SYMPOSIUM ON METHODS FOR DETERMINING BACTERIAL CONTAMINATION ON SURFACES[1]

WILLIAM G. WALTER[2]

Montana State College, Bozeman, Montana

The extensiveness of the topic under discussion was recognized at the start since many modern industries, public health agencies, and research laboratories require methods for determining bacterial contamination on surfaces. Numerous techniques have been recommended for performing such studies. In 1941, Walter and Hucker (1) reviewed some of the early literature relating to the swab-rinse technique and proposed a contact plate method patterned somewhat after the agar-disc method employed by Hammer and Olson (2) in studying contamination from churns. Various modifications of these technics have been advocated from time to time.

During the roundtable discussion, Mr. L. Flett, Mr. A. F. Guiteras, and Miss R. L. Shapiro expressed the opinion that although the swab-rinse technique, which has been standardized after considerable study by a committee of the American Public Health Association (3), has merit for some types of work, a more accurate quantitative method for determining bacterial contamination on dishes was preferable. They recommended placing a dish or dinner plate whose bacterial contamination was to be measured tightly over the bottom of a petri plate containing melted agar. Upon inversion the agar solidified in contact with the dish surface. After incubation, the dish and petri plate were turned again and a wide spatula inserted causing the agar to fall into the petri plate. The colonies were then counted in the conventional manner. Although this method required considerable incubator space, it gave a higher quantitative value than the swab-rinse method.

Mr. R. R. Barton and Mr. H. Gorfien reported that direct plating procedures in which agar was poured into mess trays, soup bowls, or cups, and in which agar was poured over silverware placed in petri dishes, gave counts 3 to 29 times as high as those obtained by the swab-rinse test. As with the previously mentioned method, some of the participants objected to the amount of incubator space required. Mr. Barton also emphasized that direct plating was of value in test work on sanitizing various types of materials. In addition, the technique has proven useful in research work on design of dishwashing machines and washing methods as well as in determining microbial distribution in biological warfare studies.

Dr. W. Litsky proposed a modified direct contact method which as yet had not been employed experimentally. A syringe-like apparatus of large diameter would be filled with an agar medium and a plunger would push the column of medium to the end of the barrel, thereby producing a layer of medium which would be brought in contact with the surface to be tested. After contact, a layer of medium would be cut off by a knife attached to the barrel and incubated in a petri dish. Advantages of such a procedure include: (a) a constant test area; (b) elimination of bulky materials as in the direct and quantitative methods; (c) possibility of employing differential or selective media; and (d) reduction in expense because of the amount of medium and equipment used.

Miss J. C. Curry described a technique she had employed in determining gross contamination in line samples of a processed food product. Cans were used in one phase of the evaluation, and greater recovery of microorganisms from the interior surfaces was obtained by vigorously swirling 50 ml of warm liquid agar and then distributing the agar by direct pouring from the can into as many petri dishes as needed. She found that in her particular situation this method was superior to a water or buffer rinse in which a dilution factor was involved.

In 1950 Higgins (4) in England found that calcium alginate swabs recovered more organisms from surfaces than cotton swabs. After

[1] Thirty-six participated in a roundtable discussion on methods for determining bacterial contamination on surfaces held on the evening of May 9, 1955, at the Society of American Bacteriologists meeting in New York City.

[2] Convenor.

swabbing an area the swab was placed in Ringer's solution and sodium hexametaphosphate added. Within a short period of vigorous shaking, the calcium alginate dissolved, thereby freeing the organisms. This accounted for counts higher than those obtained with a cotton swab in which bacteria presumably remained trapped in the fibers.

Lt. R. M. Cain, commenting on the use of calcium alginate soluble wool for swabs in connection with the bacteriological examination of cleansed eating utensils, expressed his opinion that, although the direct plate count might be superior to the swab-rinse technique for determining true total plate counts, such counts were no longer of primary importance *per se*. More emphasis should be placed on differential bacteriology since counts had been encountered of less than 100 per utensil while types of bacteria were present which should not be permitted from a public health standpoint. Such conditions had occurred because of failure of the sanitizing rinse to function properly or because of human errors in the operation of a dishwashing machine. Lt. Cain advocated the use of calcium alginate swabs and stainless steel applicators to permit a firmer swabbing action.

