Microbiological Testing of Skylab Foods

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The Skylab manned space flight program presented unique food microbiology problems. This challenge was successfully met by careful evaluation of the total Skylab food system by considering the nature of Skylab foods, their processing and handling, and Skylab food safety requirements. Some of the unique problems encountered with the Skylab foods involved: extended storage times, variations in storage temperatures, no opportunity to resupply or change foods after launch of the Skylab Workshop, first use of frozen foods in space, first use of a food-warming device in weightlessness, relatively small size of production lots requiring statistically valid sampling plans, and use of the food as an accurately controlled segment of sophisticated life science experiments. Consideration of all of these situations generated the need for definitive microbiological tests and test limits. These tests are described in this paper along with the rationale for their selection. Test results are reported which show successful compliance with the test limits.

The unmanned Skylab Orbital Workshop (SOW) is scheduled for launch early in 1973. After attaining orbit, the SOW will be manned by crews who will rendezvous from other space vehicles (Fig. 1). The first Skylab manned phase has a scheduled duration of approximately 28 days. The second and third manned phases are about 56 days each. Each crew will consist of three astronauts. An unmanned phase of approximately 60 days is scheduled between the first and second manned phase, and a 30-day unmanned phase is scheduled between the second and third manned phases.

All of the Skylab food will be stored in the SOW at the time of its initial launch. Therefore, there will be no opportunity for resupply or change of foods during flight. The Skylab food system comprises approximately 69 foods which will be stored aboard the SOW according to preselected menus. This food supply must be sufficient for 420 man-days plus a 15% allowance for menu variations. These foods have been produced at the rate of about two foods per week over a period of approximately 9 months. This lengthy production schedule was necessary because of the limited size of the specialized production and test facilities for space foods. This resulted in unavoidably long storage times (Fig. 2). This extended storage had to be taken into consideration in establishing microbiological tests and test limits.

In order to identify food production problems and allow for system design improvements, the entire sequence of Skylab foods will be produced two times. The first 9-month production sequence has been completed. These foods were used for crew evaluation, microbiological testing, storage stability studies, systems design studies, engineering systems tests, and nutrient analyses. The second production sequence began 2 months after completion of manufacture of the first. The second sequence will be used for the actual flight, detailed testing, crew training, and nutrient analyses.

Skylab foods, other than beverages, are packaged in drawn-aluminum cans with full-panel pullout lids. The astronaut will assemble these cans into meals in the Skylab food-warmer tray (Fig. 3). All food (other than beverages) will be consumed directly from the opened cans by using conventional tableware (11). Skylab beverages are packaged in a collapsible, plastic dispenser designed for convenient handling of liquids in zero gravity.

The Skylab food-warmer tray provides the first capability to heat foods during space flight. The heaters, located in the walls of the tray cavities, are electrical-resistance wires designed to heat to a maximum of 69.4 °C. Higher temperatures have been avoided for boiling must not occur in zero gravity in order to prevent food from being expelled by the boiling action. Boiling will occur near 72.2 °C in the SOW, which is approximately one-third atmosphere total pressure.

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Design requirements were set for the food-warmer tray so that it would heat frozen food \((-23.3 \pm 5.5 \, ^\circ C\) to \(65 \pm 3.3 \, ^\circ C\) within 2 hr under zero-gravity conditions. The tray was also required to keep the food at \(65 \pm 3.3 \, ^\circ C\) until consumed. These design criteria were established to prevent the growth of potential pathogenic microorganisms. The watt density design calculations of the food tray assumed heat transfer only by conduction because convection currents would be minimal in zero gravity (radiant-heat transfer was ignored as being insignificant). Ground-based testing with the complete absence of convection currents in foods could only be approximated. The possibility always remains that food heating in zero gravity will be slower than that indicated by ground-based testing. This would result from poor contact between the food and its container during weightless flight.

The Skylab flight menus have been carefully selected prior to flight for nutrient content and will be adjusted in real time as a function of astronaut preference in order to accurately control nutrient intakes during the mission. This nutrient control is a critical part of a sophisticated series of life science experiments designed to elucidate the physiological performance of man during prolonged exposure to weightlessness. The dual application of the food system as both a life support system and a component of complex experiments makes it essential that the food be both accurately defined and safe so that it will not introduce any unknown variable into the experiments.

