R. H. 1954a The study of factors which influence metabolic reactivation of the ultraviolet inactivated *Escherichia coli*. J. Bacteriol., **67**, 511–522.

HEINMETS, F., TAYLOR, W. W., AND LEHMAN, J. J. 1954b The use of metabolites in the restoration of the viability of heat and chemically inactivated *Escherichia coli*. J. Bacteriol., **67**, 5–12.

LAWRENCE, N. L., WILSON, D. C., AND PEDERSON, C. S. 1959 The growth of yeasts in grape juice stored at low temperatures. II. The types of yeast and their growth in pure culture. Appl. Microbiol., **7**, 7–11.

NELSON, F. E. 1943 Factors which influence the growth of heat-treated bacteria. I. A comparison of four agar media. J. Bacteriol., **45**, 395–403.

PEDERSON, C. S. 1936 The preservation of grape juice. I. Pasteurization of Concord grape juice. Food Research, **1**, 9–27.

PEDERSON, C. S. AND TRESSLER, D. K. 1938 Flash pasteurization of apple juice. Ind. Eng. Chem., **30**, 954–959.

PEDERSON, C. S., ALBURY, M. N., WILSON, D. C., AND LAWRENCE, N. L. 1959a The growth of yeasts in grape juice stored at low temperatures. I. The control of yeast growth in commercial operation. Appl. Microbiol., **7**, 1–6.

PEDERSON, C. S., WILSON, D. C., AND LAWRENCE, N. L. 1959b The growth of yeasts in grape juice stored at low temperatures. III. Quantitative studies on growth of natural mixed inocula. Appl. Microbiol., **7**, 12–15.

ROBINSON, J. F. AND HILLS, C. H. 1959 Preservation of fruit products by sodium sorbate and mild heat. Food Technol., **13**, 251–253.

In-Use Testing of Bactericidal Agents in Hospitals[1]

RUTH B. KUNDSIN[2] AND CARL W. WALTER[3]

Peter Bent Brigham Hospital, Harvard Medical School, Boston, Massachusetts

Received for publication July 5, 1960

The valid test of a germicidal product formulated for hospital use is its bacteriologic evaluation in the hospital under actual conditions of use. Bacteriologic monitoring of hospital floors, air, equipment, furniture, and textiles with quantitative techniques must be done continuously to capture the shifting mobile of their bacterial population.

MATERIALS AND METHODS

Air. Two types of air cultures are of value—volume air cultures and fall-out or settling plates. Both types are used to determine properly the number of bacteria-laden particles in suspension as well as the number settling out on surfaces.

Volume air cultures are made with the Wells air centrifuge, the slit sampler, or the Millipore filter. Other sampling devices are available (Public Health Service, Public Health Monograph No. 60); however, devices which collect organisms into a liquid which subsequently is plated out break up aggregations of bacteria and, therefore, do not reveal the number of bacteria-containing particles in the air; nor can the size of these particles be determined.

The Wells air centrifuge and the slit sampler are comparable and more efficient in recovering fine particles than other devices (Wells, 1955). The Millipore[4] filter (type AA) has an average pore size of 0.80 ± 0.05 μ; but, unlike the other two machines, it traps organisms in an air stream throughout the period of sampling, and care must be taken to prevent the loss of bacteria due to excessive drying. Each of these devices is portable and convenient to operate. Selective media can be used for isolation of organisms of special interest.

Settling plates simply indicate the amount of fall-out and give no indication of the organisms that remain suspended. Since a Petri dish is approximately $\frac{1}{15}$ sq ft, a 15-min exposure represents the fall-out per square foot per minute. Settling plates can therefore give quantitative results of particles with a settling velocity of 1 ft per min or greater. The positioning of these plates in an open area is another important consideration because air currents, projecting shelves, or furniture may prevent representative collections.

The two types of air cultures when taken simultaneously can serve to give valuable information. The area count in terms of organisms settling out per square foot per minute, divided by the volume count, or density of organisms, per cubic foot, gives the settling velocity in feet per minute, which in turn serves to categorize dimensions of the particle (Wells, 1955).

Floor. Floor cultures are taken by a modification of the standard method for culture of dishes (Subcommittee on Food Utensil Sanitation of the Committee on Research Standards, 1944). A sterile swab dipped in 5 ml of Butterfield's buffer, containing an inactivator as necessary, is used to rub a 5-cm^2 area of floor, outlined by an aluminum template. The swab is returned

[1] Presented at the 1960 Annual Meeting of the Society of American Bacteriologists.

