

TECHNICAL REPORT

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MICROBIOLOGICAL CONTAMINATION CONTROL IN
A CONTROLLED ENVIRONMENT FACILITY (CEEF)
FOR FOOD PROCESSING

by

Edmund M. Powers

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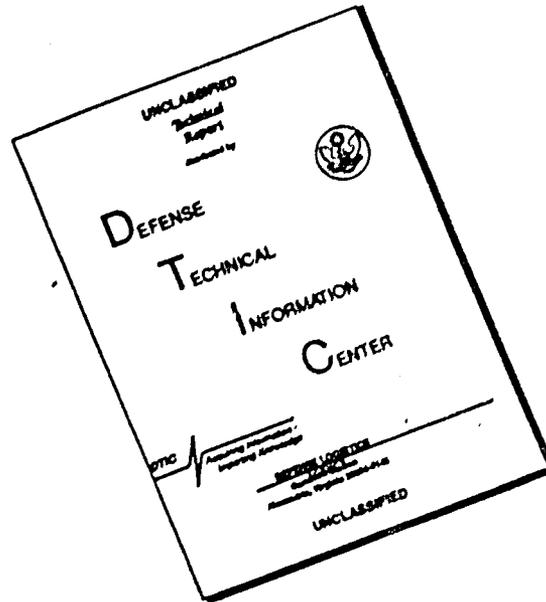
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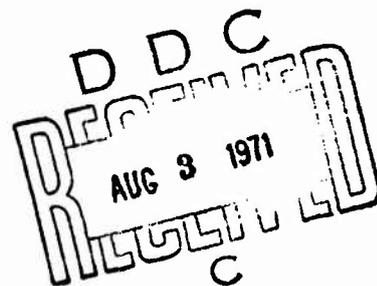
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FOREWORD

To insure that space foods meet the rigid microbiological requirements imposed on them it is imperative to closely control the food processing environment. The recent advances in the technology of laminar airflow controlled environments offers the best means of controlling microbial contamination during the processing and packaging of critical foods such as those utilized on space missions. This new technology should be explored and exploited, not only for the manufacturing of space food, but also for other select conventional foods, in general, and new convenience foods, in particular. This report offers a research and development approach toward that objective.

A special "controlled environment" facility (ChEF) was constructed at NLABS to be utilized for this research activity. The facility was supported by the National Aeronautics and Space Administration under customer order NASA, T-25041-G for food packages and containers intended for manned space flights and the Air Force School of Aerospace Medicine under their MIPR AM 6-40061 for food and packaging used for experiments simulating an Aerospace mission.

The author thanks Mr. John Swift of the Process Development Division, Food Laboratory for providing the engineering specifications.

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ABSTRACT

Experience over the past several years with space food prototypes has demonstrated the essential role of a "controlled environment" in meeting the rigid microbiological requirements during production of these critical foods. The U. S. Army Natick Laboratories has constructed a "clean room", or controlled environment facility (ChEF) for food processing, which will employ laminar airflow technology. This report presents a brief outline of a research plan for utilizing this new facility toward establishing the scientific and technological basis for assuring the microbiological wholesomeness of space food prototypes, as well as exploiting this technology toward new and useful food processes and products.

A. INTRODUCTION

One of the more rapidly developing technologies is that of the "controlled environment", or "clean room". This technology arose out of a need by the electronics industry to provide particle-free environments for the assembly of miniaturized circuits where a particle or even a microorganism could cause a short circuit (3). A breakthrough in the technology came about in 1961 when the laminar airflow concept was applied to "clean rooms" (24, 25). This concept controls particulate and microbial contamination by moving ultrafiltered air at a controlled velocity across the work area with minimum turbulence to remove airborne contaminants. This is accomplished by converting entire walls or ceilings into filter banks capable of filtering out particles of a 0.3 micron size or larger. Air flows vertically or horizontally across the room in predictable streamline paths at rates over 100 linear feet per minute. In contrast to a conventional type clean room, which can provide a maximum of 60 air changes an hour, a laminar airflow clean room can provide as many as 6000 air changes per hour (1), thus diluting the airborne contamination and carrying it away as soon as it is generated.

This new technology was soon fully exploited and further developed by the aerospace industry. It has been applied by NASA to provide biologically clean environments for the assembly of sterile planetary landing vehicles (17, 19, 20, 21). Because of the NASA policy that all planetary landing space vehicles be sterile to prevent contamination of planets by terrestrial microorganisms (18) this new technology became essential. With the development of laminar airflow work stations and cabinets of all types, portable flexible walled laminar airflow isolators, modular type laminar airflow rooms and a wide variety of accessory equipment, a multitude of diverse industries have applied this new technology to their particular needs.

Environmental control is now practiced by the pharmaceutical industry for the assembly and packaging of drugs and biologicals (4, 9). Hospitals use laminar airflow surgical tables and work stations in surgical suites (4, 5). Laminar airflow rooms and isolators are becoming widely used in wards for the protective isolation of leukemia, cancer, organ transplant and other critical patients rendered extremely susceptible to infection (4, 13). Others utilizing the controlled environment technology include: the laundry industry to supply sterile or lint-free clothing (4, 11); the plastics industry to package sterile disposable products (4); biological research laboratories to minimize cross contamination (4, 6); the weaponry industry where extreme miniaturization and close tolerances are required to meet specifications (3, 4); industries manufacturing optical instruments (4); the photographic industry (4); animal breeders (4); National Bureau of Standards (4); chemical companies (4); jet fuel companies (4); breweries (4).

Controlled environment technology can be equally revolutionary and significantly beneficial to the food industry. Food processing and packaging in a controlled environment can bring about dramatic enhancement of microbiological wholesomeness, organoleptic quality and extension of shelf-life (10). These benefits will become more significant with the current expansion of the convenience food concept and its application to military subsistence systems.

By controlling their working environment, researchers will be provided with a unique tool to discern and define the independent and interacting effects of materials, processes and environmental variables upon the microbiological profile of the packaged end-product and, hence, its potential susceptibility to spoilage or pathogenicity. This will significantly enhance the validity of experimental data basic to the development of optimal processing and packaging techniques and sound procurement specifications.

Inherent deficiencies in sampling statistics and determinative microbiological techniques make it impossible to ascertain complete absence of a given microbial pathogen. The application of the controlled environment concept will provide an excellent preventive measure that will significantly enhance the microbiological wholesomeness of the packaged end product.

Controlling the environment (in combination with other treatments) will provide an effective approach towards restricting and reducing considerably the initial or inherent load of microbial contaminants in foods of all types including raw meats, vegetables, fruits, seasoning, intermediate and finished products. This will impart to the packaged end-product a significant increase in shelf-life in the absence of terminal microbial treatments. Where terminal microbicidal treatments such as heat, chemicals or ionizing radiation are applied to the food, the required minimal dose should be significantly reduced. This, in turn, will impart a considerable improvement in the nutritional and/or organoleptic quality. It is also anticipated that in the case of aseptic processing of food (e.g., aseptic thermoprocessing), the controlled environment concept may offer a marked simplification and improvement in unit operations, namely, aseptic filling and sealing (10).

