How clean are your testing supplies?

The first aseptic products built to minimize risk—starting with the packaging itself.

Smarter, Cleaner, Convenient...Cultivate™
USP General Chapter <797>
Pharmaceutical Compounding - Sterile Preparations

The purpose of this guide is to show how Cultivate™ products support a pharmacist’s goal of complying with United States Pharmacopeia Chapter <797>. In this case, USP’s focus is on specific quality assurance activities involving compounded sterile preparations (CSPs). According to USP: “The intent of this chapter is to prevent harm and fatality to patients that could result from microbial contamination (nonsterility), excessive bacterial endotoxins, large content errors in the strength of correct ingredients, and incorrect ingredients in CSPs.”

Cultivate™ products target the prevention/detection of microbial contamination and the detection of excessive bacterial endotoxins. Areas addressed are personnel training, evaluation of aseptic manipulations, air and surface quality testing of the compounding environment, sterility testing, and detection of excessive bacterial endotoxins (pyrogen).

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USP <797> puts pharmacy prepared CSPs into 3 categories based on the difficulty of maintaining sterility and potential for patient harm: Low-risk, medium-risk, and high-risk. Refer to the definitions in <797> to determine which category describes a particular compounding operation. The frequency and complexity of Quality Assurance procedures are driven by the highest risk level. USP <797> describes the minimum QA procedures that are required. Many pharmacies routinely exceed the minimums to increase patient safety of their CSPs.
Cultivate™ Products
Frequently Asked Questions

Contact™ Test Kits

1. How long should Contact™ agar paddles be exposed to air in a laminar flow hood, barrier isolator, clean, and buffer room areas?

We suggest an exposure time of 1 hour in hoods and other areas. Exposing any Contact™ products longer than 1 hour in the air flow has the potential of drying the media. It is most important to be consistent in the exposure duration.

2. Moisture and water droplets occasionally appear on the inside of Contact™ housing. Does this have any effect on performance or shelf life?

Moisture on the inside of the housing does not harm Contact™ products. This moisture comes from the agar. Agar is mostly water and the atmosphere inside the housing stabilizes the 100% humidity.

3. How should a pharmacist purposely contaminate a Contact™ product if they want to demonstrate microbial growth?

Minimizing touch contamination is a primary goal of aseptic technique testing. Pressing finger tips on the Contact™ agar is an excellent way to inoculate the media. It also demonstrates how easily contamination can occur during a lapse in aseptic technique.

4. Can the Contact™ media paddle be used for testing sterile gloved finger tips for microbial contamination?

The Contact™ media paddle is an excellent choice for finger tip testing. The unique sealed cap with agar on paddles helps eliminate false positives often found with Petri dish media.

Clear Check™

1. Why are several repetitions of media transfers necessary to pass a validation or “competency test” of aseptic technique?

Multiple repetitions are necessary to stimulate the most complex aseptic manipulations encountered during normal workloads. Many repetitions induce the boredom that can lead to lapses in good aseptic technique.

2. Why is Clear Check™ media offered in a variety of vials and bags?

Media transfer validations should stimulate the actual manipulations typically encountered in a particular pharmacy. Examples include syringe transfers from vials to minibags, multiple additive procedures, syringe filling, and use of automated compounders. Multiple repetitive transfers from Clear Check™ vials to Clear Check™ minibags is an excellent example of a simulation of an actual procedure.

3. How important is it to exactly follow the Directions for Use that come with each box of PASS™ and PASS2™ kits?

The DFUs are written as suggestions only. They can be modified to more closely mimic the most difficult aseptic manipulations performed by a particular operator or pharmacy.
Transfer Test™, TTJunior™, and TTMicro™ Systems

1. If the IV admixture has already been infused into the patient by the time the QA Pharmacist knows the results from the contamination test, why bother?