Dr. R. D. O'Neill found the soluble swab (*Calgitex Alginate*) technique to be significantly superior to the cotton swab in studying experimentally contaminated paper surfaces such as single service cups and containers, milk and ice cream containers, and the like. Further enhancement of sensitivity was obtained by using a non-ionic surface active agent (*Carbowax* in a 1-1000 dilution) rather than water as a moistening agent in the swab-rinse method.

Mr. S. Kaye discussed a method for testing self-disinfecting surfaces in which small pieces of materials under study were inoculated and later shaken in a diluent before plating. *Bacillus subtilis* var. *niger* was sometimes employed, and distilled water and a 0.1 per cent non-ionic detergent were used as diluents.

Considerable discussion was devoted to various uses of the membrane filter for studying surface contamination. Dr. J. F. Murphy found that non-destructive sampling of meat and examination of process equipment was accomplished by placing a nutrient-soaked filter on the surface to be tested. For quantitative information, the filter was disintegrated in a dilution bottle containing glass beads or in a Waring blendor. Subsequently the sample was handled in a routine plating manner. Qualitative data were obtained by incubating the filters on pads saturated with differential media. Counts obtained by these procedures were lower than when meat samples were obtained by cork borers, but the membrane filter kept the carcass intact and gave a picture of the surface condition.

Dr. F. W. Barber reported that the membrane filter technique has been applied to the evaluation of the sanitary condition of dairy plant pipe lines cleaned-in-place. Briefly, the method consists of determining the bacterial counts of rinse water before and after circulation through the cleaned and sanitized lines and calculation of the number of bacteria removed by the rinse water by the difference. The method has been shown to be of value in instances where the condition of the pipe lines approaches sterility.

Mr. C. P. Schaufus and Mr. W. B. Krabek reported on the direct staining of microorganisms on membrane filters after several hours incubation. They advocated placing the incubated disc on a pad in a petri dish containing 0.5 per cent aqueous malachite green. After 5 seconds the filter is drained, dried, and observed under the low power of the microscope.

Other participants suggested a combination of some of the methods discussed. One recommended that a calcium alginate swab be employed for swabbing a surface. After dissolving the swab in sodium hexametaphosphate, the liquid would be poured through a membrane filter, and subsequently the disc could be incubated and stained for a direct microscopic count or incubated on a pad for determining "total" counts or differential counts.

Mr. E. H. Armbruster discussed some of the studies on radiological procedures for soil measurement which have been conducted in recent years by National Sanitation Foundation workers. Radioisotopes have been employed as a means of obtaining a quantitative measure of soil and bacteria on surfaces of cloth, china, plastics, glass, steel, and aluminum. Phosphorus (P^{32}) was used as a tag for measuring bacteria on cloth and for the first time the residual remaining after washing was measured directly. A combination of radiological and bacteriological

methods has proven invaluable in fundamental studies relating to the measurement of contamination on various types of surfaces. It was found that *Escherichia coli* was removed quite easily from cotton, while *Micrococcus pyogenes* was retained to a large degree even after repeated washings. The use of radioactive bacteria showed that the residual after cleaning was very dependent upon the type of material to be cleaned. Pertinent to the theme of the roundtable was the statement that a cotton swab would remove only a small portion of organisms from a surface with a monomolecular grease film as compared to the number it would remove from the same surface if it were grease free. Mr. Armbruster concluded his remarks by warning that unless an investigator was familiar with the limitations of the numerous procedures for determining bacterial contamination on surfaces, erroneous conclusions might be drawn.

Mr. Flett closed the roundtable discussion by stating that there were advantages and disadvantages to each of the methods discussed and that the individual had to select the one that best served his purpose.

REFERENCES

1. WALTER, W. G., AND HUCKER, G. J. 1941 The use of the contact plate method to determine the microbial contamination on flat surfaces. N. Y. State Agr. Expt. Sta. Tech. Bull., **260.**
2. HAMMER, B. W., AND OLSON, H. C. 1931 Bacteriology of butter. III. A method for studying the contamination from churns. Iowa State Coll. Agr. Expt. Sta. Research Bull. **141.**
3. TIEDEMAN, W. D., FUCHS, A. W., GUNDERSON, N. O., HUCKER, G. J., AND MALLMANN, W. L. 1948 Technic for the bacteriological examination of food utensils. Am. J. Public Health, **38,** Part II, 68–70.
4. HIGGINS, M. 1950 A comparison of the recovery rate of organisms from cotton wool and calcium alginate wool swabs. Public Health Lab. Ser. Bull. (Gr. Brit.), **9,** 50–51.

