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MICROBIOLOGICAL SURVEILLANCE OF FOOD HANDLING AT NASA-MSFC

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16. ABSTRACT

A microbiological surveillance program of cafeterias and snack bars at MSFC was conducted to supplement the inspections by NASA Medical Center personnel and to gather information for cafeteria management to pinpoint areas of possible contamination. This report summarizes the work conducted under the program from its inception in January, 1972, to its termination on September 15, 1972. Ten food handling facilities were included in the surveillance at NASA-MSFC.

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TECHNICAL MEMORANDUM X- 64734

MICROBIOLOGICAL SURVEILLANCE OF FOOD HANDLING AT NASA-MSFC

INTRODUCTION

The Process Engineering Laboratory participated in a program for microbiological surveillance of food preparation and serving areas at MSFC. Ten buildings were included in the surveillance program; namely, 4200, 4202, 4708, 4712, 4471, 4487, 4610, 4663, 4666, and 4705. The program was initiated in January, 1972, and terminated on September 15, 1972. Two microbiologists established and carried out the program throughout its existence.

Bacteriological requirements for many hazardous foods, which consist in whole or in part of milk, milk products, eggs, meat, meat poultry, shellfish, shrimp, or other ingredients capable of supporting rapid and progressive growth of infectious or toxigenic microorganisms, are not well defined, except for dairy products. Specifications regarding the coliform content of drinking water are clearly stated in the Public Health Service Drinking Water Standards, but the criteria for other potentially pathogenic microorganisms are not well defined.

The quality of food or potable water can be affected by the presence of high levels of many microorganisms which are not considered to be of sanitary importance from the view of public sanitation. However, these organisms are known to cause food spoilage and reactions in some people because of personal idiosyncrasies to the organisms or their metabolic by-product.

The subject of food storage, spoilage, and handling is of great concern. Standard methods of the Food and Drug Administration and United States Department of Agriculture were used in the program for determining microbial quality of the food products. Results of such determinations were used by the food service company in establishing handling and storage techniques to reduce spoilage and minimize the possibility of food-borne infections.

Testing of foods for microbiological quality is exacting, and a microbiological test area, separate from areas used for routine work, is highly recommended by the Food and Drug Administration and the United States Department of Agriculture. For this requirement, a room approximately 6.1 m by 6.1 m (20 by 20 ft), equipped with a class 100 clean bench, fume hood, autoclave, sink, and incubator was used.

This report: (1) describes the plan for conducting the program, (2) gives the results of seven monthly inspections, and (3) summarizes the results of the seven monthly inspections. Included in the appendixes are: (1) an assay of can opener cutting blades, (2) a plan for culturing food, and (3) Appendix C, Survey of Environmental Surfaces for Pathogenic Microorganisms, which was not performed as part of the cafeteria surveillance program. However, because of its general interest as a related area of investigation, it has been included.

SECTION I. PLAN FOR MAINTAINING A MICROBIOLOGICAL INDEX FOR SANITARY UPKEEP OF FOOD PREPARATION AND SERVING AREAS AT MSFC

A. Introduction

The Replicate Organism Detection and Counting (RODAC) plate method of culturing is used for establishing, revising, or monitoring cleaning programs and for detecting and counting microbial populations. This method is especially useful for monitoring food containers, eating utensils, cafeterias, restaurants, hospitals, and other areas of potential contamination in the public interest.

This technique was used to determine:

1. If all areas were being cleaned properly.

2. If equipment and supplies were being maintained in a sanitary condition.

3. If the correct cleaning solutions were being used.

4. If personnel were following an adequate cleaning program.

5. If the incidence of specific disease-producing microbes was on the increase.

By using a selective culture medium in RODAC plates it was possible to detect the presence of microorganisms which are capable of producing food-borne illnesses.

B. Materials and Methods

Cafeterias, food preparation areas, equipment, and supplies were cultured using the RODAC method. Sets of RODAC plates filled with Trypticase Soy Agar plus Lecithin and Tween 80 were used for determination of total surface counts. RODAC plates filled with Mannitol Salt Agar were used for detection and enumeration of mannitol fermenting Staphylococcus aureus. Desoxycholate Lactose Agar or Eosin Methylene Blue Agar was used for coliform detection.

RODAC plates were prepared in the laboratory in an environment specified in the U. S. Pharmacopoeia, 17th Edition, for aseptic filling areas. The plates were incubated for 24 hours to check for sterility before use. Representative plates from each lot of culture media were inoculated with the organism or organisms which it is intended to detect, to check for the appropriate biochemical reactions as explained under RODAC culture media below. When the cultures and sterility results of each lot of culture media were found to be satisfactory, the plates were released for use.

A series of three plates, each filled with one of the above named media, was used for sampling for each area named below:

1. Floor in food preparation area
2. Floor in food consumption area
3. Dining tables
4. Food preparation counter
5. Food chopping blocks
6. Food preparation utensils
7. Eating utensils
8. Inside refrigerators

9. Food serving counters

10. Handsoap and handwashing area.

To initiate the program three locations (articles) were cultured to give a total of nine RODAC culture plates for each area. Information gained from this culture was used to begin a baseline sanitary index. Problem areas were identified and conclusions drawn as to possible solutions.

If, on the initial culture of a specific area, it was found that low bacterial numbers were present and staphylococci and Escherichia coli were not present, the number of samples taken from that area was reduced to one series of plates. This decreased the number of cultures taken by two-thirds.

C. RODAC Culture Media

1. Trypticase Soy Agar with Lecithin and Polysorbate 80. Trypticase Soy Agar was the general purpose solid medium used for the isolation of fastidious microorganisms. Total counts were made from this medium. Lecithin and Polysorbate 80 were included in the medium to inactivate residual disinfectant cationic surface material where the specimen was being collected.

2. Mannitol Salt Agar. Manitol Salt Agar was the selective medium for the isolation of pathogenic staphylococci. Selective inhibition was obtained by means of the high salt content, and mannitol fermentation was indicated by the phenol red, contained in the medium, changing from red to yellow around mannitol positive staphylococci.

3. Desoxycholate Lactose Agar. Desoxycholate Lactose Agar was used specifically for the isolation and enumeration of coliform organisms. Growth of gram-positive organisms was inhibited by the salts, sodium desoxycholate and sodium citrate. Coliform organisms formed pink or brick red colonies.

4. Eosin Methylene Blue Agar. Eosin Methylene Blue Agar was used for the isolation and differentiation of gram-negative enteric bacilli and was especially useful in the detection of coliform organisms which formed blue-black colonies sometimes with a metallic sheen.

Reading of RODAC plates was carried out as described in Figure 1. Where some areas were not adequately sampled by RODAC plates, such as corners or edges, the swab sampling technique shown in Figure 2 was used.

