Robert W. Edwards
Martin S. Favero, Ph.D.
Robert K. Hoffman
Tom B. Lenehan
Norman H. MacLeod
Joseph J. McDade, Ph.D.
Peter Skelly
G. Briggs Phillips, Ph.D., chairman

A State of the Art Report

● The existence of viable microorganisms in, on, or around objects and materials constitutes microbiological contamination. Such contamination is like any other type of contamination because it has structure and mass. However, microbiological contamination is unlike other types of contamination in a number of important aspects. The prime reason for consideration of microbiological contamination control, apart from other types of control, is in relation to the properties that microbial contaminants possess apart from those of mass and structure. These properties are (1) ability to reproduce, (2) ability, within limits, to survive under adverse environmental conditions, and (3) ability to carry on physical and chemical processes.

There are two criteria for deciding the degree of microbiological contamination control necessary for any particular application. The first, which is the most rigid, demands elimination, to some stated degree, of living microbes plus the removal of proteinaceous residue when microbes are inactivated. The second, the less severe criterion, requires only the inactivation of all or a portion of the living microbes present in a system but does not implicitly demand elimination of the dead cellular debris resulting from biological control processes. Only the latter criterion will be considered in this presentation.

Definition of Microbiological Contamination

Microbiological contamination is defined as the presence of living microorganisms in a specified environment. In general, the microorganisms will be those classified as bacteria, fungi, viruses, or rickettsiae. Some parasites can exist in microscopic form as air and surface contaminants.

Definition of Microbiological Contamination Control

The over-riding philosophy of control of the microorganisms in any system is related to the ability to define the microbial load in the system at any particular point in time. Contamination control is achieved if the microbial load does not exceed the level established as the lowest acceptable limit. Maintenance of control, however, is complicated by the fact that, in opposition to inert contamination, the microorganisms in a population may be going through simultaneous processes of multiplication and death. Insofar as these processes are concerned, the only condition of microbiological control that can be considered stable is that of sterility — the absence of all viable microorganisms.

The General Nature of Microbiological Contaminants

For some purposes of microbiological control it may not be necessary to have extensive knowledge about microbiology. It is necessary, however, to understand that microbiology is a complex science composed of many sub-specialties and that any control endeavor must involve expert assistance or guidance at some level from a trained microbiologist.

The general nature of microbiological contaminants is emphasized by mutation and subsequent adaptation. The world of living things differs from the physical world in that there is easier recognition of the constant development and change taking place. There is a tremendous variety of microorganisms on the earth, many of which have not been described. These microorganisms can utilize as energy sources many very diverse substances ranging from basic elements such as iron and sulphur to the most complex of organic compounds. Some microbial species utilize atmospheric oxygen in their metabolism; others live without free oxygen. Microorganisms have a unique ability to survive under adverse environmental conditions. Spores, for example, represent a special form of microbial life that is more resistant to environmental influences than the vegetative form. Moreover, mutational processes enable microorganisms to develop resistance to detrimental conditions of the external environment, as in the case of organisms that become resistant to antibiotics.

Importance of Man

The significance of man in any system where microbiological contamination control is attempted deserves special consideration and understanding. Because man is an extremely prolific source of microorganisms, and because he can be extremely susceptible to those microbes that are pathogenic for him, inclusion of man in any system usually signals the weakest point in the contamination control effort. Unlike inanimate objects, man cannot be

conveniently separated from his m/crobial flora, and only with difficulty can he be inclosed in a ventilated garment to separate him from a controlled environment. These facts suggest that microbiological contamination control is simplified when man is excluded from the system and carries on his functions in relation to the system in a more or less remote manner.

Microbiological Contamination Control Barrier Systems

There are some common underlying basic concepts of barrier systems whose understanding is necessary for the selection of the most appropriate measures for microbiological contamination control.

The absolute or sterile barrier concept places the material or the work to be controlled within a gas-tight enclosure, usually a stainless steel cabinet but at times a plastic isolator. Humans are separated from the system and the work is done in attached arm-length rubber gloves. When work within the barrier is to be protected from outside contamination, the enclosure is maintained at a positive air pressure. Conversely, negative pressure is used to prevent escape of contaminants from the enclosure. According to the criteria for microbiological control, inlet and/ or outlet air may be filtered or incinerated. Prior to use of the enclosure it may be decontaminated or sterilized. Air locks, dunk baths, autoclaves, and other devices may be used to preserve the sterile integrity of the enclosure while materials are passed in and out of it. It is also possible to include many types of equipment such as incubators, refrigerators, centrifuges, etc., within the containment system.

