

USP <51> Antimicrobial Effectiveness Testing

1. If a base ingredient is designed for the purpose of compounding a specific type of preparation does this forgo the necessity to perform USP <51>?

If a base ingredient is designed for the purpose of compounding a specific type of preparation, the antimicrobial test is still necessary. The purpose of the test is to ensure that the combination of the actives, excipients, and antimicrobial preservatives are appropriate to meet the specifications for microbial reduction or prevention of proliferation depending on the organism type. An individual excipient may contain antimicrobial properties that are designed to maintain microbiological quality of a product, such as bacteriostatic water for injection. However, the antimicrobial properties may be altered when the excipient is mixed with other excipients and/or the antimicrobial properties may only be effective against a certain Genus of microorganism and not effective against others that may also be potential contaminants of the product while it is in use.

2. Is there any circumstance that would exempt a compounder from performing the USP <51> test?

There is no circumstance that would prevent a compounder from performing this test on drug products that contain antimicrobials or preservatives for the purpose of maintaining the microbiological quality of a multi-dose product during patient use.

3. In the case of non-sterile compounds when is it appropriate to conduct USP <51>?

It is appropriate to conduct USP <51> testing on any sterile or nonsterile drug product that has antimicrobial excipients and/or preservatives added, or if the active itself is antimicrobial, as a means of maintaining the microbiological quality of the multi-dose product during its use.

USP <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests and USP <62> Microbiological Examination of Nonsterile Products: Tests for Specified Organisms

1. What should happen if a product fails method suitability? Meaning that all the specified organisms would not grow.

When a product fails method suitability, the testing method should be revised, and method suitability retested until a suitable method has been confirmed.

There is some allowance for a valid testing method in USP <61> if you are unable to obtain growth with all the challenge organisms, provided you have tried all modifications (Documentation showing the methods attempted is critical here) to the test method available that do not put the results outside the acceptance range (i.e. diluting a product so that the lowest possible result is over the acceptance limit). USP <61> states the following:

"If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the article is not likely to be contaminated with the given species of the microorganism...Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion."

2. Does USP 795 state that we need to perform sterility testing on non-sterile products?

No, sterility testing is not required for non-sterile drug products. Performing USP <61> and USP <62> testing is the expectation for non-sterile products.

USP <61> and <62> (continued)

3. Is there an ISO requirement for nonsterile compounding? ISO 7 or 8 are typically your buffer and ante rooms in your sterile suite?

Per USP <1115> Bioburden Control of Nonsterile Drug Substances and Products, classified environments are not required for non-sterile product manufacturing. However, environmental monitoring of microorganisms seems to be expected in sections of the same chapter.

4. If you're compounding pursuant to a prescription when is it appropriate to perform microbial testing?

For any compounded product, microbiological quality should be built into the product by having validated aseptic and compounding processes. If the number of articles compounded is low, it's advisable to perform microbial testing on a batch by periodically increasing the number of articles compounded to provide the quantity necessary to confirm the microbiological quality of the compounded products.

5. Should the chemical manufacturer/wholesaler be measuring and reporting bioburden load on the C of A?

If the product has a compendial testing requirement, or if the customer has specific requirements for bioburden, the certificate should list each test performed in accordance with compendial or customer requirements, including the acceptance limits, and the numerical results obtained (if test results are numerical). This information can be found in Guidance for Industry, Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients. In addition to this, the facility using the ingredients should, if possible, confirm the C of A results as well.

6. For nonsterile dosage forms, what type of microbes should be tested?

See "Table 1. Acceptance Criteria for Microbiological Quality of Nonsterile Dosage Forms" in USP <1111> and the drug's monograph. The table or monograph lists the specified microorganism(s) that should be tested based on the route of administration of the non-sterile dosage form. USP <1111> also states:

In addition to the microorganisms listed in Table 1, the significance of other microorganisms ... should be evaluated in terms of the following:

- The use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract).
- The nature of the product: does the product support growth? Does it have adequate antimicrobial preservation?
- The method of application.
- The intended recipient: risk may differ for neonates, infants, and the debilitated.
- Use of immunosuppressive agents, corticosteroids.
- The presence of disease, wounds, organ damage.

Therefore, USP does not purport the organisms listed for each dosage form are exhaustive, and additional organisms, even those not listed in USP <61> and USP <62>, can be screened for if they are of concern for the product type. Organisms recovered from environmental monitoring are a good place to start to determine if, and in what quantity, they are making their way into the finished product. The organisms listed in USP <61> and <62> should be considered the minimum populations and types you should be testing for.

USP <61> and <62> (continued)

7. How often must one perform 61 and 62 tests? Must we perform microbial enumeration on every compound? Is USP <61> and <62> testing mandatory per USP regulations?

Since the chapters are sub-1000 chapters, they are considered enforceable by regulatory bodies, therefore, you should have data on your products for USP <61> and <62>. According to 21 CFR 211, each lot of component (i.e. raw material) or drug product that may become contaminated during the production must first pass microbiological testing. This would suggest that each lot of material should be tested. In addition, written procedures to prevent objectionable organisms in non-sterile drug products must be in place, as well as appropriate laboratory testing for each batch. Also, in-process materials should be tested for general microbiological quality (bioburden and objectionable organisms).

8. When would you compound a non-sterile inhalation?

While nearly all inhalation forms list the sterility test as a requirement, dry powder inhalations and inhalation aerosols are listed in “USP <5> Inhalation and Nasal Drug Products-General Information and Product Quality Tests” are listed as requiring the microbial limits tests. I don’t know of a specific case, however, where a non-sterile inhalation would be compounded.

