NASA TECHNICAL HANDBOOK

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NASA HANDBOOK FOR THE MICROBIAL EXAMINATION OF SPACE HARDWARE

Responsible Office: Science Mission Directorate

TABLE OF CONTENTS

PREFACE

This handbook is a reissued version of NHB 5340.1C, a compilation of procedures for the microbiological examination of space hardware and associated environments developed to meet the requirements of the National Aeronautics and Space Administration planetary protection and space and life sciences programs. Since issuance of the previous edition of this handbook, the development of new assay techniques and extensive field laboratory experience with the procedures have occurred, and these developments will be reflected in a future version of the document to be compiled within the next year. But for now, it is considered important to make this 1980 document available within the current directives system.

P.1 PURPOSE

This NPG describes uniform microbiological assay procedures that shall be used to:

a. Assess the degree of microbiological contamination of intramural environments where spacecraft hardware is assembled, tested and launched.

b. Assess the level of microbial contamination on spacecraft hardware in relation to the known or anticipated environments of the target planets.

P.2 APPLICABILITY

The provisions of this NPG are applicable to NASA Headquarters and Centers, including Component Facilities, and to contractors who provide microbiological assay of spacecraft hardware services to NASA.

NASA Headquarters and Centers, including Component Facilities, are responsible for ensuring contractor conformance to the procedures set forth in this NPG and for furnishing a sufficient number of copies of this publication to contractors when it is invoked in contracts.

The assay procedures in this NPG apply to all spacecraft hardware and pertinent assembly, test, and launch facilities that are required to meet planetary protection standards and/or requirements established by the NASA Planetary Protection Officer. This NPG is written primarily for use by microbiologists.

P.3 AUTHORITY

NPD 8020.7E, Biological Contamination Control for Outbound and Inbound Spacecraft, TBD date.

P.4 REFERENCES

See Appendix B.

P.5 DEVIATIONS

It is emphasized that uniformity of procedures is one of the primary objectives of this NPG. However, portions of this document have been written to include a degree of flexibility in that alternate techniques are described. Consequently, any change in these procedures, other than those specifically mentioned in alternate sections, shall be considered a deviation. Requests for approval for implementing a deviation, with a justification, will be submitted to the NASA Planetary Protection Officer, Office of Space Science, National Aeronautics and Space Administration, Washington, DC 20546. Deviation from a procedure will not be permitted unless written approval is granted.

P.6 CANCELLATION

NHB 5340.1C, February 1980, is canceled.

SIGNATURE AUTHORITY

Edward J. Weiler
Associate Administrator for
Science Mission Directorate

CHAPTER 1. Assay Procedures

1.1. GENERAL NOTES

The following general notes apply to all operations described in these procedures:

1.1.1. Any deviation from the operations or equipment specified in these procedures (or appendices) will require written approval of the NASA Planetary Protection Officer.

1.1.2. All operations involving the manipulation of sterile items and sample processing shall be performed in laminar flow environments meeting Class 100 air cleanliness requirements of Federal Standard 209B.

1.1.3. These procedures are designed primarily for the detection and enumeration of heterotrophic, mesophilic, aerobic and anaerobic microorganisms. Procedures for the detection of other microorganisms, e.g. psychrophiles and thermophiles, are included to meet the needs of specific missions. Other procedures for microorganisms that may be resistant to sterilization methods other than dry heat, and may likely survive space and planetary environments (e.g. halophiles, certain Bacillus species and extremophiles), are not included. These procedures will be provided in subsequent revisions of this document, as warranted.

1.2. ASSESSMENT OF MICROBIAL CONTAMINATION OF THE INTRAMURAL ENVIRONMENT OF SPACECRAFT HARDWARE ASSEMBLY, TEST AND LAUNCH FACILITIES

1.2.1. Assay of Airborne Microbial Contamination Accumulating on Surfaces

1.2.1.1. General

a. Stainless Steel Fallout Strips

Prepare trays of sterile, stainless steel strips (Appendix C, Sec. 1) and place them in areas to be sampled. The number and location of sampling sites shall be subject to approval by NASA management or its authorized contractor(s). When in place at each sampling site, carefully remove the aluminum foil cover of each tray. Using sterile forceps, rearrange the sterile strips to form a monolayer. The duration of strip exposure and the sampling intervals shall be subject to approval by NASA management or its authorized contractor(s). Wearing sterile gloves, collect at least six strips per tray at each sampling interval. Using separate sterile forceps, aseptically place each strip to be assayed in a separate, dry, sterile, Erlenmeyer flask or other glass container (Appendix C, Sec. 4).

It is recommended that the stopper or cap of each flask be covered with aluminum foil to minimize the possibility of contaminating the lip of the flask. The foil should extend 5-6 cm down the neck of the flask. Hold the strips contained in the flasks at 25 to 35^{o}C and begin assay procedures 24 h after collection.

Aseptically add 50 ml of sterile rinse solution (Appendix D, Sec. 1) maintained at 20 to 25^{o}C to each flask containing a strip. Make sure that the contaminated surface of the strip (marked "X") is facing the bottom of the flask. Replace the foil covering. Immediately sonicate (expose to ultrasonic energy) the sample in the following manner:

(1) Suspend flask(s) containing the strip and rinse solution in the middle of an ultrasonic bath filled to its normal capacity so that the bottom of the flask is parallel to the bottom of the bath and the bath fluid solution is above the level of the solution in the flask. Suspension shall be accomplished by any suitable means that does not alter standard sonication performance requirements (Appendix C, Sec. 5). Racks, baskets or similar devices designed to hold sample containers should not be used.

(2) Sonicate for 2.0 min. $\pm$ 5 sec.

b. Teflon Fallout Ribbons

Prepare sterile, Teflon ribbons (Appendix C, Sec. 2) and place them in areas to be sampled. The number and location of sampling sites shall be subject to approval by NASA management or its authorized contractor(s). At each sampling site, wearing sterile gloves, carefully remove Teflon ribbons from sterile glass beakers and unroll ribbons on a pre-cleaned support surface. The duration of Teflon ribbon exposure and the sampling intervals shall be subject to approval by NASA management or its authorized contractor(s). Wearing sterile gloves, collect two Teflon ribbons per sampling site at each sampling interval. Aseptically roll up each Teflon ribbon to be assayed and place in separate, dry, sterile glass jars.

It is recommended that the cap of each jar be covered with aluminum foil to minimize the possibility of contaminating the lip of the jar. The foil should extend 5-6 cm down the side of the jar. Hold Teflon ribbons contained in jars at 25 to 35^{o}C and begin assay procedures within 24 h after collection.

Aseptically add 400 ml of sterile solution (Appendix D, Sec. 1) maintained at ambient room temperature to each jar containing a Teflon ribbon. Immediately sonicate the sample in the following manner:

(1) Suspend one to four jars containing Teflon ribbons and rinse solution in an ultrasonic bath filled to it normal capacity so that the bottom of the bath and the bath fluid solution is above the level of rinse solution in the jar(s).

(2) Sonicate for 2 min. $\pm$ 5 sec.

