CETA Certification Application Guide
USP <797> Viable Environmental Sampling & Gowning Evaluation
CAG-009-00

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1.0 Revision History

1.1 **Revision 00** Effective January 23, 2012

1.1.1 Revised as follows: Initial Draft.

2.0 Background

2.1 The purpose of this guide is to establish an industry-based methodology for complying with the environmental sampling requirements addressed in USP *<797> Pharmaceutical Compounding—Sterile Preparations*. It is intended to assist certification professionals, compounders and facility managers in determining appropriate procedures which will establish a unified approach for testing facilities. For information regarding nonviable sampling, it is recommended to refer to *CAG003* and the ISO *14644* for the appropriate guidance.

2.2 This guideline has been established to create a uniform approach for field certifiers to allow consistent and repeatable testing at all facilities. The approach of this guide is to reference the applicable accepted industry guidelines, standards or recommended practices along with the specific tests within that document.

2.3 When industry guidance documents are not available for a specific procedure, guidance will be provided here for developing an appropriate procedure.

3.0 Precautions/Considerations

3.1 The individual State Boards of Pharmacy, Departments of Health, or other inspection agencies enforce the standard or their own versions of the standard. When individual state or agency standards conflict with USP *<797> Pharmaceutical Compounding—Sterile Preparations*, the standards which the facility is inspected against shall be used and clearly identified in the certification documentation.

3.2 The United States Pharmacopeia is updated and revised annually. Verify each year that no changes have been made to relevant chapters before proceeding.

3.3 The content and views expressed in this technical guide are the result of a consensus achieved by the authoring committee and are not necessarily views of the organizations they represent.

3.4 Where a contradiction is found between the text and the appendix of the USP *<797> Pharmaceutical Compounding—Sterile Preparations* chapter, the requirement in the text is used. Where contradictions are found within the text itself, the requirement deemed most suitable by this committee will be used.

3.5 USP *<797> Pharmaceutical Compounding—Sterile Preparations* was written and references USP 34 *<1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments*. This section was revised in 2011 and the title changed to USP 35 *<1116> Aseptic Processing Environments* and will be effective in May 2012. Although this guideline was written to comply with both chapters one major difference is in the method in which the concern levels are established. The new revision is geared toward aseptic processing environments where a large number of samples are taken during every shift; therefore, the manner in which concern levels are set in the new revision may be unsuited for environments that only sample a few times a year. This guideline describes the process of setting concern levels that was described in USP 34 chapter. Setting levels using the new method should be considered if it is appropriate.

3.6 Differentiation of the facility risk level with the risk assessment.

3.6.1 The facility risk level is described as high, medium and low. This is determined by pharmacy personnel and is based upon the compounding practices and materials used during compounding.

3.6.2 A risk assessment is completed while developing the sample plan and determining viable sample site locations.
FDA *Aseptic guidelines state –*

"It is important that locations posing the most microbiological risk to the product be a key part of the program. It is especially important to monitor the microbiological quality of the critical area to determine whether or not aseptic conditions are maintained during filling and closing activities. Air and surface samples should be taken at the locations where significant activity or product exposure occurs during production. Critical surfaces that come in contact with the sterile product should remain sterile throughout an operation. When identifying critical sites to be sampled, consideration should be given to the points of contamination risk in a process, including factors such as difficulty of setup, length of processing time, and impact of interventions."

Therefore, the number of sample locations is increased in areas where there is a greater potential for contamination to product.

3.7 Activity level - All viable sampling is conducted while the cleanroom is in an active or dynamic state. Do not confuse with active and passive air sampling which is described below. Facility personnel must be present and working in the cleanroom while viable sampling is performed. If there is a concern for product contamination during sampling, viable sampling can be conducted during media fills or while personnel produce product that will not be used.

3.8 Passive air sampling- Passive air sampling is conducted by opening an agar plate and exposing it to the environment for a specified period of time. The lid is closed and the plate is incubated and analyzed in the same manner. This method is a qualitative not quantitative method and it is NEITHER appropriate NOR compliant with USP *<797> Pharmaceutical Compounding—Sterile Preparations*.

Air sampling is required to be active which is conducted by drawing a known amount of air into an electronic air sampler.

For information regarding the appropriateness of an active air sampler refer to *ISO 14698-1:2003(E) Annex B Guidance on Validating Air Samplers*. You can be assured that your air sampler is compliant with this standard by requesting a copy of the ISO compliance validation document from the manufacturer of the air sampler. This document should be kept in your files for retrieval if requested by your customers or regulatory agencies.

