CONTROL OF MICROBIOLOGICAL CONTAMINATION

Submitted by

GERARD J. MCGARRITY

Institute for Medical Research
Camden, New Jersey 08103

I. INTRODUCTION

From the earliest days to the present, the greatest needs in cell, tissue and organ culture have remained: (a) improved and standardized media; and (b) freedom from microbiological contamination.

No control measures will guarantee freedom from contamination. However, certain precautions can minimize the possibility of contamination. The measures listed below have proved effective when followed strictly.

II. MATERIALS

Propipettes, Instrumentation[1]
Pi-pumps, Nos. F-37896 to F-37899, Bel-Art[2]
Chlorophen, Roch. Germicide[3]
Laminar air flow cabinets or rooms, Baker[4] and Bioquest[5]
RTV-102 sealant, Gen. El.[6]
Velometer or thermoanemometer, Alnor[7]
Temperature chart recorder, Series 1100, Fischer-Porter[8]
Autoclave tape, No. 1222, 3M[9]
Kilit spore strips, No. 12020[5]
Patapar paper, No. 27-144, Bristol Parchment[10]
Wet vacuum cleaner, with filter exhaust, Dart model, Danzig[11]
Ultipore disposable filter, No. MBY2001VRA Pall[12]
Uniforms or laboratory jackets, Angelica[13]
Disposable operating room caps, Busse Hosp. Disp.[14]
Pipette-Aid, Drummond Scientific[15]

III. PROCEDURE

A. General precautions

1. All personnel who handle cell cultures, media and sterile reagents should use careful aseptic technique.

2. Make certain that education of personnel and periodic review of aseptic procedures are carried out. Scrutinize new procedures to determine the risk of contamination to the culture and of infection to personnel.

3. Examine new equipment to determine if it might be a proximate source of contamination. Especially important are items that contain water or moist parts and small components that are difficult to clean. Bacteria can reproduce where protein and moisture accumulate.

4. Make sure facilities for cell culture work are of proper design.

5. Prohibit eating, drinking and smoking in the laboratory.

B. Specific measures

1. Use antibiotic-free media.

2. Use propipettes, pi-pumps, pipette-aid or similar hand pipetting devices in order to prohibit mouth pipetting.

3. Use quarantine/isolation facilities and techniques for handling cultures whose contamination status is unknown. If separate laboratory facilities are not available, questionable cultures should be handled after clean cultures.

4. Carefully disinfect work surfaces between handling of different cell cultures. There are many effective disinfectants available; chlorophen is used in this laboratory in a concentration of 1 oz per gallon of water. Concentration, length of exposure and presence of extraneous protein will affect the efficiency of disinfectants. Disinfectant solutions should be discarded

[1] Instrumentation Associates, New York, NY
[2] Bel Art Products, Pequannock, NY
[3] Rochester Germicide Co., Rochester, NY
[4] Baker Co., Inc., Sanford, ME
[5] Bioquest, Inc., Cockeysville, MD
[6] General Electric, Waterford, NY
[7] Alnor, Inc., Chicago, IL
[8] Fischer-Porter, Warminster, PA
[9] 3M Co., St. Paul, MN
[10] Bristol Parchment, Bristol, PA
[11] Danzig Floor Machine Corp., Dumont, NJ
[12] Pall Corp., Glen Cove, NY
[13] Angelica Uniforms, Inc., St. Louis, MO
[14] Busse Hospital Disposables, Great Neck, NY
[15] Drummond Scientific Co., Broomall, PA

immediately after use. Used disinfectant may be a rich medium for bacterial growth.

5. Discard used glass and plastic ware into a pan containing double strength disinfectant solution. Pipettes, tubes and other ware must be submerged to insure efficient disinfection. Cell culture media and other fluids must be decanted into disinfectant solution. It may be useful to use separate pans for glass and plastic ware if these are handled differently in the sterilization kitchen. Pans should be taken for terminal sterilization as soon as they are full or when work is completed.

6. Identify contaminating organisms to help determine the source.

7. Promptly autoclave contaminated cultures as opposed to continued passage in an attempt to irradicate the contamination. Such an attempt usually fails and leads to infection of other clean cell cultures.

8. Store uncontaminated cell cultures in liquid nitrogen in early passage. This permits repetition of experiments with a standardized cell culture and serves as a back-up in the event cultures are lost, mutate or become contaminated.

C. Laminar air or mass air flow cabinets or rooms

1. Use high efficiency particulate air (HEPA) filters in laminar air flow or mass air flow transfer cabinets or rooms to prevent airborne contamination during cell culture procedures, media preparation and sterility tests. These units should be checked at least yearly to insure proper filtration, air flow and air balance. Before purchase of a cabinet or room ask for a copy of the unit's operational manual. Some are most explicit and helpful.

2. Detect filter leaks by the use of an electronic particle counter.

3. Carefully scan the filter face to pinpoint leaks.

4. Examine filter seal which is a frequent site of leaks.

5. Repair small leaks with General Electric RTV-102 sealant.

6. Handle the media of HEPA filters with care since it is extremely fragile and can be damaged by even light handling.

D. Testing of laminar flow biohazard safety cabinets

1. Make sure that the average vertical velocity inside laminar flow biohazard safety cabinets is 100 (±20) ft per min (fpm) six inches downstream of the HEPA filter.

   a. Take velocity measurements with a velometer or a thermoanemometer. Most schools of engineering will have an electronic particle counter or a thermoanemometer to detect leaks in the filter and filter seal.

   b. If necessary, adjust the fan speed.

