Detecting Viruses on Nonporous Surfaces by Use of the Cotton Swab Technique

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ALTHOUGH standards of sanitation are currently predicated on bacterial assay of surfaces, bacteria represent only one group of disease-producing agents that may be transmitted via "eating utensils." A surface that has been sanitized by the application of various bactericidal agents may still present a health hazard from the viewpoint of viable residual viruses. It is well established that many bactericidal agents are not active against all viruses, consequently an eating utensil after treatment with such an agent may be relatively free of viable bacteria, while at the same time its virus population may remain unaffected.

This investigation was undertaken to determine the sensitivity of the conventional cotton swab-rinse technique in detecting viruses on nonporous surfaces. The efficient removal of viruses from such surfaces is considered to be an essential prerequisite for future studies in the sanitization of eating utensils from a virological point of view.

Little has been published on the precision of virus detection methods applicable to fomites. Parker and MacNeal (*1*), in their experiments on the persistence of influenza virus on the human hand, used a glass petri dish as a control surface. In an effort to remove the virus from both of these surfaces, they simply rinsed the sample areas with 0.4 ml. of saline and used this rinse as an inoculum for their test hosts. Their results were somewhat variable. Consequently, it is difficult to evaluate the sensitivity of this saline elution method.

Edward (*2*) studied the resistance of influenza virus to drying, using three different fomites: cloth, dust, and glass. Each material was soaked for 15 minutes in 5 ml. of nutrient broth, and the broths were assayed for virus activity. As one would anticipate, only a 10 percent recovery of virus was obtained from cloth and dust, while the recovery from glass was almost quantitative.

Chapman and Vinsel, in a paper presented at the 1956 meeting of the American Public Health Association, reported that the cotton swab method is of a high order of sensitivity in virus detection. However, their sensitivity data did not indicate the number of ID_{50} doses of influenza virus that were applied to the surfaces of the dishes before recovery by the swab was attempted. (An ID_{50} dose is that dose of virus which infects 50 percent of the test animals.) The other test virus employed by Chapman and Vinsel was bacteriophage T1. However, with bacteriophage T1, sample areas were simply estimated, not accurately demarcated.

While the work of Chapman and Vinsel did indicate the possibility of using the cotton swab for virus detection, it was felt that a more quantitative approach to the problem should be undertaken.

Materials and Methods

Two test viruses were used in this study: coliphage T1 and poliovirus type 1. Bacteriophage was selected as an indicator virus

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because it is easy to detect and its quantitative assay is relatively simple and accurate. The soft agar overlay method of assay (*3*) was used in all bacteriophage titrations. Poliovirus type 1 was arbitrarily selected as a representative of the pathogenic enterovirus group. Titrations of poliovirus were carried out in HeLa cell tissue culture tubes. The infectivity titers of the poliovirus were computed by the Reed and Muench (*4*) method, using five tubes per dilution. The results of these titrations are expressed in TCD_{50}, the dose of virus which gives rise to cytopathogenic changes in 50 percent of the inoculated tissue culture tubes. In every poliovirus titration the HeLa tubes were read every 3 days, with the final reading on the 10th day after inoculation. This type of titration is based on an all-or-none response.

The virus diluent used in all the phage experiments was a peptone broth of the following composition: Difco Bacto-peptone 0.5 percent, NaCl 0.5 percent, pH 7.2.

Contamination and Sampling of Surfaces

Bacteriophage T1. A 4-square-inch area was accurately ruled off on each of a series of clean, glazed pottery dishes and the dishes were autoclaved. Each sample area was flame seared with a bunsen burner and then contaminated with a measured volume of virus suspension of known titer. To each sample area 0.05 ml. of whole egg suspension (freshly beaten egg) was added and mixed with the virus suspension. Whole egg suspension was selected as a protein soil because it is one of the most adhesive of food soils when dry. This mixture of virus and egg soil was evenly distributed within the circumscribed sample area and allowed to air-dry for 1 hour.

Nonabsorbent cotton swabs moistened with 2 ml. of neutralizing phosphate buffer (Difco) were used to swab the sample areas. The composition of the phosphate buffer rinse was as follows: monopotassium phosphate, 0.0425 gm.; sodium thiosulfate, 0.1600 gm.; aryl sulfonate complex, 5.0000 gm.; sodium hydroxide, 0.0080 gm.; distilled water, 1,000 ml. The pH was 7.2. Each dish was swabbed four times with the same swab, which was rinsed and expressed into the phosphate buffer between swabbings. Each swabbing was at a right angle to the preceding one. Each swab was then immersed in its original tube, which contained 2 ml. of the phosphate buffer, and was shaken mechanically for approximately 1½ minutes. Aliquots of the phosphate buffer rinse were then titrated to determine the percentage of the contaminating virus that was recovered. The specific number of phage particles initially applied to each test surface area is shown in table 1. These values were determined by titrating aliquots of the contaminating phage suspension prior to applying measured volumes to the test surface areas.