5. Coagulase Test. Coagulase activity was essentially confined to staphylococci and was related to the pathogenic species of staphylococci. Coagulase activity was demonstrated when a species of staphylococci capable of producing the enzyme coagulase was added to human or rabbit plasma, and a thrombus or clot was formed. (This procedure can be performed on a glass slide or in a test tube). Reagents included plasma, human or rabbit, and fresh or rehydrated physiological saline.

6. Slide Method. The slide method was performed as described in the following steps:

a. Place a drop of physiological saline on a clean glass slide and prepare a rather heavy even suspension of staphylococci in the drop of saline.

b. Place a loopful of fresh or recently reconstituted dehydrated plasma in the suspension of staphylococci. Mix and then withdraw the loop.

c. Immediately observe for the formation of a clot. This usually occurs within a few seconds with coagulase-positive species.

7. Tube Method. The tube method was performed as described in the following steps:

a. Transfer 0.5 ml of a 24-hour broth culture of staphylococci or transfer a large loopful of growth from an agar plate of staphylococci to 0.5 ml of human or rabbit plasma in a glass tube.

b. Incubate the tube at 37° C, preferably in a water bath for 3 hours. (Since a few strains do not clot within this period, the reading should be checked after 24 hours of incubation.) Observe approximately every 30 minutes for clotting.

8. Interpretation. Interpret test results in accordance with the following descriptions:

a. Pathogenic species of staphylococci usually give a positive reaction which is evidenced when the plasma is coagulated and produces a visible clot.

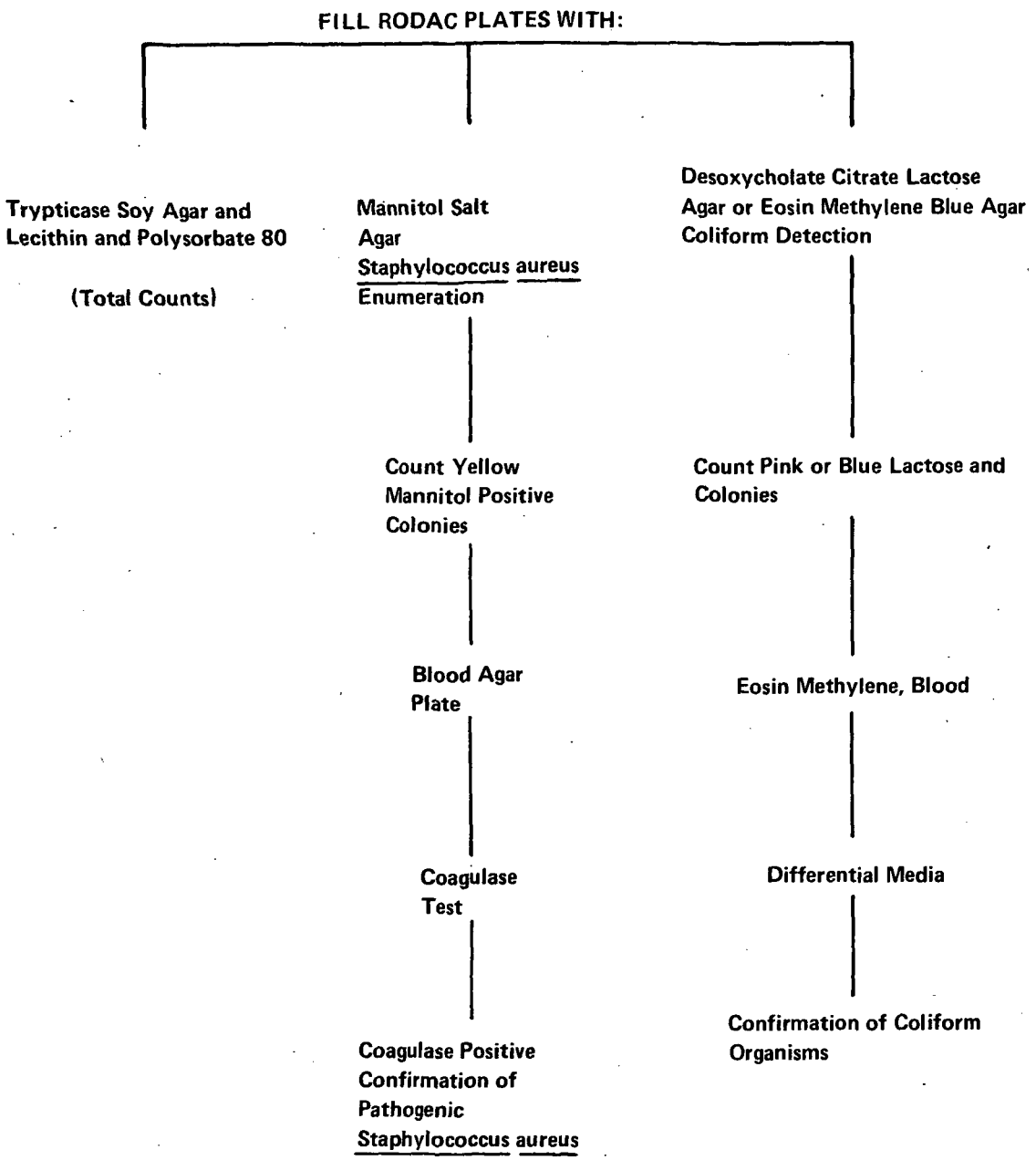
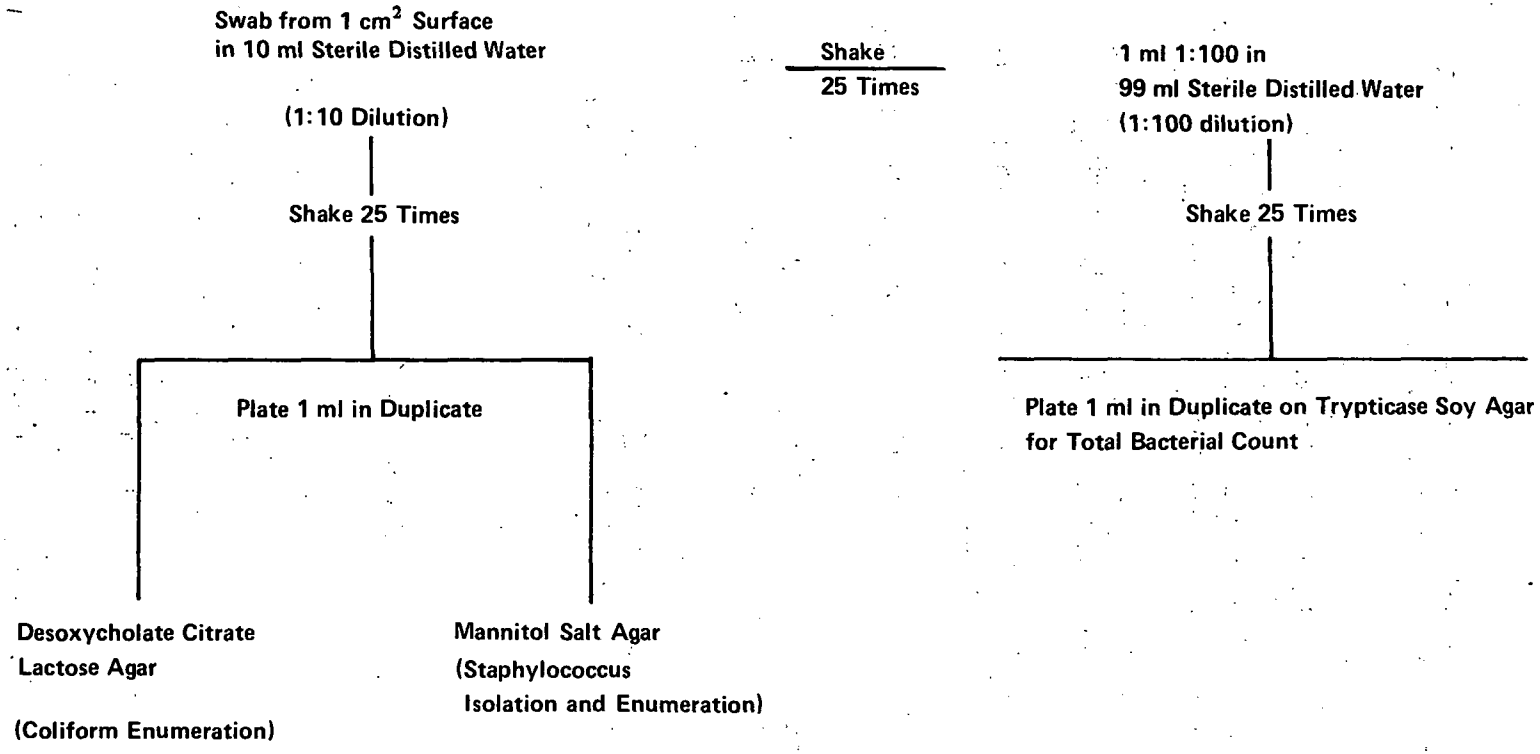