2. If the maximum velocity is still less than 80 fpm, replace the HEPA filter if it is overloaded. Cabinets and mass air flow rooms should have direct means of determining the resistance across the HEPA, a direct means of determining if a new filter is required. HEPA filters should be changed according to manufacturer's instructions.

3. If infectious or potentially infectious material is used in the cabinet, use paraformaldehyde gas sterilization of the old filter before it is removed (1).

4. Use air velocity to detect filter leaks and loose seals. Pass the nozzle of the velometer or thermoanemometer over the filter face and seal. A leak in the filter or seal will show as a large increase in air velocity.

5. Check air balance by releasing smoke one inch outside the hood and one inch inside, and determine if the smoke penetrates the air curtain. Air patterns in the hood and air balance at the front opening can be visualized by smoke. Check the balance with the hood empty and also with various equipment wares inside as it is routinely used.

6. Do not use horizontal flow laminar flow hoods that direct the air at the technician for cell culture or microbiology work.

E. Quality control testing and monitoring of other equipment and supplies

1. Use effective quality control testing to detect contamination in serum, distilled water and media before they are used to grow cell cultures. For quality control methods see Procedures 12304, 75129, and 75317.

2. Monitor the effectiveness of ovens and autoclaves by physical, chemical and microbiological means.

   a. Make physical checks through the use of a temperature chart recorder.

   b. Use autoclave tape that undergoes color change at the temperature of sterilization.

   c. Alternatively, insert specially prepared Kilit spore strips inside packages that are placed in the sterilizer. Upon removal, insert spore strips into broth tubes and incubate for signs of bacterial growth. Appropriate controls described by the supplier should be included.

3. Package sterile supplies in volumes that will be used at one sitting.

   a. Package 7-10 pipettes, depending on size. Wrap in Patapar paper for ready storage and quick use.

   b. Package tubes approximately 20 per bag.

4. Strictly enforce effective housekeeping procedures.

   a. Disinfect floors on a regular basis, preferably daily.

      (1) Obtain maximum disinfection by flooding the floor with disinfectant.

      (2) Autoclave sterile mop heads after each use because used moist mop heads can serve as a growth medium and incubator for microorganisms.

      (3) Remove disinfectant after 5 min by means of a wet vacuum cleaner equipped with a filter exhaust.

   b. Thoroughly clean water reservoirs, especially faucets, sinks and wet suction apparatus.

      (1) Empty water bath and turn off when not in use.

      (2) Use concentrated disinfectant in liquid traps for aspirated media. This disinfectant will be diluted by aspirated media and inactivated by protein as medium is introduced. An Ultipor disposable filter or equivalent should be placed in the vacuum line between the disinfectant trap and the vacuum source to remove infectious aerosols that may be generated in the trap. The trap should not become more than three-quarters full; overloading can destroy the filter.

   c. Avoid clutter in the laboratory.

   d. Store sterile supplies in dust-free cabinets.

   e. Wear clean protective clothing in the form of either full uniforms or laboratory jackets and head covering to minimize contamination by microorganisms shed by the technician or present on street clothing. This clothing should be worn only when working with cultures, changed regularly and not be worn outside the laboratory area.

   f. Do not allow any unnecessary activity in the immediate area during sterile procedures. This includes general traffic and talking.

5. Carry out periodic checking of cell cultures for microbiological contamination, including mycoplasma (see Procedure 75317).

III. DISCUSSION

Cell culture contamination may come from other contaminated cultures and the general environment. Contamination may originate from any source in the environment, but more frequently from the technician, unsterile supplies and media. The procedures outlined here are designed to prevent contamination from these sources.

Antibiotic-containing media encourage the selection of resistant contaminants that can remain undetected unless special media and culturing techniques are used. Previous experience in this laboratory has shown that antibiotics generally were present when fastidious organisms were isolated from cell cultures.

Analysis in this laboratory has shown that the major contaminants of cell cultures in antibiotic free media are microorganisms that are ubiquitous in the environment: Staphylococci, Bacillus sp., molds, pseudomonas, etc. These organisms will multiply rapidly in cell cultures and yield quick evidence of contamination, turbidity and cell destruction. In antibiotic containing media only resistant microorganisms, which are often slow growing or difficult to detect, will survive. Isolations from antibiotic containing media were characterized by more fastidious microorganisms: Hemophilus sp., mycobacteria,

anaerobic diphtheroids, slow growing yeasts and human species of mycoplasma.

Most chemical disinfectants commercially available are effective. A common difficulty encountered with disinfectants is that they are often improperly used. Concentration, as directed by the supplier, length of exposure, and presence of extraneous proteins will influence effectiveness. Proteins and other chemicals will inactivate disinfectants and may convert them to an excellent culture medium for many microorganisms.

Specific implementation of some of these recommendations will vary in different laboratories, depending on design, availability of support services and nature of the work. However, these recommendations have been used successfully in many laboratories to prevent, detect and control contamination.

IV. REFERENCES

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