PROBLEMS ASSOCIATED WITH SURFACE SAMPLING TECHNIQUES AND APPARATUS IN THE INSTITUTIONAL ENVIRONMENT[1,2]

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Several techniques and apparatus for surface sampling are critically discussed. The worker in institutional sanitation is often handicapped when he attempts to use sampling techniques commonly used in other fields. Efforts should be expanded in basic research in this field. The most important challenges lie in the areas of sampling technology, sampling statistics, epidemiological significance, and fundamental aspects of solid surface bacteriology.

The determination of microbial contamination of surfaces in institutional environments depends upon techniques and apparatus borrowed both from the dairy and food sciences and from the diagnostic bacteriology laboratory. The food and dairy laboratories are interested primarily in bacterial counts of nonporous surfaces designed to be disinfected by a rather limited spectrum of treatments. Diagnostic bacteriology on the other hand, is almost entirely a qualitative enterprise, and the bacteriological techniques available to routine medical laboratories are not designed to deal with bacterial enumeration. The investigator in the institutional sanitation field, therefore, has available to him a variety of methods, some having some application to his problem, but none being *specifically* designed to answer the *specific* questions with which he is confronted.

As a consequence of this marriage between two disciplines, each with its own tradition, technology and problems, the worker in institutional sanitation is often handicapped when he attempts to use borrowed surface sampling techniques in his unique environment. First of all, he is dealing with a wide variety of surfaces, including such diverse materials as terrazo, plastics, rubber, stainless steel, leather, textiles, wood and plaster. Secondly, many critical surfaces in the hospital environment do not permit the use of classic sampling techniques. Thirdly, these surfaces may be disinfected by a variety of chemical and physical agents at irregular and unpredictable intervals *(4)*. Above all, perhaps, the worker in institutional sanitation is frequently untrained in bacteriological and statistical theory. Consequently, invalid sampling techniques may be employed and data from these samples may be interpreted without appreciation of their drawbacks and limitations.

TECHNIQUES AND APPARATUS

The surface sampling techniques in current use can be grouped into four general categories:

1. Swabbing a known area and enumerating the organisms which adhered to the swab.
2. Rinsing a surface with a sterile liquid and culturing the dislodged contaminants.
3. Direct contact of surface with culture medium.
4. Combinations and adaptations of the above.

The principles underlying these tests are self-explanatory and do not require elaboration. However, a brief word might be in order to evaluate the various specific methods commonly employed.

Swab method

This involves the use of a sterile cotton swab usually moistened in sterile saline or water. Contaminants are picked up and transferred either directly to a nutrient medium or to an intermediate diluent which can be quantitatively assayed. This method is basic and is probably the most common single technique employed in institutions.

Unfortunately this test has many variables which are difficult to control. Some of these are: *(a)* the area of the surface to be tested; *(b)* the pressure applied during swab manipulation; *(c)* the precision and reproducibility of the swabbing technique; *(d)* the removal of all organisms from the swab to the nutrient medium.

However, even when all of these variables are controlled, the recovery of organisms by this method

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ranges from 52-90 percent, and the reproducibility of results leaves much to be desired *(2)*.

Soluble swab

To overcome objection (d) above, some investigators have used a swab composed of calcium alginate. This dissolves in the diluent and releases all the adhering organisms. Enthusiasm for this method has been expressed in some circles, but critical work by Angelloti *et al (2)*, and by Walter *et al (6)* indicate that this imaginative approach is not yet the panacea for all of our sampling problems.

Membrane filter

Workers in the meat industry apply a nutrient soaked filter to the surface to be tested, and then either incubate the filters *per se*, or disintegrate them and plate the suspending fluid. This method is essentially a static swab test, using a filter in place of a swab.

Rinse method

This system involves dislodging of surface contaminants into a sterile diluent by some type of mechanical agitation and subsequently enumerating the contaminants in the diluent. When the variables in this method are minimized *(3)*, it becomes a very useful tool, yielding both high recovery of contaminants (70%) and fair precision (71-97%). Its obvious drawback in the institutional field is its strict limitation to level, horizontal impervious surfaces.

Among the great drawbacks of both the swabbing and rinse methods are the work and the problems involved in plating the contaminated diluents. To those who work in water and dairy bacteriology, the inherent errors associated with standard plate counts are self-evident. In essence, then, we compound the experimental error of the latter technique with the errors introduced by inadequate and haphazard transfer of organisms from solid surfaces to liquids. This combination of errors often is greater than the precision of the test, thus limiting its application. The adaptation of membrane filter techniques for analysis of swab and rinse diluents has done much to minimized these errors and has improved the accuracy and precision of surface sampling.