3.9 Air samplers- Be aware that certain manufacturers have indicated that there is a MPN calculation or a statistical correction factor that must be applied to air sample data. Be sure to incorporate this factor before performing conversion calculations. Refer to the owner's manual or consult with the manufacturer before use. Be certain to inform the microbiological laboratory of this factor if the laboratory converts the data for you.

3.10 When using the duplicate plating method, be cognizant of the possibility that it is likely that bacterial colonies may be recovered on the mold and yeast plate that were NOT recovered on the bacterial plate and vice versa. This can be attributed to a multitude of reasons. By only counting the bacterial colonies on the bacterial plate and vice versa there may be times when the controlled environment fails and you will not be informed of the failure. Discuss with your laboratory how you would like to handle these situations before submitting samples. Also, for air samplers using air samples that require a MPN calculation, you will need to determine the most appropriate method to apply this calculation.

3.11 Be cognizant that there are two gloved fingertip sampling requirements in the USP *<797> Pharmaceutical Compounding—Sterile Preparations* chapter. The first is the procedure described in section **23.0 Gowning Evaluation** along with the acceptance criterion for this set. The second is the gloved fingertip sampling that is completed at the conclusion of a media fill preparation. The acceptance criterion for this sampling is not more than 3 CFU/glove.

3.12 Remain cognizant of the storage temperature of the media. Media must be stored according to the manufacturer's instructions. Most manufacturers require temperatures be maintained between 2°C and 25°C (35.6°F and 77°F). If it is possible that the media was subjected to temperatures outside this range, thoroughly inspect the media's integrity before use. Media that was frozen or became too hot must be discarded.

4.0 References

4.1 USP 35 *<797> Pharmaceutical Compounding—Sterile Preparation.*

4.2 USP *34 <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments*; USP 35 *<1116> Aseptic Processing Environments*. See section **2.4**

4.3 USP 35 *<71> Sterility Tests*

4.4 ISO 14698 *Cleanrooms and associated controlled environments — Biocontamination control —Part 1: General principles and methods*.

4.5 ISO 14698 *Cleanrooms and associated controlled environments — Biocontamination control —Part 2: Evaluation and interpretation of biocontamination data*.

4.6 FDA 2004 *Guidance for Industry Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Guide*.

4.7 IEST January 2006 - *Microorganisms in Cleanrooms*.

5.0 Definitions / Acronyms

Alert Level
: Specified microbial levels that are determined based on gathered data from the particular facility that indicate the controlled environment may be drifting out of control.

Action Level
: Specified microbial levels that are determined based on gathered data from the particular facility that indicate the controlled environment has drifted out of control. These levels when exceeded must incur an investigation into the cause as well an action that will return the environment to previously accepted values. Action levels may be obtained from the suggested levels found in USP *<797> Pharmaceutical Compounding—Sterile Preparations* until the facility can gather its data.

Backwash Area
: Areas in cleanrooms that introduce particles and/or interfere with the intended patterns of airflow due to equipment placement, personnel flow, personnel operations and/or cleanroom design.

Chain of Custody
: A chain of custody is a document that traces the deposition of viable samples from the time they are taken until they arrive at the microbiological laboratory.

Contact Plates
: A convex sampling device containing a growth media supplemented with neutralizers used to sample surfaces for viable organisms.

Dynamic State
: Compounding personnel compounding CSP or media fills.

Surface Sampling
: A quantitative sample of a surface for viable organisms.

Swab
: A sampling device used to sample uneven surfaces for viable organisms. Assure that the medium used contains an appropriate neutralizer.

Viable
: Capable of living, developing, or germinating under favorable conditions.

Viable Air Sampling
: A quantitative sample of the environment's air for viable organisms.

Volumetric Air sampler
: A calibrated instrument that draws air through the unit to capture particles.

BUD	Beyond Use Date
CAG	CETA -Certification Application Guide
CAI	Compounding Aseptic Isolator
CACI	Compounding Aseptic Containment Isolator
CFU	Colony forming unit
CSP	Compounding Sterile Preparation
FDA	Food and Drug Administration
IEST	Institute of Environmental Sciences and Technology
IPA	Isopropyl Alcohol or Isopropanol
ISO	International Organization for Standardization
PEC	Primary Engineering Control
SOP	Standard Operating Procedure
USP	United States Pharmacopeia

6.0 Equipment and Materials

6.1 Viable air sampler

6.1.1 Impaction shall be the preferred method of volumetric air sampling.

6.1.2 Calibrated and disinfected viable air sampler- Electronic air sampler capable of collecting up to 1000L or more of air with a sufficient flow rate that will not dry the media.

6.1.3 The air sampler must not interfere with the air flow patterns of the engineering controls which could introduce contamination.

