Investigations of the Swab Rinse Technic for Examining Eating and Drinking Utensils*

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AT the Annual Meeting of this Association in 1943 the Subcommittee on Food Utensil Sanitation of the Committee on Research and Standards submitted a revision of the technic for the bacteriological examination of food utensils.[1] Suggestions and criticisms were invited with a view to making further modifications so as to get the technic in generally acceptable form for standardization.

The chairman of that subcommittee reports having received many suggestions, that ranged from one to the effect that the technic was not precise enough, to one that it was too complicated for ordinary use. The suggestions for changes in the technic emphasized the need for more factual information to determine the need for standardizing various details of the technic and the values to be set.

A series of studies was suggested to secure the required data which the members of the subcommittee were not in a position to undertake. The newly organized National Sanitation Foundation undertook to sponsor this work which has been done by the group submitting this paper. The work has not been completed, but enough has been accomplished to justify this report of progress. Further comments will be welcomed and given consideration in completing the work.

At the outset it should be stated that the purpose of this work is not to recover the greatest number of bacteria possible from washed and sanitized utensils but to develop the technic that is likely to reveal the presence of relatively few bacteria on utensils and to give the most nearly uniform results under all conditions in the hands of average laboratory technicians.

Each of the four participating laboratories undertook to run a series of critical tests of the various details of the technic, particularly those that have been questioned. Among these details are the time interval between swabbing and plating, the use of wood or wire applicator rods, the number of times each surface should be covered by the swab, whether the direction of each stroke in swabbing should be the same

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or reversed, the area of plates to be covered and the need for a template to measure such area. Other details are the buffering of the distilled water to be used and the type of agar. The possible effect of caps and the applicator sticks on the pH of the rinse and therefore on the count. Another point to be investigated is the time of survival of bacteria on utensils to be swabbed.

In order to secure soiled plates, cups, glasses, forks, and spoons for testing that would have similar numbers of organisms on them a procedure was adopted for soiling them. A quantity of water was collected from the wash water tank of a dishwashing machine in a restaurant after it had been used for an hour or more for washing dishes. A sample of this water was plated in order to get an approximation of the number of bacteria present while the main portion was being held in a refrigerator to lessen the growth of bacteria. After 24 hours, when an approximation of the number of bacteria present was obtained, the water was diluted to an extent determined by trial so that the desired number of bacteria were left on test utensils. Carefully cleaned and dried and sterilized plates, cups, glasses, forks, and spoons were submerged in this diluted dish water and then allowed to drain and dry.

In much of the work an effort was made to have the counts on these soiled utensils fall somewhere near the common standard of 100 per utensil as this is the critical range in which the greatest consistency is desired. Furthermore, the significance of differences in counts is more apparent in the lower range.

Buck found that wash water with a high detergent content, that is, with a pH above 9.6, produced colonies of the spreader type and was not suitable for this work. He also reports finding thermophylic organisms in the wash water and that the recovery of organisms from utensils soiled with high count wash water was surprisingly low. Stone found the use of soiled wash water from dishwashing machines to be unsatisfactory due to the difficulty in securing it without alarming operators, to variations from meal to meal in such things as fat content, and to the fact that alkalinity and temperature determined the type of organisms surviving. Stone also had trouble with thermophiles. Furthermore, the organisms in this soiled water did not adhere well to the utensils. Stone used cultures of coliform bacteria and staphylococci in an inert gum called carboxy methyl cellulose. This gave an excellent soiling medium that adhered well to the surface and yielded nicely to swab collection after soiled rims were inverted for several minutes in a pan of water.

