

	Description	Examples	QA Monitoring
Low	<ul style="list-style-type: none"> Involves only transfer, measuring and mixing with closed or sealed packaging systems. Limited to aseptically opening ampules, penetrating sterile stoppers on vials with sterile needles or syringes, transferring sterile liquids in sterile syringes to sterile administration devices. Prepared entirely in an ISO Class 5 (see below) or better air quality environment. In the absence of passing a sterility test, storage for a maximum of 48 hours at room temperature, 14 days refrigerated, or 45 days in solid frozen state. 	<ul style="list-style-type: none"> Single transfers of sterile dosage forms from ampules, bottles, bags, and vials using sterile syringes with sterile needles. <i>(Vancomycin 1gm in NS 100ml prepared for 1 patient.)</i> Manually measuring and mixing no more than 3 manufactured products to compound drug admixtures and nutritional solutions. <i>(TPN solution compounded using gravity transfer of commercially available sterile Amino Acid and Dextrose solutions, and no more than 3 sterile additives transferred using a syringe and needles.)</i> 	<p>Media-Fill Challenge Test <i>Low-risk Media-fill Challenge</i> <u>Hardy Cat. no.:</u> HVL1 <u>Frequency:</u> Annual testing for each person who compounds low-risk sterile preparations.</p> <p>Environmental monitoring: <i>Air Monitoring:</i> <u>Hardy Cat. no.:</u> G60 <u>Frequency:</u> At least semi-annual testing of each Laminar Air Flow Workbench or barrier isolator.</p> <p><i>Surface and Glove Fingertips:</i> <u>Hardy Cat. no.:</u> P34 <u>Frequency:</u> Surface monitoring required on a periodic basis of each Laminar Air Flow Workbench or barrier isolator. Glove fingertips shall be done at initial competency evaluation and no less than three times before being allowed to compound sterile preparations. Re-evaluation is required at each media fill challenge test.</p>
Medium	<ul style="list-style-type: none"> Multiple individual or small doses are combined or pooled to prepare a CSP for administration to multiple patients or to one patient on multiple occasions. Involves complex aseptic manipulations or requires a long duration to prepare. Does not contain broad-spectrum bacteriostatic substances, and is administered over several days (e.g. worn or implanted infusion device). Prepared entirely in an ISO Class 5 (see below) or better air quality environment. In the absence of passing a sterility test, storage for a maximum of 30 hours at room temperature, 9 days refrigerated, or 45 days at solid frozen state. 	<ul style="list-style-type: none"> TPN fluids using manually or automated compounded, involving multiple injections, detachments, and attachments of nutrient source products to deliver components to a final sterile container. Filling reservoirs of injection and infusion devices with multiple sterile drug products and evacuation of air from those reservoirs before dispensing. <i>(Chemotherapy prepared for infusion over 5 days using a portable infusion device.)</i> Filling reservoirs of injection and infusion devices with sterile drug solutions that will be administered over several days at ambient temperatures between 25° and 40°C. <i>(Implanted pump reservoir filled with Preservative-Free Morphine for infusion over 4 weeks.)</i> Transfer from multiple ampules or vials into a single, final sterile container or product. <i>(Any IV solution compounded with more than 3 additives.)</i> 	<p>Media-Fill Challenge Test <i>Medium-risk Media-fill Challenge</i> <u>Hardy Cat. no.:</u> HVMI <u>Frequency:</u> Annual testing for each person who compounds medium-risk sterile preparations.</p> <p>Environmental Monitoring: <i>Air Monitoring:</i> <u>Hardy Cat. no.:</u> G60 <u>Frequency:</u> At least semi-annual testing of each Laminar Air Flow Workbench or barrier isolator.</p> <p><i>Surface and Glove Fingertips:</i> <u>Hardy Cat. no.:</u> P34 <u>Frequency:</u> Surface monitoring required on a periodic basis of each Laminar Air Flow Workbench or barrier isolator. Glove fingertips shall be done at initial competency evaluation and no less than three times before being allowed to compound sterile preparations. Re-evaluation is required at each media fill challenge test.</p>
High	<ul style="list-style-type: none"> Non-sterile ingredients are incorporated or a nonsterile device is employed before terminal sterilization. Non-sterile ingredients are incorporated, or a non-sterile device is employed before terminal sterilization. Non-sterile components are exposed for at least 6 hours before being sterilized. Exposed to air quality inferior to ISO Class 5 (see below). In the absence of passing a sterility test, storage for a maximum of 24 hours at room temperature, 3 days refrigerated, or 45 days in solid frozen state. 	<ul style="list-style-type: none"> Dissolving non-sterile bulk drug and nutrient powders to make solutions which will be terminally sterilized. <i>(TPN solutions made from dry amino acids.)</i> Measuring and mixing sterile ingredients in non-sterile devices before sterilization is performed. <i>(Ophthalmic solution filtered into a non-sterile dropper bottle.)</i> 	<p>Media-Fill Challenge Test <i>High-risk Media-fill Challenge</i> <u>Hardy Cat. no.:</u> HVH1 <u>Frequency:</u> Semi-annual for each person who compounds high-risk sterile preparations.</p> <p>Environmental: <i>Air Monitoring:</i> <u>Hardy Cat. no.:</u> G60 and W28 <u>Frequency:</u> At least semi-annual testing of each Laminar Air Flow Workbench or barrier isolator.</p> <p><i>Surface and Glove Fingertips:</i> <u>Hardy Cat. no.:</u> P34 <u>Frequency:</u> Surface monitoring required on a periodic basis of each Laminar Air Flow Workbench or barrier isolator. Glove fingertips shall be done at initial competency evaluation and no less than three times before being allowed to compound sterile preparations. Re-evaluation is required at each media fill challenge test.</p>