6.1.4 Required media filled strips or plates used in conjunction with the chosen air sampler. See section **6.3 Media** below for selection of media.

6.2 Contact plates filled with appropriate media. See section **6.3 Media** below for selection of media. Swabs may be used for sampling irregular or hard to reach areas. Swabs must contain a neutralizing buffer and must be processed by plate count method to obtain a total microbial count.

6.3 Media

6.3.1 General purpose media capable of supporting growth of a wide range of organisms. E.g. Tryptic soy agar (TSA).

6.3.2 Growth media used for surface sampling must incorporate neutralizers which are capable of neutralizing the chemicals used for cleaning the cleanroom environment. Media that is supplemented with lecithin and polysorbate 80 offers a wide range of neutralizing capabilities. However, it is important to document all cleaners which are used in the cleanroom environment and compare them to the neutralizer which have been added to the media. If the media is not capable of neutralizing the chemicals then a different additive must be used. Anytime there is a change in the cleaning chemicals used, a review of the neutralizing capabilities of the media must be conducted.

6.3.3 Media should be prepared by a known manufacturer that has quality systems in place to assure the media is appropriate for use. It should be accompanied by a certificate of analysis that documents the results of the quality control testing performed. This testing is completed by the manufacturer and is in addition to and prior to the quality control testing performed at the microbiological laboratory. It should be double or triple bagged and irradiated.

6.3.3.1 If media must be used that is not terminally sterilized (aseptically poured), each plate must be pre-incubated and visually inspected before introduction into the cleanroom. Be aware that pre-incubation may dry out the media and affect growth promotion.

6.3.4 Media must be deemed acceptable for use by performance of sterility and growth support testing as described in section **15.0 QC Testing of Media** on at least one negative and one positive control plate per media type, per lot number, per shipment and is conducted by the laboratory performing the sample analysis.

Best Practice: Include two pieces unopened of each medium type/lot used for positive and negative quality control testing to be conducted by the laboratory.

6.3.5 A general mycological media may be used in addition to the general growth media in all risk level facilities.

6.3.6 Store media prior to use according to manufacturer's directions.

6.4 Sterile wipes or alcohol pads.

6.5 Chain of custody.

6.6 Sterile 70% IPA.

6.7 Gloves- periodically disinfected with 70% IPA.

6.8 Marker or other device for labeling samples.

6.9 Tape or other device for securing samples.

7.0 Facility Risk Level

7.1 The risk level must be determined by the pharmacy director or other appropriate facility personnel and is based upon the compounding practices of the facility. The criteria for determining a facility's risk level is outlined in the current revision of USP *<797> Pharmaceutical Compounding—Sterile Preparations*.

7.2 If the facility completes compounding activities in more than one risk level then the requirements for the highest risk level must be followed.

8.0 Sampling Plan

8.1 A representative of the certifying company should meet with appropriate facility management personnel prior to testing to perform a risk assessment and develop a robust sampling plan.

8.2 Environmental sampling must also include all areas and ancillary areas where compounding occurs. For example: nursing units, ER, OR, nuclear medicine, clinics, physician office etc.

8.3 Viable Sampling Plan

8.3.1 Sites should be selected throughout the area. Areas that pose the greatest risk of contamination to the product must be included in the program.

For example, within each ISO classified area such as:

The CAI, buffer room, chemo room, ante room pass through etc. In addition, consider additional samples in backwash areas of the PEC's and pathway of CSPs within the cleanroom. Backwash areas can be identified using smoke studies and/or by individuals with expertise in cleanroom design and operations.

8.3.2 Samples must also be taken in all PECs as well as areas in close proximity; these areas would include operating room pharmacies, satellite locations, etc.

8.3.3 Most air sample locations are positioned at working height. Some locations may be higher or lower to capture microbial content in backwash or other areas where airflow may be affected.

9.0 Sampling Frequency

9.1 Semiannual basis as a minimum (twice per year). It is recommended to complete viable sampling after initial certification procedures but prior to recertification procedures to provide an "as found" result that compounding managers can utilize to show compliance for activities performed until the time of testing,

9.2 after any situation which may impact the normal operation of the cleanroom or PEC,

9.3 after movement of equipment,

9.4 after any servicing of facilities and/or equipment,

9.5 after a patient incident in which the compounded material is suspect,

9.6 after changes to the process that affect the cleanroom environment,

9.7 after observation of incorrect work practices such as: gowning, technique, material flow, cleaning etc.,

9.8 after identified problems with end product,

9.9 After significant changes in work flow or addition of new procedures or equipment.

10.0 Air Volume Selection

10.1 A sufficient volume must be taken where the level of detection is above the pass/fail criteria. To calculate the level of detection and determine if a volume of air will be appropriate for a specific divide calculate as follows:

1000[L] / Sample Volume [L]

If the result is lower than the acceptance criteria and the volume is within the specified range (400L to 1000L) then it is appropriate to use. ISO 5 areas usually require a minimum of 1000 liters per sample most other areas require a minimum of 400 liters per sample.