The partial barrier concept utilizes stainless steel enclosures or cabinets that are not gas-tight and are not completely closed systems. Containment here depends on an inward or an outward flow of air through an open working panel or through open glove ports. The inlet or outlet air may be filtered in these systems. Since only the hands and arms of the operator extend into the enclosure, the chance of spreading contamination to or from humans is minimized.

The clean room concept as applied to the control of microbiological contaminants is less effective than the concepts described above unless all personnel in the room are garbed in ventilated suits. Clean rooms are also less effective because they are not as air or gas-tight as the barrier systems. In spite of the use of masks, sterile clothing, etc., the presence of humans in a clean room minimizes its microbiological control abilities. However, some control of microbial contaminants can be achieved in controlled environment rooms where the room itself is the barrier but contains working personnel. It is recognized in fact, that with the present state of the technology and with some practices it is either impractical or impossible to remove humans or some animals from a system. The situation of the hospital operating room is a good example of this. However, it has been shown that the absolute barrier concept, with the surgeon biologically remote from the patient, can be employed even in this situation.

The principles of minimum turbulence air flow or laminar air flow have been applied in partial barriers such as cabinets and hoods and in clean rooms. This concept seeks to improve control of contamination through the use of a bank of air moving through the work area with minimum turbulence to remove airborne contaminants. The use of this technique usually means that entire walls, ceilings, or floors serve for the entrance and exit of ventilation air rather than conventional smaller air supply and exhaust ducts. In many situations it can be expected that improved microbiological contamination control in partial barriers and in clean rooms will result from the application of minimum turbulence air flow. However, a basic limitation to control is the interruption of the air flow patterns by operations being performed in the air stream. A recent book by Austin and Timmerman[1] summarizes much information on the design and operation of clean rooms and lists pertinent reference articles on conventional and laminar flow clean rooms.

The distinction between methods of achieving sterility and agents which 'decontaminate'.

It is obvious that for any particular microbiological contamination control problem, the choice of the proper barrier (absolute barrier, partial barrier, or clean room) depends upon the criterion of control. Thus if the criterion is maintenance of sterility, only a sterile absolute barrier will suffice.

Sterilizing and Decontaminating Agents

In conjunction with the above concepts it is usually necessary to apply sterilizing agents or decontaminating agents to eliminate or reduce the microbiological population.

The best means of achieving sterility is by the application of heat. Knowledge of the temperatures and exposure times necessary, the reliability of the process, and the pertinent kinetics makes this method of sterilization by far the most desirable to use wherever possible. Other treatment methods can produce sterility, but require greater attention to method of application and to statistical proof of the absence of living organisms after treatment. According to the type of contaminants involved, the materials to be treated, and oher variables, chemicals such as ethylene oxide, peracetic acid, formaldehyde, and beta-propiolactone can be considered as sterilizing agents. It must be remembered that these agents may not produce sterility under some conditions. Likewise, irradiation can produce sterility only under appropriate conditions.

Any agent or any treatment that reduces the microbial population but does not produce sterility under the given conditions is said to be a decontaminant. Included as decontaminants would be most of the common chemical disinfectants, germicidal ultraviolet radiation, etc.

Standards, Measurements, Testing, and Criteria of Control

The value of any microbiological contamination control effort is questionable unless specific standards are established prescribing the criteria of control. If a process or an item is to be maintained or rendered sterile, it should be specifically stated whether this means both internal and external sterility or only the latter. Moreover, it is necessary to establish the methods to be used for determining sterility and the exact basis of accepting or rejecting the test results.

If the control measures are not aimed at sterility but only at a reduction of a microbial flora or maintenance of a reduced flora, this should likewise be defined with exactitude. The acceptable amount of contamination in terms of the number and type of microorganisms should be specified. For airborne contaminants, particle-size data may be important. A number of physical tests and measurements may also be important in support of microbiological contamination control. The microbiological assay techniques and procedures should be specified in detail.

A partial list of the microbiological contamination control tests in common use includes:

1. Microbial air sampling — Air impaction samplers, liquid impingers, and settling plates are used most frequently. The results of impaction and impinger samples are given in terms of viable particles per cubic foot of air and/or microorganisms per cubic foot of air. The results from settling plates are expressed as viable particles per square foot per hour.

2. Particle size sampling — Liquid impinger samples with pre-impingers offer some particle size selectivity. The Andersen cascaded sieve sampler is frequently used to discriminate the

airborne viable particles in a microbiological aerosol into six particle size ranges.

3. Surface sampling — Cotton swabs or Rodac plates are usually used. Results are expressed as microorganisms per unit area of surface.

