



Microbial Growth after Filtration of Antibiotic Vancomycin Hydrochloride



#3

Application Note

#4

- Sterisart® NF
- Sterisart® NF Adapter
- Sterisart® Universal Pump

#5

#6

#7

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1. Introduction

1.1 Abstract

The following experiments were carried out to prove the growth of microorganisms in Sterisart® systems after membrane filtration of the antibiotic Vancomycin Hydrochloride.

1.2 Background

Microorganisms can propagate quickly when inhibitor substances are absent. Any residue of preservatives or antibiotics which may be contained in pharmaceutical samples for sterility testing, must be removed via rinsing before adding nutrient media into the system. Vancomycin cannot be neutralized and therefore, all parts that come in contact with the sample must be low adsorptive and designed in such a way to ensure thorough rinsing in order to remove all residues.

1.3 Description

After wetting the system with sterile physiological NaCl solution Vancomycin Hydrochloride solution is filtered through a Sterisart® system. Following a rinsing procedure, one container of the Sterisart® system is filled with soy bean casein digest medium according to the European Pharmacopoeia. The container is inoculated with 50–100 microorganisms and incubated for a maximum of 3 days. The membrane was removed from the other container and an inhibition test was carried out.

2. Materials and Equipment

Antibiotic
Vancomycin Hydrochloride
Customer Batch: A 3220187

Test Strains [ATCC No.], [DSMZ No.]
Bacillus subtilis [6633], [347]
Staphylococcus aureus [6538], [346]

Soy bean casein digest medium
Oxoid; Code No.: BO 0509M;
Lot No.: 966290
NaCl
Merck; No.: 1.06400.5000;
Lot: K 91921-400402

Pump
Sterisart® Universal Pump, order no. 16413
predecessor model of the current Sterisart®
Universal Pump 16420 (see picture)

Sterisart® units
Sterisart® NF, order no. 16466-----ACD;
Lot No: 16466 030010
Sterisart® NF adapter,
order no. 16470-----GBD

3. Preparations for the test

3.1 Sterile physiological NaCl solution

In order to prepare 0.9 % sterile physiological NaCl solution, 9 g solid NaCl is dissolved in one liter deionized water and filled into bottles with septum. Two liters were prepared. Then the solutions were autoclaved at 121°C for 40 minutes.

3.2 Dissolving of the antibiotic solution (20 g/L)

3.2.1 Background

Dissolving of the antibiotic is a very critical step in the sterility testing procedure. Undissolved residues are retained by the membrane filter and may cause growth



inhibitions during incubation. For reliable and immediate dissolving of Vancomycin Hydrochloride tests have shown that the best results are made by using physiological NaCl solution with a temperature of 30 °C. For the detailed test description and results, please refer to Sterisart® Application Note No. 1. For the following procedure a particular method for filling and shaking was developed. The physiological NaCl solution can be warmed easily in an incubator or water bath.

3.2.2 Sample preparation

10 g of the mixed sample of the antibiotic is already available in a 500 ml sterile bottle with septum (bottle A). After inserting the non-marked tube of the Sterisart® NF adapter 16470 into the pump, the long needle of 16470 with the yellow color coding is inserted into the septum of the bottle filled with 500 ml sterile physiological NaCl warmed to 30 °C (bottle B), while the short needle is inserted into the septum of the bottle A filled with the antibiotic. Bottle A is held upside down at an angle so that the antibiotic lyophilisate falls onto the neck of the bottle. The NaCl solution is then transported into bottle A which contains the antibiotic, using a pump setting of 300.

After approx. 5 sec., bottle A is brought back to its normal position so that the antibiotic settles back down to the bottom of the bottle together with the NaCl solution. The pumping pressure from the fluid is sufficient to release any antibiotic remaining stuck to the glass wall of the bottle. Bottle A is filled up to the 500 ml mark and is shaken until the antibiotic is completely dissolved. For this procedure the Sterisart® NF adapter, order no. 16470, in combination with the Sterisart® Universal Pump, order no. 16413, predecessor model of the current 16420 pump, was used. Detailed information regarding the use of the Sterisart® NF adapter 16470 is described in the package insert.

4. Test method

4.1 Membrane filtration

After placing the Sterisart® system 16466 into the pump, 100 ml of the sterile physiological NaCl solution is pumped into the

system for wetting the membranes. To eliminate the solution, sterile air is pumped into the Sterisart® unit. Next, 500 ml of dissolved antibiotic is filtered through the containers. Special care has to be taken in order to avoid that antibiotic fluid wets areas that cannot be flushed and rinsed properly afterwards. The unit is again emptied with sterile air. Subsequently, the membranes are rinsed with 5 × 200 ml sterile physiological NaCl solution to make sure that no antibiotic is left on the membranes and plastic parts, so that the growth of microorganisms is not inhibited. Detailed information regarding the use of Sterisart® NF units are described in package insert.

4.2 Filling up with nutrient media

One of the two Sterisart® containers is filled with 50 ml fluid soy bean casein digest medium. The tube is closed with the clamp and cut approx 10 cm apart from the clamp. The second container is used for the inhibition test (refer to 4.5).

4.3 Inoculation with microorganisms

Vancomycin Hydrochloride is mostly suitable for gram-positive bacteria. Therefore, *Bacillus subtilis* (gram-positive) is used. A dilution series assures that there are 50–100 cells in 1.0 ml of *Bacillus subtilis* solution. 1.0 ml of the *Bacillus subtilis* solution is added to the fluid soy bean casein digest medium.

4.4 Incubation

The Sterisart® container is incubated for three days at room temperature. The growth of microorganisms is checked daily.

4.5 Inhibition test of the membrane

The Sartochem® membrane of the second Sterisart® container is used for an inhibition test. After opening the unit and removing the membrane, it is cut into three parts:

- central membrane
- outer membrane
- punching

Each membrane part is put on an agar plate inoculated with *Staphylococcus aureus* and incubated at 30 °C for 24 hours. In case the antibiotic is concentrated in specific areas of the membrane, the growth of the bacteria would be inhibited in those regions.

5. Results

After 24 hours of incubation a slight turbidity can be observed, after three days a strong growth can be seen in the Sterisart® container. The medium is very cloudy (intense growth). Concerning the inhibition test there are no antibiotics left in the different membrane areas. The microorganisms grow on the agar plate directly at the edge of the membrane. Their growth is not inhibited.

6. Conclusion

Rinsing with 5 × 200 ml of physiological NaCl solution guarantees an adequate removal of the antibiotic. Bacterial growth tests have shown that the tested antibiotic Vancomycin Hydrochloride does not remain in the Sterisart® system where it could enhance or inhibit the growth of microorganisms.



Sterisart® Universal Pump 16420

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