NOTE: If using the duplicate plate method it is NOT acceptable to sample 500 liters for bacteria and 500 liters for mold per sample site in an ISO 5 area, each sample must be 1000 liters.

11.0 Activity Level

11.1 Samples are taken while the cleanroom is in a dynamic operating state. May be performed while the pharmacy personnel are performing their competency testing.

11.2 Surface samples are to be taken after compounding or competency testing.

11.3 In addition to dynamic sampling, static sampling may be performed for cleanroom capabilities information.

12.0 Documentation

12.1 Sampling Protocol

12.1.1 An SOP or protocol that describes the procedures for sampling along with the volume, ISO classification(s), method of collection, frequency, activity level, action levels, response to exceeded concern levels, media type(s), risk level, laboratory analysis, incubation time and temperatures must be created.

12.2 Sample Plan / Map

12.2.1 Map of the area noting the locations and types of samples to be taken. Samples must be consistently taken in the same areas each time for trending to be meaningful.

12.3 Chain of Custody

12.3.1 For each sample session the following should be documented either on the sample plan or chain of custody. Laboratories may differ slightly in the information they require to process samples, information which is generally included is as follows:

- activity level
- air sample volume(s)
- facility name or code
- technician name or initials
- date and time of sampling
- media type(s), lot number(s) and expiration date(s)
- alert and/or action levels
- ISO classification
- requested microbial testing
- name of company submitting samples
- contact information

Best Practice: Number of people present in area, equipment SN/cal date, ISO classification and time. Also, note any deviations from normal procedures that had occurred during sampling and/or transport and determine if the impact of that deviation will affect the results of the test.

12.4 Sample Plates or Strips

12.4.1 Should be labeled with the date and location or code, facility name or code (if taking samples from more than one facility that day), media type and sample type, e.g. air sample or surface sample and volume (if appropriate). Samples should be labeled on the bottom not the lid.

12.5 Report

12.5.1 total microbial count in the appropriate units

12.5.2 test results indicating any presence of mold, gram negative rod, yeast or coagulase positive staphylococcus

12.5.3 identification of all recovered organisms at least to genus level must be completed by the laboratory for all risk levels

12.5.4 results of the growth promotion and sterility quality control testing performed by the laboratory

12.5.5 media lot numbers and expiration dates

12.5.6 alert and/or action levels

12.5.7 date and time of sampling

12.5.8 activity level

12.5.9 pass or fail status

12.5.10 signature of laboratory analyst and reviewer

12.5.11 Air sample data must be transformed into CFU/m3. Refer to section **18.0 Calculations** for more information.

Best Practice: Additional information such as, equipment identification numbers, equipment calibration status, ISO classification, incubation time and temperature.

13.0 Sampling Procedures

13.1 General

13.1.1 Prior to a testing session, review the sampling plan and gather the materials needed (refer to Section **6.0 Equipment and Materials**).

13.1.2 All individuals including certifiers that enter the cleanroom must gown according to the facility protocol and/ or USP *<797> Pharmaceutical Compounding—Sterile Preparations* standards.

- Individuals must remove garments (such as scarves, hats, vests etc) makeup and visible jewelry.
- All individuals entering the cleanroom must be free from illness, skin rashes, sunburn etc.
- Clothing and shoes must be clean and long hair tied back.
- No food or beverages are permitted in the cleanroom. This includes throat lozenges, gum and mints.
- Individuals must select and facility managers must make available, proper fitting gowning materials to all individuals who enter the cleanroom.

- The individual must follow the client's protocol for gowning procedures. In the absence of a protocol USP *<797> Pharmaceutical Compounding—Sterile Preparations* gowning procedure is as follows.

- Shoe covers must be donned one at a time as the individual steps over the line of demarcation. The covered shoe shall not touch the same side of the line as the uncovered shoe.

- A beard cover (if necessary), mask and head cover must be donned. Head covers must be donned in all circumstances.

- Hands and forearms must be washed with soap and dried thoroughly.

- A proper fitting gown is then donned.

- An antiseptic hand cleansing is performed, using a waterless alcohol based hand scrub with persistent activity. Allow hands to dry thoroughly.

- Gloves are donned and disinfected.

13.1.3 Disinfect all test instruments, cart and materials thoroughly when transferring into cleanroom. Do not bring cardboard shippers or other containers which cannot be disinfected into the cleanroom.