The foregoing brief description of the application of Skylab foods outlines some of the unique situations involved in Skylab food safety. Microbiological test procedures and test limits were selected in order to insure food safety during these flight conditions. These tests have been classified into two categories: those for Skylab foods which are thermostabilized in metal cans and those for all other Skylab foods. This paper describes these microbiological test procedures, the test limits, and the rationale for their selection. The results of the microbiological testing of the first production sequence of Skylab foods are also reported and discussed.

**MATERIALS AND METHODS**

**Nonthermostabilized food:** (i) sampling. Each food package was sequentially serial numbered corresponding to the order of filling. Each microbiological test sample consisted of the first and last packages of each lot plus packages selected by use of a table of...
random numbers to make up the sample size shown in Table 1. A representative sample was aseptically removed from each package in this sample. Each sample was about equal in weight and sized so that the composite test sample totaled about 60 g. The exact weight of the complete test sample was recorded to the nearest tenth of a gram, and the sample was aseptically transferred into a sterile blender cup. A measured quantity of sterile buffered water (ca. 0.066 M PO₄, pH 7.0) (1, 2) was added to the composite test sample to produce a 1:10 dilution. This was blended for 2 min. The resultant food slurry (FS) contained the equivalent of 0.1 g of food sample per milliliter of FS. The FS was maintained between 4 and 10 C until promptly used in the test procedures.

(ii) Total aerobic count test. A 10-ml amount of FS was transferred into 90 ml of sterile buffered water, giving a final dilution of 1:100. One milliliter of the 1:100 dilution of FS was pipetted into each of five petri dishes, poured with plate count agar (1, 2), incubated at 35 C, and counted after 48 hr. A total of 500 or more colonies on the five plates constituted cause for rejection of the food lot.

(iii) Yeast and mold count test. A 1-ml amount of FS was transferred into each of ten petri dishes and poured with potato dextrose agar which had been acidified with tartaric acid to yield after pouring a pH between 3.3 and 3.7 (9). Plates were incubated at 21 C and counted after 5 days. A total of 10 or more yeast and mold colonies on the 10 plates constituted cause for rejection of the food lot.

(iv) Coagulase-positive staphylococci test. A 50-ml sample of FS was transferred into 50 ml of double-strength Trypticase soy broth (TSB) and incubated at 35 C for 2 hr. Then, 100 ml of single-strength TSB containing 20% NaCl was added to yield a final salt concentration of 10% (10). After incubation at 35 C for 24 hr ± 2 hr, 0.1 ml of the TSB culture was spread on each of two plates of Vogel and Johnson agar and incubated at 35 C. Plates were examined after 24 and 48 hr for the presence of black colonies with yellow zones. Two or more typical representative colonies were transferred to brain heart infusion (BHI) tubes and incubated at 35 C for 24 hr. The remainder of each colony was removed with a loop and emulsified in 0.2 ml of BHI, and 0.5 ml of coagulase plasma was added, mixed, and incubated in a 35 C water bath for 4 hr. At the end of 4 hr, negative tubes were noted, and the coagulase test was repeated with the 24-hr culture by using 0.2 ml of the BHI culture and 0.5 ml of coagulase plasma. A single coagulase-positive colony constituted cause for rejection of the food lot.

(v) Salmonella test. A 250-ml sample of FS was transferred into 250 ml of double-strength lactose broth and incubated at 35 C for 24 hr. After incubation, 25 ml of lactose broth was transferred to 225 ml of each of Selenite-cystine broth and tetrasulfonate broth base containing Brilliant Green (1:100,000) and incubated at 35 C for 18 to 24 hr. One loopful of enrichment culture was streaked on one plate each of three selective media: Brilliant Green sulfadiazine agar, bismuth sulfate agar, and Salmonella-Shigella agar. Brilliant Green sulfadiazine and Salmonella-

| Lot size | Sample size | Subsample size* |
|----------|-------------|-----------------|
| 8 to 150 | 8           | 1               |
| 151 to 500 | 13          | 1               |
| 500 to 1,200 | 20         | 2               |
| 1,201 to 10,000 | 32     | 3               |
| 10,001 to 35,000 | 50     | 5               |
| 35,001 to 500,000 | 80     | 8               |

*This subsample size is only applicable for the microbiological test sample of cans which have previously undergone incubation testing for adequacy of thermostabilization.