USP <71> Sterility Tests

1. Differentiate between rapid sterility testing and traditional sterility testing. Is rapid sterility testing accepted by regulatory bodies?

There are different technologies currently available that are being sold as rapid sterility testing methods. They offer faster sterility testing results, as opposed to the traditional sterility testing method that requires 14 or more days of incubation before the sterility test result can be confirmed.

Rapid sterility testing is accepted by regulatory bodies on a case by case basis. The method must be validated and show recovery of the challenge microorganisms under the conditions of the test, including in the presence of the product. The rapid sterility method validation must be shown to be equal to, or better than, the compendial 14-day test. A validated method must be demonstrated by a method suitability test for every product formulation that is tested. USP <1223> Validation of Alternative Microbiological Methods is a general information chapter that provides guidance on selecting and validating alternative microbiological methods.

2. Is random sampling of sterile products appropriate? Or should we take a sample (vial) from the start of compounding, middle of filling and at end of filling?

Where sampling is concerned, FDA guidance states “it is important that the samples represent the entire batch and processing conditions”. This is generally interpreted as taking samples from the beginning, middle, and end of the batch run, so articles created throughout production are tested.

3. What is the minimum volume considered large volume parenteral?

Per USP <71>, Injections, when the container is labeled as containing more than 100 mL, are considered a large volume parenteral. A product is considered a small volume injection, where the container is labeled as containing 100 mL or less.

USP <71> (continued)

4. How frequent do you perform sterility testing - with every batch or randomly?

In USP <797>, under the section titled "Finished Preparations Release Checks and Tests", it states the following:

"All high-risk level CSPs that are prepared in groups of more than 25 identical individual single-dose packages (e.g., ampoules, bags, syringes, vials) or in multiple-dose vials (MDVs) for administration to multiple patients or that are exposed longer than 12 hours at 2° to 8° and longer than 6 hours at warmer than 8° before they are sterilized shall meet the sterility test (see Sterility Tests <71>) before they are dispensed or administered."

This is an example of required sterility testing, and the FDA has issued 483s for pharmacies not running sterility testing on every batch. At the very least, batches that fall under the USP quote above are required to be sterility tested every time. Products not covered by the quote above should also be sterility tested, if not every time, then at predetermined intervals based on several factors. The pharmacy should have an SOP taking into account the product, storage conditions, container, environmental monitoring, in-process and raw material quality testing (USP <61> and <62>), dosage form, whether it is a multi or single use container, and the number of articles made. This list is not meant to be exhaustive for reasons to conduct a sterility test. Testing on the conservative side (i.e. more often) is never a bad idea.

USP <85> Bacterial Endotoxins Test

What instrument do you use for the endotoxin test?

For the gel-clot technique, a heat block that maintains a $37 \pm 1^\circ\text{C}$ temperature is required.

For the chromogenic or turbidimetric technique, a temperature controlled absorbance microplate reader is required.

Environmental Monitoring

1. Is there any environmental monitoring requirements for the non-sterile area?

Classified environments are not required for nonsterile product manufacturing and monitoring of unclassified environments is not required. Having said that, USP <1115> seems to make it clear that some implementation of environmental monitoring is expected in a non-sterile processing environment. The section "Microbial Assessment of Non-Sterile Product Manufacturing Environments" specifically mentions air sampling and personnel sampling depending on the level of gowning in use. A well-planned, trended, risk-based level of environmental monitoring is the suggested practice in this case. More information on bioburden control of nonsterile drug substances and products including environmental bioburden control can be found in USP <1115>.

2. What if during environmental air monitoring, the cumulative amount of microbes present on several plates exceeds "10"? Does "10" apply to a single plate only?

The number of isolates and the specified alert and action limits for each sampling location and sample type (i.e. active air, passive air, surface) can be individual or cumulative. Cumulative recoveries can be useful for a contamination recovery approach to environmental monitoring and trending. Individual CFUs per sampling event can be useful for a sampling plan that is focused on the number of colonies recovered from a given sample or plate. This should be specified in the standard operating procedure for environmental monitoring of the manufacturing facility.

Environmental Monitoring (continued)

3. Is there a comprehensive list of pathogenic bacteria?

There is not a comprehensive list of pathogenic bacteria, since not all organisms can be cultured for study, new organisms are constantly being discovered, and organisms that were once not recognized as pathogenic may become pathogens in particular medical situations. A good resource for study is the FDA's Bad Bug Book, now in its second edition. Though not meant to be comprehensive, it contains a survey of pathogenic bacteria, parasites, and viruses.

4. How would you count a "large" colony on a surface plate?

Regardless of the size of the colony, it should be counted as a single CFU. The growth speed of the organism may be the only reason it covers a large area, and may still have started as a single CFU. If there is concern that it may have started as several colonies and grown together, incubation time may need to be shortened, the incubation temperature lowered, or the frequency of the plate checks increased to ensure the plates are accurately counted.

5. Is the color of the colony significant?

Unfortunately no, although it may narrow the list of possible organisms, whether a colony represents a dangerous pathogen or not cannot be determined by the color of the colony. Many types of microorganisms have colonies of the same color. Colony color can also be variable for the same organism.

6. When you have the genus and species, where do you look to see what may be the source of this microbe?

Bergey's Manual of Determinative Bacteriology and Bergey's Manual of Systematic Bacteriology are two excellent publications that cover an extensive list of bacteria. In addition, many great resources are available on the internet, and a search of a genus-species name or even just a genus is enough to generate results and come up with some idea as to where the organism might come from (i.e. human skin flora, soil, water, etc.).