

The Golden Rules for Sterility Testing of Antibiotic Samples in a Study Using Tetracycline

When performing filtration-based sterility testing of pharmaceutical samples containing antibiotics or preservatives, three factors—the ‘golden rules’—are key to prevent false negative results: maximizing solubility of the antimicrobial agent, minimizing contact time with the membrane and optimizing the membrane rinsing procedure. Using a low-binding filter membrane like the one contained in the Steritest™ NEO device for antibiotics is key to prevent antibiotic retention. Tests were performed to find out whether it generates correct results for tetracycline-containing samples and when using *Staphylococcus aureus* as sensitive strain. All samples and positive controls, but not the negative controls, were found to yield bacterial growth after two days. The test setup and procedure thus conform to the compendial method described in the international pharmacopeias’ harmonized sterility chapters.

Some drugs are formulated with antibiotics, preservatives or other chemicals that affect microbial viability and growth. When testing samples of such drugs for sterility, it is necessary to eliminate their inhibitory effects. If not, the recovery of any microorganisms present in the samples may be reduced, thereby increasing the risk of false negative results. The European Pharmacopeia 2.6.1, United States Pharmacopeia <71> and Japanese Pharmacopeia stipulate membrane filtration as the method of choice for sterility testing whenever the nature of the product permits.

To be effective against antimicrobial activity, filtration must be combined with adapted systems and appropriate procedures. A system designed specifically for antibiotics and products with antimicrobial activity is based on our Steritest™ NEO device for antibiotics. It is a tubing and needle assembly that aseptically connects a diluent or dissolution fluid to its two closed filtration chambers, the canisters containing the low-binding Durapore® (PVDF) filter membrane (**figure 1**).

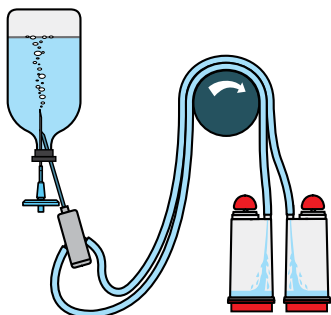


Figure 1:
Setup of a Steritest™ NEO
device for antibiotics

Rule 1: Maximizing solubility

Pure solid or liquid products are difficult to rinse away, often leaving residuals that can inhibit sterility testing considerably. Powders need to be well dissolved so they do not form aggregates because membranes tend to show an affinity for such clots. For liquid antibiotics, a dilution step is strongly recommended to reduce the concentration of the antimicrobial agent. This lowers the probability of the agent binding to the filter.

Rule 2: Minimizing membrane contact time

The propensity of molecules to adsorb to a membrane depends on its polymeric structure. For sterility testing of samples containing antibiotics or preservatives, the USP recommends a “low-binding filter material, such as polyvinylidene difluoride” (USP 39, chapter <1227>). Another important aspect is the thickness of the membrane. During filtration inhibitory residues can get trapped within the membrane. Therefore, the thinner the membrane, the lower the retention risk.

The Steritest™ NEO device for antibiotics uses a low-binding filter made of polyvinylidene difluoride (Durapore®) that has a thin membrane of about 120 to 150 µm. If multiple samples need to be filtered, they should be pooled before filtration. Our Steridilutor® NEO system is specially designed to do so without the risk of cross contamination.

Rule 3: Optimizing membrane rinsing

The construction principle of a closed sterility test device can also affect the risk of false negative results. In some of the filtration devices available on the market, the membrane is held in place by being pinched between the edges of the filtration canister and the base of the device. This can create pockets at the periphery of the membrane which may trap inhibitory products. Removing these residuals by rinsing is difficult and often impossible.

In Steritest™ NEO devices, the membrane is thermo-sealed to a specific holder (base) to prevent the formation of pockets that can trap residuals.

Sample preparation

In this study to test the suitability of the Steritest™ NEO device for antibiotics, tetracycline hydrochloride was selected. The tetracyclines are a group of broad-spectrum antibiotics used for treatment of infections of the urinary tract, respiratory tract and the intestines. They are also effective against chlamydia. They inhibit the bacterial protein biosynthesis and are therefore effective on a variety of gram-positive and gram-negative bacteria.