Shigella plates were incubated at 35 C for 24 hr and bismuth sulfite plates at 35 C for 48 hr. Two typical colonies from each plate having growth were transferred to triple sugar iron agar and an enterotube (Roche Diagnostics) and incubated at 35 C for 24 hr. Enterotube cultures with a urea-positive reaction were considered to be Salmonella negative, and the test was ended. Positive triple sugar iron agar (acid butt, alkaline slant, with or without gas and H₂S) with negative urea, and positive dulcitol, citrate, and lysine decarboxylase reactions constituted a presumptively positive Salmonella culture. Transfers from positive triple sugar iron agar slants were typed against Salmonella O polyvalent antisera. A single confirmed positive Salmonella culture constituted cause for rejection of the food lot.

(vi) Escherichia coli test. A 1-ml amount of FS was transferred to each of 10 lauryl sulfate tryptose (2) broth tubes and incubated at 35 C for 24 hr. One drop of broth from each positive lauryl sulfate tryptose tube (displaying gas) was transferred with a 1-ml pipette to an EC broth fermentation tube and incubated at 45.5 ± 0.2 C for 24 hr. Incubation (not exceeding 24 hr) was carried out in a constant-temperature bath monitored with a certified Bureau of Standards thermometer. Contents of gas-positive EC tubes were streaked on Levines eosine methylene blue agar plates and incubated at 35 C for 24 ± 2 hr. Two typical colonies were picked from each eosine methylene blue plate and transferred to a plate count agar slant and incubated at 35 C for 24 hr. Growth on plate count agar slants was confirmed for E. coli types through the establishment of the IMViC pattern according to standard procedures. IMViC patterns of + + - - or + + + + were considered confirmed E. coli types I and II. Presence of a single confirmed E. coli type I or II constituted cause for rejection of the food lot.

(vii) Clostridium perfringens test. A 0.1-ml amount of FS was spread onto each of 10 petri plates containing Shahidi-Ferguson perfringens (SFP) agar (12) and overlayed with 10 ml of SFP overlay agar (egg yolk emulsion omitted). Plates were placed into Gaspak anaerobic jars (BBL) (or equivalent) and incubated at 35 C for 24 hr. Black colonies surrounded by a zone of precipitate were counted. Typical colonies were confirmed in lactose motility
agar. Lactose motility agar was boiled for 10 min and cooled immediately prior to use. Inoculated tubes were incubated at 35°C for 24 hr. Nonmotile lactose-positive cultures in lactose motility agar were considered to be Clostridium perfringens. A total of more than 10 confirmed colonies on the 10 plates constituted cause for rejection of the food lot.

**Thermostabilized food: (i) incubation test.** The size of the sample for incubation testing is indicated in Table 1 as sample size for each given lot size. When necessary, the sample size was increased so that each retort batch in the lot was represented by an equal number of cans. The cans selected from each retort batch included at least one can from the bottom, one can from the center, and one can from the top of the batch of cans as physically located in the retort. Two separate comparable samples were drawn and incubated for 14 days. One sample was incubated at 32 ± 2°C and another at 55 ± 2°C. After incubation, the cans were manually examined for evidence of gas production. The presence of one can that became a flipper, spriger, soft swell, or hard swell (8) after incubation at either temperature constituted cause for rejection of the food lot.

(ii) Microbiological tests. A subsample (Table 1) of cans was randomly selected from the cans after their incubation. Each can in the subsample was microbiologically tested with all incubations performed at the same temperature at which the can had been incubated. About 2 g of food from each can in the subsample was aseptically transferred, in duplicate, into (i) freshly prepared fluid Thioglycollate medium and (ii) TSB with 0.1% yeast extract. Both media were prepared in 25- by 150-mm culture tubes filled to about one-half depth. The fluid Thioglycollate was incubated anaerobically by using anaerobic jars. After 9 days of incubation, the tubes were examined for indication of microbial growth. All doubtful tubes were confirmed by streaking 0.1 ml of the incubated broth on a Trypticase soy agar plate and incubating the plates for 24 hr under the same conditions (atmospheric and temperature) under which the original tubes were incubated. The presence of microbial growth in any of the microbiological test culture tubes or plates constituted cause for rejection of the food lot.