The controlled environment concept offers the best, if not the only, means of effectively controlling the processing, packaging and/or preparing of foods, to be utilized on space flights and/or for critically ill patients where the hazard of microbial contamination cannot be tolerated. Aseptic processing and packaging procedures can be determined in the CHEF which can significantly reduce hazards and improve flavor, color, nutrient value and shelf-life of aerospace foods. These factors will become increasingly important with the extended duration of the NASA flights and associated increased demands on the feeding system.

B. WHAT IS A CONTROLLED ENVIRONMENT?

Environmental control encompasses the control of temperature, humidity, pressure differential and airborne particulates. Microbiological contamination control is the control of living microorganisms in a specified environment. This definition should be extended to include dead microorganisms as far as food processing is concerned, because large dead cell populations of the enteropathogenic gram negative types may be capable of triggering symptomatic pathogenesis (12, 14). Microbial contaminants, like any other contaminant have structure and mass, but unlike other types of contaminants they have certain properties which make them more hazardous and thus they warrant special consideration. These properties are (1) ability to reproduce, (2) ability to carry on physical and chemical processes and (3) ability to survive adverse environments. These properties are particularly important in the food industry because most foods are excellent substrates for supporting microbial growth.

C. WHAT ARE THE CRITERIA NECESSARY FOR CONTAMINATION CONTROL?

To establish an adequate microbiological contamination control program in the ChEF certain criteria must be satisfied:

1. Recognize and Define the Problem (2)

When a program involving microbiological contamination control is recognized, data should be accumulated that would allow the problem areas to be predicted and the necessary control measures installed. It would then be possible to define the microbial load of a particular process and to control it. In this way losses could be prevented before they occur.

2. Establish Contamination Control Criteria (2)

The objective of a control program must be stated in microbiological terms. If sterility is the objective, the criteria should specify what procedures are required to achieve sterility and what methods will be used to determine sterility of a food item. If sterility of a food item is not the objective, the criteria should specify the maximum number and types of microorganisms allowed in the item, in the environment, on a surface, etc., and should indicate the methods to be used. Foods processed for the military and for the Space Program must be microbiologically safe for human consumption. Success of this contamination control program will depend on continued research in areas where control is needed. Only in this way can appropriate criteria and standardized testing methods be determined.

3. Employ Contamination Control Techniques (2)

a. The latest methods in modern construction and design were used in the ChEF.

b. Contamination control equipment such as laminar flow work stations, and if necessary, gas tight, absolute barrier systems will be employed. Containment equipment of all kinds are available for such procedures as shaking, grinding, centrifuging, blending, freeze-drying, mixing, steaming and weighing out of food products and ingredients.

c. Correct techniques and procedures must be used while processing food. This includes the movement of personnel while working and the use of their hands in carrying out the work. The way food items are handled and the proper sequence of handling are important in controlling contamination.

d. Sterilizing agents and germicides must be used. These will include heat, gases, liquid decontaminating agents and radiation. The extent to which these methods are employed will depend on the type of work and the food material involved. The times, temperature and doses necessary may be reduced considerably by employing aseptic techniques and utilizing environmental control approaches; reduction in sterilizing treatment levels should improve flavor, texture and acceptability of food items.

e. Effective management is essential to any microbiological contamination control effort. Management must be responsible for assigning areas of responsibility and for the proper selection of competent personnel. Likewise, management must also be responsible for providing the necessary training for employees involved in contamination control and for formulating and enforcing work regulations.

4. Microbiological Testing and Surveillance Techniques Must Be Established (2).

Monitoring procedures are needed to determine if the techniques employed achieve the microbiological control objectives. These procedures will include air sampling for viable airborne microbes and particulates; surface sampling of materials, work stations and personnel for viable microorganisms; physical and chemical tests to determine whether the temperature, time and pressure employed are maintained; testing of air filters for adequate operation and testing for leaks in barrier systems.

5. Results Must Be Analyzed and Procedures Certified (2)

The above criteria will serve as a guide for the analysis of results and certification. In analyzing results it is important to consider the following:

a. No biological detection procedure is perfect or one hundred percent valid and reliable.

b. Decontamination sterilization methods will be more effective as the microbiological load is lowered. If a microbiological contamination control process does not meet minimum standards or is shown to be out of control, corrective measures should be started immediately.

The five criteria presented above in section C must be met for effective contamination control and for an understanding of the numerous factors which contribute to food borne illnesses. The rigid microbiological requirements imposed on Space Food Prototypes and military foods and the welfare of our astronauts and soldiers make it imperative that these foods be produced under rigidly controlled conditions.

D. ENGINEERING DESCRIPTION OF THE CHEF.

The controlled environment facility at U. S. Army Natick Laboratories (Fig. 1) is a conventional type clean room equipped with horizontal laminar flow work stations. The facility has approximately 1016 sq. ft. of floor surface and consists of the following functional areas

- Air shower (13 sq. ft.)
- Pantry (115 sq. ft.)
- Change Room (50 sq. ft.)
- Airlock (14 sq. ft.)
- Processing Room (clean room A, 620 sq. ft.)
- Finishing and Packaging room (clean room B, 193 sq. ft.)
- Pass thru (11 sq. ft.)

1. Walls

Walls of the facility consist of prefabricated modular units with 1/8th inch cement asbestos board on the outside and 1/8th inch melamine coated hardboard on the inside. The walls of each area are a different pastel color.

2. Ceilings

Ceilings of the facility are 8 ft. high and constructed of 2 ft. x 4 ft. metal faced panels fitted into a suspended "T" bar grid.

3. Floors

Floors of the facility consist of 0.090 inch sheet vinyl which is cemented to a concrete floor. All corners are coved.

4. Lighting

Flush ceiling fluorescent lighting provides a minimum of 100 ft. candles at the bench level (8, 22).

