Bacteriological Examination of Glassware or China for Sanitary Quality*

C. R. FELLERS, PH.D., F.A.P.H.A.,
A. S. LEVINE AND E. W. HARVEY
Massachusetts State College, Amherst, Mass.

THE time has come when something must be done about the insanitary conditions prevailing in connection with the dispensing of beverages. Both the public and the public health officer agree on this. Although the problem has been met in some places by the use of single service paper cups, it is common knowledge that glasses are only superficially washed or disinfected between patrons. Our personal observations are that in many places dispensing beer, the glasses are not washed at all. This is probably true to a lesser extent in other beverage dispensing establishments. Sporadic efforts have been made to remedy this situation in some cities. At best, such efforts have been considered only partially successful even by their sponsors. As Calver [1] has pointed out, there is a real opportunity to better control of respiratory diseases by means of improved sanitation of glasses and other eating utensils.

However, before we can expect the health officer or his inspectors to improve conditions very much, he must be provided with certain information. First, he must have a reasonably reliable method whereby he can determine whether glasses and dishes have been improperly handled; second, he must be able to offer definite constructive suggestions as to effective washing equipment and methods. We believe that the lack of positive information on these points has delayed the carrying out of a much needed program of education and compulsion in regard to the unclean utensil situation.

METHODS IN USE

A good list of technics for the bacteriological examination of glasses has been compiled by Calver.[2] These include various modifications of the swab method, rinsing, direct plating of utensils, direct microscopic examination after staining, and several others. The agar disc method, used by Olson and Hammer [3] to study churn contamination, was considered by us to merit some study as to its possible use in public health work. The agar medium is allowed to solidify. The agar disc is then picked up with a sterile spatula,

* Read at a Joint Session of the Laboratory, Food and Nutrition, and Public Health Engineering Sections of the American Public Health Association at the Sixty-fifth Annual Meeting in New Orleans, La., October 23, 1936.

Contribution No. 258, Massachusetts Agricultural Experiment Station.

placed in a sterile Petri dish and allowed to incubate. The colonies developing on a measured area are counted and the results expressed as number per sq. cm.

COMPARISON OF VARIOUS BACTERIOLOGICAL TECHNICS

In all, 18 different methods of sampling glasses and dishes were compared for utility, accuracy, speed, and simplicity.

A method to be of use to the busy health officer or his laboratory must be relatively simple. We realize that no method will give much information as to pathogens. Of the many organisms transmitted by the mouth secretions, only the diphtheria bacillus and the pathogenic cocci can be recovered with any degree of success. We must then turn to total counts to guide us in making a decision as to the sanitary quality of glassware and its likelihood of transmitting disease.

The agar disc method was too difficult to use, resulted in too many failures, and could be applied only to small areas of the glass. However, the recovery of bacteria artificially inoculated on glasses was approximately 50 per cent. The microörganisms used were *Serratia marcescens* and *Escherichia coli.*

The making of rim impressions of glasses and other containers on agar plates was tried many times. Both firm and soft agars were used. This method possesses the disadvantage of contacting only limited portions of the container. It is subject to contaminations, is not readily carried out in restaurants or drinking places, and results cannot be easily duplicated. Furthermore, colonies tend to be confluent, and counting is difficult.

Direct plating of pieces from broken glasses and cutouts from paper cups were investigated. This method is decidedly unsatisfactory from many points of view. Direct microscopic examination of broken glass rims, stained with various stains in an effort to make the bacteria visible, was almost a total failure. In the case of paper cups, the method failed to give good duplicate results and bacteria often failed to grow from the surfaces of the waxed paper, even though they had been artificially inoculated.

The many variations of the swab method will not be enumerated here. We tried swabs of different sizes with applicators of different lengths in test tubes containing broth, saline or distilled water. Different methods of swabbing, and a number of media were also tried out. In general, all swab methods were very practical, rapid, and simple. The recovery of bacteria was always over 40 per cent and often 75 per cent with *Serratia marcescens, Escherichia coli,* and *Streptococcus hemolyticus.* In mixed cultures used as inoculating suspensions, the percentage recovery was always less than when pure cultures were used. The many data obtained were used in the formulation of the standard method here proposed. They are too numerous for reproduction in this short paper. While the method in itself is not new and various parts have been previously described, we feel that a definite procedure if adopted by health laboratories for glassware examination will yield comparable results and will serve to give an excellent idea as to the sanitary condition of glassware.

PROPOSED STANDARD METHOD

SAMPLING

The method consists in preparing medium sized cotton swabs on wooden applicators immersed in approximately 3 c.c. of saline (0.8 per cent NaCl) in ordinary 25 c.c. culture tubes. The tubes are cotton stoppered with the

applicator sticks protruding slightly above the plugs. Sterilization is accomplished by autoclaving at 10 lb. steam pressure for 20 minutes. To avoid evaporation, storage should be at low temperatures.