Regular use of the Cultivate™ end-product testers is intended to confirm that aseptic technique is being maintained. Since it is impractical to test, quarantine, and then release every admixture, the supervising pharmacist must watch for trends in the contamination testing program. If several tests exhibit turbidity over a short period of time, it would trigger an investigation. A rare, random positive would probably be tolerated. The supervisor would check to see if the multiple positives were prepared by the same pharmacist or technician. The point is to watch for trends and detect problems in validated procedures.

2. How soon after mixing a sterile drug product should sterility testing begin?

An important study determined that sterility testing should begin within 40 to 60 minutes after preparation of intravenous admixtures. Testing that began after 60 minutes decreased the recovery of Staphylococcus epidermidis and lead to increased false negatives. Contact Cultivate™ Technical Service to receive a free reprint of this research article.

3. Why should the Transfer Test™ be used with a compounder, pump, vacuum bottle, or vacuum bell when testing 3in1 TPN?

Commercial fat emulsions contain a substantial number of particles that are larger than 1 micron. These large particles can eventually occlude the Transfer Test™ filter. Compounders and vacuum systems maintain a pressure difference across the filter. This pressure overcomes the resistance caused by the large fat particles.

4. Doesn’t the residual fat emulsion in the Transfer Test™ cloud the TSB media and make seeing microbial-caused turbidity difficult?

Residual fat emulsion in the filter chamber would be a problem if we didn’t have an effective procedure for removing it. The bag of Clear Check™ media that comes with each Transfer Test™ contains approximately 100mL of sterile TSB. After filtering the admixture, an operator can perform a rinse of the filter chamber using the sterile media. This eliminates most of the residual fat emulsion. The last 20mL of TSB is left in the filter chamber to grow potential microbial contaminates.

5. How frequently should a pharmacist test IV admixtures?

Refer to USP Chapters <71> and <797> for the appropriate sampling tables.

6. Can the Transfer Test™, TTJunior™, or TTMicro™ be used to detect microbial contamination in antibiotics?

Yes. The biggest concern is reducing the concentration of residual antibiotic agents in the tester’s fluid path to a level where it does not interfere with culturing possible microbiological contaminates. USP suggests rinsing the filter sufficiently to remove these trace amounts of antibiotic agents.

Transfer Test™s and TTJunior™s unique filter design helps the rinsing process. The filter membrane is held in the plastic support by insert molding. During manufacturing, molten plastic fills the microscopic pores around the filter’s edges. Filling these pores prevents residual traces of antibiotics from being trapped in the filter’s edge during rinsing.

7. Which sterility test products meet the USP’s definition of “Membrane Filtration”, described as the method of choice in chapter <797>?

The Transfer Test™, TTJunior™, or TTMicro™ are “Membrane Filtration” sterility test devices. Examples of the second choice for sterility testing, “Direct Inoculation of the Culture Medium”, are the Double Check™ and Clear Check™ products.
Double Check™

1. **What is the purpose of the Double Check™?**

Double Check™ (#TL100) detects microbial contamination in sterile admixtures that contain suspensions or emulsions. Suspensions and emulsions are naturally turbid. That prevents visualizing microbial growth in the liquid media. Double Check™ reduces this problem by having sterile agar paddles inside the bottle of media, separate from the liquid media, on which growth can be observed.

Clear Check™ 10mL Tubes

1. **What is the purpose of the 10mL tubes of TSB (#TT010) if the preferred method of testing for sterility in compounded medications is “full titration”?**

Some drugs are dispensed in containers where it is difficult to transfer the contents through a filter test device. Respiratory drugs and eye drops are good examples. These drugs can be carefully transferred into the 10mL tubes by removing and replacing the screw cap. Some viscous drugs can not be filtered using a 0.22 micron membrane. They can be injected directly into the TSB media via the needle access port on the top of the screw cap.

Incubation of Samples

1. **Which Cultivate™ products require incubation at other than room temperature, 20 to 25°C?**

Clear Check™ liquid media may yield faster results at elevated temperatures but incubation is not needed for consistent, reliable results. Refer to USP Chapter <797> for more details. Contact™ products should be incubated between 30 to 35°C.