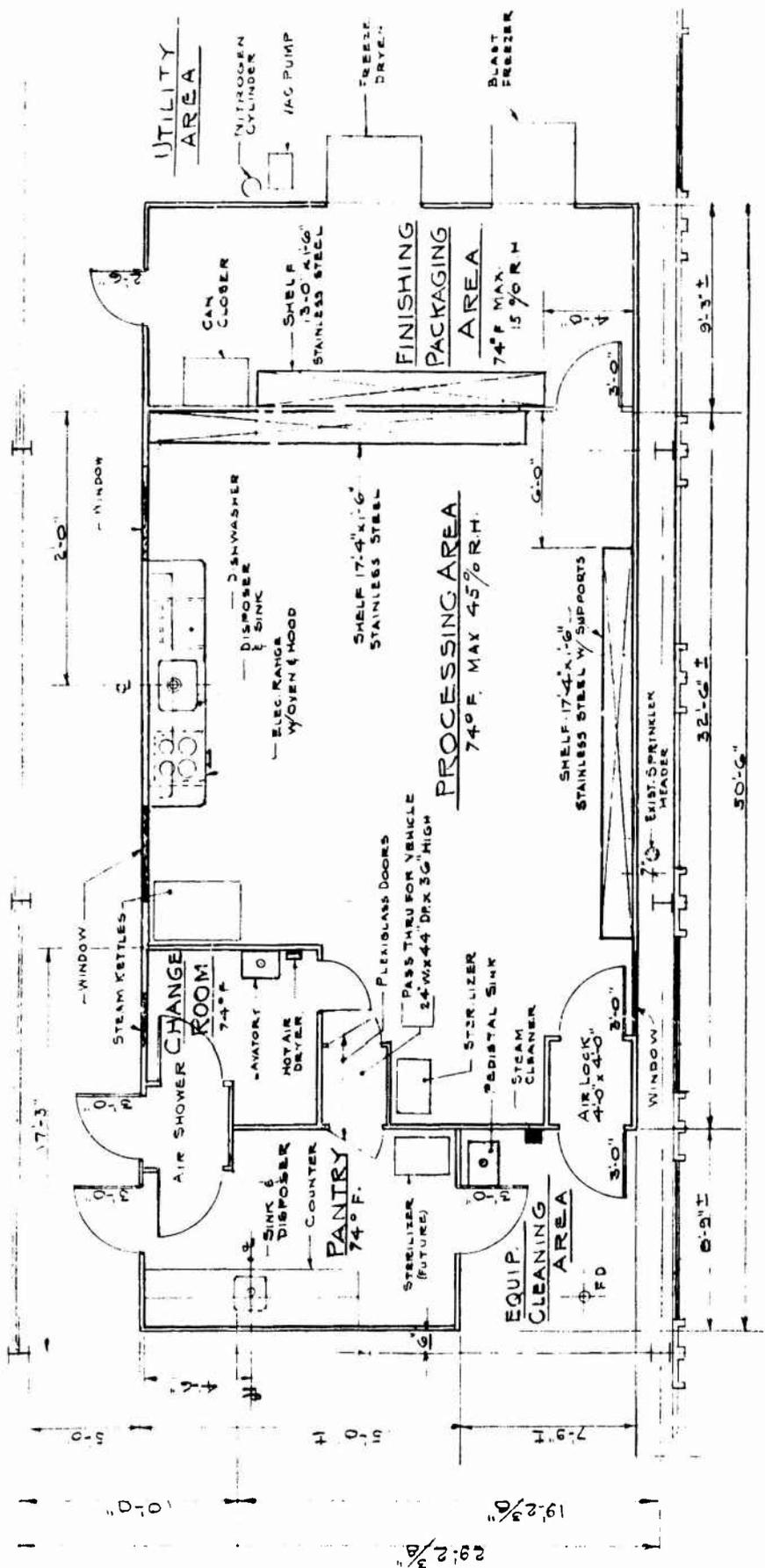


FIGURE 1. LAYOUT OF THE U.S. ARMY NATICK LABORATORIES CONTROLLED ENVIRONMENT FACILITY

5. Utilities

Most utilities are provided from overhead connections with the conduits and piping concealed within the wall modules. Electric power is supplied at 208 volts 3 phase, 208 volts single phase, and 115 volts through 200 amp and 100 amp distribution boards. Other utilities include steam, water, compressed air, nitrogen and vacuum.

6. Air Handling System

The air handling system is designed to change the room air approximately 30 times per hour. Outside air is prefiltered through furnace type fiberglass filters after which it is conditioned and enters the facility through fiberglass high efficiency particulate air (HEPA) filters located in the ceiling. Air flows into the room, through the HEPA filter, vertically at a rate of 110 ft. per minute. The HEPA filters remove 99.97% of all particles 0.3 micron in size or larger. Air is exhausted from the rooms through ducts located in the wall near the floor.

(a) Temperature. The temperature is thermostatically controlled; for optimal operation it should be maintained at 72°F.

(b) Relative Humidity. The relative humidity is uncontrolled except in the finishing and packaging room which will be maintained at a maximum relative humidity of 15%.

(c) Pressure. A minimum positive static pressure of 0.05 inch (water gauge) above ambient is maintained.

7. Clean Work Stations.

Horizontal laminar flow clean work stations (clean bench) are positioned around the processing room to provide Class 100 environments (8) for processing activities. Each clean bench is equipped with a separate air supply which is filtered through Scott foam prefilters and then through absolute filters (HEPA) adjacent to the work surface. The product or process will be bathed with ultra clean air from the filter backwall of the clean bench which is virtually free of bacteria and particulates. Air flows horizontally across the work surface at approximately 100 linear feet per minute. This rate of airflow is sufficient to protect the product from contamination shed downstream and will immediately carry away particles shed in the vicinity of the product. In addition to cleaning the air at the work surface or counter the clean bench will clean up the air of the room a distance of 10 feet from the edge of the counter (1). An area may be effectively turned into a clean room approaching the cleanliness of the work stations by

placing enough work stations in the area to give 100 recyclings an hour (22). This may be determined by the following formula (1):

$$N = \frac{AH}{27.0F}$$

where N = the number of work stations required
F = the number of 2 ft. x 2 ft. HEPA filters in the filter backwall of the work stations.
H = the ceiling height of the room
A = the area of the room in sq. ft.

E. ENTRY AND PERSONNEL FLOW IN THE CHEF

1. Air Shower

Personnel enter the facility through an air shower where they are "de-dusted" by air from 14 nozzels located on opposite walls of the shower. Air velocity from the nozzels is 90 mph. At this velocity personnel will be cleaned in the air shower for 15 seconds to remove surface contamination on outer garments and exposed skin surfaces. The duration of the air shower will be controlled by a timer. Following completion of the air shower personnel may enter the pantry or change room.

2. Pantry

The pantry is a semi clean or controlled area where raw materials and ingredients are stored and prepared for processing. After the ingredients are prepared (mixed, peeled, partially cooked, cleaned) they are transferred into the processing room (central clean room) through a pass through for further processing.

3. Change Room

The change room is entered into directly from the air shower. The room will be separated into two parts by a line painted across the floor. One side of the floor will be designated the clean side. After donning clean room garments personnel enter the clean side of the room in a prescribed manner so as to avoid undue contamination of the clean side. Personnel will then wash their hands with bactericidal soap and enter into the processing room.

4. Processing Room

The processing room is entered into directly from the change room. The most critical steps in the processing of a food are accomplished in this room. Virtually sterile environments for various processes are

provided by the utilization of horizontal laminar airflow work stations. The room itself may approach class 100 cleanliness levels by placing enough work stations in the room to give 100 recyclings an hour (see D7). The extent to which the cleanliness level of the room is challenged by the various activities will be determined.

5. Finishing and Packaging Room

The finishing and packaging room is entered into directly from the processing room. Clean work stations will be utilized in this room also, to maintain the highest possible cleanliness level. Some of the equipment located in the room includes a vacuum can closing machine, a freeze dryer, a blast freezer, a press, and a vacuum heat sealer.

6. Airlock

The equipment airlock is a small chamber which enters into the processing room. Its purpose is to maintain pressure while equipment is moved in and out of the processing room. The change room serves as a personnel airlock.

F. MISSION

Explore and generate a fund of scientific and technical knowledge that would serve both as a foundation and a guideline toward the application of clean room technology to food processing.