Best Practice: Viable sampling should occur prior to other testing criteria, except when testing a newly constructed cleanroom. It is recommended to test the cleanroom and assure it is working according to specifications. Then complete a thorough cleaning before viable sampling.

13.1.4 Disinfect the staging area prior to staging materials.

13.1.5 Examine media for microbial growth and integrity, eg:

- cracks in the media,
- missing sections of media,
- excessive dryness where the media appears to have pulled away from the edges of the plate,
- frozen agar will appear to have crystal formations and lines in the agar surface,
- excessive moisture,
- verify contact plates have a convex surface,
- Petri-dishes (or other containers) are not broken or cracked.

13.1.6 Sample in order from most clean to least clean. E.g. ISO 5 areas first then ISO 7 areas.

13.1.7 Label each sampling piece with the assigned sample site code, volume (if appropriate), date, initials, and area designation.

13.1.8 Disinfect and allow gloves to air-dry periodically (about every 5 - 6 contact samples), before and after each air sample and when anything occurs which the technician feels may have compromised the cleanliness of a glove.

13.1.9 Disinfect the air sampler head periodically and when anything occurs which the technician feels may have compromised the cleanliness of the air sampler head.

13.1.10 Sampling

13.1.10.1 Single plate method

Sample each location using a general microbiological media such as tryptic soy agar.

13.1.10.2 Duplicate plate method

Sample each location using a general microbiological media such as tryptic soy agar, then sample the same locations again using a general mycological media or a media capable of supporting growth of fungi.

13.1.11 Aseptically handle all materials. For example, do not remove lids or sleeves of media until ready to sample and position lids and sleeves in a way to prevent contamination while sampling. If the technician feels that the sample may have inadvertently become contaminated, discard that sample and resample with a new plate or strip. Do NOT discard plates, strips or swabs in the controlled environment.

13.1.12 The control plates remain with the samples and are transported to the sampling facility as well as the laboratory in the same manner and time as the samples. The samples that are used for the controls do not need to be opened by the technician, only marked as controls.

13.2 Viable Air Sampling

13.2.1 Autoclave or disinfect the air sampler head and disinfect the air sampler.

13.2.2 Select the appropriate volume of air.

13.2.3 Aseptically load the air sampler.

13.2.4 Move the air sampler to the designated location.

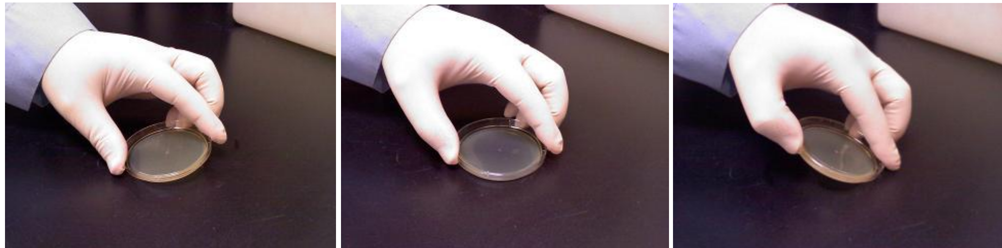
13.2.5 Operate the air sampler; refer to the operator's manual for details of its use.

13.2.6 At the completion of the cycle, aseptically remove the sample from the air sampler. Replace lid or return to its packaging and secure opening or lid.

13.3 Surface Sampling

13.3.1 Contact Plates

13.3.1.1 Carefully remove the lid and take the sample by rolling the convex agar surface in one direction across the surface of the site. Immediately replace the lid on the plate.



13.3.1.2 Apply a brief spray of 70% IPA to the site and follow with a wipe or use a wipe pre-saturated with IPA to remove nutrients.

13.3.2 Swabs

13.3.2.1 Carefully remove the swab from its container. Allow excess media to drain before removing completely from container.

13.3.2.2 Scrub the area with the swab using a twisting motion. Sample the entire area if less than 2 inch by 2 inches otherwise sample a 2”x2” area. Document the size of the sample area.

13.3.2.3 Apply a brief spray of 70% IPA to the site and follow with a wipe or use a wipe pre-saturated with IPA to remove nutrients.

14.0 Specifics for Isolators – CACI & CAI

14.1 Viable sampling (air and surface) must occur in the main chamber, pass through chamber and the environment that the isolator is located, even if the isolator is located in an uncontrolled area.

14.2 The sample equipment must be decontaminated before placing in the pass through chamber.

14.3 Move the sampling equipment into the main chamber following the manufacturer's instructions for moving equipment into the chamber.

14.3.1 Be aware of the placement of equipment into the pass through. Do NOT sample the surface in the area where equipment has been placed.