**RESULTS AND DISCUSSION**

The Skylab food microbiological test regimen was selected after evaluation of the benefit-risk ratio of each solution to problems judged in the context of the total food system (3). Furthermore, microbiological testing itself was considered as only one segment of a total food safety system. Equally essential elements of this safety system included the test procedures and test criteria for raw materials, storage, processing and shipping environment controls, personnel monitoring, intentional and unintentional additives, and examination for in-storage degradation (browning pigment accumulation, increase in hydroperoxides, and decrease in protein digestibility). It was recognized that no test regimen, per se, could assure 100% safety of Skylab food. For example, regardless of testing, food itself remains a microbiological growth media, and the safety hazard is determined by initial microbial contamination as well as by subsequent factors which impact elements of the total system.

The selection of the Skylab microbiological test sample was given careful consideration. The test examined in food microbiological testing usually represents an extremely small segment of the production lot. The statistical validity of such sampling is frequently further degraded by pooling of selected subsamples. The sampling plan for Skylab foods was influenced greatly by the unique size of the production lots. Production sequence sizes for Skylab foods ranged from only 500 to 10,000 individual food packages. The S-4 level of sampling defined in Military Standard 105D (7) was judged to be most appropriate for sampling these lots because of the previous history of low microbiological counts in space foods produced in the Skylab-type clean-room space food environments (5, 6, 10, 13).

Each Skylab food that was not preserved by thermostabilization was studied from the viewpoint of published reports of its epidemiological association with food-borne disease and its potential for supporting microbial growth. This study included processing procedures, microbiological data from similar flight foods, recommended limits established by public health organizations, and proposed crew handling procedures (including in-flight heating and refrigeration). On the basis of these studies, the requirement for testing each nonthermostabilized food for one or more of the following groups of organisms was determined: total aerobic organisms, *E. coli*, *Salmonella*, coagulase-positive staphylococci, *C. perfringens*, and yeast and mold. The test limits applied to each nonthermostabilized Skylab food are equivalent to those shown in Table 2. The results of the microbiological testing of the first Skylab food production sequence are shown in Table 3.

