



## Microbial Growth after Filtration of Antibiotic Polymyxin B Sulphate



### Application Note

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

#1

#2

#3

#4

#5

## Author

Dr. K. Pflanz  
Sartorius Stedim Biotech GmbH,  
Goettingen, Germany

## Contact

For any questions, please contact your local sales and technical support (see last page)

## 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 Polymyxin B Sulphate.

### 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. Polymyxin 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 Polymyxin B Sulphate solution is filtered through a Sterisart® system. Following a rinsing procedure, one container of the Sterisart® system is filled with fluid thioglycollate medium according to the European Pharmacopoeia. The container is inoculated with 50–100 microorganisms and incubated for a maximum of 3 days.

## 2. Materials and Equipment

Antibiotic  
Polymyxin B Sulphate  
Customer Batch: A 1411058

Test Strain [ATCC No.], [DSMZ No.]  
*Pseudomonas aeruginosa* [9027], [1128]

Fluid thioglycollate medium  
Oxoid; Code No.: BO 0510M;  
Lot No.: 964179  
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 of 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 of 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 Polymyxin B Sulphate tests have shown that the best results are obtained 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 on hand 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 solu-

tion. 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 are 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 the package insert.

#### 4.2 Filling up with nutrient media

One of the two Sterisart® containers is filled with 100 ml fluid thioglycollate medium. The tube is closed with the clamp and cut approx. 10 cm away from the clamp. The second container is used for further testing.

#### 4.3 Inoculation with microorganisms

Polymyxin B Sulphate is mostly suitable for gram-negative bacteria. Therefore *Pseudomonas aeruginosa* (gram-negative) is used. A dilution series assures that there are 50 – 100 cells in 1.0 ml of *Pseudomonas aeruginosa*-solution. 1.0 ml of the *Pseudomonas aeruginosa*-solution is added to the fluid thioglycollate medium.

#### 4.4 Incubation

The Sterisart® container is incubated for three days at 30 °C. The growth of microorganisms is checked daily.

### 5. Result

After 48 hours of incubation strong growth can be seen in the Sterisart® container. The medium is very cloudy (intense growth).

### 6. Conclusion

The 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 Polymyxin B Sulphate do not remain in the Sterisart® system where it could enhance or inhibit the growth of microorganisms.



Sterisart® Universal Pump 16420

# Sales and Service Contacts

For further contacts, visit [www.sartorius-stedim.com](http://www.sartorius-stedim.com)

## Europe

### Germany

Sartorius Stedim Biotech GmbH  
August-Spindler-Strasse 11  
37079 Goettingen

Phone +49.551.308.0  
Fax +49.551.308.3289

[www.sartorius-stedim.com](http://www.sartorius-stedim.com)

Sartorius Stedim Systems GmbH  
Schwarzenberger Weg 73-79  
34212 Melsungen

Phone +49.5661.71.3400  
Fax +49.5661.71.3702

[www.sartorius-stedim.com](http://www.sartorius-stedim.com)

### France

Sartorius Stedim Biotech S.A.  
ZI Les Paluds  
Avenue de Jouques – BP 1051  
13781 Aubagne Cedex

Phone +33.442.845600  
Fax +33.442.845619

Sartorius Stedim France SAS  
ZI Les Paluds  
Avenue de Jouques – CS 71058  
13781 Aubagne Cedex

Phone +33.442.845600  
Fax +33.442.846545

### Austria

Sartorius Stedim Austria GmbH  
Franzosengraben 12  
A-1030 Vienna

Phone +43.1.7965763.18  
Fax +43.1.796576344

### Belgium

Sartorius Stedim Belgium N.V.  
Leuvensesteenweg, 248/B  
1800 Vilvoorde

Phone +32.2.756.06.80  
Fax +32.2.756.06.81

### Denmark

Sartorius Stedim Nordic A/S  
Hoerskaetten 6D, 1.  
DK-2630 Taastrup

Phone +45.7023.4400  
Fax +45.4630.4030

### Italy

Sartorius Stedim Italy S.p.A.  
Via dell'Antella, 76/A  
50012 Antella-Bagno a Ripoli (FI)