[2] Assistant in surgery, Peter Bent Brigham Hospital, Boston, Massachusetts.

[3] Surgeon, Peter Bent Brigham Hospital and associate clinical professor of surgery, Harvard Medical School, Boston, Massachusetts.

[4] Millipore Filter Corporation, Watertown, Massachusetts.

to the buffer, the tube is shaken vigorously, and 1-ml aliquots of the buffer are plated in duplicate. The plating medium can be a nutrient agar such as Trypticase soy or tryptic digest. After 48 hr incubation, the colonies are counted; the average of the two duplicate plates is recorded as the number of organisms per square centimeter of floor.

Floor cultures are useful in testing the efficiency of floor-cleaning agents and equipment. They also reveal problems which would otherwise be unrecognized. Contaminated waxes, floor brushes, and mops have been traced by this technique. It is recommended, however, that at least three cultures be taken from different areas of the floor in a room so that an extremely high count because of spillage of soil in one area will be appreciated as such in an otherwise sanitary room.

Textiles. Cultures of textiles should be done, ideally, by grinding up a definite-size or -weight piece of the fabric to be tested in sterile buffer and plating out the suspension in nutrient medium. Since this involves destruction of the article, the number of such tests is limited by hospital economics. A method which is rapid, quantitative, and does not damage the textile has therefore been devised. If done carefully, cultures can be made of linen at the patient's bedside with no objectionable soiling.

A flat-bottomed flask, or jar, with a diameter of 8 cm is wiped with a sponge saturated with 70 per cent ethyl or 60 per cent isopropyl alcohol. The flask is air-dried. The textile is stretched over the base and pressed firmly against the blood agar surface in a Petri dish.

Masks from surgery can also be cultured in this manner. Upon removal they are placed in individual paper bags, labeled as to time worn and by whom they were worn, and then sent to the laboratory; an impression plate is then made. The side worn toward the face can be determined by examination; this is the side tested. The advantage of this technique for culturing masks lies in its simplicity in identifying carriers of staphylococci and, even more important, in detecting prolific shedders.

TABLE 1

Bacteriology of operating room floors; 6-month study

Room	Product	Bacterial Counts per Square Centimeter	
		5 Organisms or fewer	10 Organisms or fewer
		%	%
1	Quaternary A	22	50
2	Iodophor	46	81
3	Synthetic Phenolic	54	82
4	Quaternary B with TSP	31	67
5	Quaternary B with TSP	50	75
6	Quaternary B	22	82

This technique can be refined further by measuring the area of the impression and calculating the bacterial population on the basis of organisms per square foot.

Since the introduction of germicidal soaps and rinses for the laundry, tests have been developed to evaluate this treatment. The most commonly used test is the zone-of-inhibition determination. Agar plates seeded with *Staphylococcus aureus* strain 209, preferably harvested from overnight growth on agar slants to eliminate protein introduced by broth cultures, are used as a base for the immersion of swatches of the treated textile. Control pieces with no treatment may be placed on the same plate. After incubation for 24 hr, zones of inhibition are observed and recorded. The size of the zone may be measured; however, this can vary depending on the number of organisms in the seeded agar, the thickness of the agar, and the degree of leaching of the product used in the treatment of the textile.

Another test, which is more revealing, consists of cutting identically sized pieces of the textile with a cork borer, placing equal amounts of a bacterial suspension, usually *S. aureus* strain 209, upon each piece. After regular, previously determined exposure times, the pieces are dropped into known volumes of inactivating solution, shaken, and plated out in duplicate. This records the die-away of the staphylococci on the treated textile. Untreated textile is tested simultaneously as a control. This method, though considerably more time-consuming, is more meaningful. Because an inactivating solution can be used, bactericidal and bacteriostatic results are differentiated. Plotting the logarithm of the surviving organisms against time yields more conclusive information than simply the presence or the absence of zones of inhibition.

Furniture, shelves, and lights. Quantitative bacteriologic tests are difficult to perform with precision on intricate surfaces. If furniture is carefully wiped with a clean cloth wrung out of germicidal detergent, small areas of 1 cm^2 will be sterile. A semiquantitative method of swabbing a 6-in-square (6 by 6 in.) area with 10 strokes, using a moistened cotton-tipped applicator and streaking it out on a blood agar plate, will reveal the degree and the character of contamination.