| Test             | Limit                  |
|------------------|------------------------|
| Total aerobic count | Not greater than 10,000/g |
| *Escherichia coli* | Negative in 1 g         |
| Coagulase-positive staphylococci | Negative in 5 g |
| *Salmonella*      | Negative in 25 g        |
| *Clostridium perfringens* | Not greater than 100/g |
| Yeast and mold    | Not greater than 100/g   |
| Product                              | Total aerobic count | E. coli | Yeast and mold | C. perfringens | Coagulase-pos staphylococci | Salmonella |
|-------------------------------------|---------------------|---------|----------------|----------------|-----------------------------|------------|
| Rehydratable                        |                     |         |                |                |                             |            |
| Sausage patties                     | 620                 | Neg     | 12             | <10            | Neg                         | Neg        |
| Sugar-coated corn flakes            | 200                 | Neg     | NI             | NI             | Neg                         | Neg        |
| Scrambled eggs                      | 1,020               | Pos     | Neg            | <1             | NI                          | Neg        |
| Asparagus                           | 180                 | Neg     | NI             | NI             | NI                          |            |
| Salmon salad                        | 380                 | Neg     | NI             | NI             | Neg                         |            |
| Strawberries                        | 620                 | Neg     | 95             | NI             | NI                          |            |
| Mashed potato                       | 8,960               | Neg     | NI             | NI             | Neg                         |            |
| Cream of tomato soup                | 1,200               | Neg     | NI             | <10            | NI                          |            |
| Pea soup                            | 380                 | Neg     | NI             | NI             | Neg                         |            |
| Potato                              | 2,400               | Pos     | Neg            | NI             | <10                         | Ni         |
| German potato salad                 | 5,320               | Neg     | NI             | NI             | Neg                         |            |
| Shrimp cocktail                     | 2,020               | Neg     | 13             | NI             | Neg                         |            |
| Turkey-rice soup                    | 7,820               | Pos     | Neg            | NI             | <10                         | Neg        |
| Rice Krispies                       | 600                 | Pos     | Neg            | NI             | NI                          |            |
| Chicken and rice                    | 2,780               | Neg     | NI             | <10            | Neg                         |            |
| Creamed peas                        | 460                 | Pos     | Neg            | 1              | NI                          |            |
| Chicken and gravy                   | 2,520               | Pos     | Neg            | NI             | <10                         | Neg        |
| Pork and scalloped potatoes         | 700                 | Neg     | NI             | <10            | Neg                         |            |
| Mashed sweet potatoes               | 2,860               | Neg     | NI             | NI             | Neg                         |            |
| Beef hash                           | 1,900               | Neg     | NI             | <10            | Neg                         |            |
| Cream style corn                    | <20                 | Neg     | 53             | NI             | NI                          |            |
| Peach ambrosia with pecans          | 1,360               | Pos     | Neg            | 22             | NI                          |            |
| Veal and barbecue sauce            | 4,080               | Pos     | Neg            | 8              | <10                         | Neg        |
| Spaghetti and meat sauce            | 1,440               | Pos     | Neg            | 5              | <10                         | Neg        |
| Green beans                         | 1,840               | Neg     | 2              | NI             | NI                          |            |
| Macaroni and cheese                 | 880                 | Pos     | Neg            | 1              | <10                         | Neg        |
| Frozen                              |                     |         |                |                |                             |            |
| Vanilla ice cream                   | 450                 | Neg     | NI             | NI             | NI                          |            |
| White bread                         | <20                 | Neg     | <1              | NI             | NI                          | Neg        |
| Filet mignon                        | 6,400               | Neg     | NI             | <10            | Neg                         |            |
| Prebuttered roll                    | 20                  | Neg     | 7              | NI             | NI                          |            |
| Pork loin with dressing and gravy   | 2,300               | Neg     | NI             | <10            | Neg                         |            |
| Coffee cake                         | 140                 | Neg     | 26             | NI             | NI                          |            |
| Lobster newburg                     | 7,600               | Neg     | NI             | <10            | Neg                         |            |
| Prime rib of beef                   | 160                 | Neg     | NI             | <10            | Neg                         |            |
| Beverages                           |                     |         |                |                |                             |            |
| Orange crystals                     | 140                 | NI      | NI             | NI             | NI                          |            |
| Grapefruit crystals                 | 60                  | NI      | NI             | NI             | NI                          |            |
| Lemonade                            | <20                 | NI      | NI             | NI             | NI                          |            |
| Grape drink                         | 20                  | NI      | NI             | NI             | NI                          |            |
| Cocoa flavored instant breakfast    | 3,500               | Neg     | <1              | NI             | NI                          | Neg        |
| Cocoa                               | 1,600               | Neg     | 4              | NI             | NI                          | Neg        |
| Black coffee                        | 1,100               | NI      | NI             | NI             | NI                          |            |
| Tea with lemon and sugar            | <20                 | NI      | NI             | NI             | NI                          |            |
| Fruit beverage (sherry)             | 40                  | NI      | <1              | NI             | NI                          |            |
| Miscellaneous                       |                     |         |                |                |                             |            |
| Dry-roasted peanuts                 | 160                 | NI      | 14             | NI             | NI                          |            |
| Dried apricots                      | 180                 | Neg     | 1              | NI             | NI                          |            |
| Sugar cookie wafers                 | <20                 | Neg     | NI             | NI             | NI                          | Neg        |
| Cheddar cheese cracker              | 200                 | Pos     | Neg            | NI             | NI                          | Neg        |
| Mints                               | <20                 | Neg     | NI             | NI             | NI                          | Neg        |
| Ham and cheese cracker              | 120                 | Neg     | NI             | NI             | NI                          | Neg        |
| Bacon wafer                         | 40                  | Neg     | <1             | NI             | NI                          | Neg        |
| Vanilla wafer                       | 340                 | Neg     | NI             | NI             | NI                          |            |
| Hard candy                          | 20                  | Neg     | NI             | NI             | NI                          |            |
| Biscuit                             | 40                  | Neg     | NI             | NI             | NI                          |            |
| Sliced dried beef                   | 80                  | Pos     | Neg            | 18             | NI                          | Neg        |
| Catsup                              | <20                 | NI      | 9              | NI             | NI                          |            |
| Peanut butter                       | 4,380               | NI      | NI             | NI             | NI                          |            |
| Fruit jam                           | <20                 | NI      | <1             | NI             | NI                          |            |