2. **How long do samples have to remain in the incubator in order to yield reliable results?**

According to USP <797> the TouchScience should be incubated at 30 to 35°C and observed for 48 to 72 hours.

USP recommends incubating sterility tests using Soybean-Casein Digest Medium (TSB), like the Double Check™, Transfer Test™, TTV Junior™, TTMicro™, and all Clear Check™ units at 22.5°C +/- 2.5°C. They should be examined at 48 hours and daily thereafter.

Positives should be removed immediately. Units not showing growth should remain in the incubator for not less than 14 days.

Testing 70% Isopropyl Alcohol (IPA)

1. **Which Cultivate™ products are appropriate for periodically testing sanitizing agents like IPA for microbial contamination?**

Introduce an aliquot of the sanitizing agent directly into TSB media like the Clear Check™ #TT010 tube or the #TV020 vial. Observe for growth for not less than 14 days.
Contact™
Microbial Contamination Monitoring System for Surfaces Testing and Gloved Fingertip Testing
Contact™

Environmental Monitoring System

Product Information and Kit Options:

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<tr>
<th>Catalog No.</th>
<th>Description</th>
<th>Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC201</td>
<td><em><strong>x</strong></em> TSA (trypticase soy agar) growth media contact plate with easy hold finger grips</td>
<td>10 Contact Plate Testers</td>
</tr>
<tr>
<td>TC202</td>
<td><em><strong>x</strong></em> TSA (trypticase soy agar) growth media contact plate with easy hold finger grips</td>
<td>10 Contact Plate Testers</td>
</tr>
<tr>
<td>TD100</td>
<td>Double sided TSA (trypticase soy agar) growth media paddles for surface testing and gloved fingertip testing. Targets bacterial contamination. Per USP Chapter &lt;797&gt; media contains lecithin and polysorbate 80 formulated to inactivate many antimicrobial compounds.</td>
<td>10 Paddle Testers, 10 gummed labels, directions for use, and result log</td>
</tr>
</tbody>
</table>

Refer to USP General Chapter <797> for recommended incubation times and temperatures.
Glove Fingertip Sampling
Press fingertips and thumbs of both hands with enough pressure to create a light impression in the agar. Note: do not disinfect gloves directly before sampling.

Figure 1

Contact Inoculation
Press each side of the paddle firmly against the solid surface to be tested. Complete contact with the surface is accomplished with the hinged design.

Figure 2

Swabbing
A sterile swab moistened with sterile water can be used to sample hard to reach surfaces. Rotate the swab while moving it over the sample area to pick up as much sample as possible. Then, firmly roll the swab over both agar surfaces.

Figure 3

Impaction Air Testing
Using the cap as a pedestal, position one side of the paddle to be perpendicular to the air flow. Use the same exposure duration every time the test is performed. Exceeding 2 hours may dry out the agar.

Figure 4

Incubation
After the paddle has been inoculated or exposed, carefully put it back into the vial and firmly secure the cap. Incubate at (USP<797>) 30°-35°C for 48 to 72 hours.

Interpretation of Results
Carefully pull the paddle out of the vial and visually examine under good lighting. Count the number of discrete colonies, if any, and record as colony forming units (CFUs). In order to make quantitative conclusions, the same sampling technique under similar conditions must be used for subsequent tests. The agar media promotes the growth of most aerobic fungi, yeast, and bacteria.

Storage, Stability and Destruction
Contact™ kits should be kept unopened at room temperature and protected from light. Do not allow the paddles to freeze. Any unused paddles showing microbial growth and all used paddles should be discarded in accordance with State and Federal regulations.

Solid Surface Sampling
Press each side of the paddle firmly against the solid surface to be tested. Complete contact with the surface is accomplished with the hinged design. Do NOT drag the agar across the surface.