4. Surface contamination accumulation tests — Small sterile strips of stainless steel, glass, or plastic are placed in the environment. After various exposure periods, strips are collected and assayed for viable microorganisms. Results are usually expressed as microorganisms per square foot.

5. Component surface testing — Small components in systems under microbiological contamination control may be tested by complete immersion in an appropriate nutrient fluid or by washing the component in sterile saline that is then quantitatively assayed for viable microbes.

6. Internal testing of components — Information on methods for determining internal sterility of components is incomplete and adequate tests are not available. Obviously, however, these tests must be done in a sterile environment.

7. Special culture tests — Special microbial detection and assay tests may be devised for other materials such as oils, greases, powders, etc.

8. Filter and incinerator testing — Periodic microbiological testing of all air filters and incinerators used in contamination control systems is required. Testing must be done in such a manner that a break of sterility is not involved.

9. Freon testing — This test is used to validate the microbiological tightness of a containment system. Inability to leak molecules of Freon gas is equated with the inability to allow leakage of microorganisms. Helium tests are also satisfactory to insure microbial tightness, but these tests are more severe than necessary.

10. Miscellaneous measurements — To insure maximum potency it is important that chemical titrations be made and records maintained of all decontaminants such as peracetic acid, ethylene oxide, and chlorine solutions.

Records should be maintained of the temperatures and exposure times when materials are treated in autoclaves or dry-heat ovens. The temperatures on air incinerators, incubators, etc. should be periodically observed and recorded. Insofar as possible, temperature readings should be made at the most insulated or protected areas in the material being treated. Ventilation rates should be tested at regular intervals.

Areas of Application

The control of microbial contamination is important in many areas. It is not the purpose of this report to draw a dichotomy among these areas, but it is evident that in some the control of contaminants is absolutely necessary to the success of the endeavor. This would be true, for example, in the food preparation industry. The following list, which is not all-inclusive, illustrates the diverse areas of application of microbiological contamination control:

1. Space exploration and extraterrestial quarantine
2. Infectious disease research
3. Cancer research
4. Hospital management
5. Food preparation
6. Preparation of biologicals

Ten separate microbiological contamination tests are enumerated, with comments on each.

7. Storage of fuel oils
8. Paper industry
9. Optics
10. Photography
11. Tropical deterioration
12. Water industry

Stages in Achieving Microbiological Contamination Control

The remainder of this report concerns the stages to be considered in achieving microbiological contamination control and the specific decisions, approaches, and techniques available for use in each stage.

There are five essential stages of microbiological contamination control. These are shown in Table I. Any acceptable microbiological contamination control program must, in one way or another, include these five stages and at least some of the specific approaches and techniques listed under the stages.

Stage 1 — Recognizing and Defining the Problem

Problems created by lack of contamination control are often identified in retrospect — some undesired events having already occurred. Contamination control reaches its highest degree of refinement when data are accumulated that allow the problem areas to be predicted and the necessary control measures to be installed before losses occur. Once a problem involving microbiological contamination control is recognized it should then be defined as accurately as possible.

Stage 2 — Establishing Contamination Control Criteria

Any attempt to control microbiological contamination lacks significance unless the standards of control that must be achieved are defined. That is to say that there must be a definition, in microbiological terms, of the objective of the control endeavor. Many control operations, for example, require that sterility be achieved and maintained. In infectious disease laboratories the criterion may be to prevent the escape of pathogens. Water treatment systems are concerned with the elimination of pathogens to produce potable water. Food processing plants must render foods microbiologically safe for human consumption. In the hospital operating room certain air-hygiene practices are appropriate to prevent infection of patients.

Contamination control criteria should be established in a manner to facilitate validation of control processes. If sterility is the aim, the criteria should specify what procedures are to be used in testing for sterility, how many replicate tests are needed, when the tests are to be done, etc. If sterility is not the objective, the criteria should specify the maximum number and types of microorganisms allowed in an environment, in a solution, on a surface, in a component, etc., and should indicate the test methods to be used. It is most important that this concept be clearly understood. In the food industry and in the manufacture of biologicals, the control criteria are specified and controlled by certain regulatory agencies. In other areas of microbiological contamination control no widely accepted criteria have been developed. In some areas, such as in planetary quarantine, specific standards for contamination control will probably be forthcoming.

Maximum success in future contamination control activities will depend in no small part on continued research in the various fields where control is needed in order to determine appropriate criteria and standardized testing methods.

Stage 3 — Employing the Approaches and Techniques of Control

Table 1 shows five general approaches and techniques used in achieving microbiological contamination control.