Poliovirus 1. The techniques used in the experiments with poliovirus 1 were quite similar to those employed in the bacteriophage experiments, with the following exceptions: (*a*) HeLa cell tissue culture tubes were used for the virus titration; (*b*) both whole egg suspension and horse serum were used as a "sustaining medium"; and (*c*) Hanks' balanced salt solution (BSS) was used for the cotton swab-rinse instead of the neutralizing phosphate buffer. The latter was not used because of its toxicity for HeLa cells. A 0.1-ml. inoculum of the

Table 1. Recovery of bacteriophage T1 after drying 1 hour on a nonporous surface, using the cotton swab technique

Total virus particles [1]	Virus particles recovered	
	Number [1]	Percent
324,000	(2)	
32,000	(2)	
1,620	508	31
162	48	23
46	19	41
46	23	50
42	32	74
42	24	57
34	15	44
34	25	73
23	12	52
23	12	52
16	12	75
16	6	37
1.6	0	0
1.6	0	0

[1] Per 4 square inches of surface.
[2] Too numerous to count.

buffer solution produced a 4+cytopathogenic effect in 24 hours.

A notation relative to the toxic effect of neutralizing phosphate buffer on HeLa cells is believed to be of interest because the buffer rinse in question is routinely used in most sanitation studies employing the cotton swab-rinse technique. The toxic effect was observed when 0.1 ml. of the buffer was added to HeLa cell cultures containing 1 ml. of nutrient fluid. In order to eliminate bacterial contaminants, 400 units of penicillin, 400 μg. of streptomycin, and 200 μg. of mycostatin were used per milliliter of Hanks' BSS. In view of the lability of poliovirus to desiccation at room temperature (*5*, *6*), contaminated areas were sampled at time intervals of 5, 30, and 60 minutes.

In most of the poliovirus detection experiments using the cotton swab, a parallel series of experiments were performed concurrently, using a simple elution method. In the elution method, 1.5 ml. of Hanks' BSS was applied to the contaminated area and, after soaking for approximately 10 minutes, the area was gently rubbed with a ½-inch chisel-shaped rubber policeman. In all the elution experiments, the bottoms of glass petri dishes were used as the nonporous surface in order to contain the eluent. Subsequently, the eluent was pipetted off and assayed for infectivity titer.

In order to corroborate the preliminary data obtained in the initial poliovirus detection experiments using a constant inoculum, a series of clean, sterile, flame-seared glazed plates were contaminated with tenfold dilutions of poliovirus in order to determine the end point of virus detection.

In Situ Determination of Inactivation

In situ tests were performed to determine the rate of inactivation of poliovirus due to drying at room temperatures. The surfaces of sterile glass coverslips (22 by 11 mm.) were each contaminated with a measured volume of a poliovirus suspension of known titer and allowed to air-dry. At each of four different time intervals, five of the coverslips were placed in Leighton tubes, and versinated HeLa cells were added. A monolayer of HeLa cells would establish themselves on the coverslip, thus growing in intimate contact with the virus-contaminated surfaces. The presence of infective virus was evidenced by cytopathology of the HeLa cells.

Controls were set up to determine if whole egg suspension exerted any inhibitory or inactivating effect on poliovirus. Parallel serial dilutions of poliovirus were carried out in which the diluent in one series was Hanks' BSS and the other diluent was Hanks' BSS containing 20 percent whole egg. After standing at room temperature for 1½ hours, both series of dilutions were titrated; the titers were essentially the same.

Results

From table 1, it may be seen that the percentage of recovery of bacteriophage T1 by the swab-rinse technique is quite variable. The number of phage particles initially applied to the 4-square-inch test areas are given in column 1 of table 1. These values were determined by first assaying the inoculum used for each duplicate set of plates, using the soft agar overlay plaque count method (*3*). Knowing the number of phage particles contained in 1 ml. of each inoculum, we were able to compute the number of phage particles present in the 0.2-ml. aliquots that were applied to each test surface area. The percentage of recovery ranged from 23 to 75 percent, with a standard deviation of 16.8 percent. Table 1 does show,

Table 2. Percentage recovery of poliovirus 1 after drying 1 hour on a nonporous surface, using cotton swab and elution techniques

Recovery time	Poliovirus recovered by—			
	Cotton swab technique		Elution technique	
	Number [1]	Percent	Number [1]	Percent
Total	199, 000	100. 0	199, 000	100. 00
5 minutes	82, 320	41. 0	100, 960	50. 00
30 minutes	10, 096	5. 0	31, 846	16. 00
60 minutes	400	. 2	505	. 25

[1] TCD_{50} per 4 square inches.