Figure 1. RODAC plate culture method.



Incubate all plates at 35° C for 24 to 48 hours before counting. Multiply 1:10 dilution counts by 10. Multiply 1:100 counts by 100 for reporting.

Figure 2. Culture protocol for sanitary survey of surfaces using swab sampling technique.

b. In negative reations, the suspension remains homogeneous and coagulation does not take place.

c. A positive reaction, whether it be on a glass slide or in a tube, is decisive. A negative slide test must be confirmed by a tube test.

Total colony counts are made by using a Quebec Colony Counter equipped with an electronic recording device. Colonies of gram-positive cocci found to ferment mannitol are transferred to blood agar plates and incubated at 35° C for 24 hours. The coagulase test is then performed by the slide and tube method to confirm the pathogenicity of the staphylococci.

The screening procedure for the detection of Escherichia coli in ice and determination of total bacterial count were accomplished as follows:

1. Approximately 30 grams of ice were collected in sterile, foil-covered, 200-ml beakers from each cafeteria.

2. Samples of ice were returned to the laboratory and placed in the 4° C refrigerator.

3. When the ice changed to water at 4° C, 1 mil and 0.5 mil were plated in duplicate in Trypticase Soy Agar pour-plates for determination of total bacterial counts.

4. For the detection of Escherichia coli, 10 mil from each sample were added to 10 mil double strength phenol red lactose broth fermentation tubes.

5. All cultures were incubated at 35° C for 24 hours.

D. Reporting of Results

There is no fixed standard for the total number of bacteria allowed on various environmental surfaces. Hospital surfaces are rated according to counts that have been tabulated over a period of time, using before and after cleaning bacterial count figures to establish a norm. In the care of food preparation areas and fixtures of sanitary importance, it is desirable to maintain low total bacterial counts by efficient cleaning and sanitizing practices. Also, the presence of coliform organisms and coagulase-positive Staphylococcus aureus should not be allowed, because the presence of these organisms can lead to serious food poisoning. Therefore, in assessing the sanitary quality

of food preparation areas, total bacterial numbers are of importance as a criterion for cleanliness. The presence of coliform or Staphylococcus aureus is of importance in determining the possibility of food-borne infections.

Results of each month's cultures were recorded. Observations and interpretations were reported and tabulated for inclusion in the final summary, Section XIII.

SECTION II. FEBRUARY MICROBIOLOGICAL SURVEILLANCE

The RODAC plate method of culturing was used for monitoring cleaning procedures and counting microbial populations. It was observed during the culturing procedure in each area that there was a large amount of accumulated food debris on floors, equipment and in unused spaces. This accumulated food provided a source of continuous bacterial contamination to the premises and also served as a breeding ground for insect infestation of foods.

This report describes culture and identification methods used for processing RODAC plate samples. A discussion of the significance of total microbial counts and the presence of Staphylococcus aureus, mannitol positive, coagulase positive, and Escherichia coli is included for general information.

A. Materials and Methods

Materials and methods for the sanitary surveillance of surfaces were the same as previously described in Section I.

B. Results and Discussion

Escherichia coli and mannitol positive, coagulase positive staphylococci were isolated. Culture results are shown in graph form in Figure 3.

These organisms were found most frequently on food preparation surfaces and wooden or plastic chopping blocks. It was noted in most cases sliced cold cuts or precooked meat patties were prepared or stored on these surfaces. The general practice in handling sliced cold cuts appeared to be to leave them out at room temperature until used. This practice gives rise to multiplication and spread of Escherichia coli or staphylococci or both, which may be present in this type food product.