Difficult to Reach Surface Sampling
A sterile swab moistened with sterile water can be used to sample hard to reach surfaces. Rotate the swab while moving it over the sample area to pick up as much sample as possible. Then, firmly roll the swab over both agar surfaces.

Staff Training
To demonstrate the importance of handwashing, press unwashed finger tips on one side of a Contact™ Media Paddle. Wash hands and press clean finger tips on other side of the paddle. Incubate and compare results.

TSA Media Ingredients:
Tryptose, Yeast Extract, Dextrose, Agar Lecithin - Inactivates quaternary ammonium compounds. Polysorbate 80 - Inactivates phenolics, hexachloroprene and formaldehyde. Lecithin & Polysorbate 80 - synergistic effect that inactivates ethanol.

Certificate of Analysis - Available upon request.
Note: Allow medium to warm to room temperature prior to use. For the most accurate results, collect samples 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 surface sampled.

2. Hold the plate by the easy hold finger grip. Firmly press agar 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.

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.

6. Sampled surfaces should be cleaned immediately after sampling to remove residual agar.
Important Benefits

- Key to continuous quality improvement in IV pharmacies
- Pre-packaged media avoids mixing mess and false positives
- Available in several sizes and container types
- Test manual and automated processes
- Sensitive to a wide range of contaminants
- Access ports available to needle, IV, spike or luer lock connections
- Custom configurations to fit any QA program

Clear Check™
Aseptic Technique Training & Validation Aids

Product Information and Kit Options:

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<tr>
<th>Catalog No.</th>
<th>Description</th>
<th>Kit Components</th>
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<tbody>
<tr>
<td>TBV120</td>
<td>PASS™ TSB media, 20mL vials, 100mL bags</td>
<td>5 vials, 5 bags</td>
</tr>
<tr>
<td>TBVA123</td>
<td>PASS 2™ TSB media, 3mL ampules, 20mL vials, 100mL bags</td>
<td>5 ampules, 5 vials, 5 bags</td>
</tr>
<tr>
<td>TA003</td>
<td>TSB media, 3mL glass ampule</td>
<td>20 ampules</td>
</tr>
<tr>
<td>TT010</td>
<td>TSB media, 10 mL glass tube, screw cap and needle access port</td>
<td>20 tubes</td>
</tr>
<tr>
<td>TV020</td>
<td>TSB media, 20mL vial, needle port</td>
<td>20 vials</td>
</tr>
<tr>
<td>TB100</td>
<td>TSB media, 100mL bag, needle &amp; spike access ports</td>
<td>10 bags</td>
</tr>
<tr>
<td>TV100</td>
<td>TSB media, 100mL vial, needle port</td>
<td>10 vials</td>
</tr>
<tr>
<td>TV500</td>
<td>TSB media, 500 mL bag, covered needle port &amp; spike access port</td>
<td>10 bags</td>
</tr>
<tr>
<td>TS005</td>
<td>TSB media, 5cc syringe, luer lock</td>
<td>10 syringes</td>
</tr>
<tr>
<td>EV020</td>
<td>Sterile, empty 20mL vial, needle port</td>
<td>20 vials</td>
</tr>
<tr>
<td>EV100</td>
<td>Sterile, empty 100mL vial, needle port</td>
<td>10 vials</td>
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</table>

Each case contains special gummed labels and results log.
This test requires 1 Clear Check™ partially filled minibag and 1 Clear Check™ 20 mL vial, each containing TSB. The test involves adding 20 portions of the vial to the minibag and is one of the more complicated procedures the operator will perform.

2. Using standard procedures, sanitize the work area and swab the vial and bag ports.

3. If the test is to be performed within a laminar flow hood, protect the injection ports of the containers by placing them at least 6 inches within the work area. Take caution not to interrupt the clean air flow.

4. Select a sterile 3, 5, or 6cc disposable syringe. Remove the syringe from its packaging and place it in the work space. Select 20 sterile needles (18G x 1" or smaller as appropriate).