Direct surface agar plate

This technique involves pouring a liquified agar medium onto a circumscribed area of the surface to be tested, covering the agar, and incubating the test object at appropriate humidity and temperature. It is necessary to control the temperature of the agar used in this test. Obviously, if the agar is too hot, microorganisms on the surface will be destroyed. Similarly, if the agar is too cool, it will solidify and will not cover the surface uniformly. The temperature of the agar should therefore be between 43°-50° C., a condition which might be difficult to observe when testing surfaces at any distance from a laboratory tempering bath.

The system has the obvious advantage of eliminating intermediate steps such as swabs and diluents, and has demonstrated high recovery (80%) and high precision (87-98%) on non-porous surfaces *(1)*. The drawbacks to its use in institutional environment studies, however, lie in the multiplicity of textures that must be evaluated in the latter circumstances, as well as the difficulties encountered in incubating floors, walls, mattresses and bedframes!

Agar syringe method

An ingenious technique suggested by Litzky involves a syringe-like apparatus of large diameter filled with sterile agar medium *(7)*. A plunger pushes the column of medium to the end of the barrel where it comes in contact with the surface to be tested. After contact, a layer of medium is cut off by a knife or wire and is incubated in a petri dish. The advantages of this method include a constant test area, elimination of bulky and awkward equipment, and reduction in testing expense. The major drawback of this technique thus far is the difficulty of obtaining a flat smooth surface on the agar after cutting, but this disadvantage should be overcome with further testing.

Textile method

Several modifications of the direct contact method have been proposed for blanket and bed linen sampling. One involves pressing the surface of a blanket or sheet onto the sterile surface of a poured and hardened agar plate by means of a flask or beaker. The others consist of sweeping an inverted plate over a blanket, or scratching a blanket stretched over an exposed plate with a tongue blade, thereby dislodging contaminated particles onto the agar surface. Those who have used these methods report satisfaction but the quantitative aspects of these techniques leave much to be desired. Furthermore, their application is limited to flat bedclothes, and cannot be used on mattresses, pillows, clothing, and the like.

Summary of methods

As yet, no satisfactory, quantitative, bacteriological technique is available which can be used universally for the examination of various surfaces in institutions. Very little systematic work has been done outside of the food and dairy fields to evaluate the efficiency and precision of the methods now being employed. Above all, the science of institutional sanitation is in great need of technological advances in the quantitative aspects of surface sampling.

PROBLEMS ASSOCIATED WITH QUANTITATION

A discussion of surface sampling techniques would be incomplete without some consideration of the basic problems associated with bacterial quantitation in general. Mention already has been made that the institutional sanitarian has had to rely in the past on techniques borrowed from other fields. It was further pointed out that even the best of these borrowed techniques have only limited application in the institutional environment. The simple facts that institutional surfaces are of different shape, size and orientation from those in other fields; that institutional surfaces include a diverse array of textures and porosites not encountered in other fields; that institutional surfaces are treated with cleaning and disinfecting agents completely foreign to those used in other fields — these discrepancies alone preclude the simple application of borrowed techniques in the institutional environment.

Although a considerable amount of excellent work has been carried out in this field, investigators have been handicapped by the paucity of information related to basic studies. In particular, there is a great need for more serious attention to the fundamental technology of surface sampling. We do not believe that enough is known about our methodology to place excessive confidence in techniques which may be regarded as arbitrary. The field of surface contamination and disinfection has enough problems without compounding them by inadequate and semi-quantitative techniques, wherein each worker relies only on his own favorite method. This only adds to the confusion which already exists.

In which direction should our research efforts be applied? We submit that four major areas are worthy of investigation now. These are discussed below.

Basic bacteriology of surfaces

Radioisotopes have been used to measure bacterial and soil contamination. Armbruster and Ridenour *(5)* were able to demonstrate that different organisms adhere to the same surface with different tenacities and are not removed or sampled with the same ease. Furthermore, they showed that the same organisms would adhere to different surfaces with different tenacities. Pertinent to the problem of sampling was the observation that monomolecular grease films on surfaces influenced the efficiency of bacterial removal by cotton swabs. Much more attention to these phenomena and to the question of how bacteria stick to surfaces would simplify and facilitate surface sampling.

Along the same theme of basic bacteriology, studies should be made of the nutrient media and incubation conditions used for surface contaminants. The use of blood agar was borrowed from the diagnostic laboratory, and milk plate agar from the dairy people. Do we really know which media are best for floor contaminants and blanket bacteria? Is there general agreement on a proper incubation temperature? These questions deserve careful study and experimentation.