RESULTS

Five different germicidal detergents available for floor care were tested in the operating suite over a period of 6 months. Cultures were obtained in each operating room at 6:30 in the morning. The floors had been flooded with the germicidal detergents during the night (table 1). At the level of 5 organisms or fewer per square centimeter, a chi square test gave a statistically significant difference among the products ($P < 0.02$). The synthetic phenolic gave the best results, followed by the quaternary with trisodium phosphate (TSP), and then the iodophor. At the level of 10 or-

ganisms or fewer per square centimeter, no statistically significant differences could be calculated ($P = 7.5$).

Another method of statistical analysis called "ranking" (Moroney, 1953) was used. Daily evaluation was done; the floor with the lowest count for the day was given the rank of one while the highest count of the day was given the rank of six. According to this method, the best rank for the 6-month test was calculated for the synthetic phenolic; the second, for the iodophor. Quaternary B with trisodium phosphate rated third and fifth, and plain Quaternary B rated fourth; plain Quaternary A, sixth. These results were statistically significant in that the ratings showed a significant agreement from day to day for each germicide tested.

To eliminate the criticism that perhaps the bacteriology of the six operating rooms was inherently different, a synthetic phenolic was used in all the rooms. Again, the ranking analysis applied. The results showed no statistically significant difference among the rooms.

Another floor product that has been tested is germicidal floor wax. A 14-bed surgical ward was flooded with a nongermicidal detergent. The waxes to be tested were applied in bands alternating with nonwaxed areas.

Three cultures were taken on each band—right, left, and center—to determine whether any residual effect could be detected. The floors were dry-vacuumed daily so that wetting of the floor was eliminated as a factor in spreading or abetting the action of the germicide. Two such experiments conducted in this ward, one for 10 days and the other for 17 days, did not yield statistically significant results. It was then decided to use the corridor for testing to eliminate contamination from infected patients that might occur in some test areas in the ward. The corridor would still reflect the bacteriology of the hospital wards (4000 pairs of feet were counted passing through in 24 hr). One experiment conducted for 17 days and another for 27 days, with daily cultures, did not yield statistically significant results among the bare floor, a number of waxes containing a germicide, and a wax with no germicide. The sole apparent advantage in using waxes with germicides is the prevention of contamination of the wax during storage. Waxes heavily contaminated with gram negative rods have been found. This was detected during routine floor monitoring when cultures of a

TABLE 2

Bacteriology of the wash cycle (April 29, 1959)*

Process	Organisms per Ml		Temperature	Water Level
	Fill	Dump		
			F	*in.*
1st Suds	1	0	137	5
2nd Suds	0	1	141	5
1st Rinse	1	0	146	10
2nd Rinse	1	0	110	10
3rd Rinse	4	1	135	10
4th Rinse	4	4		10
Germicidal textile lubricant†	0	0		5

* Three hundred pounds of mixed, unsorted hospital linen.

† Germicidal textile lubricant used since April 25, 1959.

TABLE 3

Bacteriology of the wash cycle (April 24, 1959)*

Process	Organisms per Ml		Temperature	Water Level
	Fill	Dump		
			F	*in.*
1st Suds	4	520	138	5
2nd Suds	110	590	142	5
1st Rinse	38	440	149	10
2nd Rinse	73	460	152	10
3rd Rinse	90	360		10
4th Rinse	66	560		10
Sour†	390	430		5

* Three hundred pounds of mixed, unsorted hospital linen.

† Germicidal lubricant had not been used since March 23, 1959.

TABLE 4

Die-away of Staphylococcus aureus strain 209 on treated textiles

Time	Textile A	Textile B	Control; Home-Laundered Sheeting
hr			
0	360,000*	360,000*	360,000*
2	0	50,000	80,000
4	0	700	93,000
6	0	0	96,000

* Represents the organisms recovered from the control piece of textile at 0 time.

TABLE 5

Patient (N. W.) maintained in isolation technique for exclusion of organisms

Date of July, 1958	Volume Air* Organisms per Ft^3	Air† Fall-out per Ft^2 per Min	Floor‡ Counts per Cm^2
3	1.2	3.5	
8	0.7	1.1	1
10	1.2	3.3	4
14	1.1	2.7	2
17	2.4	5.3	2
22	0.9	2.4	2
25	3.9	6.3	5
29	0.2	1.9	1
31	1.8	5.1	4
Avg	1.5	3.5	2.6

* Volume air counts are an average of 9 to 23 individual air samples each day.

† Settling plate counts are an average of 4 plates.