NOTE: TCD_{50}—Virus dose which gives rise to cytopathogenic changes in 50 percent of the tissue culture tubes.

however, that the swab-rinse method is sensitive enough to detect a virus population as small as 16 particles per 4 square inches of surface.

Statistical analysis reveals, at the 95 percent confidence level, that the true mean recovery of T1 phage particles from nonporous surfaces by the cotton swab-rinse method lies between 40.3 and 61.1 percent.

Table 2 shows the recovery of poliovirus 1 at different time intervals using both the swab-rinse and the elution techniques. The total line in table 2 shows the number of TCD_{50} of poliovirus applied to each 4-square-inch test surface. These values were based on an assay taken from an aliquot of the inoculum used. In comparing the recoveries obtained by both methods, when sampling was done 5 minutes after contamination, when the inoculums were still wet, a difference of only 9 percent was reflected, whereas recovery at 60 minutes was essentially the same by both methods.

The chart shows the rate of inactivation of poliovirus 1 due to desiccation at room temperature. The viable residual virus present at the end of each time interval was determined by the in situ method described under "Materials and Methods." The amount of poliovirus recoverable at various time intervals indicates a logarithmic order of inactivation during the testing time. From the following formula

$$K=\frac{\log B-\log b}{\text{time}}$$

the death rate constant was computed to be 0.04.

Rate of poliovirus inactivation with desiccation

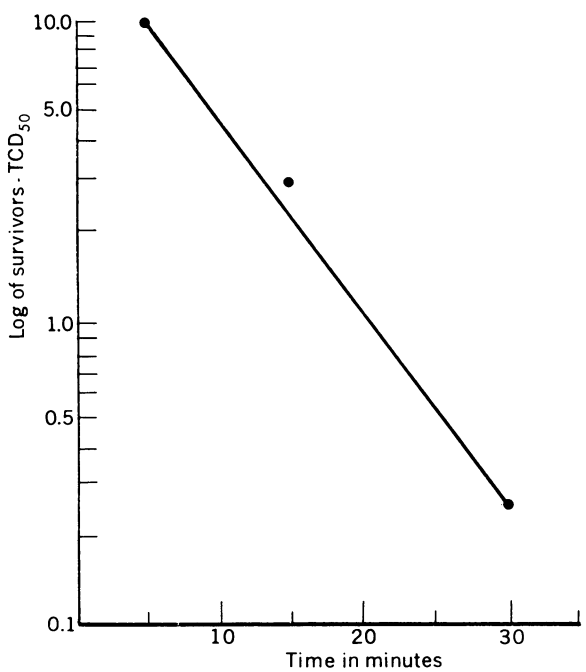

Table 3. Recovery of virus from plates contaminated with log decrements of poliovirus 1, using the cotton swab technique

Contaminated titer		Recovery of virus at—	
		5 minutes	
Number TCD_{50}[1]		CPE number tubes	Positive for virus
126,200		5/5	+
12,620		5/5	+
1,262		5/5	+
126		5/5	+
12		2/5	+
1		0/5	0
Number TCD_{50}[1]	Estimated TCD_{50}[1] after 1-hour drying [2]	60 minutes	
63,100	250.00	5/5	+
6,310	25.00	2/5	+
631	2.50	0/5	0
63	.25	0/5	0
6	.025	0/5	0

[1] Per 4 square inches.

[2] $K=\frac{\log B-\log b}{t}$; $0.04=\frac{4.800-x}{60 \text{ min.}}$

NOTE: TCD_{50}—Virus dose which gives rise to cytopathogenic changes in 50 percent of the tissue culture tubes.

CPE—Cytopathogenic effect.

Table 3 shows the sensitivity of the swab method in detecting poliovirus 1 on glazed plates contaminated with log decrements of the virus. The upper half of the table shows that a concentration of virus as small as 12 TCD_{50} per 4 square inches was detectable 5 minutes after the plates were contaminated. The lower half of table 3, which shows recovery of virus after 60 minutes of drying, reveals that the end point of virus detection occurs between contaminating inoculum titers of $10^{-2.8}$ and $10^{-3.8}$ per 4 square inches.