1. Facility Design Features

Modern construction criteria applied in the construction of facilities can do much to control mirobial contamination. Some of the features that have been suggested for inclusion in new or renovated facilities to control contamination are:

a. Use of ventilated cabinets, chambers, cages, etc., to achieve

an absolute or partial barrier to contain microorganisms at their point of use or to exclude them from a specific work area.

b. Use of clean rooms to exclude microorganisms from a particular environment.

c. Use of differential air pressures within a facility so that air moves from clean areas toward areas of higher microbial contamination.

d. Use of appropriately effective microbiological filtration of air supplied to and/or exhausted from rooms, cabinets, chambers, cages, etc.

e. Change rooms, water shower rooms, or air shower rooms for personnel.

f. Use of ultraviolet air locks and door barriers to separate areas of unequal risk.

g. Treatment of microbiologically co :taminated liquid effluents.

h. Room arrangement or layout to achieve traffic control within the facility along a clean-contaminated axis.

i. Use of an effective intercommunication system to avoid unnecessary movement of personnel from area to area.

For those faced with initiating a design plan of a facility where microbiological contamination control is needed, the problem is one of determining which of the above items are to be used and to what extent. Moreover, it is usually necessary to make these determinations before the planning stage of a new or renovated facility. This is a difficult problem whose dangers are that the facility will provide more contamination control features than are necessary. Fewer features than are necessary will fail to protect surrounding communities or areas from the contamination, or will be too inflexible in the future to accommodate changes in the contamination control requirements or the scope of the work.

The best approach to the design of a facility for contamination control requires consideration of some basic policy decisions before the design is begun. As an example, a comprehensive list of such policy questions relating to the design of laboratory facilities for infectious disease research has been published[2]. The most important major decisions in selecting engineering features for microbiological contamination control should be based on the fact that control should begin at the work surface or area where the contamination originates or where the item to be protected is located.

2. Use of Containment Equipment

Experimental evidence and practical experience have shown that handling techniques alone cannot be depended upon for consistent control of microbiological contamination. As the criteria for control become more exacting, aseptic handling techniques fail to provide sufficient containment. Engineering developments, however, have provided devices that provide efficient microbiological and physical separation between environments. As discussed elsewhere in this report, the most important type of containment and isolation equipment and the type capable of meeting the most severe control criteria is the gas-tight, absolute barrier enclosure. Ventilated work cabinets and animal cages are representative of this type of equipment. A number of recent references describe the design and use of ventilated cabinets and animal isolation equipment[3-6]. Many different types of absolute barrier enclosures have been developed. Moreover, cabinets and enclosures have been designed for the partial barrier concept wherein microbiological contamination control is achieved by controlling the direction of the air flow in or out of an open panel on the cabinet. Some laminar flow cabinets perform essentially the same function.

According to the containment requirement, various engineering and performance standards can be established for containment equipment. Such requirements relate to (i) leak tests for the enclosure, (ii) ventilation rates, (iii) filtration or incineration of air supplies or exhausts, and (iv) provisions for decontam-

inating or sterilizing the interior of the enclosure and the air filters.

An important aspect with regard to containment equipment is the selection of the proper type of equipment in relation to the type of contamination control or the criteria for control. For example, for use with infectious disease agents used in research laboratories, recommendations have been made that correlate the type of disease agent and the level of risk of the research with the type of protective cabinet needed[2].

In addition to cabinets, chambers, and animal cages, other types of containment equipment are available or can be designed for specific procedures. For example, containment equipment has been used for procedures such as centrifuging, grinding materials, shaking, blending and lyophilizing.

3. Use of Correct Techniques

Even in the presence of adequate facilities and good containment equipment, the success of most attempts to control microbiological contamination depends in no small part on the work techniques of the involved personnel. Although no inclusive list of correct techniques would be appropriate for all areas of application of microbiological contamination control, it is possible to discuss some fundamentals that suggest correct techniques and some general types of procedural rules that can be considered.

It is important to emphasize that microbial contamination can exist and yet be not readily detectable in the usual sense; the contamination may be odorless, tasteless, and invisible. Moreover instantaneous monitoring devices for microorganisms, comparable with devices for detecting radioactive contaminants, are not yet available. Next, it is important to understand the ease with which microorganisms can be made airborne, and their ability to remain airborne in small particulate form and to move from place to place in air currents. Finally, it is significant that the physical state of a microbiological contaminant is related to the ease or difficulty of containment. Thus dried, micronized, powdered, or lyophilized microbial preparations are much more difficult to contain than contaminants in a wet or fluid state.