1.2.1.2. Stainless Steel Fallout Strip Assay Procedure 1

a. Heated Portions

Aseptically pipette 25 ml of the rinse solution into the bottom of a separate, sterile test tube (approximately 25 x 150 mm) taking care not to contaminate the upper inside surface of the tube. When the pipetting procedure requires more than 10 min., place the tube containing 25 ml of rinse solution in an ice bath for no longer than 45 min. The rack containing the tubes shall then be placed in the water bath at 80 $\pm$ 2^{o}C and remain for 15 min. after the temperature in the pilot tube reaches 80 $\pm$ 2^{o}C. Make certain the water bath level is above the level of the liquid content of each test tube. After heat-shock, the tubes shall be cooled rapidly by immersion in cold water (10 to 15^{o}C) or by any other suitable means which affords rapid (within 2 min.) cooling of the rinse solution to 30 to 35^{o}C. Aseptically pipette 5.0-ml portions of the heat-shocked liquid from each tube into four petri plates. Add approximately 20 ml sterile, molten (48 to 50^{o}C) TSA to each plate, mix the contents by gentle swirling and allow the mixture to solidify.

b. Unheated Portions

Pipette 5.0-ml portions of the solution remaining in the flask into four petri plates. Add approximately 20 ml sterile, molten (48 to 50^{o}C) TSA to each plate, mix the contents by gentle swirling and allow the mixture to solidify. Using sterile forceps, aseptically remove each strip from its flask, rinse it completely in a gentle stream of sterile water for 3 to 5 sec. and place it, contaminated side (marked "X") facing up, in a sterile, dry petri plate. Add enough sterile, molten (48 to 50^{o}C) TSA to completely cover the strip and then allow the medium to solidify.

c. Incubation

Aerobically incubate two plates containing heated samples and two plates containing unheated samples at 32^{o}C for 72 h in an inverted position. Anaerobically incubate (Appendix C, Sec. 12) the remaining four plates at 32^{o}C for 72 h in an inverted position. Aerobically incubate the overlaid strip. Scan plates of the aerobically incubated samples at 24 and 48 h; count if growth indicates. Perform and record colony counts at 72 h. Do not remove the petri plate covers until the final count is made. Perform colony counts of the anaerobically incubated samples after 72 h.

In accordance with the assay group's needs or, if applicable, as required by NASA management or its authorized contractor(s), plates should be incubated at 3^{o}C for 14 days to encourage the selection of psychrophilic organisms. To encourage the selection of thermophilic organisms, incubation should be at 55^{o}C for 24 to 48 h.

1.2.1.3. Stainless Steel Fallout Strip Assay Procedure 2

When it is desirable or required that the assay be directed toward one group of microorganisms (i.e., aerobes, aerobic spores, or anaerobic spores) and when the level of microbial contamination is considered to be extremely low, the following assay procedure may be used:

a. Unheated Portions

After sonication aseptically pour two 25-ml portions of rinse solution into two large, sterile petri plates (150 x 25 mm). Gently swirl the solution in the plate, quickly add 75 ml of sterile, molten (50 to 53°C) concentrated TSA (Appendix D, Sec. 3), and *immediately* resume gentle swirling until the contents are thoroughly mixed. Assay the strip in the same manner described in Assay Procedure 1. Standard membrane filter methods may be used as an alternative to the above procedure.

b. Heated Portions

After sonication, place the flask directly in a water bath at 80±2°C for 15 min. as determined by a pilot flask containing a thermometer. Make sure the water bath level is above the liquid contents of the flask. After heating, immediately cool the contents of the flask to approximately 45 to 50°C and then plate as described above (Unheated Portion, Fallout Strip Assay Procedure 2).

c. Incubation

Incubate and perform colony counts in the same manner described in Fallout Strip Assay Procedure 1.

1.2.1.4. Stainless Steel Fallout Strip Assay Procedure 3

When an assay laboratory is adjacent to or close to the test area, the following may be permitted: instead of retrieving each strip and placing it in a sterile, dry flask, place it directly in a flask containing 50 ml of cold (0 to 6°C), sterile rinse solution. Handle the flask in such a manner as to maintain the temperature of the rinse solution between 0 and 6°C. Initiate the actual assay within 20 min. from the time of strip pickup. From this point proceed in the same manner described in Fallout Strip Assay Procedure 1 or 2.

1.2.1.5. Teflon Fallout Ribbon Assay Procedure 1

a. Heated Portions

Aseptically pipette 30-ml portions of the rinse solution into four separate, sterile test tubes (approximately 25 x 150 mm) taking care not to contaminate the upper inside surface of the tubes. When this procedure requires more than 10 min., place the tubes containing 30 ml of rinse solution in an ice bath immediately after the pipetting procedure. They shall be held in the ice bath no longer than 45 min. before placing in an 80±2°C water bath for 15 min. as determined by a pilot tube containing a thermometer. Be certain that the water bath level is above the liquid level in each test tube. After heat shock, the tubes shall be cooled by any suitable means that affords rapid (within 2 min.) cooling of the rinse solution to 30 to 35°C. Placement into 10 to 15°C water is recommended. Aseptically pipette 25 ml portions of the heat-shocked liquid from each tube into four petri plates (150 x 25 mm). Add sterile, molten (48 to 50°C) TSA to each plate; mix the contents by gentle swirling and allow to solidify.

b. Unheated portions

Aseptically pipette 5-ml portions of the solution remaining in the jar into four petri plates. Add approximately 20 ml sterile, molten (48 to 50°C) TSA to each plate; mix the contents by gentle swirling and allow to solidify.

c. Incubation

Aerobically incubate two plates containing heated samples and two plates containing unheated samples at 32ºC for 72 h in an inverted position. Anaerobically incubate (Appendix C, Sec. 12) the remaining plates at 32ºC for 72 h in an inverted position. Scan plates of the aerobically incubated samples at 24 and 48 h; count if growth indicates. Perform and record colony counts at 72 h. Do not remove the petri plate covers until the final count is made. Perform colony counts of the anaerobically incubated samples after 72 h. In accordance with the assay group's needs or, if applicable, as required by NASA management or its authorized contractor(s); plates should be incubated at 3ºC for 14 days to encourage the selection of psychrophilic organisms. To encourage the selection of thermophilic organisms, incubations should be at 55ºC for 24 to 48 h.

1.2.1.6. Teflon Fallout Ribbon Assay Procedure 2

When the level of microbial contamination is considered to be extremely low, or the assay is directed toward one group of microorganisms, the following assay procedure may be used:

a. Heated Portions

After sonication, place the jar containing a ribbon and the 400 ml of rinse solution in a water bath at 80±2ºC and allow to remain for 15 min. after the rinse solution has reached a temperature of 80±2ºC. A pilot jar containing 400 ml of the rinse solution and a thermometer shall be used to determine when the solution reaches temperature. After heating, immediately cool the contents of the jar to 30 to 35ºC and filter according to standard membrane filter procedures. Place the filter from each rinse solution onto the surface of a fresh, newly prepared plate of TSA.

b. Unheated Portions

After sonication, aseptically pour the entire 400 ml portion of rinse solution through a membrane filter as described. Plate as described above in heated portions.

c. Incubation

Incubate and perform colony counts in the same manner described in Fallout Ribbon Assay Procedure 1.