SELECTED REFERENCES PUBLISHED IN 1950–1954

AMERICAN PUBLIC HEALTH ASSOCIATION 1953 Tests for sanitization of equipment and containers. *Standard methods for the examination of dairy products,* 10th ed., Chap. 8. New York, N. Y.

ANNEAR, D. I. 1951 The inoculation, cultivation, and enumeration of bacteria on glass surfaces. Australian J. Exptl. Biol. Med. Sci., **29,** 147–151.

ARMBRUSTER, E. H., AND RIDENOUR, G. M. 1952 Detergency measurement. Soap Sanit. Chemicals, **28,** 83–86.

BARBER, F. W., BURKE, C. P., AND FRAM, H. 1954 The millipore filter technique in the dairy industry. J. Milk and Food Technol., **17,** 109–112.

BARTON, R. R., GORFIEN, H., AND CARLO, R. M. 1954 Determination of bacterial numbers on tableware by means of direct plating. Appl. Microbiol., **2,** 264–267.

BURKE, C. P. 1953 New bacteria spotter. Food Eng., **25,** (9), 47; 178–180.

CAIN, R. M., AND STEELE, H. 1953 The use of calcium alginate soluble wool for the examination of cleansed eating utensils. Can. J. Public Health, **44,** 464–467.

FLETT, L., AND GUITERAS, A. 1952 Microbiological cleanliness of dishes washed with anionic detergents under practical conditions. Proc. Chemical Specialties Manufacturers Assoc., 38th Mid-Year Meeting, 120–122.

FLETT, L., AND GUITERAS, A. 1952 Bacteria removal by hand dishwashing detergents. Soap Sanit. Chemicals, **28,** (10), 48–50.

GUITERAS, A. F., FLETT, L. H., AND SHAPIRO, R. L. 1954 A quantitative method for determining the bacterial contamination of dishes. Appl. Microbiol., **2,** 100–101.

HIGGINS, M. 1950 A comparison of the pour plate and surface plate methods in estimating bacterial infection of table crockery and kitchen utensils. Public Health Lab. Ser. Bull. (Gr. Brit.), **9,** 52–53.

HIGGINS, M., AND HOBBS, B. C. 1950 Kitchen hygiene: the effectiveness of current procedure in cleansing tableware. Public Health Lab. Ser. Bull. (Gr. Brit.), **9,** 38–49.

HUCKER, G. J. 1954 The adherence of organisms and soil to surfaces of eating utensils. J. Milk and Food Technol., **17,** 48–51.

HUCKER, G. J., EMERY, A. J., AND WINKEL, E. 1951 Adherence of film to plastic and china surfaces. J. Milk and Food Technol., **14,** 95–97.

KLARMANN, E. G., WRIGHT, E. S., AND SHTERNOV, V. A. 1953 Prolongation of the antibacterial potential of disinfected surfaces. Appl. Microbiol., **1,** 19–23.

MALLMANN, W. L., AND KAHLER, D. 1952 A commentary on the efficiency of machine dishwashing. J. Milk and Food Technol., **15,** 149–154, 161.

MALLMANN, W. L., KAHLER, D., AND BUTT, S. 1954 Studies on the cleaning and sanitizing of melamine plastic and vitreous china dinnerware. Modern Sanitation, **6**, 20–25; 48–52.

MARTH, E. H., HUNTER, J. E., AND FRAZIER, W. C. 1954 Bacteriological studies of a farm bulk milk handling system. J. Milk and Food Technol., **17**, 86–90.

OWEN, W. L. 1953 Pinning down bag-borne bacteria. Food Eng., **25**, (1), 57–58; 132; 134.

RIDENOUR, G. M. 1952 The use of isotopes for measuring cleanliness of surfaces. Modern Sanitation, **4**, 61–63.

RIDENOUR, G. M., AND ARMBRUSTER, E. H. 1953 Bacterial cleanability of various types of eating surfaces. Am. J. Public Health, **43**, 138–149.

RIDENOUR, G. M., ARMBRUSTER, E. H., AND MILONE, N. A. 1952 A bacteriological study of automatic clothes washing. National Sanitation Foundation Bull., M.C.W. **1**, 1–116, Ann Arbor, Mich.

STEDMAN, R. L., KRAVITZ, E., AND BELL, H. 1954 Studies on the efficiencies of disinfectants for use on inanimate objects. I. Relative activities on a stainless steel surface using a new performance test method. Appl. Microbiol., **2**, 119–124.