14.3.2 Be aware of placement of all sampling equipment in the main chamber. Do NOT perform surface sampling in an area where sampling equipment has been staged.

14.4 Set up the air sampler. Once the air sampler is ready to be started, the compounding personnel enter the isolator ports, don sterile gloves and perform competency testing or simulate compounding practices while the air sampler is running.

14.4.1 If the compounding personnel are performing their competency testing at the same time, the technician waits until the competency testing is completed before intervening again.

14.4.2 When the simulated / competency testing is completed, the compounding personnel will complete the gloved fingertip sampling on both hands before the technician intervenes. Do NOT disinfect the gloves prior to sampling. See **section 23.3.6 through 23.3.9**

Best Practice: If the isolator has three glove ports and only two ports are routinely used the unused glove port can be replaced with a port cover. However, all gloves that are present should be tested.

14.5 Once the air sampler has completed its cycle, and any competency testing has been completed as well as the gloved fingertip sampling, the compounding personnel remove themselves from the isolator to allow the technician to perform surface sampling. Compounding or sampling personnel do not disinfect the surface prior to sampling.

14.6 Once viable sampling has been completed in the main chamber, perform surface sampling in the pass through chamber from the main chamber. Do NOT sample the surface in the same area that equipment was placed during transfer. Be sure that the door to the outside of the isolator is closed properly.

14.7 Aseptically load the air sampler and move it into the pass through and sample that area. Ensure both pass through doors are closed properly.

14.8 When the air sampler is finished, move the air sampler back into the main chamber for removal of the media. All equipment can then be transferred back into the pass through and removed from the isolator. Disinfect the any areas that can in contact with the sampling equipment.

NOTE: There is a difference in opinion and confusion regarding the ISO classification of the negative pressure and on-demand purge isolator pass through. If the sampling is performed correctly and the isolator is operating correctly it is NOT unreasonable to expect it to pass ISO 5 criteria. However, if this cannot be routinely met trending of the data to set appropriate levels is required. Regardless of data trending results the criteria should not be set above ISO 7 classification if the isolator is located in an ISO 7 area or ISO 8 criteria if it is located in an unclassified area.

15.0 Transporting Samples

15.1 Samples must be repackaged in a manner that prohibits them from being contaminated during transport. Package so the plates are stacked together and lids do not come off during transport. After sampling is completed, samples should be kept cool but not frozen prior to and during transport to the laboratory. Samples may be placed in a cooler with ice packs during transportation. DO NOT use dry ice. DO NOT allow the ice pack to come in contact with the media, this will freeze the media. Place some type of material between the ice packs and media such as shipping peanuts or bubble wrap. Plates can be repackaged in the bag supplied by the manufacturer. Secure the opening with a twist tie or tape.

Best Practice: Place all samples in a plastic sealed bag when shipping to the laboratory rather than packing loose plates in the shipping box. This will help prohibit contamination and assure that the laboratory removes all plates from the box.

15.2 Plates should be transported and stored inverted to minimize condensation in the lids of plates.

15.3 If plates are being shipped via a third party, plates must be shipped over night.

15.4 Samples should arrive at the laboratory and be placed on test the same or next day. In the case of late sampling, weekends or holidays, plates should not be held longer than 4 days and must be refrigerated (approximately 2°C to 8°C (35.6°F and 46.4°F) prior to being shipped to the laboratory.

15.5 Samples must be packaged in a manner that prohibits contamination during transportation to the facility as well as the laboratory.

16.0 QC Testing of Media

16.1 Seed lot culture maintenance techniques must be used so that the viable organisms used are not more than five passages from the original master seed lot. Commercially prepared calibrated cultures that comply with this requirement may be used. Strains of test microorganisms which are suitable for use in growth promotion testing.

16.1.1 *Staphylococcus aureus* - ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276

16.1.2 *Bacillus subtilis* – ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134.

16.1.3 *Pseudomonas aeruginosa* – ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275.

16.1.4 *Candida abicans* – ATCC10231, IP 48.72, NCPF 3179, NBRC 1594.

16.1.5 *Aspergillus brasiliensis* – ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

16.2 Growth Promotion Testing – Single Plate Method

16.2.1 Positive control plates are inoculated with less than 100 CFU of *Aspergillus brasiliensis, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* in individual portions of the media. Inoculated media is incubated for not more than three days for bacteria at 30°C to 35°C and not more than five days for mold and yeast 20°C to 25°C. Media is suitable for use if clearly visible typical growth occurs for each organism within the allotted time.