From the plate initially contaminated with 6,310 TCD_{50} per 4 square inches, the approximate amount of virus recovered was 0.1 percent. This is in the same order of recovery as shown by table 2, wherein 0.2 percent of the initial virus was recovered after 60 minutes of drying.

Discussion

In this preliminary study, two test viruses were employed: bacteriophage T1, a bacterial virus; and poliovirus 1, an animal virus. The two agents differ not only in morphology and size but also in their rates of inactivation during desiccation. It was believed that the phage virus would serve as an ideal indicator virus because of its stability and the fact that it can be titrated with a relatively high degree of accuracy. Under ideal conditions, phage has an infectivity ratio of one, which means that it may be assumed that a single plaque represents one phage particle.

In the phage experiments, all contaminating soils were permitted to air-dry in order to simulate conditions one would encounter in field sampling of "mechanical vectors." In sampling dried (1 hr.) contaminated nonporous surfaces with the swab-rinse method, a marked difference in recovery is observed between bacteriophage and poliovirus 1 (50 percent and 0.2 percent, respectively). The data indicate that this disparity is primarily due to the rapid inactivation of poliovirus by desiccation, rather than the inability of the cotton swab to "pick up" the poliovirus. This is evidenced by the following:

- When the test surfaces were sampled only 5 minutes after contamination, approximately 50 percent of the poliovirus was picked up by the swab.
- The percentage of poliovirus recoverable at various time intervals by both the swab and elution techniques approaches a logarithmic order of inactivation.
- The death rate of poliovirus 1 as determined by the in situ method indicated a logarithmic order of inactivation.

In the first phase of this investigation, the elution method was run in parallel with the cotton swab technique to compare differences in recovery of virus particles. In the second phase, however, it was decided to use the cotton swab-rinse technique only. This decision was based on the following reasons: (*a*) In view of the fact that only a slightly higher recovery of poliovirus was obtained with the elution method, it seemed reasonable to assume that the small difference in recoveries between the two methods was not statistically significant; (*b*) even if we assumed that the difference in recovery obtained by the two techniques, swab versus elution, represents a real difference, the versatility of the swab method in field sampling of irregular surfaces, such as forks, spoons, glasses, and so on, would compensate for the small loss in percentage of recoveries.

It is interesting to compare the results obtained in this study with the data reported by Angelotti and co-workers (*7*) in the detection of bacteria. In the current study, the mean value of phage virus particles recovered by the cotton swab-rinse method was 50 percent, with a 95 percent confidence interval of 40 to 61 percent, and the mean value obtained by Angelotti for the recovery of the test bacteria *Micrococcus pyogenes* was 52 percent, with a 95 percent confidence interval of 42 to 61 percent. It is to be emphasized, however, that the foregoing comparison of percentages of recovery is computed on the basis of the titers of the contaminating inoculums that were initially applied to the test surfaces. Angelotti used an in situ method, direct surface agar plate, for determining the number of bacteria present on the test surface prior to swab sampling.

Summary

A preliminary study was performed to determine whether the standard cotton swab-rinse technique for detecting bacterial contaminants could also be used for the recovery of viruses from nonporous surfaces. The results indicated that, under the experimental conditions described, the percentage of recovery of bacteriophage T1 virus approximates the precision obtained in detecting bacterial contaminants.

The data show that the cotton swab-rinse method is sensitive enough to detect virus when as few as 16 particles are initially applied to a 4-square-inch test surface area and air dried for 1 hour.

As few as 12 TCD_{50}, the virus dose which gives rise to cytopathogenic changes in 50 percent of the tissue culture tubes, of poliovirus 1 per 4 square inches were detected, using five HeLa cell culture tubes per sample, when sampling was done 5 minutes after the test surfaces were contaminated.

When the test surfaces were contaminated and then allowed to air-dry for 1 hour, the end point of poliovirus detection occurred between contaminating inoculum titers of between 6,310 TCD_{50} and 631 TCD_{50} per 4 square inches.

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Wider Use of Research Findings Urged

"The Costly Time Lag," a publication containing facts about the unnecessary loss of life, health, and money that results from failure to use new research findings has been released by the Public Health Service.