1.2.1.7. Calculation of Results

Results shall be expressed initially as number of microorganisms per strip or Teflon ribbon (i.e., aerobes, anaerobes, aerobic spores, or anaerobic spores per strip). The general categories of microorganisms shall be reported as individual groups (i.e., aerobes, aerobic spores, etc.) and shall not be added in any combination. It is suggested that the mean and range be calculated for each series of strips or ribbons. However, all extrapolations of data shall be done in accordance with the assay group's needs or, if applicable, as required by NASA management or it authorized contractor(s).

1.2.1.8. Controls

a. Process a minimum of six sterile stainless steel strips as sterility control check items on the entire assay procedure. Processing shall be during one of the assays conducted each day or more often as prescribed by NASA management or its authorized contractor(s).

b. Process a minimum of two sterile Teflon ribbons as sterility control check items on the entire assay procedure. Processing shall be during one of the assays conducted each day or more often as prescribed by NASA management or its authorized contractor(s).

1.2.2. Microbiological Sampling of Environmental Surfaces

1.2.2.1. Swab-Rinse Method

Aseptically remove a sterile cotton swab from its container (Appendix C, Sec. 7) and moisten the head of the swab in 10.0 ml of sterile distilled water. Express excess moisture from the swab against the interior wall of the tube.

Hold the swab so that the handle makes about a 30-degree angle with the surface to be sampled. Rotate the head of the swab slowly and thoroughly over a measured surface area of no more that 26 cm^2. Change the direction of the swabbing motion 90 degrees and again swab the surface. Complete a third coverage of the surface by again changing the direction of the swabbing motion by 90 degrees. Return the head of the swab to the original tube of solution, breaking off the head of the swab below any portion of the handle that was touched by the sampler. Allow the swab head to drop into the liquid and replace the screw cap. Transport samples to the laboratory and process within 24 h.

Prior to sonication, place each tube containing the water and the swab on a vortex mixer and agitate at maximum power for 5-6 sec.

Suspend the tubes containing swab heads in the middle of an ultrasonic bath filled to normal capacity. Not more that 12 tubes shall be placed into the bath at one time. Make sure that the bath fluid is above the liquid level in the tubes and then sonicate for 2 min. $\pm$5 sec. After sonication, plate the vortexed solution as described in one of the following methods:

a. Swab Assay Method 1

(1) Unheated Portions

Aseptically pipette 2.0-ml portions into two petri plates. Add approximately 20 ml of sterile, molten (48 to 50ºC) TSA to each plate and mix the contents by gentle swirling. Allow the mixture to solidify.

(2) Heated Portions

Heat-shock the remaining rinse solution in each tube by placing the tube with the vortexed solution and the swab head in a 80$\pm$2ºC water bath for 15 min. as determined by a pilot tube containing a thermometer. Tubes containing swab heads shall be placed in an ice bath immediately after the unheated portions are plated if the entire procedure requires more than 10 min. They may be stored for no longer than 45 min.

Make certain the water bath level is above the level of the liquid content of each tube being heated. After the heat-shock, rapidly (within 2 min.) cool the tubes so that the water/swab solution is from 30 to 35ºC. Aseptically pipette 4.0 ml portions into two petri plates. Add approximately 20 ml of sterile, molten (48 to 50ºC) TSA to each plate and mix the contents by gentle swirling. Allow the mixture to solidify.

(3) Dilutions

If necessary, make appropriate dilutions of the rinse fluid in sterile rinse solution (Appendix D Sec.1) after sonication. Plate portions of each dilution as described above in Unheated Portions and Heated Portions.

(4) Incubation

Aerobically incubate one unheated sample and one heated sample from each swab assay at 32ºC in an inverted position. Anaerobically incubate (Appendix C, Sec 12) the remaining unheated and heated samples from that swab at 32ºC in an inverted position. Scan plates of the aerobically incubated samples after 24 and 48 h incubation; count if growth indicates. Do not remove the plate covers until the final count is made at 72 h. Perform colony counts of the anaerobically incubated samples after 72 h.

In accordance with the assay group's needs or, if applicable, as required by NASA management or its authorized contractor(s), plates should be incubated at 3ºC for 14 days to encourage the selection of psychrophilic organisms. To encourage the selection of thermophilic organisms, incubation should be at 55ºC for 24 to 48 h.

b. Swab Assay Method 2

When it is desirable or required by NASA management or its authorized contractor(s) that the assay: (1) be directed toward one group of microorganisms (i.e., aerobes, anaerobes, aerobic spores, anaerobic spores, psychrophiles, etc.); (2) be performed when the level of microbial contamination is considered to be extremely low; (3) be directed toward two

groups of microorganisms; or (4) yield duplicate plates, the following assay procedures may be varied to yield the required information.

(1) Unheated Portions

After sonication as described in Swab Assay Method 1, pipette as much of the 10-ml water/swab solution as necessary into a petri plate(s). Add sterile, molten (48 to 50ºC) TSA and mix the contents by gentle swirling. Allow the mixture to solidify.

(2) Heated Portions

After sonication, place the tubes containing the swab heads and the water/swab solution in a water bath at 80±2ºC, hold at temperature for 15 min. as determined by a pilot tube containing a thermometer. After heat shock, cool the tubes rapidly so that the fluid contents are from 30 to 35ºC. Plate as described above under Unheated Portions.

(3) Incubation

Incubate in same manner described above under Swab Assay Method 1.

c. Calculation of Results

Express the results as number of microorganisms per cm^2 of surface. When applicable, general categories shall be reported as individual groups (i.e., aerobes, aerobic spores, etc.) and shall not be added in any combination. It is suggested that the mean and range be calculated for each series of swab samples. However, all extrapolations of data shall be done in accordance with the assay group's needs or, if applicable, as required by NASA management or its authorized contractor(s).

d. Controls

A minimum of 10% of the total swab samples taken shall be utilized as sterility control check items on the entire assay procedure. Processing shall be during one of the assays conducted each day or more often as prescribed by NASA management or its authorized contractor(s).