16.3 Growth Promotion Testing – Duplicate Plate Method

16.3.1 Bacterial Media

16.3.1.1 Positive Control plates are inoculated with less than 100 CFU of *Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus* in individual portions of the media. Inoculated media is incubated for not more than three days for bacteria at 30°C to 35C°C Media is suitable for use if clearly visible typical growth occurs for each organism within the allotted time.

16.3.2 Fungal Media

16.3.2.1 Positive Control plates are inoculated with less than 100 CFU of *Aspergillus brasiliensis* and *Candida albicans* in individual portions of the media. Inoculated media is incubated for not more than five days for mold and yeast at 20°C to 25°C. Media is suitable for use if clearly visible typical growth occurs for each organism within the allotted time.

16.4 Media Sterility Testing

16.4.1 Controls for sterility are incubated along with the samples. Media is suitable if no growth of organisms occur. Note: USP *<71> Sterility Tests* calls for media to be incubated for 14 days since sterility tests are incubated for this length. Since environmental samples are only incubated for up to seven days this is the duration usually completed for media sterility testing which will be used for environmental sampling.

17.0 Sample Incubation

17.1 Single Plate Method:

17.1.1 Incubate samples inverted at 30˚C to 35˚C for 48 hours to 72 hours and then incubate at 26˚C to 30˚C for 5 to 7 days.

17.2 Duplicate Plate Method:

17.2.1 General microbiological media.

17.2.1.1 Bacterial recovery incubation is 30˚C to 35˚C for 48 hours to 72 hours. Invert plates.

17.2.2 Mycological media or other media capable of supporting the growth of fungi.

17.2.2.1 Fungal and yeast recovery incubation is 26˚C to 30˚C for 5 to 7 days. Invert plates.

18.0 Analysis of Samples

18.1 Single Plate Method:

18.1.1 After initial incubation, the total number of colonies recovered are counted and documented.

18.1.2 Samples are re-incubated at 26˚C to 30˚C for an additional 5 to 7 days.

18.1.3 Remove from incubator and count all recovered colonies.

18.2 Duplicate Plate Method:

18.2.1 After incubation remove plates from the incubators.

18.2.2 Count the recovered bacteria colonies on the plates that were incubated at the 30˚C to 35˚C temperature.

18.2.3 Count the recovered mold and yeast colonies on the plates that were incubated at the 26˚C to 30˚C temperature.

18.2.4 Add the bacterial count and the mold yeast count from the corresponding plates for each location.

18.3 Refer to section **18.0 Calculations** for calculating the total microbial count in the appropriate units.

18.4 Each colony type must be identified at least to genus level.

18.5 Microbiological methods must be employed to determine if any recovered Staphylococcus colonies are coagulase positive.

18.6 A review of the organisms found must be conducted to determine if any mold, yeast, coagulase positive staphylococcus or gram negative bacteria rods were recovered.

19.0 Calculations

19.1 Viable Air Samples

Note: Certain impaction air samplers have an MPN or a statistical correction factor that needs to be applied. Refer to the manufacturer's instructions.

19.1.1 Total Microbial Count/ Cubic Meter

19.1.1.1 Data must be transformed into CFU/m^3. The total microbial count is determined by adding the total bacterial count and the total yeast and mold count. This number is then multiplied by 1000 and divided by the volume taken.

$$\frac{(\text{Total bacterial count on sample [CFU]} + \text{Total fungal count on sample [CFU]})*1000\ [L/m^3]}{\text{Volume sampled [L]}}$$

19.2 Surface Samples

19.2.1 The total microbial count is determined by adding the total bacterial count and the total yeast and mold count.

19.2.2 The total microbial count for swab samples is determined by adding the bacterial count and the total yeast and mold count multiplied by the dilution factor.

20.0 Data Expression

20.1.1 Viable air samples will be expressed as CFU per cubic meter.

20.1.2 Contact plate surface samples will be expressed as CFU per plate.

20.1.3 Swab samples will be expressed as CFU/swab or area sampled.

20.1.4 If no colonies are recovered, the results will be reported as less than the detection level. For example, zero cfu recovered on a surface sample will be reported as "<1 cfu/plate." Refer to section **9.0 Air Volume Selection**.

21.0 Pass/Fail Criteria

21.1 Viable data collected over time is evaluated by appropriate statistical tools and concern levels are determined. These levels when set may not significantly deviate from the suggested levels in USP. In the interim of collecting data the suggested levels in the USP *<797> Pharmaceutical Compounding—Sterile Preparations* chapter may be used.

