PART 3

Environmental Sampling Guidance

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Photo courtesy of Fred Massoomi

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Compounding safe sterile preparations relies on a complicated and interconnected system of people, processes, and engineering controls. The performance of this intricate service necessitates a defined environmental monitoring plan that encompasses a range of test methodologies to understand the compounding environment. An environmental monitoring program must be robust enough to provide useful information on personnel and processes to guide decisions on the safety of compounded products. USP Chapter <1116> clearly defines the ISO classifications and microbial status for compounding to ensure compounding areas are operating in the optimal state of control and provides detailed guidance on sampling techniques, number of required samples per area, and example surface sample location. The optimal environmental monitoring is able to confirm "consistent, high-quality environmental conditions at all times."[1]

Criteria and Certification

The minimum criteria required is outlined in the current (2008) version of USP Chapter <797> with pending proposed changes highlighted in **TABLE 1**.[2] It is important to note that USP sampling plans will not capture all potential events of contamination and is an episodic representation.

Most hospitals have established formal environmental monitoring programs with some managed through outsourced certifying companies that provide certification of classified areas along with air and surface microbiological monitoring.[3] The certification of the classified areas should be performed by the Controlled Environment Testing Association's (CETA's) National Board of Testing (CNBT) qualified individual(s), with additional accreditation by National Sanitation Foundation (NSF) for biological safety cabinets. Refer to certification procedures as outlined in the CETA Certification Guide for Sterile Compounding Facilities (CAG-003-2006) for classified areas for details.[4]

Choosing Equipment

USP <797> and <1116> specify the equipment and processes required to conduct microbiological sampling.[1,2]

Growth Media

USP states that a general microbiological growth media like soybean-casein digest medium (also known as trypticase soy agar [TSA] and trypticase soy broth [TSB]) is appropriate for conducting viable air and surface sampling and is suitable for culturing aerobic bacteria and fungi. The media must be able to promote and support the growth of pathogens that would best represent the intended purpose ("suitability"). USP <71> *Sterility Tests* clarifies what microorganisms are suitable for use in the growth promotion test of TSA/TSB media (see **TABLE 2**).[5]

USP <797> (2008) notes that malt extract agar or other fungi supportive growth media must be used in high-risk compounding environments; the revised USP <797> gives the option to use two types of media for sampling locations regardless of risk or category.[2] Choosing a media in solid or liquid form requires sites to consult the Certificate of Analysis (COA) document to ensure that the pathogens are represented and the incubation temperatures match USP requirements. Media for surface sampling must have neutralizing agents to ensure detection and growth of microbes in the presence of disinfecting agents. The media's storage and expiration dating must be

Part 1, Design Tips for Creating a USP <800> Compliant Cleanroom, and Part 2, PEC Placement in the Cleanroom, can be accessed at **pppmag.com/white_papers**.

followed and products must be inspected to ensure the media is intact and meets the description noted in the COA. Do not assume that all TSA/TSB meet USP standards.

Prior to conducting sampling, staff must be trained on how to properly handle, label, document, and incubate the media devices. Competencies for hand hygiene, garbing, and aseptic technique should be a minimal requirement for staff engaging in an environmental monitoring competency assessment in order to ensure the collection of meaningful data while minimizing the risk for false positives and false negatives. Sampling conditions (personnel collecting samples, temperature, humidity, etc) of the environment must be documented at the time of sampling.

Surface Sampling Equipment

Contact plates, RODAC plates, and paddles are used for flat surfaces (eg, within PECs), work surfaces, and walls, while sterile swabs with TSB can be used for irregular surfaces such as crevices, keyboard keys, gas ports in PECs, equipment handles and switches.

Viable Air Sampling Equipment

Personnel and materials represent the primary contaminating sources and continually shed into the air and on surfaces while moving within the compounding spaces and transferring materials through the spaces. For viable air sampling, USP <1116> outlines the various types of equipment that can be used. Approved devices include a sieve impactor, slit-to-agar sampler, centrifugal sampler, sterilizable microbiological atrium, surface air system sampler, gelatin filter sampler, and traditional settling plates.

USP <797> clearly states that the preferred method of volumetric air sampling is impaction,[2] an active monitoring process that physically draws a specified volume of air into a sampling head by a pump/fan and the air is accelerated through a perforated cover (ie, a sieve sample) or a slit on the cover (ie, a slit sampler).

Sieve impaction samplers create laminar airflow over the media surface. Once the air enters the chamber and meets the media, the air tangentially changes direction, and any suspended viable particles are moved out by inertia, thus impacting onto the media. Conversely, a slit sampler has a slit located above a turning agar plate or spinning agar strip with the ultimate function of depositing viable particles over the media. Once the programed volume of air is moved across the media, the unit will cease airflow over the media. USP <797> states that a sufficient volume of air is required for testing of each location at 400 to 1000 liters; the revised USP <797> states the required volume of air is 1000 liters.[2]

Personnel need to be properly trained on how to use the equipment to ensure no false positive or negatives result. In addition, devices must be serviced and calibrated according to manufacturer guidance. For sites embarking on in-sourcing any microbiological processes, collaboration with infection control and microbiology departments is beneficial.