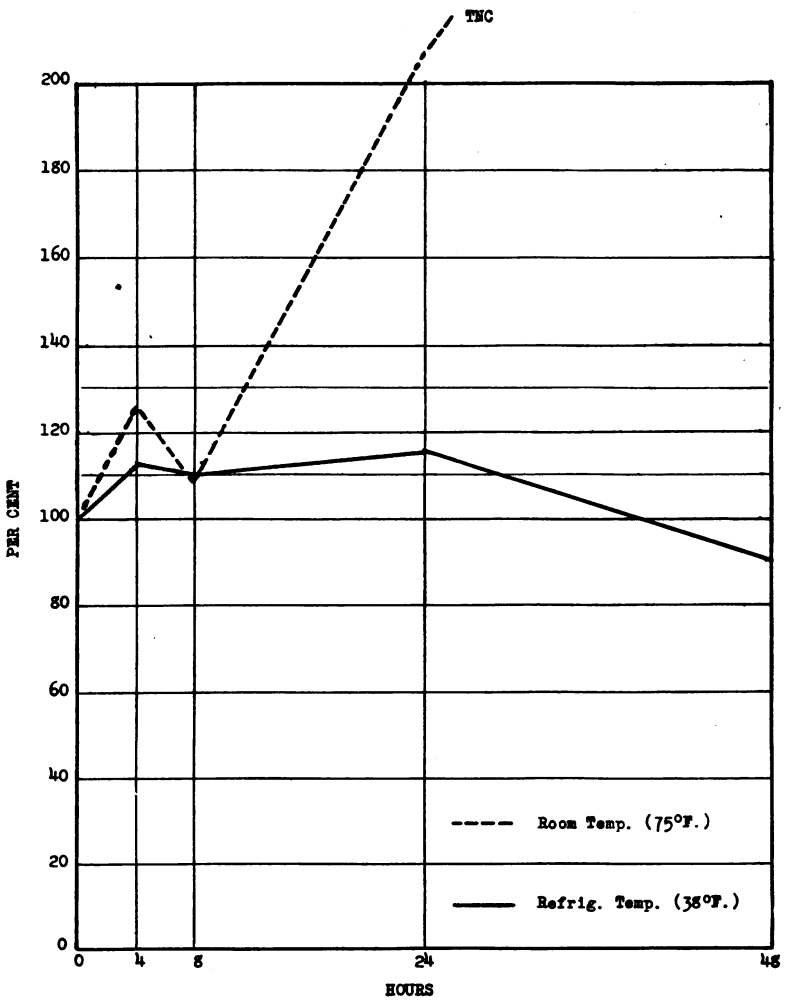
The proposed technic required the plating of samples of the rinse water within 4 hours after swabbing. This precludes the shipment of rinse samples to laboratories and limits the usefulness of the test. This provision was based on work which showed that a single species of bacterium died off in the rinse water. As has been indicated wash water from commercial dishwashing machines or a mixed culture of coliform organisms and staphylococci was used in making this test. These are organisms more likely to be found on utensils washed by machine than that used in previous tests. Generally thermoduric or thermophylic organisms predominate. The work of all the investigators on the delayed plating of such rinse water shows that when held at refrigerator temperature the count at the end of 24 hours did not vary greatly from that at the end of 4 hours. This was true whether the count was low or high. There were variations in individual tests. The differences between counts obtained by plating portions of any one sample at 4, 8, and 24 hours

are close enough, however, to make it unimportant when the plating is done within a 24 hour period. It is necessary that the sample be refrigerated because at about 75° F. growth was found to be profuse after 8 hours. Figure 1, prepared by Buchbinder, gives a summary of the results on all utensils of four experiments with rinse samples held at refrigerator temperatures and of three experiments with rinse samples held at about 75° F.

Considerable doubt has been expressed as to the length of time that organisms left on utensils during the washing and sanitizing processes will remain viable. In two experiments with low count utensils, Buchbinder found that plates stored unstacked in an open room showed a marked increase in count during the first 3 hours whereas all other utensils showed little variation during the first 24 hours. Three experiments with high count