Source of risk level information: www.uspnf.com

The contact agar method was one of several methods developed in the 1930's to monitor surfaces for contamination. A petri dish with a diameter of 60mm is slightly overfilled with agar medium. Surface contact is made possible by raising the meniscus above the rim.

Revisions to USP Chapter <797> Pharmaceutical Compounding Sterile Preparations, Environmental Monitoring section, requires surface sampling of ISO Class 5 Sources and sampling of glove finger tips of compounding personnel. Contaminated or inadequately disinfected surfaces and/or glove fingertips can also cause microbial contamination of sterile preparations. Assessment and verification of the adequacy and quality of the environment in which sterile preparations are compounded is essential. Trained individuals must perform evaluation of surface contamination in ISO Class 5 controlled environments such as Laminar Air Flow Workbenches (LAFW) and barrier isolators. Samples may be collected using sterile swabs and performing plate counts or by using contact plates. The area chosen for sampling should be done at sites most prone to contamination during compounding activities. It is suggested, that these areas include one bottom, one side and one upper surface location of each Laminar Air Flow Workbench and barrier isolator. These evaluations are performed at regular intervals. Frequent sampling provides an early means to detect loss of environmental control. Perform sampling when no other activities are occurring in critical areas.

Personnel monitoring of glove tips is required because it is the most likely source of contamination to CSPs. Glove fingertip sampling shall be used to evaluate the competency of personnel in performing hand hygiene, garbing procedures and proper work practices, which includes frequent and repeated glove disinfection during actual compounding of CSPs. Sample glove fingertips after compounding CSPs immediately after leaving the ISO Class 5 environment (sampling must be done outside the ISO Class 5 area). Do not disinfect gloves prior to sampling glove fingertips.

All compounding personnel shall successfully complete an initial competency evaluation and glove fingertip/thumb sampling procedure (zero cfu) no less than three times before being allowed to compound CSPs for human use. Re-evaluation of this competency is required for all personnel during media fill challenge testing.

The number of discrete colonies present on the surface of the media is counted and reported as Colony Forming Units (CFU). This provides a measurement of the level of microbial contamination of the surfaces used within the tested environment. If the number of CFUs exceeds recommended action levels, operational procedures, cleaning procedures and solutions and air filtration efficiency within the compounding area must be examined.