In general, "correct techniques" as used in this discussion relate to the movements of people in the working environment insofar as these movements can minimize the spread of contamination through the air or on surfaces. These techniques, more-

TABLE 1. STAGES, APPROACHES, AND TECHNIQUES OF MICROBIOLOGICAL CONTAMINATION CONTROL

Stage 1—RECOGNIZE AND DEFINE THE PROBLEM

Stage 2—ESTABLISH CONTAMINATION CONTROL CRITERIA

  Maximum number of organisms allowed, types of organisms, where located, how detected, and other criteria.

Stage 3—EMPLOY APPROACHES AND TECHNIQUES OF CONTROL

Facility Design Features	Use of Containment Equipment	Management Functions	Use of Correct Techniques	Use of Sterilizing Agents, Germicides and Other Control Measures

Stage 4—MICROBIOLOGICAL TESTING AND SURVEILLANCE

Air Sampling	Surface and Component and Sampling	Physical and Chemical Tests and Measurements	Testing of Filters, Incinerators, Sewage, Water	Freon Leakage Testing

Stage 5—ANALYSIS OF RESULTS AND CERTIFICATION PROCEDURES

  Recording results, statistical tests, use tests of items, formal or informal certification.

over, relate principally to the movement of the hands in carrying out work. An analogy can be made to the techniques of the surgeon and surgical nurse who must at all times be aware of how materials are to be handled aseptically. How materials are handled and the proper sequence of handling are important in controlling contamination. Techniques that involve violent movements, aspiration of fluids, spraying of materials, foaming or bubbling of liquids, and overflow or leakage of materials signal the need for specifying exactly how the technique is to be carried out to achieve minimum spread of microbial contamination. A list of techniques recommended by Wedum[5] illustrates how these considerations apply in the infectious disease laboratory.

4. Use of Sterilizing Agents and Germicides

This section presents a digest of methods for destroying microbial contaminants. In spite of the extensive literature and continued research in this field, practical problems continue to arise for anyone employing routine sterilization procedures — problems for which there may be no clear-cut answer. This seeming dilemma is due to the fact that heat, the most reliable means of inactivating microbes, cannot be applied in many situations where the contaminants exist on or in thermolabile materials. It therefore often becomes necessary to resort to less reliable means of sterilization, or to accept something less than sterilization, generally referred to as disinfection or decontamination.

It has long been recognized that chemical decontamination is made difficult by the existence of species differences in susceptibility. In addition, the velocity of the process of sterilization by different chemicals depends to a variable degree on dilution, temperature, presence of organic matter, hydrogen ion concentration, extent of penetration, surface tension, and other environmental factors. The application of germicidal radiation such as ultraviolet light is likewise limited by its low penetrating power.

Of the numerous physical and chemical means of sterilization or inactivation of microorganisms, those that are most widely applicable may be classified under one of four main headings: (a) heat, (b) vapors and gases, (c) liquid decontaminants, and (d) radiation.

a. Heat

It is generally accepted that the application of heat, either dry or moist, is the most effective method of inactivating microorganisms. The exposure temperatures and times required for sterility are known and can be readily controlled. Whenever possible, heat should be used to sterilize materials. Current texts adequately specify conditions for the application of heat for sterilization[7,8]. Recent research on the kinetics of heat inactivation of microbial spores has empasized lower temperatures for longer exposure times for the sterilization of spacecraft and spacecraft components[9].

b. Vapors and Gases

A variety of vapors and gases possess germicidal properties. Among these are ethylene oxide[10,11], formaldehyde[10], propylene oxide[12], beta-propiolactone[13,14], and methyl bromide[15]. When these agents are employed in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can result. Under controlled conditions, ethylene oxide is a highly penetrating and effective sterilizing gas, convenient to use, versatile, non-corrosive, and effective at room temperature. However, the gas is slow in killing microorganisms and must be used mixed with other gases to avoid explosion hazards. Ethylene oxide is widely used to treat many items not suitable for heat sterilization. Its use in treating foods is limited because it reacts with and destroys some vitamins and because some of it hydrolizes to ethylene glycol.

This has accelerated the use of propylene oxide as a sterilizing gas for foods. Some of the foods decontaminated with the oxide are cocoa powder, dried vegetables, dry food mixes, dried egg and milk products. Propylene oxide is slower acting than ethylene oxide but it presents less toxicity problems.

