A LABORATORY PERSPECTIVE OF <USP 797> GUIDELINES

In 2004, The US Pharmacopeia published Chapter <797> - Guidebook to Pharmaceutical Compounding – Sterile Preparations. It outlined a program to ensure that products made by sterile compounding practices are of the highest quality and patients are not compromised due to contaminated preparations. Revisions were made in December 2007 and became official June 1, 2008. Additional proposed revisions are currently ongoing with a public comment period likely.

From the microbiology perspective the program involves Environmental Monitoring in the anteroom, buffer areas and critical areas in addition to a robust program to validate compounder competency. Until USP <797> there were no compulsory accreditation/inspections for pharmacies and currently many states board of pharmacy are adopting the Chapter <797> guidelines. See IACPrx.org. Some individual states have enhanced the existing <USP 797> guidelines.

In January 2012 CETA published a consensus document, CAG-009-2012, Environmental Sampling & Gowning Evaluation guidelines, an industry based methodology for complying with environmental sampling as described USP <797> viable environmental sampling.

SAMPLING CONSIDERATIONS

A thorough and well defined sampling plan is key for securing meaningful results. A visit to the sampling facility prior to the sampling event insures every detail is addressed prior to the Environmental Monitoring (EM).

For microbial sampling these details include correct media format and types of media for air and surface sampling, equipment choice and condition and ancillary supplies such as parafilm, Chain-of-Custodies, etc for your microbial assessment.

In addition to the prescribed areas in the pharmacy, EM should be done throughout the areas especially those which pose greatest risk of contamination, such as entries to the pharmacy suite, product storage, areas around the sink, backwash area, etc.

If the facility completes compounding activities in more than one risk level then the sampling in highest risk level should be considered or must be done. Sampling is done at least semi-annually for recertification of equipment and facilities.

AIR SAMPLING

Air sampling for all compounding risk levels are done by certified individuals to evaluate airborne microorganisms. Prior to sampling make sure you are following all the guidelines for cleanliness, equipment's sterilization and using the proper attire/covering gears. A single or double-headed impactor sampler such as a SAS or RCS, must be calibrated and pull required air volumes (400-1000 L), onto suitable agar media plates. The action levels for different ISO environments dictate total air volume. See the chart for Recommended Action Levels. ISO 5 requires a total of 1000 liters to reach a Detection Limit of 1 cfu/m^3. Typically 500 liters is pulled for ISO 7 and ISO 8 environments

Total volume tested should be between 400 to 1000 liters to maximize sensitivity. Microbial media for air sampling include Soybean Casein Digest medium or TSA (Tryptic Soy Agar) for bacterial growth and MEA (Malt Extract Agar) or SDX (Sabouraud Dextrose Agar) for mold or yeast growth. It is required media filled strips or plates be used in conjunction with the chosen air sampler.

Contact your laboratory to discuss situational based sampling. Distinct temperatures are dictated for the TSA (bacteria) at 30˚C to 35˚C for 48 to 72 hours. MEA/SDA (fungi) should be incubated at 26˚C to 30˚C for 5 to 7 days. Unique temperature environments and incubation periods allow bacteria and fungi to grow at temperatures that optimize recovery of both environmental organisms and clinical pathogens thereby increasing the sensitivity of each analysis.

SURFACE SAMPLING

Sampling is done using contact plates (RODAC-Replicate organism detection and counting)) or swabs. Sampling is done after compounding, cleaning and disinfection and after any media fill procedure. Surface samples using contact plates are taken by gently touching the sample area and rolling the plate across the sample area. RODAC or Contact plate ranges from 24-30cm2 .

Surface sampling media should include additives to neutralize the effects of the disinfecting agents like TSA (Tryptic Soy Agar) with lecithin and polysorbate 80 for bacteria and MEA (Malt Extract Agar) with lecithin and polysorbate 80 or SDX (Sabouraud Dextrose Agar) with lecithin and polysorbate 80 for mold and yeast.

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.

PROCESSES AND PROCEDURES AFTER SAMPLE RECEIPT IN LABORATORY

When the samples are received at the laboratory they are unpacked and compared with the chain of custody. The samples are examined by laboratory personal during this process and any samples that look compromised will be rejected. The samples are then logged into a laboratory information system (LIMS) and each plate is labeled with a unique number that can be traced back to the chain of custody. From this point on the samples are incubated per the USP 797 protocol. Below is a general outline of how the samples are incubated and processed.