1.2.2.2. Wipe-Rinse Method

In a clean laminar vent hood, wearing sterile gloves, aseptically remove a sterile wipe (Appendix C, Sec. 9) from its wrapper (Appendix C, Sec. 9) and place the individual wipe into a clean transport jar or sterile petri dish. Moisten the wipe with approximately 15 ml sterile, distilled water. Place the wipe flat on the sample surface and rub over the entire surface using a firm, steady pressure. Refold the wipe by reversing the direction of the open fold so the contaminated surface is interior to the new configuration. Rub the wipe over the sample area a total of three times, rotating the direction of motion 90 degrees after each complete coverage of the sample area. Transfer the wipe into a dry, sterile, glass jar with appropriate capacity to accommodate the rinse solution volume. For each six samples collected, a handling control will be taken. These controls will be utilized to check possible contamination picked up during the sample procurement. Remove the sterile wipe from its wrapper, moisten with approximately 15 ml sterile, distilled water, and insert into a dry, sterile, glass jar. Rinse hands with filtered sterilized 70% isopropyl alcohol between each sample, and change gloves at least once every 24 samples. Transport samples to the laboratory and process within 24 h Add 200 ml of sterile, buffered rinse solution (Appendix D, Sec. 2) to each sample and reseal the jar. Place sample jars (not to exceed four) in ultrasonic bath, making sure the liquid level in the bath is above the liquid level in the sample jars. Sonicate 2 min.±5 sec. After sonication, plate the rinse solution as in one of the following methods:

a. Wipe-Assay Method 1

(1) Unheated Portions

Using a sterile pipette, agitate the wipe in the sample jar for approximately 10 sec. Aseptically pipette 4.0 to 10.0-ml portions of rinse solution into sterile petri plates. A total of 48 ml of solution should be plated. Add sterile, molten (48 to 50ºC) TSA to each plate and mix the contents by gentle swirling. Allow the mixture to solidify.

(2) Heated Portions

Using a sterile pipette, agitate the wipe approximately 10 sec. Aseptically pipette two 25.0 ml portions of rinse solution from each sample into 25 x 150 mm screw cap tubes and place in a 80±2°C water bath for 15 min. as determined by a pilot tube containing a thermometer. Transfer heat-shocked tubes to a cold water bath and cool rapidly (within 2 min.) to 30 to 35°C. Aseptically pipette 4.0 to 10.0-ml portions of the rinse solution into sterile, petri plates. A total of 24 ml from each tube should be plated. Add approximately 20 ml sterile, molten (48 to 50°C) TSA to each plate and mix contents by gentle swirling. Allow the mixture to solidify.

(3) Dilutions

If necessary, make appropriate dilutions of the rinse fluid in sterile rinse solution (Appendix D, Sec. 1) after sonication. Plate portions of each dilution as described above in Unheated Portions and Heated Portions.

(4) Incubation

Aerobically incubate one unheated sample and one heated sample from each wipe assay at 32°C in an inverted position. Anaerobically incubate (Appendix D, Sec. 12) the remaining unheated and heated samples at 32°C in an inverted position. Scan plates of the aerobically incubated samples after 24 and 48 h incubation; count if growth indicates. Do not remove the plate covers until the final count is made at 72 h. Perform colony counts of the anaerobically incubated samples after 72 h. In accordance with the assay group's needs or, if applicable, as required by NASA management or its authorized contractor(s); plates should be incubated at 3°C for 14 days to encourage the selection of psychrophilic organisms. To encourage the selection of thermophilic organisms, incubation should be at 55°C for 24 to 48 h.

b. Wipe-Assay Method 2

When it is desirable or required that the assay be directed toward one group of microorganisms (i.e., aerobes, anaerobes, aerobic spores, or anaerobic spores) and or when the level of microbial contamination is considered to be extremely low, the following assay procedure may be used:

(1) Unheated Portions

After sonication as described above in Wipe-Rinse Method, use a sterile pipette to agitate the wipe for approximately 10 sec. then aseptically pipette four duplicate 25.0-ml portions of rinse solution into 150 x 25 mm plates. Add sterile, molten (55°C), concentrated (Appendix D, Sec. 3) TSA to each plate and mix the contents by swirling.

(2) Heated Portions

After sonication as described above in Wipe-Rinse Method, use a sterile pipette to agitate the wipe for approximately 10 sec. Aseptically pipette four 25.0-ml portions of rinse solution from each sample into sterile, screw cap, 25 x 150 mm tubes. Place tubes in a 80±2°C water bath and allow to remain at temperature for 15 min. as determined by a pilot tube containing a thermometer. Transfer tubes to cold water bath and rapidly (within 2 min.) cool to 30 to 35°C. Make four pour plates by adding the contents of each tube to each plate (150 x 25 mm). Add sterile, molten (55°C) concentrated TSA to each plate and mix the contents by swirling.

(3) Incubation

Incubation shall be carried out in the same manner as in (d) Wipe-Assay Method 1.

c. Calculation of Results

Express the results as number of microorganisms per cm^2 of surface. When applicable, general categories shall be reported as individual groups (i.e., aerobes, aerobic spores, etc.) and shall not be added in any combination. It is suggested that the mean and range be calculated for each series of wipe samples. However, all extrapolations of data

shall be done in accordance with the assay group's needs or, if applicable, as required by NASA management or its authorized contractor(s).

d. Controls

Process a number of sterile wipes equal to 10% of the total number of wipes processed as check items on the entire procedure. Processing shall be during one of the assays conducted each day or more often as prescribed by NASA management or its authorized contractor(s).

1.2.3. Microbiological Sampling of Intramural Air

1.2.3.1. Slit Sampler Agar Impaction Devices

a. Method

For sequential sampling of intramural air in "clean" assembly environments, a slit sampler capable of collecting at least 850 *l* of air on a 150 x 25 mm agar plate in a single revolution should be used. The volume sampled with each plate should not exceed 3500 *l*. Other desirable features for "clean room" sampling include oil-less pumps and manual, spring-wound clock motors.

b. Incubation

Aerobically incubate all plates at 32ºC for 72 h in an inverted position. Scan plates at 24 and 48 h; count if growth indicates. Perform and record colony counts at 72 h. Do not remove the petri plate covers until the final count is made at 72 h.

c. Calculation of Results

Figure 1 illustrates a sample data sheet that may be used in conjunction with the S-T-A Air Sampler. The example illustrates an electric clockwise rotation. Manual spring-wound models would have a counter-clockwise rotation and the numbers would therefore run clockwise.

Each segment (1/30) represents a 2 min. sample when a 1 rev/h clock motor is used. Results shall be reported as the number of colony forming units per *l* of air or, when applicable, may be prescribed by NASA management or its authorized contractor(s).

1.2.3.2. Membrane Filter Field Monitors

a. Method

(1) Single Samples

When desirable, membrane filter field monitors may be used for the sampling of intramural air, especially in areas where larger slit sampler agar impaction devices are restricted. Each field monitor will use a 0.45 or 0.8 µm membrane filter (Appendix C, Sec. 11) attached to a vacuum source via an aerosol adapter with a 10 *l*/min. flow-limiting orifice. The volume of air sampled with each field monitor shall not exceed 300 *l*. The membrane filter shall be aseptically removed from the field monitor and placed onto the surface of a TSA plate.