For the tetracycline samples and the negative controls, 6 g of tetracycline were dissolved in 808 mL 0.01 M HCl solution with Milli-Q® ultrapure type 1 water. For the positive controls, the tetracycline was omitted. The whole filtration and rinsing process was done in a laminar flow hood.

The test method

To filter the tetracycline samples, a Steritest™ pump and Steritest™ NEO devices for antibiotics were used. For all following rinsing steps a spare vent was used to minimize the risk of carrying over tetracycline into the rinsing fluids. All filtration steps were performed using a pump rotation speed of 25, except the tetracycline samples (speed 65) in order to minimize antibiotic binding.

Merck's liquid culture media Fluid A and Fluid K, which are manufactured according to the pharmacopeias' formulations, were used to perform the following test method.

The membrane of the Steritest™ NEO device was pre-wetted with 50 mL of Fluid K (step 1) to prevent antibiotic binding on the dry membrane. Immediately after, the samples were filtered through the membrane (step 2). For rinsing, the canisters were filled up to 100 mL three times with Fluid K to remove possible droplets of tetracycline solution from the canister's inner surfaces (step 3). After each filling step, the fluid was removed using the red stoppers.

Additional rinsing was performed twice with 100 mL of Fluid A (step 4). To inoculate each sample, approximately 25 cfu of *Staphylococcus aureus* ssp. aureus (ATCC® 6538 Vitroids™ 50 CFU) were pipetted into the final 100 mL of Fluid A.

Once emptied, the canisters were closed with the yellow stoppers and filled with 100 mL of Fluid Thioglycollate Medium (FTM, step 5). All canisters were then incubated for 7 days at 32.5 °C (± 2.5) (step 6). After 2, 3, 4 and 7 days the canisters were visually checked for turbidity.

The test method described above was repeated using Steritest™ NEO devices from 3 different lots, with 12 canisters tested per lot. For the positive and negative controls, 2 canisters from each lot were used.

No growth inhibition

Microbial growth was deemed to have occurred if the media in the canister showed at least slight turbidity or a concentrated microbial growth pattern, either within the media or directly on the membrane (see figure 2).

Figure 3 shows the results of canister readings performed after two days of incubation for the tetracycline samples with subsequent inoculation, the positive controls and the negative controls. The concentration of the *Staphylococcus aureus* inoculum used for each of the lots is also indicated.



To place an order or receive technical assistance

In the U.S. and Canada, call toll-free 1-800-645-5476

For other countries across Europe and the world, please visit: [EMDMillipore.com/offices](https://www.EMDMillipore.com/offices)

For Technical Service, please visit: [EMDMillipore.com/techservice](https://www.EMDMillipore.com/techservice)

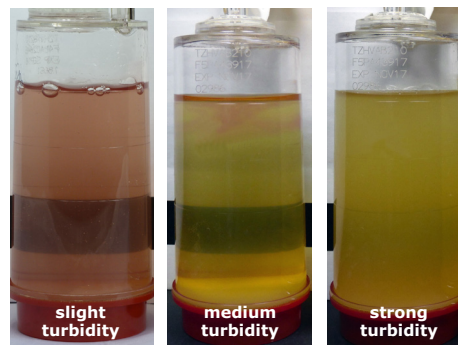


Figure 2: Examples of positive growth in Steritest™ NEO canisters

All 12 inoculated sample canisters showed bacterial growth for each of the three lots tested. As expected, all positive controls (bacteria but no antibiotic) were also found to be positive, whereas all negative controls (antibiotic but no bacteria) remained perfectly clear.



Figure 3: Number of positive (visible growth) and negative (no visible growth) canisters after 2 days

Full conformity

This study showed that the method used to test tetracycline-containing samples, when also using devices containing Durapore® (PVDF) filters, prevent antibiotic residues from binding to the membrane. Good solubilization of the antibiotic and reduced contact time with a low binding filter membrane ensure perfect rinsing efficiency. This method based on the Steritest™ NEO devices for antibiotics thus complies with the 'golden rules' and conforms to the compendial test method described in the US, EU and Japanese pharmacopeias.

