THE SCANRDI® STERILITY TEST – SUITABILITY CONSIDERATIONS

By: William J. Zolner, PhD | Chief Scientific Officer | Eagle Analytical Services, Houston, TX
Suitability testing using the ScanRDI method

Recently there has been considerable confusion over the requirements for a “Suitability” test when testing compounded sterile preparations for sterility using the ScanRDI method. This short paper should help clear this confusion and explain how we demonstrate the suitability of the method proposed by Eagle Analytical using the ScanRDI to test compounded sterile preparations for sterility.

Most people are familiar with the USP <71> Sterility Test procedure, which calls for a Method Suitability Test on each sterile preparation from each pharmacy to verify that the method is suitable for testing that specific preparation. This test used to be called the Bacteriostasis and Fungistasis test and can be thought of as a process to test the stasis of the overall sterility test method.

Since the USP <71> Sterility Test requires growth of any suspect contaminating microorganism, the Method Suitability Test procedure confirms that the sterility testing process will not inhibit microbial growth. For example, if you were testing an antibiotic, would the test procedure that you chose allow any microorganisms that were in the preparation to grow in the media used in the sterility test?

As specified in the USP <71> monograph, to perform this suitability or Bacteriostasis and Fungistasis test, six sterility tests are performed and a different microorganism is added to the last rinse step in each of the tests. Then the six sterility tests are allowed to proceed to the incubation step. Growth of the organisms must be observed within five days in each of the tests for the method to pass. If the organisms do not grow, no observable indication is present, the test fails, and the sterility method must be modified until growth of all the organisms is evident.

In essence, the suitability test verifies that microorganisms will grow in the test media if they are present in the preparation using the specified testing method process.

“Now comes the catch-22 – how do you perform a sterility test that demonstrates growth but does not require microbial growth? One answer: ScanRDI”
For those not familiar with the ScanRDI method, a quick review is as follows:

1. The ScanRDI system employs a combination of direct fluorescent labeling and solid phase laser scanning cytometry to rapidly calculate viable microorganisms. **The system has sufficient sensitivity to detect a single viable microorganism in 2-3 hours without the need for growth or cell multiplication.**

2. Initially the sample is filtered through a 0.4 µm polyester membrane filter. Sample volumes similar to the USP <71> method can be tested.

3. The filter is treated with a combination of background and viability stains consisting of a membrane-permeant, non-fluorescent substrate, which freely crosses any cell membrane. The substrate is cleaved by non-specific esterases into a membrane-impermeant chromophore. As a result, viable cells with intact membranes accumulate the chromophore in the cytoplasm while nonviable cells are unable to retain the fluorescent probe.

4. The filter is then transferred into the cytometer where the filter is efficiently scanned by a high-speed 488 nm argon laser. Fluorescent light is detected by multiple photomultiplier tubes and processed through a number of discrimination parameters that enable the instrument to differentiate between microorganisms and background noise. The result of this scan is displayed in the form of a membrane scan map that identifies the position of each fluorescent event.

5. The “Events” are then visually inspected using an epifluorescent microscope with an automated motorized stage by a degreed microbiologist to determine if they are viable microorganisms or artifacts. Those events that possess morphology consistent with microbial cells are subsequently considered viable microorganisms.

6. If one or more microorganisms are detected, the sample fails the test for sterility.

7. Artifacts, which can be particulates that have picked up the dye or other things such as silicone oil, which auto-fluoresces, are invalidated. If the cytometer notes more than 100 artifact events, the test is labeled “Incompatible” for testing with the ScanRDI. (Occurs after inspecting the filter to ensure that the events are not microbes, which would indicate a gross contamination of the sample.)

As you can see from the above, there is no growth step for the organisms, as the ScanRDI is capable of detecting any microbe without the necessity of amplification by growth.
There have been several suggestions that to demonstrate “Suitability” in the ScanRDI, one could add known microorganisms to the preparation then test to determine if the organism is recovered in the method. This would be really unneeded (explained below).

If a microorganism is added to a compounded preparation, and then the sample solution is tested using the ScanRDI, only five outcomes are possible:

1. The organism is killed by the compounded preparation and we cannot detect. This is fortunate as we now know that this organism cannot contaminate the preparation. (We would check first to make sure that the ScanRDI can see the organism if it was alive and not in the presence of the preparation.)

2. The preparation is loaded with particulates and the resulting ScanRDI test “Events” is greater than 100. When this happens, we microscopically inspect the filter to make sure that the Events are not all microbes and then classify the test as “Incompatible.” In effect, we have performed a suitability test on the sample and found that for this specific sample, the ScanRDI procedure is not suitable.

3. Any preparation residue left on the filter interacts with the chemicals used in the ScanRDI labeling process. This condition is clearly observable during the visual microscopical inspection of the filter, and again we label the ScanRDI test as “Incompatible,” as the ScanRDI test method is not suitable for this preparation.

4. The microorganisms are alive and trapped by the filter and labeled with the fluorescent dye for detection. Then only one question remains: will the ScanRDI respond to that microorganism? We have shown that the ScanRDI responds to all six of the standard USP organisms and several others of importance (for example, *Wangiella dermatitidis* – the organism that was implicated in the North Carolina compounding incident several years ago.) The ScanRDI has been shown to be capable of detecting over 3,000 microbiological strains. In addition, the basic underlying fluorochrome marker process is a widely used and proven technology in microbiology.

5. No microorganisms are present on the filter after inspection of all the Events identified by the laser scan. In this case, the sample tested is reported as “Sterile.”

CONCLUSION:

It has been suggested that the above process is really two tests in one ScanRDI test method. The first is a Suitability Test where it is determined if the ScanRDI method is suited for the particular sample under investigation, the second is the actual sterility test. If the first test for suitability is judged “Incompatible,” then the second test for sterility is terminated and never completed, and another sterility test can be performed on additional units or vials in the lot or batch.

Therefore, when questioned concerning ScanRDI suitability test, Eagle Analytical affirms that a suitability test is actually performed on each and every sample as it is being tested. If the suitability of a specific compounded sterile preparation does not pass this test, it is reported as “Incompatible” or not suited to being tested by the ScanRDI method.
• Dedicated to the unique needs of compounding
• Microbial Detection using the bioMerieux ScanRDI® Protocol
• Potency to USP protocols for accurate compositional analysis performed using HPLC systems

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