(2) Manifold Method

When sampling larger volumes of intramural air, or when isolation of different types of microorganisms by the use of selective isolation media is desired, a manifold capable of holding up to six membrane filter field monitors may be used. Each manifold shall be equipped with a flow meter calibrated to collect air at the rate of 10 *l*/ min. per field monitor up to a maximum of 60$\pm$.02 *l*/min. per manifold. The volume of air sampled with each manifold shall not exceed 300 *l* per field monitor, or 1800 *l* per manifold. The membrane filters shall be aseptically removed from the field monitors and placed onto the surfaces of TSA plates or onto the surface of selective growth media.

b. Incubation

Incubate in same manner as described above under Slit Sampler Agar Impaction Devices.

c. Calculation of Results

The number of colonies growing on the surface of the membrane filter represents the number of airborne viable particles per 300 *l* of air. This value divided by 300 would represent the mean for one *l* of air. Results shall be reported as the number of colony forming units per *l* of air or, when applicable, may be prescribed by NASA management or its authorized contractor(s).

d. Controls

Process a number of sterile membranes equal to 10% of the total number of cloths processed as check items on the entire procedure. Processing shall be during one of the assays conducted each day or more often as prescribed by NASA management or its authorized contractor(s).

1.2.4. Assessment of Microbial Contamination on Spacecraft Hardware

1.2.4.1. Piece-parts

a. Surfaces

(1) General

Aseptically place the piece-part to be assayed in a flask containing 50 ml of sterile rinse solution at $25 \pm 5^{o}C$ (Appendix D, Sec. 1). Each flask should have its stopper or cap covered with aluminum foil and the foil should extend 5-6 cm down the neck of the flask. Immediately sonicate the sample in the following manner:

(i) Suspend each flask containing the piece-part and rinse solution in the middle of an ultrasonic bath filled to its normal capacity so that the bottom of the flask is parallel to the bottom of the bath and the bath fluid solution is above the level of the rinse solution in the flask. Suspension shall be accomplished by any suitable means. Racks, baskets, or similar devices designed to hold sample containers should not be used.

(ii) Sonicate for 2.0 min. $\pm$5 sec.

(iii) At the discretion of the assay group, irregularly shaped piece-parts may be sonicated for 2 min. in each of several positions to ensure adequate exposure of all surfaces to ultrasonic energy.

(iv) For large piece-parts that will not fit into an Erlenmeyer flask, a suitable container, which conforms to the performance test for removing microorganisms from surfaces specified in Appendix C, Sec. 5, shall be used.

(2) Piece-part Assay Method 1

Use the same method described above under Fallout Strip Assay Procedure (section 1.2.1.2).

(3) Piece-part Assay Method 2

Use the same method described above under Fallout Strip Assay Procedure 2 (section 1.2.1.3).

(4) Calculation of Results

Each piece-part shall be identified and the total exposed surface area shall be determined and recorded. Results shall be expressed initially as number of microorganisms per piece-part (i.e., aerobes, anaerobes, aerobic spores, or anaerobic spores per piece-part). The general categories of microorganisms shall be reported as individual groups (i.e., aerobes, aerobic spores, etc.) and shall not be added in any combination. It is suggested that the mean and range be calculated

for each type of piece-part. However, all extrapolations of data shall be done in accordance with the assay group's needs or, if applicable, as required by NASA management or its authorized contractor(s).

(5) Controls

Process a minimum of 10 sterile piece-parts or 10 sterile stainless steel strips as sterility control check items on the entire assay procedure. Processing shall be during one of the assays conducted each day or more often as prescribed by NASA management or its authorized contractor(s).

(6) Test for Inhibitory Substances

After sterilization with dry heat (Appendix C, Sec. 1), perform an inhibitory test on each type of piece-part assayed. After sterilization, aseptically place the sterile part in a flask containing 50 ml of sterile TSB (Appendix D, Sec. 5). Add 80 to 120 spores of *Bacillus subtilis* var. n*iger*. Sonicate the flask containing the part for 2 min.$\pm$5 sec. and then allow it to stand for 30 min. at room temperature (22 to 27^{o}C). Aseptically remove the part and incubate the flask at 32^{o}C for 72 h. Perform a viability check on the spore inoculum by inoculating a tube of sterile TSB and incubating it at 32^{o}C for 72 h.

1.2.4.2. Space Hardware: Modules, Components, Subsystems, Systems and Landing Capsules

a. Swab-Rinse Method

Use the same techniques described above under Swab-Rinse Method (Microbiological Sampling of Environmental Surfaces).

b. Wipe-Rinse Method

Use the same techniques described above under Wipe-Rinse Method (Microbiological Sampling of Environmental Surfaces).

c. Detachable Strip Method

Hardware design should provide for the inclusion of detachable strips on the surface of each flight item. The time of attachment, location and the number of strips shall be determined by the assay group's needs or by NASA management or its authorized contractor(s). The method of attachment shall be such that it does not compromise the physical integrity of the space hardware or the validity of the microbiological assay. Use the method described above under the section describing assay of airborne microbial contamination accumulating on strips.

APPENDIX A: ABBREVIATIONS AND SYMBOLS

C	degree centigrade
cm	centimeter
cm^2	square centimeter(s)
cm^3	cubic centimeter(s)
diam	diameter
deg	degree
FEP	fluorinated ethylpropylene resins
g	gram
h	hour
HCl	hydrochloric acid
Hg	mercury
H_2S	hydrogen sulfide
kHz	1,000 cycles per second
ID	inside diameter
l	liter
l/min	liters per minute
m	meter
m^2	square meter(s)
m^3	cubic meter(s)
*m*m	micrometer
mg	milligram
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
NaOH	sodium hydroxide
pH	effective hydrogen ion concentration
%	percent
rev	revolution
sec	second
sq	square
S-T-A	slit to agar
TSA	Trypticase soy agar
TSB	Trypticase soy broth
USP	United States Pharmacopeia
vol/vol	volume to volume
w/cm^2	watt per square centimeter

APPENDIX B: REFERENCES

1. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1071. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Washington, D.C.

2. Anspach, W.E. 1974. Local airflow protection of surgical wounds to prevent airborne contamination. National Academy of Sciences, Washington, D.C.

3. Brazis, A.R., W.S. Clark, Jr., and William Sandine. 1972. Standard plate count method, p. 71-87. In William J. Hausler, Jr. (ed.), Standard methods for the examination of dairy products, 13th ed. American Public Health Association, Inc., Washington, D.C.

4. Brewer, J.H. and D.L. Allgeier. 1966. A safe, self-contained carbon dioxide-hydrogen anaerobic system. Appl. Microbiol. 14:985-988.

5. Favero, M.S. 1968. Microbiological sampling of surfaces. J.Appl. Bact. 31:336-343.

6. Favero, M.S. 1971. Microbiologic assay of space hardware. Environmental Biology and Medicine 1:27-36.

7. Feagin, J.A., Jr., 1974. The case for clean air. National Academy of Sciences, Washington, D.C.

8. Fields, N.D., G.S. Oxborrow, J.R. Puleo and C.M. Herring. 1974. Evaluation of membrane filter field monitors for microbiological air sampling. Appl. Microbiol. 27:517-520.

9. Hall, L.B. and M.J. Hartnett. 1964. Measurement of the bacterial contamination on surfaces in hospitals. U.S. Public Health Report 79, pp. 1021-1024.

10. Miller, J., G. Richards, G.R. Murphy and J. Prentis. 1974. Personal envelop system in the control of operating room air contamination. National Academy of Sciences, Washington, D.C.