* Neg, negative; Pos, positive; NI, test not indicated.
This table also indicates which foods were tested for each group of microorganisms and which tests were judged unnecessary. Each judgment as to the necessity for each test was made on the basis of three factors: (i) the nature of the food item and its characteristic microflora; (ii) the likelihood of destruction of the microorganism during processing; and (iii) the likelihood of growth of that organism under the conditions of Skylab if that organism were present in the given food.

The total aerobic count was imposed primarily as a monitor of overall sanitation of food production procedures. All of the food items were found to be within the requirement of less than 10,000 aerobic organisms per gram. The yeast and mold requirements were included to assist the quantitation of and limitation of spacecraft contamination as well as for food safety considerations. With the exception of strawberries and cream style corn, all of the foods examined possessed relatively low counts. The requirements for E. coli, coagulase-positive staphylococci, and Salmonella were selected in order to limit known pathogens rather than to test for "indicator" organisms. Thirteen of the foods were presumptively positive for E. coli. However, these foods were negative when examined in EC broth. None of the foods were positive for coagulase-positive staphylococci or Salmonella.

C. perfringens tests were performed on the 15 foods which required warming prior to consumption and in which it was judged that C. perfringens might be present (see Table 3 for food items tested). The requirement of not greater than 100 per g was based upon the assumptions that (i) a total dose of 10^4 viable organisms are required to produce detectable signs or symptoms; (ii) there would be no lag phase; (iii) a generation time is 20 min; and (iv) any one meal would not contain more than 100 g of contaminated food (foods involved are either entrees which appear only once per meal or rehydratable foods which contain about 30 g per serving, dry weight). Based on these assumptions, it would require any one meal to be held for approximately 2 hr in the temperature range for growth of C. perfringens before it could cause a clinical episode. SFP agar was the method selected for enumeration of C. perfringens. SFP agar is less selective than sulfite-polymyxin-sulfadiazine or tryptone-sulfite-neomycin agar (4). This nonselectivity somewhat increases the conservativeness of the test limit. Fifteen of the food items which required warming prior to consumption were examined for the presence of C. perfringens, and all were essentially negative.

The adequacy of the thermostabilization process was verified by two test procedures. First, incubation tests determined whether or not gas was produced in the sealed cans during 32 and 55°C incubation. Subsequent to incubation, the cans were examined microbiologically to detect any microbial growth which may have occurred without gas production. The extended Skylab storage time and variations in storage temperature necessitated a more rigorous microbiological testing plan than normally applied for thermostabilized foods. None of the 12 Skylab thermostabilized foods (ham salad sandwich, butterscotch pudding, tuna salad sandwich spread, lemon pudding, chili with meat, pineapple, turkey and gravy, applesauce, hot dogs with tomato sauce, peaches, pears, stewed tomatoes) produced gas or microbial growth when subjected to this test regimen. Three food items, catsup, peanut butter, and fruit jam, could be considered to be preserved by thermostabilization. However, these foods were tested via the methods outlined for nonthermostabilized foods due to the relatively low temperatures involved in the thermostabilization of these products.

The results of the microbiological examination of the first production sequence of Skylab food demonstrated that all of the established microbiological safety requirements were satisfied. In evaluating these results, it should be emphasized that these products are not off-the-shelf items. All products were produced following strict specifications. Compliance with requirements was verified at numerous inspection points during the production.

The foods involved were processed by a wide variety of methods including: freeze-dehydration, spray drying, intermediate moisture, thermostabilization, and freezing. These microbiological test results reveal the types and numbers of microorganisms important to public health which are associated with this wide variety of foods produced under ideal conditions. As such, these test data contribute to information needed to establish realistic food safety standards. The Skylab food safety standards represent standards designed to provide maximal practical consumer protection derived from realistic appraisal of the benefit risk ratios involved in the total system.

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