Boxes or packets of these tubes are taken by the inspector who secures his samples as follows:

Remove the plug; lift the applicator above the surface of the liquid; press against the side of the tube and twist so as to squeeze out as much excess saline from the swab as possible. Remove the applicator and thoroughly swab the inside and outside lips and rims of glasses. Replace the applicator in the culture tube and insert the cotton plug. A number of glasses should be swabbed in each establishment in order to obtain a fair average. Tubes should be labeled and returned to the laboratory for plating within a few hours, preferably 2 hours.

PLATING OF THE SAMPLES

Upon arrival at the laboratory the tubes are agitated by holding firmly in the hand and striking 25 times. Remove the plug, squeeze the cotton swab as before to remove excess liquid, and discard. The liquid contents of the tube are poured into a Petri dish. There is actually only a very small amount of liquid retained by the swab. At this point, the swab may be used to inoculate Loeffler's blood serum slants in an effort to isolate pathogens.

Next 7 c.c. of nutrient agar (2 per cent agar) is poured in the Petri dish and mixed thoroughly. Incubation is at 37° C. for 48 hours. Counts are recorded for individual glasses and not as averages.

With slight modifications the above

TABLE I

TEST OF PROPOSED METHOD USED IN SOME EATING PLACES

Establishment	*Bacteria per Glass Individual Counts*	*Average*	*Method of*		
			Cleaning	*Rinsing*	*Drying*
Soda Fountain 1	3, 0, 2, 1, 9, 8, 1, 3	3	soap and hot water	hot water	metal racks
" " 2	3,900; 3,700; 670; 3; 20,700; 280	4,880	soap and tepid water	sozzle	shelf under counter
" " 3	2; 3; 580; 5; 120; 13,300; 9,300	4,720	dirty dish water	sozzle	metal racks
Restaurant 1	5,600; 360; 460; 3,300; 3,300; 2,100	2,520	dirty dish water	tepid water	dirty towel
" 2	150; 10; 45; 20; 23; 25	48	soap and hot water	hot water	clean towel and tray
" 3	134; 11; 400; 500	260	soap and hot water	hot water	dish towel
" 4	50; 15; 10; 8; 16	20	soap and hot water	hot water	dish towel
" 5	10; 12; 45; 9; 3; 4	14	steam washing machine	unnecessary	dish towel
Bar 1	2,800; 11,000; 50; 40; 160; 1,900; 20	2,280	warm water no soap	warm water	none
" 2	too numerous to count approximately 350,000	350,000	merely rinsed	cold water	none

method is easily adapted to the examination of dishes and silverware.

While occasional plates may be overgrown or colonies too numerous to count, the writers feel that plating the whole sample is better than making routine dilutions. At least, the results give an excellent idea as to the sanitary quality of the glassware examined. The method is also useful in the study of bacteriological effectiveness of mechanical dish washers and cleaning and sterilizing compounds.

Table I summarizes the results of a practical test of the proposed method used in some public eating and drinking establishments. A good correlation is shown between the care in cleansing and the number of viable organisms left on the glassware. These results should not necessarily be viewed with alarm, but they can be taken as good indications of the general sanitary conditions of the individual establishments. High counts should warrant more critical routine inspection by the health departments.

SUMMARY

A comparison of various methods for the bacteriological determination of sanitary quality of drinking glasses and eating utensils showed the swab method to be most satisfactory. From 40 to 80 per cent of the organisms present are recovered by this technic.

Specific directions are given for preparing sampling tubes, taking and handling samples, and recording results. These can be carried out at small expense in any health laboratory.

Practical tests of the method show it to be rapid, simple, and reliable. Health laboratories can use the method in a routine way to check cleansing and sterilization of utensils in food and beverage dispensing establishments.

REFERENCES

1. Calver, H. N. A Neglected Opportunity for the Control of Respiratory Disease. *A.J.P.H.*, 25:8:953 (Aug.), 1935.

2. Calver, H. N. Techniques for the Bacteriological Examination of Glasses. *Mimeograph.* The Public Health Committee of the Cup and Container Institute, 30 Rockefeller Plaza, New York, 1935.

3. Olson, H. C., and Hammer, B. W. The Agar Disc Method for Studying the Contamination from Metal Surfaces. Iowa Agri. Exper. Sta., *Bull.*, 300:323 (May), 1933.

Novel Purification Design

. . . The principal novel features of Burnt Mills, Md., water purification works pertain to the treatment unit assembly, containing a pipe vault, 4 filters, each of 1¼ m.g.d. nominal rating, a filter control house, a coagulating basin having a 55 minute detention period, and a 275,000 gal. filtered water reservoir. These are built of steel, cylindrical in form, and arranged concentrically. The flat continuous bottom of the structure rests on a thin concrete base lightly reinforced and the outside frame of the reservoir is surrounded by a loose rock collar to a height of 4′, beyond which there is earth fill. All seams are welded with the exception of the roof of the filtered water reservoir which has a thickness of 3/16″, all plating is 5/16″ thick. No deterioration of the steel work in the plant has been found at the end of approximately 1 year's operation. At the present time a second filter plant of 5 m.g.d. capacity, is under construction.—*Annual Report of the Bureau of Sanitary Engineering of the Maryland State Board of Health—Year 1935.*