Formaldehyde and beta-propiolactone are used primarily as decontaminants for room and building interiors. Formaldehyde, the slower acting of the two, has the undesirable property of condensing, when sprayed, and polymerizing. The polymer, once formed, requires long aeration (sometimes a week or so) for removal. Beta-propiolactone holds great promise as a space decontaminant. In the vapor state, it acts rapidly against bacteria, rickettsiae, and viruses, and has no adverse effect on most materials. It is much faster acting than formaldehyde and does not leave an undesirable residue after spraying. A serious deterrent to the use of this chemical is its toxicity.

Methyl bromide is about one-tenth as active against microorganisms as is ethylene oxide. The bromide has found greatest use in soil sterilization, especially to eliminate fungi.

Peracetic acid[16] is also bactericidal in the vapor state; however, its primary use is as a liquid decontaminant. The chemical has value in decontaminating enclosures or other areas where a vapor as well as a liquid is required to sterilize the item. Because peracetic acid is corrosive to metals, care must be exercised in the selection of materials treated with this chemical.

c. Liquid Decontaminants

There are many misconceptions concerning the use of liquid decontaminants. This is largely due to a charateristic capacity of such liquids to perform dramatically in the test tube and to fail in a practical situation. Such failures often occur because too little consideration is given to such factors as temperature, contact, pH, concentration, and the presence of organic material at the site of application. Small variations in these factors may make large differences in germicidal effectiveness. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants.

Hundreds of decontaminants or germicides are available under a variety of trade names. Most, however, may be classified as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydic compounds, and other organic preparations. None is equally useful or effective under all conditions.

In the decontamination of large areas or rooms the mechanical removal of microorganisms by washing with water or disinfectants plays an important part. For this reason, surface-active agents are often incorporated in germicidal solutions. The most frequently used liquid disinfectants are chlorine solutions, iodoforms, phenol and related acids, mercuric chloride, formalin, quarternary ammonium compounds, and sodium hydroxide solutions. Solutions of soap must not be overlooked for decontamination purposes.

When decontamination with chemical solutions is required, viruses and rickettsiae present special problems. The evaluation of the virucidal and rickettsiacidal action of chemicals is difficult. Most tests take place under various conditions of time, temperature, pH, and organic material that may be hard to duplicate. Moreover, complete inactivation is difficult to determine because of the methods used for virus detection and assay.

Current texts on decontamination and disinfection contain much useful information on practical chemical disinfection. Phillips et al[17] have presented several summary tables suggesting exposure times, temperature, and concentration of some commonly used chemicals for inactivation of various types of microorganisms.

d. Radiation

Ultraviolet radiation, X-rays, gamma-rays, high-energy electrons, protons, alpha particles, and neutrons are examples of forms of ionizing radiation capable of destroying microorganisms. The most common methods presently used for the sterilization of materials (surgical supplies, laboratory supplies, packaged foods, etc.) are: (i) high-energy electrons from a particle accelerator and (ii) gamma-radiation from a radioactive source. Although microorganisms vary in their resistance to radiation, a dosage of around 2.5 megarads usually is sufficient to sterilize surgical

materials[18]. Irradiation sterilization with gamma-rays or high-energy electrons is used mostly with packaged goods.

In certain specific applications, germicidal ultraviolet (UV) radiation at 2537A is an effective means of decontaminating air and surfaces. It is sometimes used for the treatment of water and other liquids. Used in air-locks or doors barriers, UV radiation can isolate areas of differing levels of contamination within a building[19]. It is also useful for reducing extraneous contamination in rooms. Window-type air conditioners used in contamination control areas may be fitted with UV lamps to decontaminate recirculated air. UV radiation has limited penetrating power and thus is most effective on exposed surfaces or in air. Proper concentration, contact time, and maintenance are also critical. Phillips and Hanel[20] have adequately described the use of UV for practical decontamination applications.

5. Management Functions

Management's policies, directives, and other actions are essential to any microbiological contamination control effort. Obviously programs of contamination control are initiated, funded, and supported at the management level. At the time programs are initiated management must assign responsibilities. Persons at various levels in the organization who will be responsible for the outcome of the contamination control efforts must be identified. But beyond this, management at various levels must also concern itself with other essential functions. For example, management must be responsible for the proper selection of employees. This refers not only to technical competence and skills but also to the fact that it may be undesirable to employ persons with certain physical conditions or diseases for certain types of work involving microbial contaminants. Management, likewise. should be concerned with providing the necessary training for employees involved in contamination control activities, for formulating work regulations, and for enforcing them.

Stage 4 — Microbiological Testing and Surveillance

In the fourth stage of microbiological contamination control, Table I depicts five types of procedures for testing and surveillance. In any control endeavor one or more of these techniques is needed to assess whether the techniques employed (stage 3) achieved microbiological control that meets the criteria established (stage 2).