FIGURE 1

EFFECT ON COUNT OF TIME OF PLATING RINSE SOLUTION IN PERCENTAGE OF INITIAL COUNT (BUCHBINDER)



dishes showed a similar increase in count on plates during the first 3 hours followed by a decrease to about the original number during the next few hours, and then a fairly constant status for 24 or 48 hours. Other utensils with high counts, however, showed a decrease in count during the first 6 hours, averaging about 70 per cent, which leveled for utensils stored in a closet. The low count utensils which are of special interest to enforcement officials, generally showed proportionally higher counts after storage than did those with original high counts. Comparative tests on utensils stored without stacking out of the direct sunlight in an open room and in a dark closet showed little difference in the general trend. Both high and low count plates, whether kept in a room or in a closet, increased in count considerably during the first 3 hours. However, high count plates stored for 24 hours gave within 30 or 40 per cent of the original count whether kept in a room or dark closet. Increases in counts on storage must indicate either deposition of air-borne bacteria or growth. Buck reports that as many as 300 organisms were deposited in 24 hours on an open agar Petri plate. However, he too found a considerable decrease in the number of organisms remaining on utensils after 24 hours.

In practice most utensils would be examined at the time of an inspection made between breakfast and lunch or between lunch and dinner or within 4 hours after they are washed. This work indicates that if a utensil were entirely free from organisms after being washed and sanitized and were stored in a clean place as required, it should not pick up more than the usually allowable 100 organisms per utensil from the air. If more should accumulate, however, the air-borne organisms would be objectionable since their presence would indicate improper handling. Furthermore, the rate of dying off of organisms, whether stored in a light or dark place, is not so great that a utensil with an initial count much greater than 100 is likely to show a satisfactory count after standing for 4 hours. Moderately high counts might change, however, to passing counts in this time.

The proposed technic specifies the use of wooden applicator sticks the lengths of which are not fixed. These sticks are broken off before shaking, which allows sufficient disintegration of the cotton swab during shaking. Some workers were reluctant to change from stainless steel wire to the use of wood for this purpose without more factual evidence of the need for changing. The use of cotton is specified without saying what kind of cotton, although this may make considerable difference. Tests were run using wooden applicator sticks and stainless steel wire ones. The cotton was removed from the wire rods with sterile forceps for a third comparison. In 59 tests on various utensils Buchbinder found that in 39 instances those tests made with wood applicators gave higher counts than the metal ones, the same count in 4 instances, and lower counts in only 16 trials. This constituted a significant difference in favor of wooden applicators. When the cotton on the metal applicators was removed with sterile forceps to permit more complete disintegration, the tests made with wooden applicators gave higher counts in only 29 instances, the same counts were obtained in 11 tests, while tests made with metal rods gave higher counts in 19 instances. This difference is not mathematically significant. However, the work of detaching the cotton takes considerable time and Buchbinder, therefore, favors the use of wooden applicators broken off before shaking. The work of the other investigators was in agreement and they also concluded that the use of wooden applicators is desirable. Buck and Kaplan [2]

have proposed the use of a simple sterile cutting device for this purpose. Stone points out that the flexibility of the wood in conforming to the shape of the utensil also is an advantage in making good contact with the whole surface to be swabbed.

Stone conducted some experiments designed to compare absorbent cotton, non-absorbent cotton, and glass wool as swab material. It is clear that the material used must perform the somewhat conflicting tasks first of snaring bacteria and then of releasing them as completely as possible in the rinse water. At best many organisms are retained in the cotton after shaking. Stone's work indicates that non-absorbent cotton gives the most uniform results of any of the three materials tested.

The proposed technic does not include a requirement as to the pH of the rinse solution after sterilization which appears to be of considerable importance. When bakelite caps on screw cap vials were substituted for aluminum ones Black found that some of these caps would not withstand sterilization and that many of them changed the pH of the sterilized rinse solution. Black found a source of mineral filled bakelite type caps which stood the test of withstanding sterilization without releasing any chemical that would affect the bacteria in the rinse solution. Similarly, Phelps reports that both applicator sticks and cotton in the swab may affect the pH of the rinse solution to increase the acidity of the rinse solution. He found a standard baby swab that had little if any effect on the pH. It may be necessary to specify materials and to require the spot checking of the pH of the rinse solution in vials after sterilization with the applicator and cotton in place.