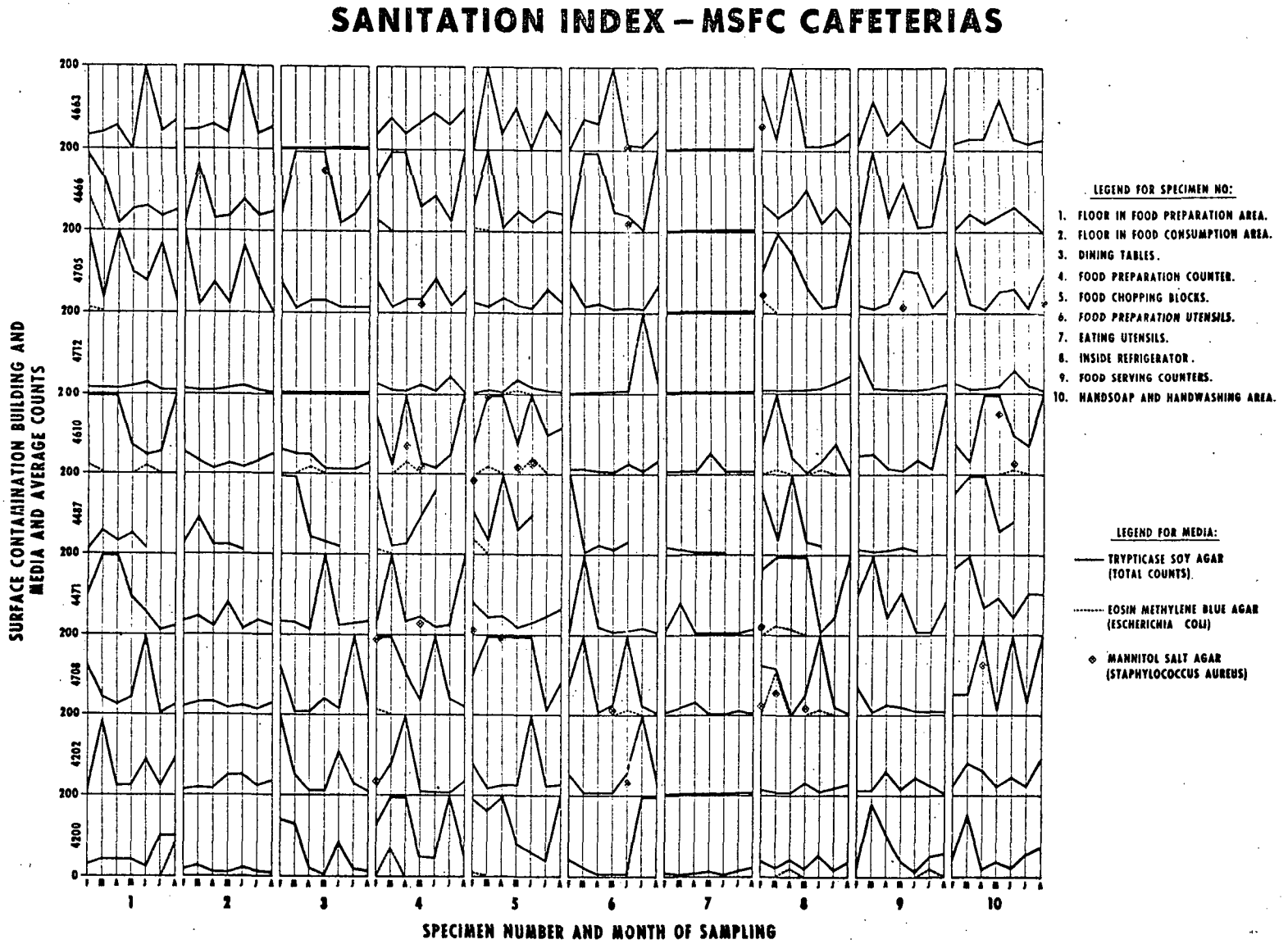


Figure 3. Sanitation survey sampling index.

Cultures made from the inside of opened precooked sausage and beef patty boxes grew staphylococci which may have been introduced after the boxes were opened. Usually precooked meat products contained less than 10 viable microorganisms per gram if properly cooked, stored, and handled.

It should be noted that from the ten cafeterias cultured Pseudomonas aeruginosa was isolated from the chopping block of one of the cafeterias. This microorganism is found on some vegetables and in the soil. Although it is not associated with food poisoning it is an opportunistic pathogen capable of producing personnel infections, and the presence of Pseudomonas aeruginosa is a sign of inadequate sanitation.

Organisms isolated from most of those table tops which showed high bacterial counts were from the group which is indigenous to the human skin and respiratory tract. These organisms may multiply rapidly on cleaning cloths which contain wiped up food and are allowed to "lay around" between uses. Some of these skin and respiratory tract bacteria were found on the raised splashboards behind sinks where it may be common practice to toss cleaning cloths after use.

SECTION III. RECHECK OF SURFACES IN CAFETERIAS IN BUILDINGS 4708 AND 4487

Cafeterias located in buildings 4708 and 4487 were recultured in areas previously showing the presence of Escherichia coli or Staphylococcus aureus. Other areas were cultured which were observed to present potentially hazardous conditions. During this recheck it was noted that potentially hazardous foods were being processed or stored in a manner which could easily give rise to the growth of toxigenic bacteria such as Staphylococcus aureus. This organism was found on food preparation surfaces on the February Microbiological Surveillance.

A. Materials and Methods

Materials and methods for the sanitary surveillance of surfaces were the same as previously described in Section I.

B. Results and Discussion

In most cases cultures from the building 4708 cafeteria showed a decrease in bacterial counts. The exceptions were the meat slicer which had a total count of 200 bacteria per RODAC plate. However, no staphylococci were isolated as there were in February. Coagulase positive staphylococci were isolated from a storage box containing precooked beef patties. Coliform organisms were also isolated from this box.

Cultures from building 4487 had a high incidence of Staphylococcus aureus which appeared to be associated with the food slicing procedure and cold roast beef.

Coliforms were found on raw, floured chicken storage trays next to the stove and on "clean" food preparation utensils. A large number of "clean" utensils were found to be wet, greasy to the touch, or laden with food debris. Coliforms were isolated from the exterior of the powdered milk storage can in this cafeteria also.

The table on which the meat slicer was stored contained a drawer. This drawer was heavily laden with old food scraps and crumbs. Cultures from the drawer yielded high counts of fungi and soil-borne bacteria, an indication of infrequent cleaning.

SECTION IV. MARCH MICROBIOLOGICAL SURVEILLANCE

Cultures were taken from surfaces of sanitary importance in the 10 cafeterias at MSFC. Results indicate that the microbial counts on surfaces can be significantly reduced by the use of more stringent cleaning methods.

Escherichia coli was cultured from the insides of some refrigerators and food preparation counters. Staphylococcus aureus was also found in some refrigerators. Both organisms were associated with salad making processes and cold roast chicken slicing.

A. Materials and Methods

Materials and methods for the sanitary surveillance of surfaces were the same as previously described in Section I.

B. Results

Culture results compared with those obtained in February showed that some areas appeared to have improved in the methods used to sanitize surfaces. Others show increased bacterial counts which offset some of the improvement. Culture results are shown in graph form in Figure 3.