1. Air Sampling

Air sampling test and surveillance procedures provide quantitative data on the presence of viable airborne microbes. According to the sampling devices used, assessment can be based on microorganisms per unit volume of air, or on microorganism-containing particles per unit volume of air. Settling plates can provide data on viable particulates falling on a unit area of surface per unit of time (e.g., particles per square foot per hour). Other types of samplers can provide estimates of the particle sizes of viable airborne particles. A recent monograph by Wolf et al[21] is an excellent summary of air-sampling techniques and devices. The use of selective culture media in air samplers may provide an opportunity to test for specific types of microorganisms. Also, microorganisms obtained from the air during sampling can be subjected to further testing for specific identification.

2. Surface and Component Sampling

Moistened cotton swabs or Rodac plates are usually used to detect microbiological surface contamination[22]. If possible, the results of surface sampling should be expressed on a quantitative basis (e.g., microorganisms per unit area of surface). Sterile strips are used to quantitate the accumulation of microorganisms on surfaces over periods of time. Small components may be tested by complete immersion in an appropriate nutrient fluid or by washing the component in a sterile fluid that is quantitatively assayed for viable microorganisms. Information on methods for determining the internal sterility components is incomplete. Ob-

viously, however, these tests must be done in a sterile environment. Special microbial detection and assay tests may be devised for other materials such as oils, greases, powders, etc.

3. Physical and Chemical Tests and Measurements

According to the nature of the microbiological contamination control endeavor, a number of physical and chemical tests and measurements may be done. In some instances these tests are critical to the surveillance program and in other instances they provide presumptive evidence that the control criteria are being met. Whenever wet heat is used for the sterilizing procedure a record of the temperature, pressure, and treatment time should be maintained. With dry heat sterilization the temperature and treatment time must be recorded. When liquid or gaseous decontaminants are used these should be periodically assayed chemically to assure proper chemical concentration and pH.

4. Testing of Filters, Incinerators, Sewage, and Water

Whenever microbial air filters, air or solid waste incinerators, or sewage or water treatment systems are a part of a contamination control procedure, these systems must be tested to assure adequacy of operation. It is particularly important to test systems prior to their being put into routine operation. In some instances microbiological tests with tracer microorganisms will be appropriate and in other instances temperature measurements and other tests are applicable. Testing must be done in such a manner that a breck of sterility or containment is not involved. Decker et al[23] have prepared a comprehensive monograph on air filtration and air filters that is recommended for use by anyone concerned with the filtration of airborne microbial particles.

5. Freon Leakage Testing

Freon testing should be used to validate the microbiological tightness of any absolute barrier system. Inability to leak molecules of Freon gas is equated with the inability of microbes to enter or escape from the barrier. For a cabinet or similar enclosure one ounce of Freon gas is admitted for each 30 cubic feet of space. Using compressed air or an inert gas, the pressure is raised to 6 inches water gauge. There must be no leakage when tested with a G.E. Halogen Leak Detector operating on high sensitivity range.

Stage 5 — Analysis of Results and Certification Procedures

It is obvious that the control criteria that are established in the second stage are the guidelines for the analysis of results and certification. Moreover, it follows that corrective actions should be started when a microbiolgical contamination control ,process is shown to be out of control or not meeting the minimum standards. In establishing methods for the analysis of results the following are cardinal considerations:

1. No biological detection procedure has perfect validity and reliability.

2. Within certain limits, sterilization and decontamination procedures improve as the challenge microbial load is lowered.

3. Sampling statistics are a major tool in analyzing the results of microbiological testing.

Conclusions

Techniques of microbiological contamination control have been used for many years. Lord Joseph Lister became one of the earliest practitioners when in the 1870's he sprayed carbolic acid in operating rooms to prevent surgical sepsis. Today microbiological contamination control finds application in many diverse areas. Moreover, the direction of man's science and technology signals an ever-increasing need for the control of microbial contaminants. The ultimate in microbiological contamination control is found in the achieving and maintenance of sterility, but this condition is usually definable only in statistical terms on the basis of a sample of the treated population and on the known characteristics of microbes in their reaction to inactivating treatments.

Man's reaction to a microbiologically contained system is such that control is better maintained if he manipulates the system remotely; thus, enclosure of the technique or operation is more convenient than enclosure of man himself.

The general principles of microbiological contamination control and the requirements for measurement and testing discussed herein illustrate the need for the involvement of a trained microbiologist in every control endeavor. They also illustrate the need for additional summarized information on the standards of control, the techniques required to achieve control, and means of microbiologically determining when control is reached. Most, if not all, microbiological contamination control efforts should contain elements from the five stages shown in Table 1. Finally it must be realized that the entire field of microbiological contamination control is dynamic and that new techniques and new solutions will be required as new problem areas arise.