The formulation of Tryptic Soy Agar (TSA) with Lecithin and Tween[®] 80 is prepared according to the United States Pharmacopeia (USP) standards formula for Soybean-Casein Digest Agar Medium.⁽¹³⁾ It is also included in approved procedures in the *Compendium of Methods for the Microbiological Examination of Foods* and *Standard Methods for the Examination of Water and Wastewater*.^(4,8)

Hardy Diagnostics Tryptic Soy Agar (TSA) with Lecithin and Tween[®] 80 contains digests of soybean meal and casein that provide amino acids and other nitrogenous compounds making it a nutritious medium for many microorganisms. Sodium chloride is added to maintain the osmotic equilibrium. Lecithin and Tween[®] 80 are added to the formulation to neutralize germicidal or disinfectant residues. Neutralization of these residues reduces their inhibitory effect which would ultimately result in the lowering of the microbial count. Quaternary ammonia compounds are neutralized by lecithin while phenolic disinfectants and hexachlorophene are neutralized by Tween[®] 80. Together, lecithin and Tween[®] 80 neutralize ethanol.^(4,8)

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Peptic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Tween [®] 80	5.0gm
Lecithin	0.7gm
Agar	15.0gm

Final pH 7.3 +/- 0.2 at 25 degrees C.

*Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Upon receipt store at 2-8 degrees C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Products are light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date applies to each product in its intact packaging when stored as directed.

90 Days: P34 TSA with Lecithin and Tween[®] 80

Refer to the keyword "Storage", in the Hardy Diagnostics software program HUGO™, for more information on storing culture media.

PRECAUTIONS

For laboratory use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified personnel. Sterilize all biohazard waste before disposal.

Refer to the keyword "Precautions", in the Hardy Diagnostics software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE

Allow medium to warm to room temperature prior to use. Consult listed references for information concerning testing procedures.^(2,4-8) Surface monitoring results will be most accurate if samples are collected after cleaning and disinfection procedures are performed. This will most closely simulate the environmental conditions present prior to preparing a sterile compound.

1. Use a separate contact plate for each sample site selected.
2. Hold the plate with thumb and second finger and use index finger to gently press plate bottom firmly against the selected test surface. The same amount of pressure should be applied for every sample. Do not move plate laterally. Lateral movement spreads contaminants over the agar surface, thus making resolution of colonies difficult. A rolling motion may be used for slightly curved surfaces i.e. sampling glove fingertips.⁽⁴⁾
3. Sample areas of the Laminar Air Flow Workbench (LAFW) or isolator, at least 6 inches inside the work area using a separate contact plate for one bottom, one side, and one upper surface location.
4. Incubate the plates aerobically at 30 to 35 degrees C. for 48 to 72 hours.

5. Using adequate light and magnification, count the number of colonies within the squares of the grid area. Take care not to count a square more than once. Using a Bactronic or a stereoscopic (dissecting) microscope colony counter, count colonies and record as the number of colonies per contact plate or number of colonies per square centimeter.⁽⁸⁾
6. Sampled surfaces should be cleaned immediately after sampling to remove residual agar.

INTERPRETATION OF RESULTS

Similar appearing colonies growing in close proximity, but not touching, should be counted as individual colonies. Colonies with different morphology or color should be counted as individual colonies. When spreading colonies are present, a representative portion of colonies in a spread-free area should be counted. This is only done if the area covered by the spreaders does not exceed one-half of the plate area.

Table 4. Recommended Action Levels (Counts) of Microbial Colony-Forming Units (cfu) per Cubic Meter of Air or Contact Plate

ISO Class of Sampled Location	Sampled Sources and Their Action Levels (Counts) of Microbial cfu ^a		
	Active Air ^b	Glove Fingertip	Surface Sample (contact plate) (cfu per plate)
5	>1	>3*	>3
7	>10	Not applicable	>5
8	>100	Not applicable	>100

^aThe cfu action levels are obtained from USP <797> Guidebook to Pharmaceutical Compounding – Sterile Preparations.

^b At least one cubic meter, m³, or 1000 liters, L, of air must be sampled.

*0 cfu allowed at initial competency evaluations.

Results for fingertip sampling shall be reported separately as the number of cfu per employee per hand (left hand, right hand). The cfu action level for gloved hands is based on the total number of cfu on both gloves, not per hand. When gloved fingertip sample results exceed action limits, a review of hand hygiene and garbing procedures as well as glove and surface disinfection procedures and work practices must be performed.

Because of the inherent variability of environmental sampling methods, it is more useful to trend contamination recovery results rather than to focus on the number of colonies recovered from a particular sample. Action should be required when the contamination recovery rate trends above the recommended action levels for a significant time.