SECTION V. APRIL MICROBIOLOGICAL SURVEILLANCE

The sanitary surveillance program which was conducted during the month of April indicated that in general food serving counters, floors, and dining tables were within quantitative and qualitative microbiological limits which indicated a sanitary condition. However, items such as the interior of refrigerators, meat slicers, and some wooden chopping blocks showed results that need a higher level of sanitation.

A. Materials and Methods

Materials and methods for the sanitary surveillance of surfaces were the same as previously described in Section I.

B. Results

RODAC culture results are given in graph form in Figure 3.

All samples of ice meet the requirements for pure ice except those from buildings 4471 and 4202. These two ice samples were taken from storage areas which exposed the ice to maximum patron handling. From the low counts and absence of Escherichia coli from the other eight samples, it appears that ice produced at MSFC cafeterias is suitable for human consumption and problems may arise from handling procedures.

SECTION VI. MAY MICROBIOLOGICAL SURVEILLANCE

A. Methods

Materials and methods for the sanitary surveillance of surfaces were the same as previously described in Section I.

B. Results

The sanitary surveillance conducted in the month of May indicated a continuing upgrading of the bacterial sanitary level in most cafeteria areas. In some instances bacterial counts were high (200 or more) on items such as tables which were visually clean but located under very dirty air conditioning ducts. Other areas of high counts were from places not in continuous use but used only infrequently for food storage. Culture results are shown in Figure 3.

SECTION VII. JUNE MICROBIOLOGICAL SURVEILLANCE

Escherichia coli continued to be isolated from the floor and interior of refrigerators in several cafeterias.

Staphylococcus aureus was recovered from the outside surfaces of mayonnaise dispensing pans in two snack bars.

Pseudomonas aeruginosa, an opportunistic food spoilage organism and potential pathogen, was isolated from refrigerators and food chopping blocks, indicating a possible situation for dairy product spoilage and general insanitation.

The floor of a mobile snack bar was cultured and the results indicated that unsanitary conditions existed in this area.

A. Materials and Methods

Materials and methods for the sanitary surveillance of surfaces were the same as previously described in Section I.

B. Results

Culture results showed no definite trends. Pseudomonas aeruginosa on environmental surfaces found during the testing was mostly likely connected with the advent of warm weather. This organism can cause rapid spoilage of dairy products and is mostly of economic importance in food work. Figure 3 shows the culture results in graph form.

Escherichia coli was found in the container of frozen shrimp in building 4708 as expected, since frozen seafood has been reported (in the literature) to contain high numbers of Escherichia coli and other enteric organisms.

Escherichia coli was isolated from the floor in building 4610 and was probably caused by the floor drains backing up into the kitchen. This situation was noted previously.

Coagulase positive staphylococci was isolated from the outside surface of bulk mayonnaise dispensing pans in buildings 4666 and 4663. This situation could lead to prolific growth of staphylococci in this potentially hazardous food product, since it is held and served at room temperature.

SECTION VIII. JULY MICROBIOLOGICAL SURVEILLANCE

A. Materials and Methods

Materials and methods for the sanitary surveillance of surfaces were the same as previously described in Section I.

B. Results

The cafeteria in building 4487 was undergoing renovation and was not sampled. All cafeterias and snack bars in general were found to be in a sanitary condition. It was noted in building 4200 that the mold count on the floor of the sandwich line was high. This was due to the large number of crumbs which had apparently been left there over the weekend.

High counts were obtained from the exterior surfaces of pans containing cooked meat, however no pathogenic bacteria were isolated. Culture results are shown in graphic form in Figure 3.

SECTION IX. AUGUST MICROBIOLOGICAL SURVEILLANCE

A. Materials and Methods

Materials and methods for the sanitary surveillance of surfaces were the same as previously described in Section I.

B. Results

Cultures of surfaces were made from nine cafeterias and snack bars. RODAC plates were used to detect the presence of Escherichia coli, Staphylococcus aureus and to establish total counts.

Escherichia coli was isolated from the kitchen floor in building 4200. It was noted that when trash cans were emptied, they were allowed to drip on the floor. Culture results are shown in Figure 3.

SECTION X. SUMMARY OF MICROBIOLOGICAL SURVEILLANCE FROM FEBRUARY 1972 THROUGH AUGUST 1972

This section presents the work performed during the 7-month microbiological surveillance program and discusses the results.

A. Materials and Methods

Materials and methods used for the program were outlined in Section I.

B. Results

The results of RODAC plate sampling during the period February 1972 through August 1972 from cafeterias and snack bars located in buildings 4200, 4202, 4708, 4471, 4487, 4610, 4712, 4705, 4666, and 4663 are shown graphically in Figure 3. Please note that the June surveillance was the last one performed in building 4487 because after that date the cafeteria was closed for remodeling.

In Figure 3 under the heading specimen number and month of sampling Escherichia coli was isolated during the 7-month period occasionally from floors in the kitchen of buildings 4200, 4610, 4705, and 4666. This was in part caused by improper floor drainage and also by allowing trash cans to drip on the floor after emptying. Erratic total counts from month to month were caused by differences in the time samples were taken during the day and the amount of traffic in the area. Floor counts and bacterial quality of areas 1 and 2 on the chart were generally acceptable.

Dining tables showed a general decrease in bacterial count toward the end of the sampling period. This may be caused by standardization of cleaning techniques.

Total bacterial counts on food preparation counters and chopping blocks fluctuated because of usage. However, the presence of Escherichia coli and Staphylococcus aureus is not desirable. More rigid cleaning and scraping techniques in these areas are needed.

CONCULSION

The Surveillance of Food Handling Program at MSFC provided information that led to improvement in the sanitation level of the food handling facilities. This was accomplished by designating areas where improvement was needed and disclosing methods for overcoming deficiencies. On an overall basis, rapid improvement was noted soon after the program was initiated. The sanitation level appeared to reach a plateau where it was maintained for the duration of the program.

APPENDIX A

BACTERIOLOGICAL ASSAY ON CUTTING BLADES OF CAN OPENERS IN VARIOUS BUILDINGS

Bacteriological assays were made from the cutting blades of can openers located in 11 MSFC buildings. During the process of obtaining culture samples from the openers it was noted that most of the cutting blades were coated with old grease and metallic particles. Culture results showed that the opener in building 4481 was contaminated by a coagulase positive Staphlococcus aureus. Thorough scrubbing in hot soapy water and a hot water rinse followed by drying in a 140° F oven for 2 hours was effective in decontaminating the can opener. This was verified by a followup culture.

A. Materials and Methods

Samples were taken from cutter blades on can openers in the buildings listed in Table A-1. The following procedure was used in taking samples:

1. A sterile cotton swab was moistened in a sterile 10-ml water blank identified by building number.

2. Each surface to be sampled, approximately 1 cm^2, was swabbed using a rolling motion.

3. The swab was reinserted in the same 10-ml water blank in which it was moistened and the upper part of the swab was snapped off. This gives a 1:10 dilution of the area which was sampled.