21.2 USP 34 *<797> Pharmaceutical Compounding—Sterile Preparations* recommended microbial action levels

Fingertip (Post fill) CFU/plate	Fingertip (Gown Validation) CFU/plate
>3	>0

Classification	Air CFU/m3	Surface CFU/plate
ISO Class 5	>1	>3
ISO Class 7	>10	>5
ISO Class 8	>100	>100

Recovery of any mold, yeast, coagulase positive staphylococcus or gram negative rods in any area is considered not to be in compliance with USP *<797> Pharmaceutical Compounding—Sterile Preparations* standards and immediate remediation and investigation into the cause must be conducted.

22.0 Evaluation of Data

22.1 If the reported value(s) exceeds the Action level(s) and/or objectionable organisms have been detected, the following steps should be taken:

22.1.1 Report results immediately to the personnel responsible for results e.g. Pharmacy Director.

22.1.2 Appropriate facility personnel are responsible for appropriate action(s) which will return the facility into compliance.

- A cleaning of the affected areas followed by a re-sampling.
- An investigation into the probable cause of the exceeded concern level.
  - Areas of investigation may be determined by the type of organisms recovered.
  - If a cause is found, procedures are implemented to prevent recurrence.

23.0 Review

23.1 Pharmacy personnel are responsible for reviewing and analyzing the data generated by the environmental sampling program periodically and evaluating for trends.

23.2 Pharmacy personnel must analyze the data periodically (usually yearly) and set appropriate alert and action levels based on the data generated by the program. These levels may differ from the USP suggested levels but may not deviate significantly.

24.0 Gowning Evaluation

24.1 Equipment and Materials

24.1.1 Contact Plates -filled with a general growth media with added neutralizers.

24.1.2 Sterile gloves and appropriate Gowning Materials.

24.1.3 Appropriate Audio/Visual training.

24.1.4 Checklist.

24.1.5 Written test.

24.2 Frequency

24.2.1 All risk levels – Initial, before they begin to perform compounding practices, each personnel must pass three trials successfully.

24.2.2 Low and medium risk levels – annually thereafter, one trial successfully completed.

24.2.3 High risk levels – semiannually thereafter, one trial successfully completed.

24.3 Procedure

24.3.1 Specifics for Isolators

24.3.1.1 Compounding personnel will garb (if required) and don gloves of the isolator in the same manner they complete for compounding practices.

e.g. If it is their procedure to replace the gloves of the isolator and use those gloves for compounding then they follow that practice and continue with section 23.3.6 below.

If it is practice to don sterile gloves inside the isolator then that procedure is followed and continue with section 23.3.6 below.

24.3.2 Individuals must be trained by expert personnel in theoretical principles and practical skills of garbing using audio– visual instructional sources and professional publications.

24.3.3 Individuals must complete a written test.

24.3.4 Individuals must be observed as they gown. Trainers must assure the proper procedure is followed noting any discrepancies with the procedure.

24.3.5 Once the individual has donned correctly without any discrepancies, including sterile gloves, follow step 23.3.6.

24.3.6 Have the individual touch all four gloved fingers and the thumb on the surface of a media plate. The pressure of the touch should be enough to leave a slight impression but not so much as to crack or break the integrity of the agar surface. Repeat this procedure with the other hand.

24.3.7 Immediately have the person spray or wipe their hands with 70% IPA and degown. This procedure must be repeated three times for the initial validation.

24.3.8 Samples must be marked with the date, initials, trial number and left or right hand.

24.3.9 Samples are incubated at 30°C to 35°C for 48 hours to 72 hours.

Best Practice: Samples should also be incubated at 20°C to 25°C for an additional 5 to 7 days for recovery of mold.

Note: In addition, if cleaning and disinfecting procedures are performed by other support personnel, they must be trained in proper hand hygiene, garbing and cleaning and disinfection. This can be completed by a qualified compounding expert. After completion of training support personnel shall routinely undergo performance evaluation of proper hand hygiene, garbing and cleaning and disinfection.

24.4 Pass / Fail Criteria

24.4.1 A person passes the initial validation when all of the following are met:

24.4.1.1 Three trials result in no discrepancies from the gowning procedure.

24.4.1.2 Three trials result in no growth of microorganisms on his/her gloved fingertip samples.

24.4.1.3 The individual receives a passing grade on the written test.

24.4.2 A person passes the revalidation when all of the following are met:

24.4.2.1 One trial results in no discrepancies from the gowning procedure.

24.4.2.2 One trial results in no growth of microorganisms on his/her gloved fingertip samples.

24.4.2.3 The individual receives a passing grade on the written test.

24.5 Failure of Evaluation

24.5.1 If an individual fails one or more of the requirements they shall be re-instructed and re-evaluated by the trainer and repeat the failed portion of the requirements.

24.5.2 Compounding personnel shall pass all evaluations prior to resuming compounding practices.