If action levels have been identified, a thorough investigation into the adequacy of personnel work practices, operational procedures, cleaning procedures and solutions and air filtration efficiency within the compounding area must be made. Once changes have been made, monitoring procedures must be repeated to determine if the changes made were effective. Documentation of all monitoring results, remedial action and follow-up monitoring must be maintained. The Results Log Sheet, included in this document, may be used for this purpose.

Consult listed references for more detailed information concerning plate count methods.⁽⁸⁾

LIMITATIONS

Accurate counting can be made difficult by molds or spreading colonies.

Sampling challenges may occur as a result of irregular, porous, rough or textured media surface.

Contact Plate Media is not recommended for sampling crevices or irregular surfaces.

Rare fastidious organisms may not grow on Tryptic Soy Agar with Lecithin and Tween[®].

Refer to the keyword "Limitations", in the Hardy Diagnostics software program HUGO™, for more information regarding general limitations on culture media.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as counting devices, thermometers, incinerators, incubators, etc., as well as serological and biochemical reagents are not provided.

QUALITY CONTROL

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> ATCC [®] 8739	J	3-5 days	30-35°C	Aerobic	Growth
<i>Bacillus subtilis</i> ATCC [®] 6633	J	3-5 days	30-35°C	Aerobic	Growth
<i>Candida albicans</i> ATCC [®] 10231	J	3-5 days	30-35°C	Aerobic	Growth
<i>Staphylococcus aureus</i> ATCC [®] 6538	J	3-5 days	30-35°C	Aerobic	Growth
<i>Aspergillus brasiliensis</i> formerly <i>A. niger</i> ATCC [®] 16404	J	5-7 days	25-35°C	Aerobic	Growth
<i>Pseudomonas aeruginosa</i> ATCC [®] 9027	J	3-5 days	30-35°C	Aerobic	Growth
<i>Salmonella enterica</i> ATCC [®] 14028	J	3-5 days	30-35°C	Aerobic	Growth

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable). Refer to the following keywords, in the Hardy Diagnostics software program HUGO™, for more information on QC: "Introduction to QC", "QC of Finished Product", and "The CLSI (NCCLS) Standard and Recommendations for User QC of Media". Also see listed references for more information.⁽¹⁻⁷⁾

* Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics software program HUGO™, for a description of inoculation procedures.

PHYSICAL APPEARANCE

TSA with Lecithin and Tween® 80 should appear clear to slightly hazy, and light amber in color.

Media is contained in a 15x60mm Contact Plate. Plates are slightly overfilled with medium so that the meniscus is slightly above the rim of the plate. Each contact plate has a specified grid molded into the bottom of the plate.

REFERENCES

1. Anderson, N.L., et al. 2005. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.2.
2. Murray, P.R., et al. 2007. *Manual of Clinical Microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., et al. 2007. *Bailey and Scott's Diagnostic Microbiology*, 12th ed. C.V. Mosby Company, St. Louis, MO.
4. American Public Health Association. 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. APHA, Washington, D.C.
5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
6. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media, M22*. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
8. Vanderzant, C. and D.F. Splittstoesser, (ed.). 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. APHA, Washington, D.C.
9. USP General Chapter <797> Pharmaceutical Compounding - Sterile Preparations. United States Pharmacopeial Convention Inc., Rockville, MD.
10. USP General Chapter <797> Pharmaceutical Compounding - Sterile Preparations. 1st Supplement. United States Pharmacopeial Convention Inc., Rockville, MD.
11. www.uspnf.com.
12. Newton, D.W. Proposed Revisions to USP Chapter <797> Pharmaceutical Compounding Sterile Preparations, *Drug Standards*, www.ashp.org.
13. The Official Compendia of Standards. 2008. *USP27-NF22*. United States Pharmacopeial Convention, Rockville, MD.
14. USP General Chapter <797> Pharmaceutical Compounding, Sterile Preparations Revision Bulletin, 2007. p. 1-61. United States Pharmacopeial Convention Inc., Rockville, MD. www.usp.org/pdf/EN/USPNF/GeneralChapter797.pdf

ATCC is a registered trademark of the American Type Culture Collection.

Tween is a registered trademark of ICI Americas, Inc.

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