G. OBJECTIVES

1. Establish the technical evidence necessary for the development, engineering, production and control of space food prototypes, processed and packaged in a controlled environment.
2. Explore the applicability of a controlled environment in the development of new and improved food processes and products.
3. Discern and define the effect of product, process and environmental variables upon the microbiological profile of the final product and its potential susceptibility to spoilage or pathogenicity.

H. ULTIMATE GOALS

1. New scientific knowledge
2. New food technology
3. In-plant and in-process inspection and control guides and/or manuals.

4. For each new prototype food product:
 - a. Production guide and/or manual
 - b. Procurement end-product specifications
5. Microbiological Handbook for controlled environment, food processing and packaging.
6. Engineering and Production Handbook for controlled environment, food processing and packaging.
7. Quality Inspection and Control Handbook for controlled environment, food processing and packaging.

I. EXPERIMENTAL APPROACH

1. Conduct comparative microbiological studies of processing operations in the ChEF versus operations in a non-controlled environment.
2. Determine optimal workflow patterns for the processing of food in the ChEF.
3. Determine shelf life of products prepared in the ChEF as compared to products prepared in a non-controlled environment.
4. Optimize procedures for measuring the microbial load in foods.
5. Improve and optimize methods for measuring the microbial profile of the clean environment (air, surface and on personnel).
6. Determine the effect of short sterilization times on the organoleptic properties and wholesomeness of foods processed in the controlled environment. The thermal processing times will be determined by the microbial population measured in the food items.
7. Determine the microbial loading of food items due to handling by personnel with and without gloves.
8. Determine the microbial levels of various types of food prepared in the ChEF:
 - a. Dehydrated
 - b. Precooked frozen foods
 - c. Precooked food

9. Evaluate the effect of pretreatment methods on the microbial population of raw products processed in the ChEF:

a. Blanching

b. Peeling

10. Determine the effect on the microbial population of the time elapsed between the preparation of the product and the start of the dehydration process in the ChEF.

11. Determine the effect of time and temperature of dehydration on the microbial population of food products processed in the ChEF.

12. Develop procedural guides for the ChEF.

a. Standard operating procedure (SOP) for the environmentally controlled facility.

b. Equipment operation manuals:

(1) Correct use of equipment

(2) Cleaning, decontamination and sterilization of equipment

(3) Entry and removal of equipment from the ChEF

(4) Dismantling of equipment

c. Food processing instructions or production guides for the ChEF

(1) Food processing operations

(2) Packaging

d. Monitoring handbook for the ChEF

(1) Monitoring requirements

(2) Monitoring equipment

(3) Monitoring techniques

(4) Data reporting

13. Establish standards and specifications for clean room processes and products.

- a. Microbial requirements for space foods
- b. Microbial requirements for military foods
- c. Microbial requirements for a controlled environmental food processing facility
- d. Packaging specifications
- e. Thermal processing requirements
- f. Rules and regulations for food handlers

J. ENVIRONMENTAL SURVEILLANCE OF THE CHEF

1. Microbiological Monitoring

- a. Determine the basic microbiological profile of the controlled environment facility (ChEF):
 - (1) Microbial load in the air (occupied and unoccupied)
 - (2) Microbial load of surfaces (occupied and unoccupied)
 - (3) Particulate count of the air (occupied and unoccupied)
- b. Determine the effect of laminar flow work stations on the basic microbial profile of the ChEF.
- c. Routine microbiological monitoring during food processing operations:
 - (1) Measure microbial load of the air
 - (2) Measure microbial load of surfaces
 - (3) Measure microbial load on personnel
 - (4) Measure microbial fallout on surfaces over extended periods of time
 - (5) Evaluate clean work stations

2. Environmental Control:

- a. Evaluate food processing operations to determine their potential for generating microbiological contamination
- b. Determine the effect of personnel on the microbial profile of the controlled environment:

- (1) At rest
- (2) At work
- (3) Wearing street clothes
- (4) Wearing clean room garments

c. Determine efficiency of cleaning and sanitation procedures on reducing the microbial contamination on surfaces of equipment and other work surfaces:

- (1) Rodac contact sampling of surfaces
- (2) Swab sampling of surfaces

d. Determine types and numbers of microorganisms generated by personnel and activities.

3. Quality Control:

a. Conduct comparative microbiological studies of processing operations in the ChEF versus operations in a non-controlled environment.

b. Determine optimal workflow patterns

c. Determine shelf life of products prepared in the ChEF as compared to products not prepared in a controlled environment.

d. Measure the microbial load of predominant raw materials or ingredients used during food processing operations.

e. Measure the microbial load of intermediate products during food processing operations.

f. Measure the microbial load of the finished products.

g. Optimize procedures for measuring the microbial load in foods, equipment and personnel.

K. FOOD RESEARCH AND DEVELOPMENT

Food research and development is a dynamic and ever increasing activity at NLABS and continues to be a prime mission of the Food Laboratory which is recognized as a leader by the food industry in the United States and abroad. The ChEF will be utilized and exploited as a tool to advance the state of knowledge and to set the pace for future research and development of new processes and products. These will include the following:

1. Processes

Typical processes which will be involved in food research and development will include compression, freeze-drying, thermostabilization, microwave cooking, baking, frying, roasting, flash freezing, steaming, packaging and package sealing.

2. Products

Products involved will include baked items (pastry and bread), meats, synthetic foods, vegetables, fruits, precooked frozen foods, space foods (thermostabilized and dehydrated), low moisture foods, canned foods and irradiated foods. Flow diagrams of typical processes are presented in the Appendix. An example of the microbiological support for these activities is presented in Appendix A (1). The microbiological requirements for space foods and the analytical methodology is presented in Appendix B.

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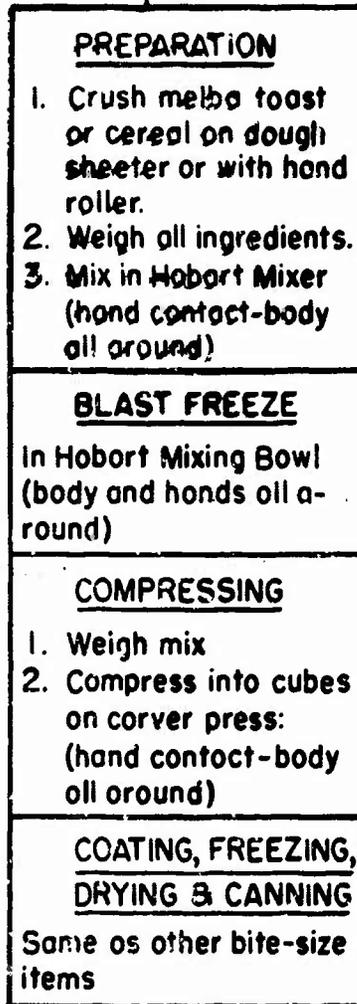
APPENDIX A