Quantitative technology

Efforts should be made by several laboratories working cooperatively to evaluate quantitatively and qualitatively the several surface sampling techniques commonly used in institutions today. Studies should be made on artificially contaminated surfaces in the laboratory and in the field to determine the actual efficiency and precision of these methods and their applicability for the many surfaces that must be tested.

Attempts should be made to develop new techniques. Perhaps it will be possible to evolve a universal method, suitable for testing all surfaces with comparable precision. Perhaps more attention should be focused on specific methods for specific surfaces. Certainly adaptations should be made of those methods which show promise in the food sanitation field, such as the rinse method and the direct contact method.

At the University of Minnesota, we have been experimenting with an impression plate method that shows promise. Essentially it consists of aluminum milk bottle covers filled with agar. These plates can be applied directly to a surface, removed, and incubated. It is similar in principle to Litsky's wafer, but has the advantage of a larger area, and a consistently uniform flat surface. Furthermore, the aluminum caps are inexpensive and in plentiful supply. The technique is applicable to horizontal and vertical surfaces, textiles, skin, plastics, as well as to floors, walls, and furniture. It combines the advantages of the swab method and the direct surface agar plate, provides a picture of contamination *in situ* and is easy to use. Unfortunately, it is still in the experimental stage and we know very little about its recovery efficiency and precision.

Some further approaches that have engaged our attention are the use of moistened replicate discs and pressure sensitive tape. The discs consist of a cardboard backing to which is attached a piling of thousands of perpendicularly oriented fibres. Preliminary work with these discs shows excellent precision, but poor recovery.

The pressure tape method might also be suitable, but involves the preparation of soluble tape with a bacteriologically compatible adhesive. This is still in the research stage of development.

Ultimately, the problems condense into attempts to develop sampling methods specifically designed for the environment under study.

Statistics of surface sampling

Perhaps the statistical problems of surface sampling have been our greatest frustration. Here again we have been guilty of borrowing assumptions and methods from other fields and trying to apply them without serious consideration as to their applicability. In fact, the statistics of surface sampling should be one of our greatest challenges. Where should a sample be taken from a floor in a 100 square foot room? How many samples should be taken to yield a representative picture? How often should these samples be taken? How can we justify a bacteriological standard for an area in which the contamination exists as discrete, non-uniform and dynamic entities?

In the laboratory we can contaminate a surface in a fairly uniform manner. In the field we dare not assume that we are studying similar phenomena until enough work is done and enough data are analyzed to support these assumptions.

Epidemiological significance of contaminated surfaces

This problem is broad enough to merit a discussion of its own. It is well beyond the scope of this brief presentation. It is nonetheless of fundamental importance to all of us, and is decidedly a fruitful area of future research. It needs the interdisciplinary approach of bacteriologists, medical and surgical staff, and sanitation technologists. We must ultimately, in an honest and objective manner, establish the significance of fomite borne contamination. Perhaps we are already doing too much in surface decontamination and should spend our funds and resources in other endeavors. On the other hand, perhaps we should be redoubling our efforts in this field and attempt to establish standards. But above all, we must know in which direction we are heading.

SELECTED REFERENCES

1. Angelotti, R. & Foter, M. J. A Direct Surface Agar Plate Laboratory Method for Quantitatively Detecting Bacterial Contamination on Non-Porous Surfaces. Food Research **23**: 170-174. 1958.

2. Angelotti, R.; Foter, M. J.; Busch, K. A. & Lewis, K. H. A Comparative Evaluation of Methods for Determining the Bacterial Contamination of Surfaces. Food Research **23**: 175-185. 1958.

3. Buchbinder, L.; Buck, T. C.; Phelps, P. M.; Stone, R. V. & Tiedman, W. D. Investigations of the Swab Rinse Technique for Examining Eating and Drinking Utensils. **37**: 373-378. 1947.

4. Reddish, G. F. Antiseptics, Disinfectants, Fungicides & Sterilization. 2nd Edition, 1957. Lea and Febiger, Philadelphia.

5. Ridenour, G. M. The Use of Isotopes for Measuring Cleanliness of Surfaces. Modern Sanitation **4**: 61-63. 1952.

6. Walter, W. G.; Angelotti, R.; Armbruster, E. H.; O'Neill, R. D. and Tennant, A. D. Recent Developments in Determining Bacterial Surface Contamination. 87th Annual Meeting, Am. Public Health Assoc. Laboratory Section, Atlantic City, New Jersey. 1959.

7. Walter, W. G. Symposium on Methods For Determining Bacterial Contamination on Surfaces. Bact. Rev. **19**: 284-287. 1955.