An Emergency Method For Estimating Bacterial Populations

(A Preliminary Report)

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IF the surface of a suitable solid medium is flooded with a liquid such as milk containing dispersed microorganisms, and the liquid then poured off, there will remain adhering to the surface a film of the liquid in which the dispersed organisms are retained. If such cultures, prepared in suitable dilutions, are incubated, discrete colonies will develop on the surface and may then be counted.

The area of the surface and the thickness of the film are the two factors determining the total amount of liquid adhering to the surface of the medium and consequently the number of organisms so entrapped. The surface area may be fixed by preparing the medium in containers of determined size. The thickness of the film depends upon the conditions under which the last traces of the liquid are removed: conditions which affect its viscosity such as temperature, the time allowed for draining, and of course the position of the slab. By controlling these conditions it is possible to obtain reproducible colony counts apparently within the limits affected by random distribution, over-crowding, etc. Then, by comparison of such colony counts with actual plate counts, the relationship between them may be noted and perhaps expressed as a factor for future use under like conditions.

For example, we have found that the relationship noted when using milk at room temperatures for the liquid vehicle carrying the organisms and Standard Agar slabs (2 percent agar) at the same temperatures, having surface-areas of about 20.5 square centimeters, is surprisingly like the factor used in reporting counts from a 1 to 100 dilution. From one sample of pasteurized milk two duplicate plates developed 5 colonies and 2 colonies from a 1 to 100 dilution, while three agar slabs flooded with the undiluted milk developed 1, 3, and 4 colonies respectively. A pasteurized, homogenized milk with a single plate developing 113 colonies from a 1 to 100 dilution, developed on the agar slabs 116, 123, and 129 colonies respectively. A pasteurized skim milk, plated in two 1 to 100 dilutions, developed in one plate 329 colonies and in a second plate 419 colonies, while using this same skim milk to flood the agar slabs in three bottles, 280 colonies developed in the first, 368 in the second, and 218 in the third with several spreading colonies. Using a raw mixed milk but agar slabs with slightly larger surfaces (about 23.5 square centimeters) two plates from a 1 to 100 dilution developing 55 and 64 colonies, the flooded slabs developed 68, 66, and 56 colonies respectively. A sample of milk from a single cow in 1 to 100 dilution developed in plates 24 and 30 colonies. Agar slabs flooded by this milk at different temperatures, produced colonies as follows: at 40° F., 22 colonies; at 70° F., 28 colonies; at 85° F., 16 colonies; at 100° F., 17 colonies; cooled from 100° F. to room temperature and warmed again to 85°, 24 colonies.

An orange staphylococcus culture from a milk sample was plated out from water dilutions and compared with slabs where the dilutions used for flooding were made in recently boiled milk, with results as shown in Table 1.

Sterile skim milk is recommended for making dilutions.

Since samples for the first flooding must be shaken very violently to break up clumps, it is necessary that these bottles be only partially filled.

TABLE 1

Dilution	*Colonies in Plate* (from water dilutions)	*Colonies on Slab* (from milk dilutions)
1 to 1,000	*13,624	.....
1 to 10,000	1,442	1,014 (24 hour count)
1 to 100,000	158	128 (24 hour count)
1 to 1,000,000	14	8 and 1 Spreader

* Estimated from the average counts of several representative areas.

Considerable difficulty with spreaders has been encountered. Since surface colonies grow so rapidly, perhaps 24-hour counts will to some extent obviate this difficulty, and still be sufficiently accurate. The advantages of such earlier information will appeal to many engaged in practical control work. Possibly some growth accelerants may be added to the medium or to the milk which will further shorten the time which must elapse before counts can be made. For many purposes, such as roughly grading milk, checking sterility of equipment, etc., accurate counts are often unimportant although both time and labor-consuming. A glance will determine whether few or many organisms are present.

This work has been done using 10 milliliters of agar media (2 percent agar) sterilized in 2 oz. square-bottomed, flint glass bottles fitted with molded bakelite caps. To avoid injury to the cap-liners, caps should not be screwed down tightly until after sterilization. While the agar is still melted the bottles are laid on their sides until the media has hardened.

The counting of colonies is facilitated by the use of a magnifying glass, by spotting with a pen, or by marking the bottle into suitable squares with a red pencil. It is recommended that the bottle be held toward a light with the bottom of the agar slab nearer to the eye.

This report is presented with some hesitancy because of insufficient experimental material and the uncompleted study of the principles involved, as well as the most satisfactory methods for their application. The urgent needs of workers in the laboratories of the Armed Services, Public Health, Milk Plants, etc.—even of Producing Dairymen themselves—for methods requiring a minimum of time, skilled labor and laboratory facilities, is offered in extenuation. It is sincerely hoped that others will develop the possibilities and limitations of this method as quickly as possible for use in the national emergency. It may be particularly suitable for traveling and field laboratories.

SUMMARY

(1) It has been found that reproducible colony counts within the usual limits may be obtained by flooding, draining and incubating slabs of solid nutrient media with a suspension of bacteria in a vehicle such as milk or skim milk if constant conditions of the area of the slab and the drainage of the vehicle are maintained.

(2) These quantitative results may be correlated with an actual plate count, and a factor expressing the relationship may be obtained for future use under like conditions.

(3) A report has been made of the

results of several experiments utilizing the adherent film of the vehicle in lieu of formal measurements and dilutions, and correlating these results with actual plate counts.

(Note: Dr. R. V. Stone, Department of Health, Los Angeles, California, points out that the usefulness of the method does not necessitate a comparison of the counts obtained with those by standard methods. The sterile medium surfaces can be stored for ready use in the event of, say, bombing, requiring immediate action on milk and water supplies even though normal facilities for conducting laboratory work have been interrupted. The counts on these bottles can be compared against each other, and serve as a "screening" procedure to determine whether or not the samples are relatively good, fair, or potentially unsafe.—*Editor.*)

CADMIUM POISONING *

There has been a pronounced increase in the use of cadmium in electroplating materials and alloys. It is important that the plating industry, in its search for substitutes for tin and other valuable war materials, be cautioned that cadmium is not a suitable substitute for the plating of utensils for food. Prior to 1941 there have been reported in the literature 20 cases of cadmium poisoning due to the ingestion of cadmium. Since January, 1941, there have been reported 315 cases definitely caused by this metal.

Owing to the greatly increased technical use of cadmium in the manufacture or repair of various types of containers used for food, several instances have occurred from this cause. It is also possible that cases of cadmium poisoning have been mistaken for food poisoning owing to the similarity of the symptomatology of cadmium poisoning to ordinary so-called "food poisoning."

* Cadmium Poisoning, *Pub. Health Repts. 57,* 601 (1942).

FOOD SERVICE EQUIPMENT *

In February, 1941, a proposed simplified practice recommendation covering sizes, dimensions, and details of construction of cooking and serving equipment used by hotels, restaurants, cafeterias, hospitals, etc., was submitted to those concerned for consideration and acceptance as standard practice. This proposal, which was developed by a committee of the Food Service Equipment Industry, Inc., was modified by the manufacturers and users, and then promulgated through the National Bureau of Standards, U. S. Department of Commerce.

* Food Service Equipment. Simplified Practice Recommendation R182-41, October 1, 1941.