TABLE 1

USP's Minimum Criteria

Element	2008 Frequency	Proposed Frequency
Site Requirements: Sterile Compounding Areas		
Temperature	Document daily	Document daily
Relative humidity	Not required	Document daily
Pressure differentials (continuous monitoring) -Cleanroom suite -Segregated compounding area -Containment segregated compounding area	 Document daily Not required **	 Document daily Not required Document daily
Certification Requirements: Classified Areas*		
Airflow test	6 months	6 months
HEPA filter integrity test (ie, PEC and ceiling HEPAs)	6 months	6 months
Dynamic smoke pattern test	6 months	6 months
Total airborne particle sampling	6 months	6 months
Microbiological Monitoring: Classified Area		
Volumetric active viable air sampling in each classified area	6 months	6 months
Viable surface sampling	6 months	Monthly
Microbiological Monitoring: Personnel Processes		
Personnel gloved finger and thumb testing *(Assessment of hand hygiene and garbing)*	Initially and annually	Initially and 6 months
Personnel media fill assessment *(Assessment of aseptic technique)*	Initially and annually for low- and medium-risk Initially and 6 months for high-risk	Initially and 6 months for categories 1 and 2

**Recertification of classified areas must take place under the following circumstances: redesign and construction of the compounding area; replacement or movement of a primary engineering control; change or addition of equipment in the compounding area that may alter air movement; major facility service changes that may impact compounding area.*

***Containment Segregated Compounding Areas (C-SCAs) were introduced in the final version of USP <800> (2016).*

Interpretation of results from growth media devices should be managed by the microbiology department, noting that further speciation of colony forming units (CFUs) may need to be employed.

Sampling Locations

Microbial sampling should occur when personnel and materials are in the areas while processing activities are ongoing, with the full complement of personnel working in the environment. Caution must be taken to ensure the sampling process does not contaminate or impede defined operations. Sites that have historically used an outsourcing certifier to conduct air and surface sampling can review the certifier's sampling maps to establish their sampling locations (see **FIGURE 1**). Creating a sampling map for air and surface sampling provides a visual guide to staff and ensures consistency in result trending.

For locations without a preexisting map, sampling areas should include those in close proximity of the products, containers, and product contact surfaces. Sites should be selected based on human activity during product movement and compounding. Consider high-touch areas within the workspace such as staging carts/work surface, poor airflow areas, and areas where dust accumulates.

Monitoring Within the PEC

The highest risk point for compounding sterile preparations is the direct compounding area located within the PEC. USP <797> has strict action level limitations for allowable CFUs in and around the PEC (see **TABLE 1**).[2] Regardless of the compounding room type (eg, cleanroom suite, SCA, C-SCA), air and surface sampling must take place within the PEC workspace at least every 6 months or sooner if the PEC is moved or undergoes repairs that may alter the airflow pattern. For sites with zoned PECs for the entry of materials (ie, clean zone), compounding workspace (ie, working zone), and waste and exit of final products (ie, dirty zone), surface sampling can take place within each zone to provide information regarding the impact on surfaces as personnel move products within the PEC (see **FIGURE 2**). If the PEC is not zoned, select surfaces that could pose a risk to product integrity.

TABLE 2

Specific Microorganism Strains Suitable for Use in Growth Promotion[5]

Aerobic Bacteria	
Staphylococcus aureus	ATCC 6538, CIP 4.83, NTC 10788, NCIMB 9518, NBRC 13276
Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134
*Pseudomonas aeruginosa**	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275
Anaerobic Bacterium	
*Clostridium sporogenes***	ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437, NBRC 14293
Fungi	
Candida albicans	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594
Aspergillus niger	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

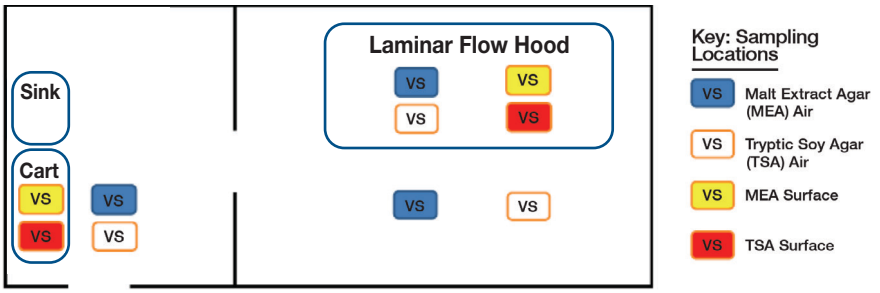
**Alternative Kocuria rhizophila (Micrococcus luteus), ATCC 9341*

***Alternative (if non-sporing indicated) Bacteroides vulgatus, ATCC 8482.*

FIGURE 1

Sampling Map

The map summarizes where sampling occurs, the type of sample, and the media used for viable sampling (VS).