11. National Aeronautics and Space Administration. 1967. Standards for clean rooms and work stations for the microbially controlled environment. U.S. Government Printing Office, Washington, D.C.

12. National Aeronautics and Space Administration. 1976. Quarantine provisions for unmanned extraterrestrial missions. National Aeronautics and Space Administration, Washington, D.C.

13. Puleo, J.R., M.S. Favero, G.S. Oxborrow and C.M. Herring. 1975. Method for collecting naturally occurring airborne bacterial spores for determining their thermal resistance. Appl. Microbiol. 30:786-790.

14. U.S. Government Printing Office, 1973. Clean room and clean work station requirements, controlled environments. U.S. Government Printing Office, Washington, D.C.

15. Vesley, D. 1963. Surface sampling techniques for the institutional environment. Proc. Nat. Conf. Inst. Acquired Inf., PHS Pub. No. 1188, 1964, pp. 101-103.

APPENDIX C: PREPARATION AND STERILIZATION OF EQUIPMENT

1. *STAINLESS STEEL SURFACES*

a. Type

Stainless steel strips shall conform to the following specifications: cold roll; type 302 or 304; No. 4 finish, 2.5 x 5.1 cm; 22 to 26 gauge (0.08-0.05 cm).

b. *Cleaning*

Wash in hot tap water (80 ± 10^{o}C) containing a nonionic detergent. Rinse three times with distilled water. Rinse with reagent grade absolute isopropyl alcohol. Drain dry.

c. *Marking*

Each strip shall be marked in one corner with a small "x" (no part shall exceed the limits of a 1.3 cm diam circle), using a diamond tipped scriber or equivalent.

d. *Preparation for Sterilization*

Using forceps, place clean, dry, stainless steel strips (marked side up) on flat, non-corrosive metal trays (approximately 28 x 38 cm). These trays shall not have raised edges.

e. *Degreasing Aluminum Foil*

Degrease aluminum foil used for covering trays of strips by wiping with a lint-free wipe wetted with reagent grade absolute methyl alcohol or equivalent.

f. *Sterilization*

Arrange the strips in a monolayer on each tray. Wrap each tray with degreased aluminum foil. Make certain that the entire tray is covered and the foil is not punctured in the process. Sterilize the wrapped trays with dry heat at 175 ± 2^{o}C for 2 h. The sterilization time shall begin when the tray temperature reaches 175 ± 2^{o}C as determined by thermocouples. If more than one tray is sterilized at a time, the thermocouples shall be attached to the tray nearest the center of the oven.

2. *TEFLON RIBBON SURFACES*

a. *Type*

Teflon ribbons shall conform to the following specifications: Teflon, FEP Type 500A Transparent, Roll 7.6 by 1646 cm.

b. *Cleaning*

Wash in hot tap water (80 ± 10^{o}C) containing a nonionic detergent. Rinse three times with distilled water. Drain dry.

c. *Preparation for Sterilization*

Using gloves, roll up clean, dry Teflon ribbon, and place in clean 250 ml glass beaker. Cover each beaker with degreased aluminum foil.

d. *Sterilization*

Sterilize the Teflon ribbons in glass beakers with dry heat at 175±2°C for 3 h. New ribbons are preconditioned by two exposures to dry heat for 3 h each at 175±2°C to allow for degassing of Teflon prior to a third heating interval for sterilization. The sterilization shall begin when the ribbon temperature reaches 175±2°C as determined by a thermocouple attached to the Teflon ribbon inside of a glass beaker, located nearest the center of the oven.

3. *FORCEPS*

Clean the stainless steel forceps (at least 15 cm long) as described in section 1b above and package them individually in degreased aluminum foil or metal-capped test tubes. Sterilize with dry heat at 175±2°C for 2 h. The time for sterilization shall be determined in the same manner as described in 1f above.

4. *ERLENMEYER FLASKS*

a. *Type* - Borosilicate glass

(1) 250 ml

(2) 125 ml, wide mouth

b. *Cleaning*

Wash flasks and stopper in hot tap water (at least 80°C) containing a nonionic detergent. Rinse five times with tap water and one time with distilled water. Drain dry.

c. *Stoppers and Caps*

White or black No. 6-1/2 stoppers shall be used.

When black or white rubber stoppers are used for the first time, the following procedures shall be used to remove toxic substances:

(1) White stoppers (No. 6-1/2)

(a) Prepare 0.5 normal NaOH in distilled water (20 g/*l*).
(b) Boil rubber stoppers for 30 min. in this solution.
(c) Rinse 15 min. in hot (80±10°C) tap water.
(d) Place stoppers in 0.5 normal HCl (18 ml of concentrated HCl/*l*).
(e) Boil 30 min. in this solution.
(f) Rinse 15 min. in hot tap water (80±10°C).
(g) Rinse five times with distilled water.

(2) Black stoppers (No. 6-1/2)

(a) Soak rubber stoppers in a nonionic detergent for approximately 12 h.
(b) Rinse in tap water for 10 min.
(c) Boil 30 min. in fresh nonionic detergent.
(d) Rinse with hot tap water for 30 min.
(e) Rinse three times with distilled water.

d. *Sterilization*

Cover each flask with a layer of aluminum foil 5-6 cm down the neck of the flask. Sterilize the aluminum foil covered flask with dry heat at 175±2°C for 2 h. Sterilize the stoppers in an aluminum foil covered beaker or equivalent in an autoclave at 121°C for 15 min. Aseptically place the sterile stoppers on the sterile flasks. Replace the aluminum foil

over the stoppered flasks. From each lot of 50 processed flasks, select at random six flasks and to each aseptically add 50 ml of sterile TSB (Appendix D, Sec.5). Incubate these sterility controls at 32ºC for 72 h.

5. ULTRASONIC BATH

a. *Specification*

All sonication procedures shall conform to the following specifications:

(1) The frequency shall be 25 kHz or greater.

(2) The rated power output in relation to the bottom surface area of the bath should be at least 0.35 w/cm^2.

(3) If the ultrasonic bath is not automatically tuned, tuning shall be performed according to the manufacturer's directions.

(4) The inside surfaces of the bath shall be stainless steel

(5) Each Erlenmeyer flask containing a strip or piece-part to be assayed shall be suspended individually in the middle of a full bath and sonicated for 2 min. $\pm$5 sec.

(6) The bath fluid shall be an aqueous solution of 0.3% vol/vol Tween 80. This solution shall be made up fresh each day.

(7) The temperature of the bath fluid shall be at least 20ºC and shall not exceed 32ºC.

(8) Prior to sonicating samples to be assayed, the bath shall be operated for at least 5 min. to de-gas the bath fluid.

(9) The bath fluid shall be above the level of the liquid in the flasks being sonicated.

(10) It is suggested that ultrasonic baths approximately 23 cm wide, 25 cm long and 25 cm deep be used. The use of larger baths is discouraged but not prohibited.

b. *Performance Requirements for Modified Sonication Procedures.*

(1) *Purpose*

The sonication of more than one flask in an ultrasonic bath at one time and/or the use of containers other than flasks for the sonication procedure shall be permitted if the modified procedure meets the standard performance requirements specified herein.