‡ Floor counts are an average of 3 daily samples plated in duplicate.

freshly flooded and waxed floor gave too-numerous-to-count pour plates because of a pseudomonad. The contamination of a floor wax with *S. aureus* has also been described (W. C. Freeman, Personal Communication).

Impression plates of clean linen yield but 1 organism per plate or, extrapolated, 20 organisms per square foot. Clean blankets show the same bacteriology. Blankets are usually not exposed to temperatures high enough for bacterial destruction during the washing procedure, nor are they ironed or tumbled. For this reason they must receive either a presoak in germicide or a final rinse in germicide; they must also be protected from bacterial fall-out during drying. British workers (British Launderers' Research Association and the International Wool Secretariat, 1959) and a Melbourne hospital (T. A. Pressley, Personal Communication) have reported a boiling-water washing for woolens, using an anionic detergent at a neutral or slightly acid pH and have found no shrinkage after 200 such launderings.

Plate counts of the wash water during the laundry cycle indicate the degree of bacterial contamination of the incoming linen. A germicidal rinse will inhibit proliferation of bacteria in moist, soiled linen as it awaits laundering. The bacterial counts of the wash water will reflect this inhibition as well as the effect of temperature, pH, sour, and bactericidal rinse upon the organisms at various stages in the wash cycle. Table 2 shows the results of cultures made during a typical laundry cycle of linen which has been repeatedly washed with hexachlorophene-containing tallow soap and treated with a germicidal textile lubricant. Table 3 shows laundry-cycle bacteriology when no germicidal rinse was used for 1 month. Table 4 represents the results of an experiment in which uniform, circular swatches (12 mm in diameter) of treated pieces of sheeting are inoculated with *S. aureus* strain 209 and cultured by the pour plate method after inactivation.

The final and most important test of all germicidal products is in the resultant composite bacteriology of a room in actual use, a room which has been cleaned by spraying walls with germicidal detergent and squeegeeing dry, wiping furniture, and flooding the floor with germicidal detergent; a room in which the bedding has been sterilized with ethylene oxide and ventilation controlled by intake of filtered outdoor air, casual contamination by droplet nuclei from the hospital area being prevented by a curtain of ultraviolet light over the door. Patients can be, and have been, kept in rooms with the bacteriology shown in table 5.

Operating room bacteriology is comparable. Eighteen operations were followed from the preparation of the patient to the closing of the wound. The average air sample showed 5 organisms per cubic foot of air with a fall-out of 4 bacteria-carrying particles per square foot per minute. This bacteriology characterizes the most aseptic environment achieved in a hospital with 12 changes of air an hour, of which 8 per cent is recirculated through an electrostatic air cleaner.

Ward bacteriology followed for a period of 4 years has shown that air counts of 20 or fewer organisms per cubic foot with a fall-out of 20 or fewer can be attained in an unventilated area by the use of germicidal detergents for floors, walls, furniture, and equipment; germicidal textile rinses; and training of personnel in techniques for reducing bacterial dissemination. Standards have been established on the basis of data collected in this 4-year study (Walter, 1958).

SUMMARY

The population of bacteria in any hospital environment is the resultant equilibrium of the numbers introduced by patients, personnel, and air currents and their removal by cleaning procedures and ventilation. If the methodical removal of organisms does not keep pace with the increments, bacterial counts rise. For this reason bacteriological monitoring is indispensable for evaluating adequate cleaning procedures.

Properly used effective germicides will show a statistically significant difference in bacterial counts on the surface on which they are used and will also lower volume air and fall-out bacterial counts by decreasing the number of organisms available for dissemination.

A hygienic environment can be defined by its bacteriology.

REFERENCES

British Launderers' Research Association and the International Wool Secretariat June, 1959 High temperature laundering of woollen hospital blankets, London, England.

MORONEY, M. J. 1953 *Facts from figures*, ch. 18. Penguin Books Ltd., Harmondsworth, Middlesex, England.

Public Health Service 1959 Sampling microbiological aerosols. Public Health Monograph No. 60.

Subcommittee on Food Utensil Sanitation of the Committee on Research Standards 1944 Progress report for 1943: A proposed method for control of food utensil sanitation. Am. J. Public Health, **34**, 255–258.

WALTER, C. W. 1958 Sepsis—a problem for every physician. Minnesota Medicine, **41**, 584–585.

WELLS, W. F. 1955 *Airborne contagion and air hygiene*. Harvard University Press, Cambridge, Massachusetts.