SAMPLING - DUAL PLATE PROTOCOL

Separate bacterial and fungal samples are taken for each location. The bacterial samples are incubated at 30˚C to 35˚C for 48 to 72 hours after receipt at the laboratory. After this period the plates are examined and the bacteria are enumerated and identified. The fungal plates are incubates at 26˚C to 30˚C for 5 to 7 days after receipt at the laboratory. After this period the fungi are enumerated and identified. The optimum media for bacterial sampling is TSA. The optimum media for fungal sampling is MEA. Sabouraud Dextrose Agar (SDA) is acceptable for fungal analysis but is less desirable to MEA in that vegetative growth is promoted while sporulation is suppressed. Sporulation of the fungi in most circumstances is required for identification.

This approach will allow final results to be reported within the 5 – 7 day period. Interim "alert" emails should be expected from the laboratory when a "Highly Pathogenic Microorganism" is isolated so you can contact your client about their environment.

After sampling is done seal the individual plates preferably with parafilm, pack the plates carefully in a manner that prohibits them from being contaminated. If multiple projects in one shipping container, place each projects plates in a clean Ziploc bag to separate projects. Keep the plates cool but do not freeze prior to and during transport to laboratory. Keep the plates inverted while packing in a sealed plastic bag to avoid/minimize the condensation in the lids of plates. Plates must be packed with ice packs, make sure ice pack should not be in direct contact with plates. Place the COC along with samples in a different plastic bag. Samples must be shipped over night to the laboratory. In case of weekend/holidays make sure samples must be refrigerated until shipped. Plates should not be hold more than 4 days. The laboratory can be contacted for Saturday delivery options.

This approach would result in final reports at a minimum of 5 days to maximum of 7 days.

SAMPLING - SINGLE PLATE PROTOCOL

The CETA CAG-009 references a method that uses a single plate of TSA – a general media used for bacteria. Incubate samples at 30˚C to 35˚C for 48 to 72 hours (for environmental and clinical bacterial pathogens) and then plate must be moved to incubate at 26˚C to 30˚C for 5 to 7 days (for environmental and clinical pathogenic yeast and molds).

This approach would result in final reports at a minimum of 7 to maximum of 10 days.

The single plate protocol also raises concerns based on laboratory observations and experience.

- Many fungi will grow on it but the characteristics the lab rely on for the identification are often distorted and make identification problematic.
- A variety of bacteria will grow rapidly during the initial incubation period at 30˚C to 35˚C. Most fungi are suppressed at this temperature. If a bacteria grows over a colony forming unit of fungi, the fungi may never develop or its development can be inhibited and never develop into a visible colony. If it is able to grow into a visible colony it may grow in an atypical way making identification difficult to impossible.
- The initial incubation temperature of 30˚C to 35˚C is very stressful for most environmental fungi. This in itself can retard the growth of fungal colony forming units even after the initial incubation period is over. When further development takes place the fungi may develop in an atypical fashion making identification problematic or impossible

GLOVE FINGERTIP AND GARBING SAMPLING

Microbial contamination of CSP's is mainly from environmental contact, hand hygiene and garbing. Garbing and glove fingertip competency are done to evaluate the compounding personnel's hand hygiene and garbing procedures. All compounding personnel has to pass initial competency (no less than 3 times) before compounding and there after annually (High risk CSP's) or semiannually (Low and medium Risk CSP's).

Sterile contact plates ranging from 24 to 30 cm2 with ingredients to neutralize disinfectants (TSA for bacteria and MEA/SDA for fungi) are recommended for glove fingertip sampling.
Glove fingertip Sampling should be done before cleaning the gloves with 70% IPA. Each hand should be done on separate plates and imprints of all fingers should be on the media. It is preferable to do control plate of the media used along with each sampling. Plates should be labelled with sampling location (right or left hand), sampling date and initials. Plate should be closed or sealed tight immediately after sampling inside the ISO 5 to avoid opening of the plate lids during transit to the lab. Plates are incubated at 30-35° C for bacteria count and as referenced in CAG -009 Best Practice for an additional 5 to 7 days for mold./yeast colony count. NOTE: Certifier must note the analyses requested for Glove Fingertip on the COC, i.e., Total Bacterial Count and/or Total Fungal Count.