REFERENCES CITED

1. Austin, P. R. and S. W. Timmerman. 1965. Design and operation of clean rooms. Business News Publishing Company, Detroit, Michigan.

2. Wedum, A. G. and G. B. Phillips. 1964. Criteria for design of a microbiological research laboratory. Amer. Soc. Heating Refrig. Air Conditioning 6:46-52.

3. Gremillion, G. G. 1959. The use of bacteria-tight cabinets in the infectious disease laboratory. p. 171-182. In Proceedings of the Second Symposium on Gnotobiotic Technology. Univ. Notre Dame Press, Notre Dame, Indiana.

4. Blickman, B. I. and T. B. Lanahan. 1960. Ventilated work cabinets reduce lab risks. Safety Maintenance 120:34-36.

5. Wedum, A. G. 1964. Laboratory safety in research with infectious aerosols. Public Health Rept. 79:619-633.

6. Phillips, G. B. 1965. Microbiological hazards in the laboratory — Part One — Control. J. Chem. Educ. 42:A43-A48. Part Two — Prevention. Ibid. A117-A130.

7. Perkins, J. J. 1956. Principles and methods of sterilization. Thomas C. Thomas — Publisher. Springfield, Illinois. 340 p.

8. Reddish, G. F. (ed.). 1957. Antiseptics, disinfectants, fungicides, and sterilization. 2nd ed. 975 p.

9. Bruch, C. W., M. G. Koesterer and M. K. Bruch. 1963. Dry heat sterilization: Its development and application to components of exobiological space probes. Developments in Industrial Microbiology 4; Washington, D.C. American Institute of Biological Sciences.

10. Phillips, C. R. 1957. Gaseous sterilization. p. 746-765. In G. F. Reddish (ed.) Antiseptics, disinfectants, fungicides and sterilization (2nd ed.). Lea and Febiger. Philadelphia, Penns.

11. Schley, D. G., R. K. Hoffman and C. R. Phillips. 1960. Simple improvised chambers for gas sterilization with ethylene oxide. Appl. Microbiol. 8:15-19.

12. Bruch, C. W. and M. G. Koesterer. 1961. The microbiocidal activity of gaseous propylene oxide and its application to powdered or flaked foods. J. Food Sci. 26:428-435.

13. Hoffman, R. K. and B. Warshowsky. 1958. Beta propiolactone vapor as a disinfectant. Appl. Microbiol. 6:358-362.

14. Spiner, D. R. and R. K. Hoffman. 1960. Method for disinfecting large enclosures with B- propiolactone vapor. Appl. Microbiol. 8:152-155.

15. Munnecke, D. E., R. A. Ludwig and R. E. Sampson. 1959. The fungicidal activity of methyl bromide. Canadian J. Botany 37:51-58.

16. Barrett, J. P., Jr. 1959. Sterilizing agents for Lobund flexible film apparatus. Proc. Animal Care Panel 9:127-133.

17. Phillips, G. B., E. Hanel, Jr. and G. G. Gremillion. 1965. Practical procedures for microbial decontamination. J. Amer. Assoc. Contamination Control (to be published).

18. Symposium. 1961. Sterilization by ionizing radiations. p. 7-56. In Sterilization of surgical materials. The Pharmaceutical Press, London.

19. Wedum, A. G., E. Hanel, Jr. and G. B. Phillips. 1956. Ultraviolet sterilization in microbiological laboratories. Public Health Rept. 71:331-336.

20. Phillips, G. B. and E. Hanel, Jr. 1960. Use of ultraviolet radiation in microbiological laboratories. United States Library of Congress. P.B. 147 043. Listed in U.S. Gov't. Res. Rept. 34(2), August 19, 1960. p. 122.

21. Wolf, H. W., P. Skaliy, L. B. Hall, M. M. Harris, H. M. Decker, L. M. Buchanan and C. M. Dahlgren. 1959. Sampling microbiological aerosols. Public Health Monograph No. 60, Public Inquiries Branch, U.S. Public Health Service, Washington 25, D.C. 53 p.

22. Hall, L. B. and M. J. Hartnett. 1964. Measurement of bacterial contamination on surfaces in hospitals. Public Health Rept. 77:1021-1024.

23. Decker, H. M., L. M. Buchanan, L. B. Hall and K. R. Goddard. 1962. Air filtration of microbial particles. Public Health Service Publ. No. 953, U.S. Govt. Printing Office, Washington, D.C. 43 p.