The proposed technic provides for running the swab slowly and firmly 3 times over the surface to be examined. Questions have been raised as to whether the direction should be reversed between strokes and as to whether better uniformity might be obtained by increasing the number of strokes. In a series of two experiments with high count utensils and three with low count utensils Buchbinder found that counts increase progressively with the number of strokes. He found that with high count utensils the mean count with 5 strokes was about 60 per cent greater and with 10 strokes, 80 per cent greater than the mean count obtained with 3 strokes. Similarly on low count utensils the mean count with 5 strokes was approximately 20 per cent greater and with 10 strokes, 30 per cent greater than that obtained with 3 strokes. It also was found that with high count utensils (between 40,000 and 100,000), reversing the direction of strokes decreased the count in all tests, whereas with low count utensils (between 800 and 1,200), reversing the direction between strokes increased the count from 5 to 15 per cent. In covering an area of 4 square inches Buck recovered more than 3 times as many organisms using 10 strokes reversing directions between strokes as he obtained with 10 single strokes in a single direction. It is evident that further standardization of this detail of the method is desirable. It also is suggested that additional thought be given to making the areas swabbed on utensils of different types nearly equal within practical bounds.

Among the criticisms of the proposed technic that were received was one to the effect that the entire surface of plates should be swabbed. This point is to be investigated but the work has not been completed.

Another point criticised was that the use of metal templates, which require flaming between use, to measure a 4 sq. in. area was too laborious. It was suggested that an approximation would do as well. Buchbinder found that counts made by swabbing 4 sq. in.

by template and 4 sq. in. by guess were similar. He noticed a tendency to overswab by guess, but this had no serious effect on the results. Buck also reports little difference between counts on plates similarly soiled with results obtained by approximating the 4 sq. in. area and by fixing the exact area by template. In this connection if exact measurement should seem desirable the use of single service sterilized templates made of Kraft paper has been suggested.

Stone reports some work on the composition of the medium to be used in plating the rinse solution. The proposed technic specifies the use of standard tryptone glucose extract agar (without milk). Media suggested for investigation are the old nutrient agar consisting of 3 per cent beef extract, 5 per cent peptone and agar without dextrose, the same with tryptone and dextrose, the same with tryptose and dextrose, and the same with neo-peptone and dextrose.

Some work in the investigation of the composition of the buffered distilled water has been done and more is contemplated. Special attention is being given to the possibility of adding a neutral wetting agent.

SUMMARY

An important decision reached is that if the samples of rinse water are kept at 40° F. or less without freezing, plating at any time within 24 hours after sampling is satisfactory. This makes the test available to those who have found that the best time to collect samples at bars is during the evening rush hours and also to those who must ship samples to the laboratory. The work also shows that there are sound reasons for requiring the use of 3 inch long wooden applicator sticks broken off before plating; for specifying the use of non-absorbent cotton; for controlling the pH of the rinse water as ready for use, that is taking into account any effect of the cap, applicator stick, and cotton. Enough of the bacteria on dry utensils will persist for 4 hours or more, or for the usual interval between the morning and noon, and noon and evening meals, to make it possible to detect the use of ineffective washing and sanitizing processes. It is recognized that some deposition of airborne bacteria on such utensils is likely to occur.

It also is evident that increasing the number of strokes in swabbing increases the number of bacteria recovered from the surfaces of utensils, and it makes a slight difference whether the strokes are reversed or are all in the same direction. In other words this detail must be standardized. The work indicates that whether the area swabbed on plates is measured by template or approximated is not of great importance. The use of a template involves extra equipment and work. Also areas on cups and glasses are approximated.

The work is not completed but there is a good prospect of getting it done within the next year. Although the final determination of the medium to be used for this work may be influenced greatly by the media used in public health laboratories, for other work a farther comparison of media is desirable. Further standardization of a soiling medium for this work also is desirable as is more work on the composition of the buffered distilled water.

After the Subcommittee on Food Utensil Sanitation has reached a decision as to the details of the revised technic some critical work should be done to determine the degree of uniformity that may be expected in the use of the technic.

REFERENCES

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