The passing criterion for initial competency of compounding personnel glove fingertip is 0 cfu (colony forming units). The action level thereafter is less than 3 cfu and based on the total number of cfu on both gloves together as per <USP 797>. As per USP <1116>, acceptable limits for garbing areas should not exceed 5 cfu for ISO 5 and 20 cfu for ISO 7. The colony counts relate to compounding personnel's hand hygiene and aseptic procedure followed. Exceeding the action level will require revaluation and retraining of the personnel before he can compound CSP's for human use.

DOCUMENTATION

Each sample session must be documented on Chain of Custody (COC) form provided by your laboratory noting analysis requested, date of collection, technician collecting sample, PO or Project Name, type of media, ISO class, air volume and surface area and any additional information noted during sampling which can help later while interpreting results.

HOLD TIME

Samples must be shipped over night to the laboratory. The samples should get to the lab in the shortest possible time, ideally within 24 hours of sampling. In case of weekend/holidays samples must be refrigerated until shipped. The laboratory can be contacted for Saturday delivery options. As per CAG-009-00, samples should NOT be held for more than 4 days after sampling. Samples should be refrigerated at 4-8°C. Refrigerating samples for longer period of time may cause the media to freeze and result in loss of colony count. Also the longer they are in transit they more likely to be exposed to temperature fluctuations as well as poor handling by package delivery services which may result in sample damage and result in resampling

TRANSPORTATION

When sampling is complete it is critical that the samples get to the lab in good condition and in a timely manner. If using contact plates they should be taped shut to avoid the lids coming loose in transit. If using the larger petri dish type plates, the plates they should be sealed shut around the entire circumference of the plate where the top and bottom portions come together with petri-film or elastic tape. This will prevent any contaminants from gaining entry to the plates during transit. The plates should be packed carefully with packing materials to avoid any movement of the samples within the package with the lid of the plate face down in a thermally insulated container such as a cooler. Ice packs may be necessary during hot weather. The ice packs should not come in direct contact with the samples inside the container. Using a thermally insulated container will help maintain a constant temperature during transit will reduce the risk of condensation inside the plates. The plates packed lid side down with the agar portion of the plate facing down so that any condensation that may develop on the lid will not drip on the surface of the agar. Any water that drips on the agar will cause any microorganisms that may be present to spread across the plate potentially ruining the sample.

The samples should get to the lab in the shortest possible time. Ideally within 24 hours of sampling. The longer they are in transit they more likely they are to being exposed to temperature fluctuations as well as poor handling by package delivery services.

DATA INTERPRETATION

RECOMMENDED ACTION LEVEL FOR MICROBIAL CONTAMINATION

ISO CLASS	Air samples (cfu*/m^3)	Surface samples (cfu/contact plate**)	Glove fingertip (cfu/plate)
ISO Class 5	>1	>3	>3
ISO Class 7	>10	>5	N/A
ISO Class 8 or Worse	>100	>100	N/A

Source: USP<797> Pharmaceutical Compounding-Sterile Test
*CFU: colony forming units

** Contact plates may vary from 24 to 30 cm^2.

QC TESTING OF MEDIA

Media must be acceptable for use by performance of sterility and the ability to support growth. At least one TSA plate and one MEA plate per lot number should be submitted for a negative control and one TSA plate and one MEA plate per lot number should be submitted for positive growth control. (same for any type of fungal media). Each plate should be included on the chain of custody with appropriate test noted.

QUALITY ASURANCE PROGRAM

The purpose of a quality assurance program is to provide a system for monitoring, evaluating, correcting, and improving activities and processes. A quality assurance program should review and analyze objective data and use these data to develop action plans. Quality assurance measures each risk level compounded in a facility, include routine cleaning and disinfection and air quality testing, and preparations to ensure accuracy of compounded products, and visual inspection of final CSPs to confirm the absence of particulate matter.

IN SUMMARY

The <USP 797> and CETA CAG-009 offer guidelines and industry based definition for professionals in the certification and the support laboratory industry, Those in both disciplines must readily admit that following these guidelines and recommendations is not as easy as it sounds but we strive to follow the information as outlined. The laboratory will do everything possible to support the certifiers before they go into the field and after they have collected their samples. By understanding just how important it is to collect the most appropriate and defensible sample, document it accordingly, ship it properly then you can be confident that once your samples arrive at your laboratory, they will be handled and analyzed by strict adherence to SOPs by highly qualified microbiologists.

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