(2) *Methods*

Obtain standard 2.5 x 5.1 cm stainless steel strips contaminated with *Bacillus subtilis* var. *niger* spores on the surface marked with an "X". Using sterile forceps place one strip in each container to be used in the modified procedure. The strip should be placed so that the contaminated surface faces the transducers during sonication. Aseptically add the quantity of sterile rinse solution used in the modified procedure to each container. Place the container or containers in the ultrasonic bath according to the modified procedure and sonicate for 2 min.$\pm$5 sec. Pour all the rinse liquid in each container into separate 150 x 25 mm petri plates. No more than 25 ml of liquid should be poured into any one plate. Gently swirl the solution in the plate, quickly add 75 ml of sterile, molten (50 to 53ºC) concentrated TSA (Appendix D, Sec. 3), and *immediately* resume gentle swirling until the contents are thoroughly mixed. Allow the mixture to solidify. Using sterile forceps aseptically remove the stainless steel strip from the container, rinse it completely in a gentle stream of sterile distilled water for 3 to 5 sec. and place the strip with surface marked with an "X" facing upward in a sterile, dry, 100 mm diam petri plate. Add enough sterile, molten (48 to 50ºC) TSA to completely cover each stainless steel strip. Aerobically incubate all samples at 32ºC. Scan plates of the samples after 24 and 48 h incubation; count if growth indicates. Do not remove the plate covers until the final count is made at 72 h.

(3) *Calculations*

Divide the total colony count from all the rinse fluid in a container by the sum of the total colony count from all the rinse fluid in that container plus the colony count from the plated stainless steel strip taken from that container. Multiply this quotient by 100 to obtain the percent of spores removed from the strip in that container.

(4) *Requirements*

The number of standard contaminated stainless steel strips assayed by the modified procedure shall be determined by the following:

(a) If five or more containers are sonicated in one ultrasonic bath at one time, at least three sets of standard strips shall be assayed. Each set shall contain a number of strips equal to the maximum number of containers sonicated at one time with the modified procedure.

(b) If less than five containers are sonicated in one ultrasonic bath at one time, a total of at least 12 standard strips shall be assayed. The strips shall be sonicated singly or in sets according to the modified procedure.

The modified procedure meets performance requirements if: in (a) above, the mean percent of spores removed is 95% or greater for each of the three sets; in (b) above, the mean percent of spores removed from the 12 standard strips is 95% or greater.

6. *PETRI PLATES*

Commercially available, disposable sterile 100 mm diam and 150 mm diam petri plates or reusable glass petri plates of similar diameter are equally acceptable. Glass petri plates should be washed according to the procedure outlined in Appendix C, Sec. 4. Place clean, dry, glass petri plates in metal petri plate containers. Sterilize packaged petri places with dry heat at 175ºC for 2 h.

7. *COTTON SWABS*

Use commercially available absorbent cotton swabs firmly twisted to 5 x 19 mm long over one end of a 15 cm wooden applicator stick. The swabs shall be packed individually in a protective container such as sterile test tubes with slip-tops or cotton plug closures. Swab heads should be directed away from the closure. Sterilize swabs by autoclaving and allow them to dry prior to use.

8. *WIPES*

Use commercially available 100% polyester bonded clean room wipes, 23 x 23 cm. No more than five folded wipes shall be placed in individual surgical sterilization bags (or equivalent). Sterilize wipes by autoclaving and allow them to dry prior to use.

9. *SLIT SAMPLER*

a. *Calibration and Preparation*

Each sample shall be equipped with a calibrated in-line flowmeter. A vacuum source 50% greater than the designated sampling rate is required for each sampler. Before initial operation and semi-annually thereafter, calibrate all samplers to draw the designated volume $\pm$5%.

b. *Preparation of Volumetric Air Sampler Plates*

Use sterile, 150 x 25 mm petri plates. Prepare each plate to contain 85.0$\pm$2.0 ml of sterile TSA. Wrap prepared batches of plates securely to prevent dehydration. Incubate prepared and wrapped plates at 32ºC for 72 h. Unwrap and discard contaminated plates. Rewrap and refrigerate plates at 4 to 6ºC until 12 to 18 h prior to use. Again unwrap plates and leave at room temperature until used. Use sterile plates within 18 h after unwrapping and within 30 days of initial preparation.

c. *Data Recording*

Figure 1 shows a sample sheet, which may be used for reporting results, obtained with the long-term slit sampler.

10. *MEMBRANE FILTER FIELD MONITORS*

Commercially available, disposable, sterile, clinical field monitors shall be used.

a. *Calibration and Preparation*

Insert a 10 *l*/min flow-limiting orifice to the outlet end of an aerosol adapter. With a clinical field monitor attached to the inlet of the adapter and the outlet connected via vacuum hose to a minimum vacuum source of 500 mm Hg, a constant flow rate will be obtained. To ensure accuracy, the orifice should be calibrated with a flowmeter at the actual working vacuum level.

When a manifold is used, it shall be equipped with a calibrated in-line flowmeter. A minimum vacuum source of 75 *l*/min free air is required for each manifold. Before initial operation and at monthly intervals thereafter, calibrate all manifolds to draw air at a velocity of 10 *l*/min per field monitor up to a maximum of 60$\pm$.02 *l*/min per manifold.

11. *PREPARATION OF ANAEROBIC CONDITIONS*

Establish and maintain anaerobic conditions in one of the Brewer jars, either the electric or newer cold catalyst system. If the electric Brewer jar is used, gaseous hydrogen and a catalyst shall be used to remove residual oxygen. Place a reducing indicator (such as methylene blue) in each jar. One may include the anaerobic indicator organism *Clostridium novyi* type B and optionally the aerobic indicator organism *Alcaligenes faecalis*. If the *A. faecalis* shows visible growth after 72 h at 32°C or the indicator remains blue after 12 h at 32°C, the environment cannot be considered anaerobic. If *C. novyi* shows visible growth and the indicator is white, anaerobic conditions are considered to be established. If *C. sporogenes*, or any other organism thought to produce H_2S, is grown, the catalyst pellets should be replaced before another run. When using a cold catalyst system below room temperature for the incubation of psychrophiles, the jar should be set up and allowed to set at room temperature for 30 min before placing into a low temperature incubator, otherwise the non-heated catalyst will not be active.

APPENDIX D: PREPARATION AND STERILIZATION OF CULTURE MEDIA

1. *RINSE SOLUTION*

Prepare a 0.02% vol/vol solution of certified (i.e., certified non-toxic to microorganisms) Tween 80 in buffered distilled water. The final pH shall be 7.2±0.1. Sterilize by autoclaving according to Appendix D, Sec.8.

2. BUFFERED DISTILLED WATER

To prepare a stock buffer solution, dissolve 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water, adjust to pH 7.2±0.1 with one normal NaOH, and dilute to one *l* with distilled water. To prepare a working solution of buffered distilled water, add 1.25 ml of the stock buffer solution to one *l* of distilled water.