From estimates based on the U.S. National Health Survey and other data collected by the Service, the publication states that:

- 40,000 cancer deaths which could have been prevented occur every year.
- 40 million children are growing up without the protection from tooth decay afforded by fluoridated water.
- 20,000 persons die each year from preventable attacks of rheumatic fever and rheumatic heart disease.
- 60,000 new cases of tuberculosis occur every year although ways of preventing its spread are well known.
- 17 million persons have some type of chronic disability and a high proportion fail to receive care that could reduce handicapping effects.

The publication is designed to encourage wider use of the health-saving knowledge that has resulted from the findings of medical research during the past two decades.

The publication (PHS Publication No. 813) is on sale by the Superintendent of Documents, Government Printing Office, Washington 25, D.C., at 20 cents a copy.

Federal Publications

The Arden House Conference on Tuberculosis. *PHS Publication No. 784; 1960; 68 pages; 45 cents.*

Last fall, the National Tuberculosis Association and the Tuberculosis Branch of the Public Health Service held the Arden House Conference on Tuberculosis at Harriman, N.Y. Eighteen distinguished panelists were invited to discuss ways to use present knowledge to hasten the decline of tuberculosis in this country.

This volume presents the conference recommendations, a summary of the deliberations, and information about tuberculosis in the United States, which spells out in detail some aspects of the epidemiology of the disease.

Your Children and Their Gangs *Children's Bureau Publication No. 384; 1960; by Edith G. Neisser and Nina Ridenour; 34 pages; 20 cents.*

Directed to parents, this booklet is intended to explain why youngsters will give their greatest interest and even loyalty to a gang or club at some periods and seem to become almost strangers in their own homes.

It discusses the meaning of groups, how they help children grow, what is meant by the group "code," and the forms which groups may take. Constructive ways in which parents can express their interest in children's activities are also outlined.

Indians on Federal Reservations in the United States. A digest. Phoenix Area. *PHS Publication No. 615, pt. 6; 1961; 58 pages.*

This is the sixth in a series of digests of selected information about Federal Indian reservation groups which for health purposes are under the jurisdiction of the Division of Indian Health, Public Health Service.

The digest covers Indian groups in the Phoenix Indian Health Area, comprising Arizona, California, Nevada, and Utah. The subject matter includes a brief description of location, ownership, and topography of reservation land; population groups; social characteristics including homes, education, and income sources; and health services and health status.

VD Fact Sheet, 1960. Basic statistics on the venereal disease problem in the United States. *PHS Publication No. 341 (17th revision); 1961; 23 pages.*

Directed to public health specialists, physicians, and those concerned with statistics and data relevant to venereal diseases, this booklet presents up-to-date information on the incidence and prevalence of syphilis and gonorrhea.

It also discusses casefinding activities, mortality and insanity due to syphilis, and penicillin in the treatment of venereal diseases.

Statistical tables show figures for cases, costs, and treatment schedules.

Field Rodents. Public health importance and control. *PHS Publication No. 797; 1960; by Harold George Scott; 4 pages; 5 cents.*

A training aid, this leaflet lists diseases transmitted by field rodents and discusses recognition of rodents, population dynamics, and surveys. Shipping specimens for identification, rodent and ectoparasite control, and trapping and excluding rodents are also covered.

Instructions for field sanitation, use of chemical exterminators, and avoidance of hazards are given.

A Report on Social Security Programs in the Soviet Union. *Social Security Administration Publication (unnumbered); 1960; 157 pages; $1.*

Economic, social, and demographic backgrounds, development, and financing of the social security system are described. Outlines of provisions and administration of the general pension system are given. Personal, service, war, cash sickness, maternity, industrial injury, and disease pensions, and determination of disability for various disablement programs are discussed.

This report also covers family allowances, social security on collective farms and for producers' cooperatives, statistical data and analysis of the social security system, and welfare services and assistance.

The training of social security personnel and banking, insurance, and other aspects of economic security are considered. Health conditions and fertility analysis, opinions and views of the populace on social security, and related matters are reported on.

A Study of Student Nurse Perception of Patient Attitudes. *PHS Publication No. 769; 1960; by Rena E. Boyle; 77 pages; 45 cents.*

Methods and findings of a 2-year research project on the ability of student nurses to recognize which hospital nursing services are most important to their patients are reported. Also included is a description of the prior pilot study conducted to develop appropriate study methods and techniques.

Data are given for questionnaire or interview responses by 386 undergraduates of 7 schools of nursing and their 9 affiliated hospitals and 290 patients selected by the students as those they "knew best."

This report will be of interest to nurse educators and others engaged in planning and developing curriculums for nurses.

This section carries announcements of new publications prepared by the Public Health Service and of selected publications prepared with Federal support.

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