1–5,000 Triton X–100 solution was 30.1 per cent greater than that of buffered dilution water. The percentage increase in area is only slightly greater (2.2) than that of a 1–20,000 solution.

To measure the toxic effect of various concentrations of Triton X–100 on colony growth on drop plates five samples of raw and pasteurized milk were diluted in buffered dilution water containing various concentrations of Triton X–100, i.e., 1–40,000, 1–20,000, 1–10,000 and 1–5,000. One-tenth ml portions in quadruplicate were tested on drop plates containing TGE agar. A concentration of 1–5,000 decreased the bacterial counts 58.8 per cent, 1–10,000, decreased the count 37.7 per cent, whereas concentrations of 1–40,000 and 1–20,000 gave a decrease of less than 5 per cent. Accordingly, a 1–20,000 concentration of Triton X–100 was adopted for the remainder of this study. Using Triton X–100 buffered dilution water an even distribution of colonies occurred on the drop plates and the peripheral build-up was eliminated.

Although in the presence of 1–20,000 concentration of Triton X–100 in the dilution water there was only a slight toxicity on the drop plate, there was a marked reduction in numbers of colonies when pour plates were used. In many cases there was as much as 75 per cent reduction as compared to pour plates made from dilution water not containing Triton X–100.

In the early study of drop plates confluent growth occurred frequently, due undoubtedly to improper drying of the agar surfaces. To check the effect of drying, eight sets of plates were dried in the incubator for periods ranging from one to eight hours. Additional freshly poured agar plates were added to the series and five samples of milk were planted in quadruplicate on the plates in 0.1 ml quantities of 1–10 dilutions of the milk. The plates were incubated at 35° C and counts were made after 36- and 48-hour periods. Pour plate tests were made in duplicate. When drying periods of less than three hours were used the agar surfaces were not sufficiently dry as evidenced by confluent colonies. Counts made on plates dried from three to eight hours did not show any significant variations nor were there any significant differences between the 36- and 48-hour counts.

Having developed what appeared to be a satisfactory technic, tests were run routinely on samples of both raw and pasteurized milk, using the standard pour plate method for comparison. To demonstrate the relationship 57 pasteurized milk samples were checked by standard pour plate technic by a regulatory laboratory. The milks were then tested in our laboratories by the drop plate technic.

The average logarithmic counts were 7,480 for the drop plates and 7,820 for the pour plates giving a ratio of 1 to 1.005. Forty-five samples were in agreement based on a $\pm$ 25 per cent variation, seven samples gave higher counts on drop plates and five gave higher counts on pour plates.

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Procedure—For Use of the Drop Plate Technic

1. Twelve–15 ml of tryptone glucose extract agar is poured into standard Petri dishes.
2. These are dried in a 35° C incubator in an inverted position with the bottom elevated slightly for approximately four hours. For convenience a week's supply of plates may be poured, stored in a refrigerator, and dried prior to use.
3. The agar plates are then divided into four quadrants.
4. Milk dilutions of 1–10 and 1–100 are made in a buffered saline water blank containing 1–20,000 concentration of Triton X-100.
5. Using a 0.2 ml pipette, 0.1 ml of each dilution is delivered to a single quadrant. In this manner two milk samples diluted 1–10 and 1–100 may be drop plated on one Petri dish. These dilutions are suitable for pasteurized milk. For raw milk higher dilutions may be necessary.
6. Care must be exercised in that the pipette should be drawn along the inner rim of the dilution blank to remove any excess.
7. In delivering the drop the pipette is rested on the surface of the agar and is allowed to drain slowly.
8. To prevent the drops from running together, allow at least one hour before moving the plates.
9. When the drop has been completely absorbed by the agar the plates are then incubated in a 35° C incubator right side up. The colonies may be counted in 36–48 hours.

In summary—a method for determining bacterial population in milk using the drop plate technic is presented.

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Florida Poisoning Control Program

What is reported to be the only state that "has a complete group of poisoning control centers serving the entire state" is described in a leaflet prepared by the Florida Chapter of the American Pediatric Society and the Florida Pediatric Society.

A total of 15 poisoning control centers are strategically located in 15 hospitals of as many cities throughout the state. Here are filed the names of 1,500 poison or poison-containing products and the ingredients of each, as well as the names of 400 toxic or chemical poisons, their sources, degree of toxicity, symptoms of poisoning, and treatment. Each center is covered for every full 24-hour day. Here it is expected that physicians will report any new poisons coming to their notice. Here also are reference books on the subject.

In making the announcement credit is given for the help of the Florida State Board of Health and its epidemiologist, James Bond, M.D., and "nationally known poisoning control expert," Edward Press, M.D., field director of the American Public Health Association. Chairman of the Poisoning Control Committee is Robert Grayson, M.D., of Miami Beach.

An exhibit on this state's poisoning control centers was recently shown at a meeting of the Florida Medical Association. Because of a demand for a wider showing, it will be among the scientific exhibits at the 84th Annual Meeting of the American Public Health Association in Atlantic City in November.