Flow Diagrams of Typical Food Processes

APPENDIX A

(I) PREPARATION OF COMPRESSED CUBES AND MICROBIOLOGICAL SURVEILLANCE

MIX

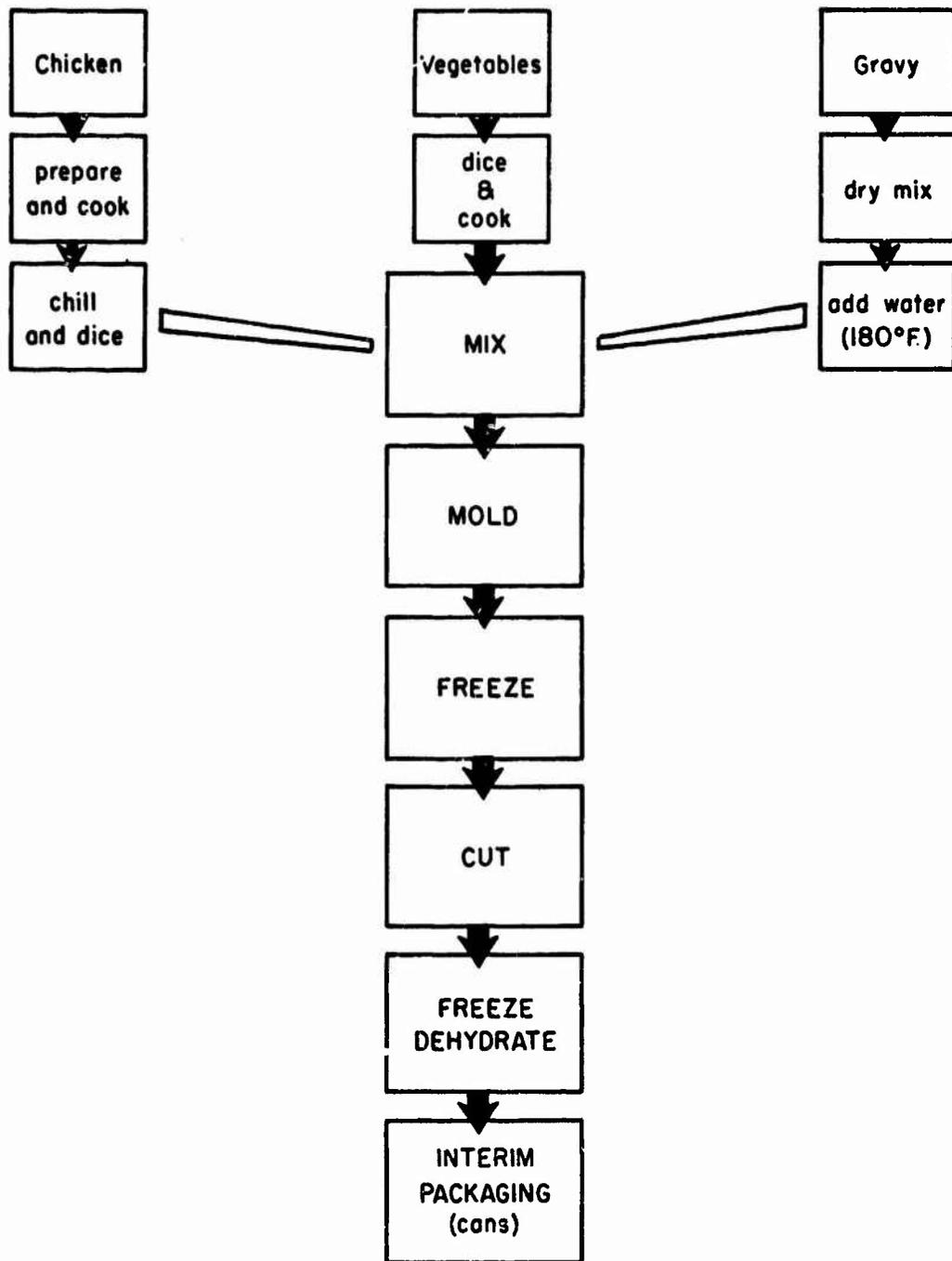


Perform microbiological assays on cubes handled with human hands & compare microbiol count w/cubes not handled w/human hands.

1. Continuous air sampling during processing.
2. Surface sampling periodically of:
 - (a) Table top
 - (b) Hobart mixer
 - (c) Food handler
3. Microbiological analysis of:
 - (a) Toast
 - (b) Mix
4. Microbiological analysis of mix after blast freezing:
 - (a) determine survival of microbial population
5. Sample surface of Carver press to determine the microbial load on the press
6. MICROBIOLOGICAL ANALYSIS of COMPRESSED CUBES.
7. Microbiological analysis of coating material.
8. Microbiological analysis of dried cubes.
9. Microbiological analysis of coned product.