Incubation Requirements

Once samples have been collected and labeled, they must be incubated at the proper temperatures. Sites choosing to purchase and manage an incubator must not place the incubator in a buffer room and must ensure the device is routinely cleaned and calibrated to the manufacturer's specifications. The proposed version of USP includes modified incubation temperatures and times from the 2008 version. The 2008 version of USP <797> requires that samples be incubated at 30˚ to 35˚C for 48 to 72 hours for TSA and at 26˚ to 30˚C for 5 to 7 days for malt extract agar. Conversely, the proposed version states that TSA samples are to be incubated at 30˚ to 35˚C for no less than 48 hours followed by 20˚ to 25˚C for no less than 5 days, which is adequate for both bacterial and fungi growth.

Post-Sampling Cleaning

As facilities embark on insourcing microbiological sampling, it is extremely important to include a documented cleaning process. Surfaces that have come into direct contact with growth media must be cleaned with the approved cleaning agent followed by a disinfectant; this removes any residual growth media which could promulgate growth of microorganisms. Sites that outsource surface sampling must have an understanding of the post-sampling cleaning procedures used by the outsourcing

FIGURE 2
A Zoned PEC
With a zoned PEC, surface sampling data can be used to evaluate the impact of personnel and product workflow.

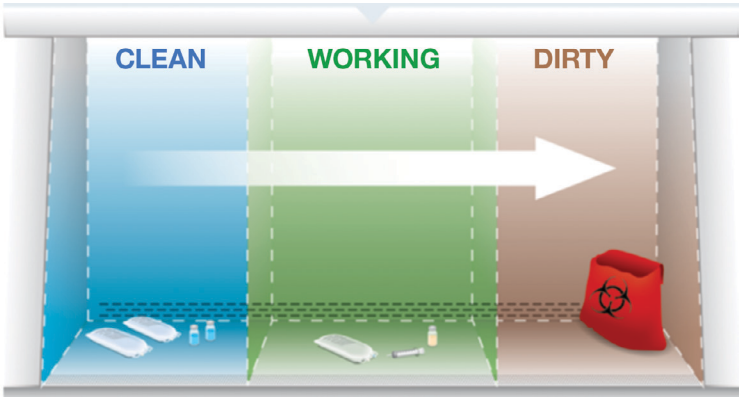


Photo courtesy of NuAire

vendor to ensure it meets the expectations defined by the facility's SOPs. Do not assume outsourced certifying companies are trained in cleaning processes or the use of site-specific cleaning and disinfecting solutions.

Documentation Requirements

USP <797> clearly states that all microbiological air and surface processes, results, and any subsequent investigative processes with corrective actions must be documented in order to demonstrate compliance.[2] Sites should develop paper or electronic sampling forms that ensure consistency of data that can then be trended to provide information if an action level is exceeded. Sampling forms should include the name of each individual involved, sampling date, sample type (ie, air or surface), sample location, sampling devices, expiration date/lot number of sampling devices, calibration date of equipment (if used), time period of sampling, number of personnel in sampling area, and temperature/humidity of sampling area. Staff must not erase, white-out, or scribble out any unwanted or incorrect information. All data must be stored to allow for quick access while preventing deterioration and/or loss. The documentation must comply with state and federal laws and be stored for at least 3 years or longer if directed by oversight agencies.

Trending Data

As the amount of data collected accumulates over time, a single data point must be summarized and evaluated to determine whether the production environment is in a state of control. Isolated recoveries of microorganisms should be considered a "normal phenomenon" in conventional cleanrooms, since they are not sterile environments. Isolated incidents generally do not require specific corrective action, unless they exceed the action levels for CFUs defined by USP.

When a CFU count exceeds its corresponding action level, sites must immediately undertake a formal investigation following the steps outlined in USP <797>. A formal SOP must be in place to document the investigation and summarize corrective and preventative actions. Investigations should review personnel processes, cleaning procedures, and documentation for any gaps as well as historical certification reports to supplement findings. During the investigative and resampling period, changes to assigned beyond-use dates may need to be implemented and/or the PEC may need to be taken out of service for repair. If the source of the problem cannot be identified and eliminated, the site may need to engage its infection control department and certifying company to assist with the investigation. USP notably states, "The source(s) of the contamination must be corrected, and....the investigation and resulting corrective actions must be documented."[2]

Collecting and trending data will help in the investigation of an excursion. If no historical patterns are noted, an excursion point could be considered a one-time incident; conversely, if historical data shows a trend toward the current excursion point, it indicates that the issue has been brewing over time.

Conclusion

The environmental monitoring program is a key performance tool in pharmacy's efforts to demonstrate a state of control within the compounding environment. The data that results from such a program is intrinsic to ensuring the effectiveness of personnel, processes, and environmental controls. In the future, the advent of rapid testing methods (which are noted in USP <797>) may facilitate immediate environmental sampling results, providing compounding personnel with critical decision-making information prior to dispensing products to patients.

References

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