Samples were cultured immediately upon return to the laboratory, using the protocol described in Figure 1. A 1:10 dilution was made in the original sample tube. This dilution was used for coliform and mannitol positive staphylococcus enumeration. The 1:100 dilution was used for determining total bacterial count on 1 cm^2 of surface.

B. Results and Discussion

There is no fixed standard for the total number of bacteria allowed on various environmental surfaces. Hospital surfaces are rated according to

TABLE A-1. CULTURE RESULTS FROM MSFC CAN OPENERS AND OTHER SITES

Culture Results and Counts per cm^2				
Building Number	Trypticase Soy Agar	Mannitol Salt Agar	Desoxycholate Citrate Agar	Comments
4705	100	Zero	Zero	
4708	600	Zero	Zero	
4491	900	Zero	Zero	
4485	1000	Zero	Zero	
4481	500	410	Zero	Coagulase Positive Staphylococcus aureus
4250	600	Zero	Zero	
4207	800	Zero	Zero	
4207	300	Zero	Zero	Electric Opener
4483	700	Zero	Zero	
4487	400	Zero	Zero	
4487	600	Zero	Zero	Opener on Coke Machine
4663	900	Zero	Zero	
4561	800	Zero	Zero	
Tr 5 Complex	200	Zero	Zero	
4663	300	Zero	Zero	Second Opener
4481	Zero	Zero	Zero	After Washing and Scrubbing

counts which have been tabulated over a period of time using before and after cleaning bacterial count figures to establish a norm. In the care of food preparation areas and fixtures of sanitary importance it is desirable to maintain low total bacterial counts by efficient cleaning and sanitizing practices. Also, the presence of coliform organisms and coagulase positive Staphylococcus aureus should not be allowed because the presence of these organisms can lead to serious food poisioning. Therefore, in assessing the sanitary quality of food preparation areas, total bacterial numbers are of importance as criteria of cleanliness and presence or absence of coliform and Staphylococcus aureus is of importance.

Results shown in Table A-1 show that no coliform organisms were present on the surfaces cultured. The can opener blades in building 4481 had a low total bacterial count in comparison to most of the other openers cultured. However, coagulase positive Staphylococcus aureus was the predominant organism. This opener was brought to the laboratory, scrubbed with a brush and hot detergent, dried, and recultured. No Staphylococcus aureus was isolated. This indicates that a thorough cleaning with soap and water is a good means for maintaining the can openers in a sanitary condition.

APPENDIX B

SAMPLING AND CULTURING FOOD

A plan for sampling and culturing food from cafeterias at MSFC to supplement the index plan in Section I was outlined. Methods for culturing were the most recent published by the Food and Drug Administration and the U. S. Department of Agriculture.

A. Materials and Methods

1. Organoleptic Examination. The term "organoleptic" refers to the use of the senses in determining the fitness of a product. When questions related to spoilage arise, the organoleptic results are of primary importance; bacteriological results are corroborative and substantiating. Every sample or portion of every sample is examined as to appearance and odor. Any abnormality is noted and recorded on the laboratory report.

2. Bacteriological Examinations of Fresh or Prepared Foods. This procedure is applicable to the routine bacteriological examination of samples collected in Federally inspected meat and poultry establishments producing fresh, frozen, cooked, smoked, or dehydrated meats and poultry; refrigerated salads; prepared or pre-cooked products (such as pot pies, lunch meats, dinners, breaded cutlets, dehydrated soups, etc.); and the ingredients incorporated with meat and poultry products (such as spices, vegetables, breading milk powder, dried egg, etc.). Do not make a composite of the components of items such as frozen dinners into a single sample. Examine, as separate samples, the vegetable servings and meat servings deposited in separate compartments of the same tray. The examinations were as described in the following steps:

a. Examination of Fresh or Prepared Foods. The procedures for determining aerobic plate count and numbers of Staphylococcus aureus are the same as the American Organization of Agricultural Chemists (AOAC) procedures. The procedures for determining numbers of coliforms and Escherichia coli differ from the AOAC procedures as follows:

(1) Single tubes of Lauryl Sulfate Tryptose broth per dilution, rather than a three-tube minimum probability number (MPN), are used.

(2) Inoculated Lauryl Sulfate Tryptose and inoculated Escherichia coli broth are incubated 24 ± 2 hours.

(3) Gassing Lauryl Sulfate Tryptose and gassing Escherichia coli broth are considered positive for coliforms and Escherichia coli respectively, with no further testing.

b. Equipment and Materials.

(1) Quebec Colony Counter and Tally Register

(2) Balance; Capacity, 2k; sensitivity, 0.1 g

(3) Blender and sterile blender jars

(4) Sterile forceps, spoon, etc.

(5) Sterile 10-ml and 1- or 5-ml pipettes

(6) Incubator at 35 ± 1° C

(7) Water bath at 45.5 ± 0.5° C

(8) Water bath at 37 ± 1° C

(9) Simple stain reagents

(10) Standard Petri dishes, glass or plastic, sterile

(11) Desiccated coagulase plasma, rabbit

(12) Transfer loop, 3 mm

(13) Microscope

(14) Butterfield's phosphate diluent (20.1)[1]

c. Media. Plate Count Agar (20.2); Lauryl Sulfate Tryptose Broth (20.3); Escherichia coli Broth (20.4); Vogel-Johnson Agar (20.5); Brain Heart Infusion Broth (20.6); Trypticase Soy Broth with 10 percent Sodium Chloride (20.7).

1. Numerical references are paragraph numbers of the Bacteriological Analytical Manual, Food and Drug Administration.

d. Preparation and Dilution of the Food Homogenate. Whenever convenient, weigh portions of frozen samples without thawing so that the effect on microorganisms of thawing and re-freezing is avoided if a re-examination is necessary; otherwise partially thaw at 2 to 5° C for around 18 hours or by immersing water-tight sample containers in cold water for 1 to 2 hours.

Using sterile spoons, forceps, scissors, etc., weigh aseptically 50 g ± 0.1 g of the sample into a sterile blender jar. Add 450 g sterile diluent (20.1) and blend 2 minutes, to give 40 000 to 50 000 revolutions. This is the 10^{-1} dilution. Not more than 15 minutes should elapse from time sample is blended until all dilutions are in approporiate media.

If sample consists of less than 50 g, weigh about one-half the sample and add amount of diluent required to make the 1:10 dilution (nine times the weight of the portion of sample used). Total volume in blender jar must completely cover blades.