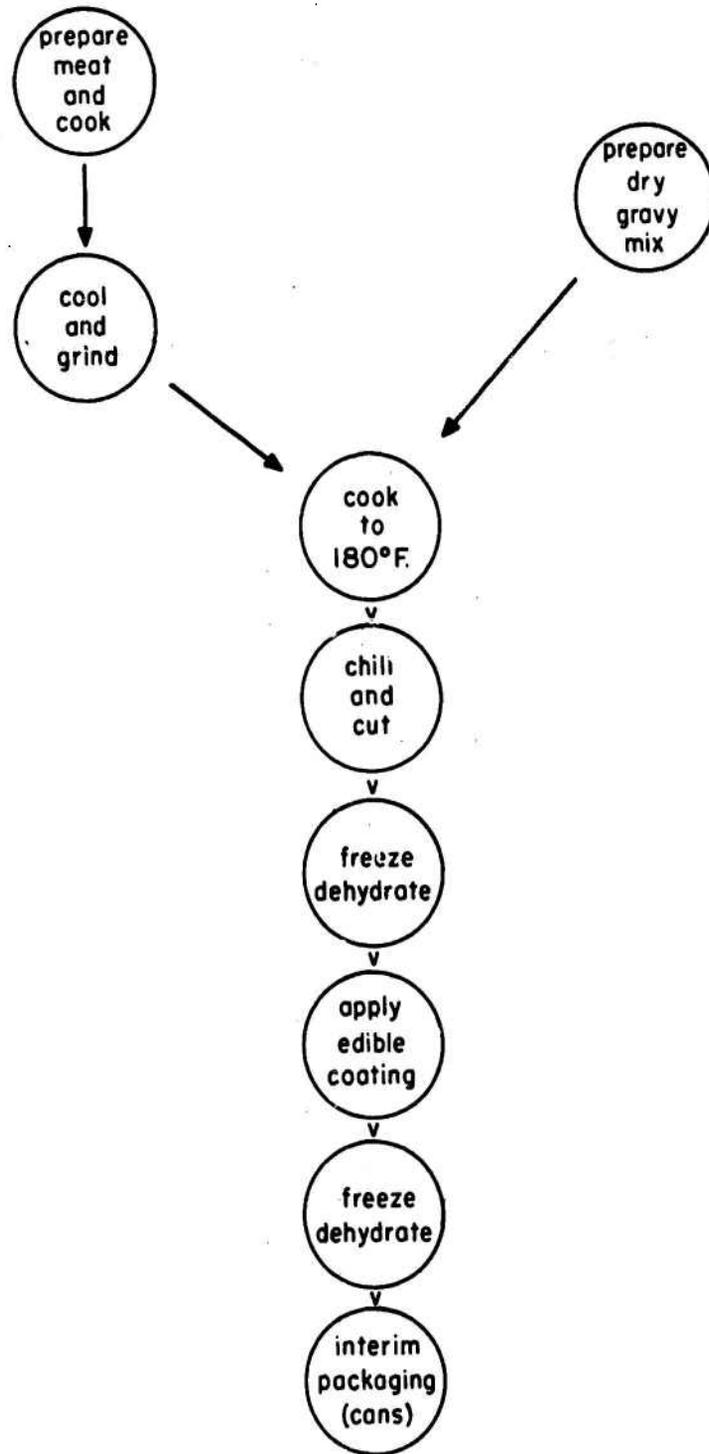
APPENDIX A

(2) PREPERATION OF MEAT VEGETABLE BAR



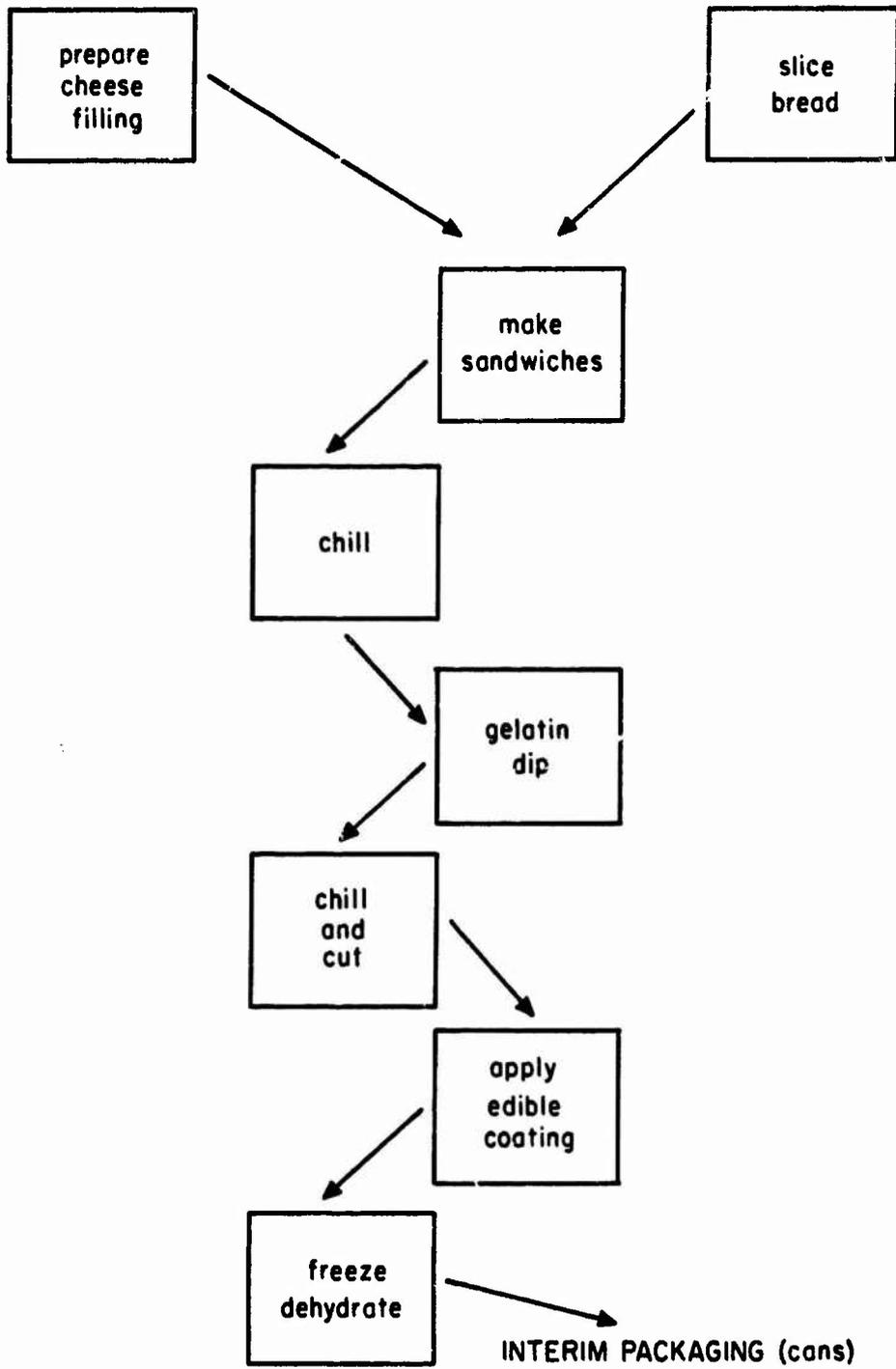
APPENDIX A

(3) PREPARATION OF MEAT BITES



APPENDIX A

(4) PREPARATION OF CHEESE SANDWICHES



STANDARD FOR SPACE FOOD PROTOTYPES

APPENDIX B

Microbiological Requirements for Space Food Prototypes

APPENDIX B

Space Food Prototype
Production Guide
Addendum No. 1E
U. S. Army Natick Laboratories
Natick, Massachusetts
27 March 1969
Superseding Addendum No. 1D
1 December 1967

MICROBIOLOGICAL REQUIREMENTS FOR SPACE FOOD PROTOTYPES

I. Microbiological Specifications

Total Aerobic Plate Count	Not greater than 10,000/gm
Total Coliform Count	Not greater than 10/gm
Fecal Coliform Count	Negative in 1 gm
Fecal Streptococci Count	Not greater than 20/gm
Coagulase Positive Staphylococci	Negative in 5 gm
Salmonellae	Negative in 10 gm

II. Methodology

A. Preparation of Slurry:

1. Twenty-five (25) grams of the dehydrated sample are aseptically transferred to a sterile blender cup. Add 225 ml of chilled buffered water (SBW: PO_4 M/15, pH 7.0)² and blend for two minutes. This slurry constitutes a 1:10 dilution and contains the equivalent of 0.1 gm food sample per ml. Hereafter this dilution shall be termed Extract A.

2. Extracts shall be maintained at no greater than 5°C until promptly used as prescribed in the following tests.

B. Total Aerobic Plate Count:

1. Transfer eleven (11) ml of Extract A into a 99-ml SBW, giving a final dilution of 1:100.

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2. Transfer one (1)-ml of the 1:100 dilution into each of five (5) Petri-plates and pour with "Tryptone Glucose Yeast" agar (Plate count agar - Difco).^{1,3}

3. Incubate plates at 35 C and count after 48 hours.

4. The total number of colonies on the five (5) plates shall not exceed 500.

C. Total Coliform Count:

1. Transfer one (1)-ml of Extract A into each of ten (10) Petri-plates and pour with "Violet Red Bile" agar (VRB).^{1,3}

2. Stratify solidified plates with 5 ml of same agar.

3. Incubate plates at 35 C and count typical coliform colonies (dark red, 0.5 mm or more in diameter) after 18 to 24 hours.