3. TSA CULTURE MEDIUM

To prepare Tryptic Soy Agar dissolve commercially certified Tryptic Soy Agar Mix in water per the manufacturer instructions. Final reaction should be pH 7.3. Autoclave at 121ºC for length of time suggested in Appendix D, Sec. 8. Immediately after autoclaving, place the containers of sterile TSA in a 48 to 50ºC water bath or allow to cool to 50 to 55ºC before placing in a 48 to 50ºC holding oven. The medium shall be used within 6 h.

In accordance with the assay group's needs or, if applicable, as required by NASA management or its authorized contractor(s), media may be removed from the autoclave and allowed to solidify. Flasks containing media shall be stored at ambient room temperature for 72 h and observed for evidence of contamination. Discard any flask containing contaminated agar. Store remaining flasks containing agar at 4ºC. Media not used within 14 days from date of sterilization shall be discarded. Prior to the assay operation, place flasks containing solidified agar in the autoclave. Allow to remain at temperature for 10 min. Immediately after autoclaving, place the flasks of sterile agar in a water bath at 48 to 50ºC or allow cooling to 50 to 55ºC before placing in a 48 to 50ºC holding oven. The medium shall be used within 6 h.

Prepare concentrated TSA in the same manner described above using 20 grams of agar per liter volume of distilled water. Use a water bath or holding oven set at 50 to 55ºC.

4. *TSA WITH NEUTRALIZERS*

To prepare Tryptic Soy Agar with Neutralizers dissolve commercially certified Tryptic Soy Agar with Neutralizers containing Tween 80 and lecithin to make concentrations of 0.5% and 0.07% vol/vol respectively. Mix in water per the manufacturers instructions Mix thoroughly and sterilize at 121ºC for the length of time suggested in Appendix D, Sec. 8. Use within 30 min.

5. TSB

To prepare Tryptic Soy Broth dissolve commercially certified Tryptic Soy Broth Mix in water per the manufacturer instructions. Mix thoroughly and sterilize at 121ºC for the length of time suggested in Appendix D, Sec. 8. Final reaction should be pH 7.3.

6. *TSB WITH NEUTRALIZERS*

To prepare Tryptic Soy Broth with Neutralizers dissolve commercially certified Tryptic Soy Broth with Neutralizers containing Tween 80 and lecithin to make concentrations of 0.5% and 0.07% vol/vol respectively. Mix in water per the manufacturer instructions and sterilize at 121ºC for the length of time suggested in Appendix D, Sec. 8. Use within 30 min.

7. *D/E NEUTRALIZING AGAR*

Suspend the following quantities of dehydrated ingredients in one *l* of distilled water:

Tryptone	5 g
Yeast Extract	2.5 g
Dextrose	10 g
Sodium Thioglycollate	1 g
Sodium Thiosulfate	6 g
Sodium Bisulfite	2.5 g
Lecithin (Soy Bean)	7 g
Polysorbate 80	5 g
Agar	15 g
Brom Cresol Purple	0.02 g

Mix until a uniform suspension is obtained. Heat with frequent agitation and boil for one minute. Dispense as required and autoclave at 121ºC for the length of time suggested in Appendix D, Sec. 8. Final reaction should be pH 7.6.

8. *STEAM STERILIZATION OF LIQUIDS*

Autoclave time, at the desired autoclave temperature, is the most critical parameter for adequate sterilization. The time and temperature required for a given load varies with the size and shape of the container, the volume of liquid per container, the number of containers per load, the thickness of the container walls, and the thermal conductivity of the container. The amount of space between the containers is also of importance in that a tightly packed load may increase sterilization time by interfering with free circulation of steam. The influence of variations in sterilizers and ancillary equipment such as steam supply, pipe size and pressure regulating systems also affects sterilization time. The effect of some of these factors is shown in Tables 1 and 2. These data are shown only as examples.

In practice, each autoclave should be tested with indicator organisms under various conditions of loading and with various materials to establish a profile of sterilization cycle times, temperatures and pressures for that sterilizer.

Table 1. STEAM STERILIZATION OF LIQUIDS- EFFECT OF VOLUME PER CONTAINER (ERLENMEYER FLASKS) ON TIME REQUIRED TO REACH 121ºC (SINGLE CONTAINER LOAD)

Size of container (ml)	Milliliters of liquid per container	Chamber temp (C) at initiation of cycle	Liquid temp (C) at initiation of cycle	Mins for chamber to reach 121º C	Mins for center of liquid to reach 121º C	Total time (mins) of cycle
50	25	110	25	2	4	14
125	75	110	25	2	5	15
200	150	110	25	3	7	17
500	400	110	25	3	10	20
1000	800	110	25	3	14	24
2000	1500	110	25	6	19	29
3000	2500	110	25	7	25	35
5000	4500	110	25	8	33	43
6000	5500	110	25	8	44	54

Table 2. STEAM STERILIZATION OF LIQUIDS- EFFECT OF VOLUME PER CONTAINER AND NUMBER OF CONTAINERS ON TIME REQUIRED FOR LIQUID TO REACH 121ºC

Liters of liquid per container	No. of containers per load	Chamber temp (C) at initiation of cycle	Liquid temp (C) at initiation of cycle	Mins for chamber to reach 121ºC	Mins for center of liquid to reach 121ºC	Total time (mins) of cycle
0.5	30	27	29	10	19	29
1.0	20	27	26	12	34	44
1.5	15	56	26	12	36	46
2.0	10	46	27	13	37	47
2.5	10	66	26	15	40	50
3.0	8	46	26	15	43	53
3.5	6	46	26	12	50	60
4.0	5	43	26	12	52	62
4.5	5	44	26	14	58	68
5.0	5	46	26	15	60	70
5.5	5	42	26	17	60	70
6.0	4	42	26	15	62	72

Chart or external thermometer readings reflect the temperature of the chamber, not the load. Variations in load and equipment necessitate the use of a temperature-sensing device in the center of the load. Consequently a thermocouple and potentiometer or similar device shall be used to determine the sterilization cycle. Once the cycle has been established there is no need to use thermocouples for daily use. It is recommended however, that the cycle be checked once every two weeks.

Colony counts per 2 min segment. (In this example count/segment divided by 100 = colony count per liter of air.)

PLACE FIGURE 1 HERE

Date: May 15, 1978 Technician: Ed Fiola

(I) Sampler location: KSC Hangar AO-Shuttle assembly area
(2) Time of operation: on: 0800 off: 0900 Total time: 60 min.
(3) Bacteriological medium: TSA Sampling rate: 50 liters/min.
(4) Incubation of sample: Aerobic Temperature: 32°C Time: 72 hr.
(5) Mean colonies/liter: Total count/3000=120/3000=0.05

Remarks: Sampler located 1 m from floor and approximately 2 m from Shuttle. Personnel activity moderate.

Figure 1. Sample sheet for reporting results obtained with long-term slit sampler.