Pipet 10 ml of the blended 10^{-1} dilution into a 90-ml dilution blank for the 10^{-2} dilution. Repeat to prepare dilutions of 10^{-3}, 10^{-4}, etc. Shake all dilutions 25 times in a 1-ft arc. Use a separate 10-ml pipette to prepare each dilution.

Pipettes must deliver accurately required volumes. Do not deliver less than 10 percent of their volume. For example, to deliver 1 ml, do not use a pipette of more than 10-ml volume.

Hold reserve of samples in freezer at or below 5° F (unless the product is stored normally at ambient temperature) until it is determined that a repeat examination is not necessary.

e. Aerobic Plate Count. Pipet 1 ml from 10^{-2}, 10^{-3}, 10^{-4}, etc., dilutions, using separate sterile pipettes for each dilution. Use additional dilutions when expecting higher bacterial levels. Place 1 ml of appropriate dilution into each of duplicate petri dishes, and add molten plate-count agar (cooled to 45 ± 1° C in water bath).

Mix by swirling or tilting plates to disperse the inoculum throughout the medium. Incubate 48 ± 2 hours at 35 ± 1° C. Using the Quebec Colony Counter, count duplicate plates in a suitable range. If plates do not contain 30 to 300 colonies, record dilution counted and the number of colonies found. Average the counts obtained from duplicate plates and report aerobic plate count per gram. Report incubation temperature used.

f. Coliform Group and Escherichia Coli. Pipet 1 mil from the dilutions into a single Lauryl Sulfate Tryptose broth tube per dilution, using separate pipettes for each dilution. Begin with the 10^{-1} dilution. Maximum dilutions of sample must be sufficiently high to yield negative end point. Incubate 24 ± 2 hours at 35 ± 1° C for gas formation as evidenced by displacement of liquid in insert tubes or by effervescence when tubes are shaken "gently". Consider those dilutions of Lauryl Sulfate Tryptose producing gas to contain coliforms, and report coliforms per gram according to the highest dilution of gassing Lauryl Sulfate Tryptose. When a "skip" occurs, report by utilizing the "Phelps Index." For example, if the 10^{-1}, 10^{-2}, and 10^{-4} dilutions produce gas but the 10^{-3} dilution tube is nongassing, report "1000 coliforms per gram."

Transfer, using a 5-mm loop, from every gassing Lauryl Sulfate Tryptose tube to Escherichia coli broth tubes. Incubate Escherichia coli tubes 24 ± 2 hours at 45.5 ± 0.05° C in covered water bath. (To determine both psychrophiles and mesophiles, incubate a duplicate set of plates at 20 to 25° C for 4 or 5 days.) Submerge Escherichia coli tubes in bath so that the water level is above the highest level of medium in the tubes. Record every tube producing gas as evidenced by displacement of liquid in insert tube or by effervescence when tubes are shaken "gently". Report Escherichia coli per gram according to the highest dilution of gassing Escherichia coli, utilizing the "Phelps Index" when a "skip" occurs.

g. Coagulase Positive Staphylococci (Staphylococcus Aureus). Pipet 1 ml from the dilutions into a single Trypticase Soy broth (with 10 percent NaCl) tube per dilution, using separate pipettes for each dilution. Begin with the 10^{-1} dilution. Maximum dilutions of samples must be sufficiently high to yield negative end point. Incubate tubes 48 ± 2 hours at 35 ± 1° C.

Using 3-mm loop, transfer loopful from each growth-positive tube and from the next higher dilution tube to previously prepared Vogel-Johnson agar plates. Streak in a manner as to give well isolated colonies. Incubate plates 48 ± 2 hours 35 ± 1° C.

Pick two or more representative colonies, which have reduced tellurite, from all Vogel-Johnson plates showing growth to plate-count agar slants. Incubate slants 18 to 24 hours at 35 ± 1° C. Examine cultures microscopically (simple stain) and discard noncoccal forms.

Transfer asceptially 0.2-ml portions of Brain Heart Infusion broth into 13 by 100 mm test tubes and inoculate with "small" amount of growth from the plate count agar slants. Incubate Brain Heart Infusion broth 24 hours at 35 ± 1° C.

Just before use, reconstitute desiccated coagulase plasma according to directions on label. Add 0.5 ml reconstituted plasma to the Brain Heart Infusion cultures and mix thoroughly. Place tubes in 37 ± 1° C water bath and examine each hour for 4 hours for clot formation. Test known positive and negative cultures simultaneously with sample.

Record every dilution containing coagulase positive staphylococci. Report coagulase positive staphylococci per gram according to the highest dilution found, utilizing the "Phelps Index" when a "skip" occurs.

B. Reporting of Results

Total bacterial counts, coliform counts and Staphylococcus aureus counts were reported as number of viable cells per milliliter or gram of sample.

Results of organoleptic examinations were reported describing the physical appearance of the food and order if present.

APPENDIX C

SURVEY OF ENVIRONMENTAL SURFACES FOR PATHOGENIC MICROORGANISMS – BUILDINGS 4471 AND 4494

A study of possible human infection by disease causing fungi and bacteria was undertaken when workmen working in the ceiling of building 4471 uncovered birds nesting in the eaves, ventilation fans, and air conditioning ducts. Dust generated by the overhead work filled the air and covered most work surfaces. Feathers and feces from birds were found in the ceiling insulation.

It is known that the birds, especially pigeons and chickens, harbor resistant spores of disease-causing fungi and pass these into the environment in their feces.[2] Also feathers or nest material may harbor fungal spores. Dried feces mixed with dust particles can be an excellent means of transmitting fungal diseases and also bacterial diseases. Histoplasma, Coccidioides and Cryptoccoccus are some of the pathogenic fungi which were checked for by culturing. The means of infection by these fungi are not completely understood, so special care is needed in culturing these organisms. Each of these fungi can cause infection of the skin, lungs, and other parts of the body. Respiratory infections were of prime interest since the amount of dust in the air was great.

A. Materials and Methods

Sterile swabs, spatulas, and petri dishes were used to collect samples of dust, feces, straw, and ceiling insulation.

Media used to culture for microorganisms were Brain Heart Infusion Agar (DIFCO Laboratories, Inc.) for total microorganisms present; Mycosel Agar (Baltimore Biological Laboratories) for fungi; Sabouraud's Dextrose Agar (DIFCO Laboratories, Inc.) for fungi; Tryptic Soy Agar (DIFCO Laboratories, Inc.) for all microorganisms; Blood Agar to test for hemolysis; and Sabouraud's Medium with added yeast nitrogen base (DIFCO Laboratories, Inc.) for yeast and molds.