Phone +39.055.63.40.41  
Fax +39.055.63.40.526

### Netherlands

Sartorius Stedim Netherlands B.V.  
Edisonbaan 24  
3439 MN Nieuwegein

Phone +31.30.6025080  
Fax +31.30.6025099

### Spain

Sartorius Stedim Spain SA  
C/Isabel Colbrand 10,  
Oficina 70  
Polígono Industrial de Fuencarral  
28050 Madrid

Phone +34.90.2110935  
Fax +34.91.3589623

### Switzerland

Sartorius Stedim Switzerland GmbH  
Lerzenstrasse 21  
8953 Dietikon

Phone +41.44.741.05.00  
Fax +41.44.741.05.09

### U.K.

Sartorius Stedim UK Limited  
Longmead Business Park  
Blenheim Road, Epsom  
Surrey KT19 9 QQ

Phone +44.1372.737159  
Fax +44.1372.726171

## America

### USA

Sartorius Stedim North America Inc.  
5 Orville Drive  
Bohemia, NY 11716

Toll-Free +1.800.368.7178  
Fax +1.631.254.4253

Sartorius Stedim SUS Inc.  
1910 Mark Court  
Concord, CA 94520

Phone +1.925.689.6650  
Toll Free +1.800.914.6644  
Fax +1.925.689.6988

Sartorius Stedim Systems Inc.  
201 South Ingram Mill Road  
Springfield, MO 65802

Phone +1.417.873.9636  
Fax +1.417.873.9275

### Argentina

Sartorius Argentina S.A.  
Int. A. Avalos 4251  
B1605ECS Munro  
Buenos Aires

Phone +54.11.4721.0505  
Fax +54.11.4762.2333

### Brazil

Sartorius do Brasil Ltda  
Av. Dom Pedro I, 241  
Bairro Vila Pires  
Santo André  
São Paulo  
Cep 09110-001

Phone +55.11.4451.6226  
Fax +55.11.4451.4369

### Mexico

Sartorius de México S.A. de C.V.  
Circuito Circunvalación Poniente No. 149  
Ciudad Satélite  
53100 Naucalpan, Estado de México

Phone +52.5555.62.1102  
Fax +52.5555.62.2942

## Asia | Pacific

### China

Sartorius Stedim Beijing  
Representative Office  
No. 33, Yu'an Road,  
Airport Industrial Zone B, Shunyi District  
Beijing 101300

Phone +86.10.80426516  
Fax +86.10.80426580

Sartorius Stedim Shanghai  
Representative Office  
Room 618, Tower 1, German Centre,  
Shanghai, PRC., 201203

Phone +86.21.28986393  
Fax +86.21.28986392.11

Sartorius Stedim Guangzhou Office  
Room 704, Broadway Plaza,  
No. 233-234 Dong Feng West Road  
Guangzhou 510180

Phone +86.20.8351.7921  
Fax +86.20.8351.7931

### India

Sartorius Stedim India Pvt. Ltd.  
10, 6th Main, 3rd Phase Peenya  
KIADB Industrial Area  
Bangalore – 560 058

Phone +91.80.2839.1963|0461  
Fax +91.80.2839.8262

### Japan

Sartorius Stedim Japan K.K.  
KY Building, 8-11  
Kita Shinagawa 1-chome  
Shinagawa-ku  
Tokyo 140-0001

Phone +81.3.3740.5407  
Fax +81.3.3740.5406

### Malaysia

Sartorius Stedim Malaysia Sdn. Bhd.  
Lot L3-E-3B, Enterprise 4  
Technology Park Malaysia  
Bukit Jalil  
57000 Kuala Lumpur

Phone +60.3.8996.0622  
Fax +60.3.8996.0755

### Singapore

Sartorius Stedim Singapore Pte. Ltd.  
10, Science Park Road, The Alpha  
#02-25, Singapore Science Park 2  
Singapore 117684

Phone +65.6872.3966  
Fax +65.6778.2494

### Australia

Sartorius Stedim Australia Pty. Ltd.  
Unit 5, 7-11 Rodeo Drive  
Dandenong South Vic 3175

Phone +61.3.8762.1800  
Fax +61.3.8762.1828