4. The total number of typical colonies on all ten (10) plates shall constitute the "Total Coliform Count" per 1.0 gm of food and shall not exceed ten (10).

D. Fecal Coliform Count:

1. Transfer each typical VRB colonies (see C. 3.) into phenol red lactose broth fermentation tubes.

2. Incubate at 35^oC for 18-24 hours.

3. Transfer two loopfulls (3-mm diameter) of broth from each positive tube (displaying acid and gas) into an "EC" broth fermentation tube.^{3,4,5}

4. Incubate at 45.5 ± 0.2 C for 24 hours. Both temperature and time are critical for this differential test. Hence, incubation shall be carried out in a constant-temperature bath, monitored with a certified Bureau of Standards thermometer or equivalent. Incubation time shall not exceed 24 hours. "EC" tubes displaying gas production are considered positive for "Fecal Coliforms". A single "EC-positive" culture shall constitute rejection.

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5. Where merited, the analyst may further confirm the EC-positive cultures for the Escherichia coli type through the establishment of their IMViC pattern according to Standard Procedures.

E. Fecal Streptococci Count:

1. Transfer one (1)-ml of Extract A into each of ten (10) Petri-plates and pour with "KF Streptococcus" agar.^{3,6}

2. Incubate plates at 35°C and count typical colonies (dark red or those having a red or pink center, 0.3 to 2 mm in diameter) after 48 hours.

3. The total number of typical colonies of all ten "KF" plates shall constitute the "Fecal Streptococci Count" per 1.0 gm of food and shall not exceed twenty (20).

4. Where merited, the analyst may further confirm the KF-positive colonies through:

- a. Microscopic examination
- b. Gram stain
- c. Catalase test
- d. Growth in Ethyl Violet Azide (EVA) broth

F. Coagulase Positive Staphylococci:

1. Transfer a 50-ml of Extract A into 200 ml of cooked meat medium with 10% NaCl.³ The medium is prepared overstrength by adding 31.25 gm of cooked meat medium and 24.5 gm of NaCl to 200 ml of distilled water. The final concentration in 250 ml will be 12.5% and 10% respectively.

2. Incubate at 35°C for 24 hours.

3. Spread one-tenth (1/10)-ml of cooked meat medium on each of five (5) plates of Tellurite Glycine Red Agar (Vogel & Johnson).⁸

4. Incubate VJ plates at 35°C and examine after 24 and 48 hours for the presence of black colonies with yellow zones.

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5. Transfer three (3) black colonies (with yellow zones if present) to trypticase soy broth (TSB) tubes, and incubate at 35°C for 24 hours.

6. Remove the remainder of the colony with a loop and emulsify in 0.2 ml of TSB. Add 0.5 ml of coagulase plasma, mix and incubate in a 35°C water bath for 4 hours (if time does not permit, use the 24 hour culture).

7. Note those tubes which are negative and repeat the coagulase test with the 24 hour culture.

8. A single coagulase positive colony shall constitute rejection.

G. Salmonellae:

1. Transfer 100 ml of Extract A into 100 ml of double strength lactose broth.

2. Incubate at 35°C for 24 hours.

3. Transfer 25 ml of lactose broth into 225 ml of each of Selenite-Cystine broth and TT broth [modified TETRATHIONATE BROTH containing brilliant green (1:100,000)]^{3,9,10}

4. Incubate at 35 C for 18-24 hours.

5. From each enrichment culture streak a loopful on two plates each of three (3) selective media: Brilliant Green Sulfadiazine (BGS) agar, Bismuth Sulfite (BS) agar, and Salmonellae-Shigellae (SS) agar.

6. BGS and SS plates are incubated for 24 hours and BS plates for 48 hours at 35°C. Typical colonies are pink to deep fuschia on BGS, black on BS, smooth and colorless on SS).

7. Pick with a needle two typical colonies from each plate having growth. Inoculate "Triple Sugar Iron" agar (TSI) slant by stabbing the butt and streaking the slant and streak on "Christensons Urea" agar (CU) slant.

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8. Incubate all slants at 37°C for 24 hours.
9. Observe CU slants periodically every 4 to 6 hours. If culture shows an urease positive reaction (reddening of agar) the respective colony is Salmonellae negative and test is ended.
10. Positive TSI (acid butt, alkaline slant, with and without gas and H₂S) associated with urease negative reaction shall constitute a presumptively positive Salmonellae culture.
11. Transfers from positive TSI slants are typed against salmonellae O and H polyvalent antisera. Positive reaction constitutes confirmed presumptively positive salmonellae in the test sample.
12. Presumptively positive TSI cultures are further confirmed through reactions in the following:
 - a. dulcitol (+)¹⁰, malonate (-) broths.¹⁰
 - b. Lysine decarboxylase broth (+).
 - c. KCN broth (-).
 - d. Indole broth (-).
13. A single confirmed positive Salmonellae culture shall constitute rejection.

H. General Provision:

1. Due to the special nature of certain dehydrated menu components, the prescribed dilution schedule may be modified in order to facilitate appropriate pipetting of the test material. Higher dilutions may be prepared and used for subculturing, providing the total equivalent mass of the subcultured test component remains the same as currently prescribed.

APPENDIX B

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APPENDIX C

Clean Room Procedures

APPENDIX C

I. PERSONNEL RULES APPLICABLE TO FOOD PREPARATION IN THE CHEF (23)

A. Health

1. Food handlers health certificates are to be renewed annually and are required by all personnel engaged in handling food, or food processing equipment.
2. No person while affected with a communicable disease, or while a carrier of such disease, or while afflicted with boils, infected wounds, or an acute respiratory infection shall work in food preparation, or service areas in any capacity which is likely to transmit the disease to fellow workers, or contaminate food contact surfaces with pathogenic organisms.
3. Personnel shall be screened regularly by a supervisor to prevent the above infractions from occurring.

B. Clothing

1. Garments of all personnel, including dishwashers, engaged in handling food or food containers and utensils shall be clean.
2. Shoe coverings and head coverings or effective hair restraints shall be used by personnel engaged in the preparation of food.
3. Appropriate clean room garments shall be worn.

C. Cleanliness

1. Wash hands frequently with bactericidal soap.
 - a. Before starting work
 - b. Before handling cooked food
 - c. After handling raw and cooked foods
 - d. After every break

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- e. After visiting the toilet
 - f. During working hours at 2 hour intervals or as often as necessary to remove soil and contamination.
2. Keep finger nails clean and neatly trimmed.
 3. Male workers should be clean shaven.
 4. Keep hair free of dandruff.
 5. Fingernail polish and other cosmetics including face powder, rouge, eye makeup and hair spray are prohibited.

D. Tobacco

1. Tobacco is prohibited in any form while in food preparation, or while in the equipment and utensil washing or food preparation areas.

E. Other Practices

1. Personnel shall maintain a high degree of personal cleanliness and shall conform to good hygienic practices during all working periods.
2. Avoid insanitary personal practices such as scratching the head and body, placing the fingers in or about the mouth or nose, or uncovered sneezing or coughing.
3. Use fingers as little as possible on cooked food.
4. Personnel suffering from sunburn are prohibited from entering the ChEF.
5. Personnel suffering from hay fever or other allergies which are likely to contaminate food or food contact surfaces are prohibited from entering the ChEF.