2. Conant Norman F., et al.: Manual of Clinical Mycology. Second Edition, 1962, pg. 121.

Methods of collection of samples for testing were to brush or scrape dust, dirt particles, feces, feathers, or insulation fiber into sterile petri dishes using sterile swabs or spatulas. Samples were collected from desks and cabinets in an area where work was not being done overhead at the time of sampling and from areas where work was being done. Also, dirt, feces, etc., were scrapped from areas of bird nesting, and a sample of ceiling insulation, with one side covered with nest material, was collected. All samples were sealed in the petri dishes by use of cellophane tape and transported to the laboratory for culturing.

To culture, 1 g of each of three samples was added to separate tubes containing 9 ml of sterile distilled water to give a 1:10 dilution (weight/volume). The dilutions were shaken vigorously by hand for 1 minute and then allowed to stand for 10 minutes. The dilutions were again shaken for 1 minute and further dilutions of 1:100 and 1:1000 were made. One ml of each dilution of each sample was placed in sterile 150-mm petri dishes and 40 ml of either Brain Heart Infusion Agar or Mycosel Agar (45° C) were added. The plates were incubated for 7 days at room temperature (25° C). Colonial types were picked and inoculated onto Sabouraud's Dextrose Agar, Tryptic Soy Agar, and Sabouraud's yeast medium. Also Lactophenol cotton blue staining of cells of each colony was performed and the cells viewed microscopically at 600X and 1500X.

A colony of each pure culture was inoculated to slants of blood agar. Inoculated tubes of blood agar were incubated at 37° C and checked daily.

Special care was taken at all steps of sample handling and culturing to avoid contamination of the laboratory or infection of personnel.

B. Results

Table C-1 shows the number of colonies found on each plate of each sample. The sample "dust in the air" was a fine powder brushed off of desks and cabinets which contained approximately 10 500 colony-producing microorganisms per gram. The sample "bird dirt" contained feathers, feces, dirt, and straw, and approximately 600 colony-producing organisms per gram. The ceiling insulation sample contained approximately 50 colony-producing organisms per gram. All the above counts were made on Brain Heart Infusion Agar. Results from the Mycosel Agar plates show very low counts of fungi which are resistant to the antibiotics in this medium.

TABLE C-1. PLATE COUNTS ON ISOLATION MEDIA

Source	Colony Count/Plate		Total Count/g
Dust from Air	Brain Heart Infusion Agar	Mycosel	
1:10	500	5	> 5 000
1:100	129	2	12 900
1:1000	9	1	9 000
Bird Dirt			
1:10	75	4	750
1:100	5	1	500
1:1000	1	0	< 1 000
Ceiling Insulation			5
1:10	5	1	5
1:100	2	0	200
1:1000	0	0	0

Colonies which grew on plates of Brain Heart Infusion Agar were found to be predominately yeast or yeast-forms of Mycelial fungi. This was especially true of the "air dust" Brain Heart Infusion Agar plates. On these plates, three colonial types of yeasts were found, and one of these types of yeast had a Mycelial phase. Two of the three types of yeast resemble Rhodotorula and Candida; the yeast Mycelial Fungus is still unidentified.

Molds of the genera Mucor, Aspergillis, Penicillium and Rhizopus were found on most plates of Brain Heart Infusion Agar, especially the "bird dirt" plates. Bacteria found and identified were Bacillus species and Actinomycetes.

None of the organisms, yeasts or bacteria, tested were hemolytic on blood agar, although all grew.

C. Discussion

The high quantity of dust in the air and on environmental surfaces of areas of building 4471, coupled with a relatively high count of microorganisms per gram of dust, leads one to believe a problem could arise. Nasopharyngeal and respiratory discomfort or infection could arise from the inhalation of airborne dust with its accompanying microorganisms.

The microorganisms isolated from the tested sources are not considered highly pathogenic, but are opportunistic and can cause secondary infections or irritations.

D. Identity of Organisms Isolated from Building 4471 by Center of Disease Control in Atlanta

A sample of the dust in building 4471 and two cultures of fungi isolated from this dust were sent to the Center for Disease Control (CDC) in Atlanta. The two cultures were also sent to Birmingham for testing and identification. In this laboratory the cultures had been tentatively identified as a Rhodotorula and a possible Cryptococcus.

The CDC report indicates that the possible Cryptococcus is a Cephalosporium and that the dust contained no pathogenic fungi. The laboratory in Birmingham agreed on the Rhodotorula and identified the second organism as a nonpathogenic Cryptococcus.

All three of these fungi, Rhodotorula, Cephalosporium, and non-pathogenic Cryptococcus, are considered not to be disease causing and are classed as Saprophytes.

E. Survey of Environmental Surfaces in Building 4494

Shower stalls (walls and floor), locker room floor, lavatory, and judo mat were survey-sampled. The methods of sampling were the RODAC plate and sterile swab methods. Media used in RODAC plates were Trypticase Soy Agar with Polysorbate 80, Mannitol Salts Agar and Eosin Methylene Blue Agar. The plates were read after 24-hour incubation at 35° C.

The swab method was used to sample crevices in the walls and floor of the shower stalls and also to sample the folds of the judo mat. After sampling, the swabs were placed in 1 ml of sterile distilled water for transfer to the laboratory. In the laboratory, the swabs were aseptically placed in tubes containing 10 ml of Trypticase Soy broth and incubated at 35° C. The tubes were checked after 24-hour and 72-hour incubation periods.

Table C-2 shows the counts from the RODAC plates. Identities are given of important organisms from swab sampling. As can be seen from the data, the only area showing high counts are the shower stall floors. The swab cultures showed numerous fungi and bacteria. No athelete's foot fungus, Trichophyton, was isolated, even on blood agar plates inoculated from the Trypticase Soy Agar broth tubes.

TABLE C-2. MICROBIOLOGICAL SURVEILLANCE PROGRAM RODAC PLATE REPORT

Specimen Number	Location of Tested Surface	Media and Average Counts			Comments
		TSA	EMB	MSA	
1	Floor in shower No. 1	200	0	80	Staphylococcus aureus
2	Floor in shower No. 2	200	0	85	Staphylococcus aureus
3	Shower No. 1 wall	20	0	0	
4	Shower No. 2 wall	45	0	0	
5	Lavatory	5	0	0	
6	Locker room floor	5	0	0	
7	Judo mat	25	0	3	
8	Swab — shower No. 1	Broth 10^4/ml			
9	Swab — shower No. 2	10^3/ml			
10	Swab — judo mat	10^2/ml			Bacillus, Staphylococcus aureus Clostridium, Fungi

APPROVAL

MICROBIOLOGICAL SURVEILLANCE OF FOOD HANDLING AT NASA-MSFC

By F. J. Beyerle

The information in this report has been reviewed for security classification. Review of any information concerning Department of Defense or Atomic Energy Commission programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

This document has also been reviewed and approved for technical accuracy.

3/19/73

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