5. Aseptically attach a needle to the syringe. Withdraw 1 mL of TSB from the vial and inject it into the minibag. Change the needle.

6. Repeat step (#5) 19 times, using 19 different needles with the same syringe and receiving minibag. After the final transfer there will be approximately 120 mL in the minibag.

The complexity of the above procedure can be increased by transferring the contents of the minibag into another empty container at the end of step (#6). The receiving container should be a frequently used size. The transfer is accomplished using gravity and a standard sterile pharmacy tubing set.

7. Immediately inspect the TSB in the final container for particulates, corings, and fibers. These particles should not be recorded as microbial growth.

8. Label the final container and incubate at a temperature of 20° - 25°C or 30° - 35°C for 14 days.

9. Examine the TSB daily for turbidity. If turbidity is observed, the test is positive. A positive test sample indicates that the operator has introduced microorganisms into the “product” and has failed the test. If the TSB is clear, the test is negative and the operator has passed the test.

10. After 14 days, complete the required data in the PASS™ log.

11. Reevaluation should take place using the procedure above. The frequency of which depends upon the risk level being simulated.
<table>
<thead>
<tr>
<th>Comments</th>
<th>Organisation Identified</th>
<th>Initials</th>
<th>Incubation Temperature</th>
<th>Incubation Media Lot</th>
<th>Process Tested Solution or Process Tested</th>
<th># Hood</th>
<th>Prepared by</th>
<th># Sample</th>
<th>Test Date</th>
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<td>NEGATIVE</td>
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<td>POSITIVE</td>
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**RESULTS**
PASS 2™
Personal Aseptic Sampling System
For Low and Medium Risk Levels
Cat. #TBVA123

1. This test requires 1 Clear Check™ ampule, 1 Clear Check™ partially filled minibag and 1 Clear Check™ 20 mL vial, each containing TSB. The test involves transferring the contents of an ampule to a vial and then adding 20 portions of the vial to the minibag. It is one of the more complicated procedures the operator will perform.

2. Using standard procedures, sanitize the work area and swab the vial and bag ports.

3. If the test is to be performed within a laminar flow hood, protect the injection ports of the containers by placing them at least 6 inches within the work area. Take caution not to interrupt the clean air flow.

4. Select a sterile 3, 5, or 6cc disposable syringe. Remove the syringe from its packaging and place it in the work space. Select 20 sterile needles (18G x 1” or smaller as appropriate).

5. Aseptically attach a needle to the syringe.

6. Draw up contents of the ampule and inject into the vial. Shake to mix indicator dye.

7. Withdraw 1 mL of TSB from the vial and inject it into the minibag. Change the needle.

8. Repeat step (#7) 19 more times, using 19 different needles with the same syringe and receiving minibag. After the final transfer there will be approximately 120 mL in the minibag.

   The complexity of the above procedure can be increased by transferring the contents of the minibag into another empty container at the end of step (#6). The receiving container should be a frequently used size. The transfer is accomplished using gravity and a standard sterile pharmacy tubing set.

9. Immediately inspect the TSB in the final container for particulates, corings, and fibers. These particles should not be recorded as microbial growth.

10. Label the final container and incubate at a temperature of 20° - 25°C or 30° - 35°C for 14 days.

11. Examine the TSB daily for turbidity. If turbidity is observed, the test is positive. A positive test sample indicates that the operator has introduced microorganisms into the “product” and has failed the test. If the TSB is clear, the test is negative and the operator has passed the test.

12. After 14 days, complete the required data in the PASS™ log.

13. Reevaluation should take place using the procedure above. The frequency of which depends upon the risk level being simulated.
<table>
<thead>
<tr>
<th>Test</th>
<th>Sample</th>
<th>Solution or Process Tested</th>
<th>Hood</th>
<th>Media</th>
<th>Lot #</th>
<th>Initials</th>
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### RESULTS

#### NEGATIVE

- Organism Identified
- Incubation Temperature
- Prepared by

#### POSITIVE

- Comments