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6. Keep surroundings clean, neat and tidy. Spills on the floor shall be cleaned up immediately and hands washed immediately following the operation.
7. NO ONE SHALL ENTER THE CHEF UNLESS HE HAS A PROVEN NEED. PROVEN NEED SHALL CONSIST OF THE LABORATORY DIRECTOR'S WRITTEN APPROVAL FOR THOSE WHO ARE NOT REGULARLY EMPLOYED IN THE CHEF.

II. CHEF PERSONNEL ENTRY PROCEDURES

- A. Remove all outer clothing before entering clean room facility and empty all pockets.
- B. Vacuum shoes and step onto sticky mat.
- C. Enter air shower one at a time.
- D. Turn slowly with arms raised over head and feet spread apart until air shower stops.
- E. Enter change room.
- F. Don fresh, clean, lint-free clothing taking care not to dirty them with shoes or from the floor.
- G. Proceed to bench and put on one boot without touching the dirty side of the floor. As soon as the boot is on, place the foot on the clean side of the floor and don the other boot. Care should be taken not to step on the "dirty" side of the floor with the booties on, or on the "clean" side without the bootie on.
- H. Wash hands and arms thoroughly as directed with hexachlorophene containing soap.
- I. Dry hands with lint-free towels and enter the clean room. Keep hands out of pockets and away from face and hair.

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- J. Sanitize hands in disinfectant solution. Each time a worker has contact with uncooked items, he must wash hands before handling cooked items.
- K. Wear rubber or plastic disposable gloves where appropriate.

III. ChEF SANITATION PROCEDURES (16)

- A. Houskeeping personnel assigned to the ChEF must abide by all personnel regulations established for the facility.
- B. Materials used for cleaning will be as follows:
 - 1. Tap water
 - 2. Non residue forming detergent.
 - 3. Commercial window cleaners in pressurized cans. (windex with Ammonia is recommended).
 - 4. Clean room approved lint-free wipers.
 - 5. High grade non-flaking plastic buckets.
 - 6. Cellulose sponges.
 - 7. Cellulose sponge mops with anodized aluminum handles.
 - 8. Anodized aluminum ladders.
 - 9. Central vacuuming system (Exhaust outside of facility). Portable vacuum may be used in Clean Room only if exhaust is equipped with absolute filters.
- C. All work areas including tops of benches (including laminar flow benches) and tables shall be cleaned by operating personnel daily, with a disinfectant.
- D. Damp mop floors daily before normal work shift with disinfectant and vacuum dry.

APPENDIX C

- E. Damp mop floors weekly with tap water and detergent sanitizer, and vacuum dry.
- F. Do not wax floors.
- G. Wipe walls weekly with a damp sponge using detergent-sanitizer and vacuum dry.
- H. Wash walls with tap water and detergent and vacuum dry whenever necessary to remove visible dirt deposits
- I. Wash windows weekly and wipe dry with a lintless wiper inside and outside.
- J. Wipe ceilings weekly with a damp sponge using a disinfectant and vacuum dry.
- K. Wash ceilings with detergent-sanitizer and tap water and vacuum dry whenever necessary to remove visible dirt deposits.
- L. Clean overhead light troffers with a detergent-sanitizer as often as necessary to remove visible dirt deposits.
- M. Wipe overhead light lenses weekly with a damp sponge using a disinfectant and vacuum dry.
- N. All daily cleaning with the exception of the floors may be performed during the normal work shift by operational personnel.
- O. Weekly cleaning by the custodial service shall be performed during hours when the room is not in normal use.
- P. The Room Supervisor will determine when the tacky or sticky mats shall be changed.

APPENDIX C

IV. HAND DISH WASHING (15)

- A. Scrape and preflush whenever possible with cold or lukewarm water.
- B. Wash with measured amount of detergent solution at 52^oC.*
- C. Rinse in clear water at 60^oC.
- D. Sanitize by one of the following methods.
 1. Immersion for one minute in clean hot water at a temperature of at least 80^oC.
 2. Immersion for one minute in an aqueous solution containing 50 ppm of available chlorine at a temperature of at least 24^oC.
- E. Air dry, do not towel.

V. POT AND PAN WASHING (15)

- A. Soak in standard detergent solution at 50^o - 60^oC, then scrape.*
- B. Brush-wash in standard detergent solution at 52^oC.
- C. Rinse with clear running water at 60^oC.
- D. Sanitize by one of the following methods:
 1. Immersion for one minute in clean hot water at a temperature of at least 80^oC.
 2. Immersion for one minute in aqueous solution containing 50 ppm of available chlorine at a temperature of at least 24 C.
- E. Air dry, do not towel

* Measure detergent each time according to manufacturer's instructions.

VI. CLEANING LARGE EQUIPMENT (15)

- A. Schedule operations so that all processing utensils and equipment can be thoroughly cleaned daily. Dissassemble equipment as much as possible.
- B. Wash with stiff brush using a standard hand dish washing or pot washing detergent at 60°C.*
- C. Rinse with 80°C water from hose.
- D. Sanitize with steam from a hose or with boiling water; or spray or sponge with a solution containing 100 ppm available chlorine.
- E. Air dry, do not towel.

VII. CLEANING OVENS

Ovens may be cleaned with "Micro Clean" oven cleaner (Lanewood Laboratory, Framingham, Mass.). Follow manufacturer's instructions.

VIII. CLEANING REFRIGERATORS AND FREEZERS

- A. Maintain mechanical parts in good working conditions. Have refrigerators and freezers inspected by an expert.
- B. Inspect doors for faulty gaskets, hinges and latches.
- C. Keep drains clean at all times.
- D. Defrost refrigerators before cleaning. Refrigerators should be defrosted weekly. Freezers need defrosting when the frost builds up to 1/4 inch.
- E. Before cleaning refrigerators, food items, as well as shelves, hooks, and other accessories, should be removed.
- F. Clean with a standard detergent solution and rinse with clear water.
- G. After cleaning flush with baking soda in warm water.

* Measure detergent each time according to manufacturer's instructions.

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- H. Refrigerators should be checked daily and freezers at regular frequent intervals to insure that the correct temperature is maintained and that the food is being stored in appropriate containers or securely wrapped.

IX. STORAGE OF CLEAN EQUIPMENT AND UTENSILS

- A. Food-contact surfaces of cleaned and sanitized equipment and utensils must be kept clean and prevented from being recontaminated:
 - 1. Knives, forks, spoons, whips, spatulas and other implements should be picked up by the handle and stored away from dust.
 - 2. Pots and pans should be handled so that fingers and thumbs do not contact inside surfaces.
 - 3. Pots and pans and other containers should be inverted.
 - 4. All small equipment and utensils should be stored on airy racks off the floor and be protected from dust.
 - 5. Food contact surfaces of fixed equipment should be protected from splash, dust and other contamination.
 - 6. Utensils should be air dried before being stored in a self draining position.