



Concentration and Diafiltration of Tetanus Vaccine



#10

Application
Note

#11

Purification by
Crossflow Filtration
Hydrosart® 0.2 µm MF
Hydrosart® 30kD UF

#12

#13

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Autor

Dipl.-Ing. Frank Meyeroltmanns
Peter Schmidt
Sartorius Stedim Biotech GmbH,
Goettingen, Germany

Abstract

This trial was performed to investigate the downstream processing of Tetanus vaccine and also to assess the suitability of the Sartorius Stedim Biotech Cross-Flow-Equipment for a tetanus vaccine process.

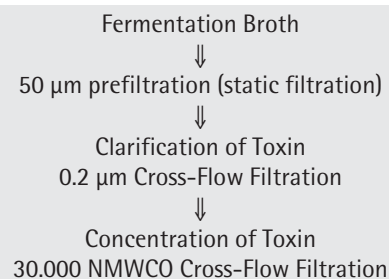
1. Background

To prevent blocking of the cassette inlet channels and damage to the pump from any foreign objects it was decided to prefilter the tetanus culture by using a 20 µm depth filter Sartopure PP 2 (cat. No. 5592520P1).

A batch of 60 liter Tetanus culture was prefiltered. After use the prefilter cartridge was disposed. The filter was only coloured in a very small area and was cleanable easily by backflushing with water.

2. Description of Trial Procedure

The following protocol was adopted for the trial:



3. Materials and Methods

Crossflow system

Sartocon 2+ ultrafiltration setup, the pump used to run the system was a peristaltic pump, double jacketed feed vessel cooled with cold WFI in order to ensure temperatures below 10°C. The temperature was carefully monitored during the whole experiment.

MF-Membrane

Hydrosart® 0.2µm,
order no. 3021860706W--SG,
membrane area 0.6 m².

UF-Membrane

Hydrosart® 30 kD,
order no. 3021445906E--SG,
membrane area 0.6 m².



4. Investigation Trials

4.1. Clarification of Tetanus Toxin (MF)

4.1.1. Set up of Microfiltration Membrane

One 0.2 μm Hydrosart[®] cassette with 0.6 m² filtration area (cat. No. 3021860706W--SG, lot no. 00070136) was installed into a Sartocan 2+ holding device. The cassette was first rinsed with 5 liters of DI-water, both retentate and permeate lines were directed down the drain.

After rinsing the system was conditioned with 0.9% NaCl. This was done by rinsing the system with 5 liters of NaCl (retentate and permeate to feed tank), recirculation for 5 minutes. The purpose of this conditioning was to equilibrate the membrane surface in order to minimize non specific binding of toxin on to the membrane surface.

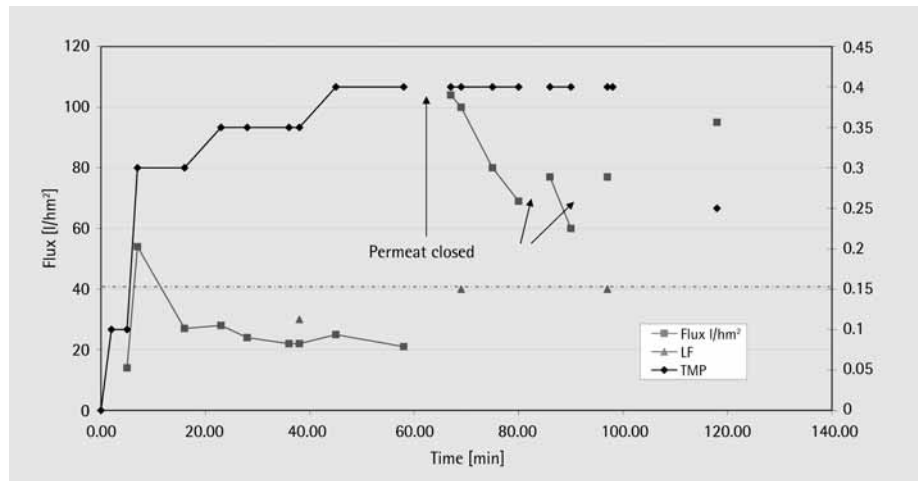
4.1.2. Operating Parameters

It was decided to run the clarification step at a minimum operating pressure, this pressure can be seen in table 1. The experience of Sartorius Stedim Biotech is that operating at minimal pressure would minimize losses of toxin.

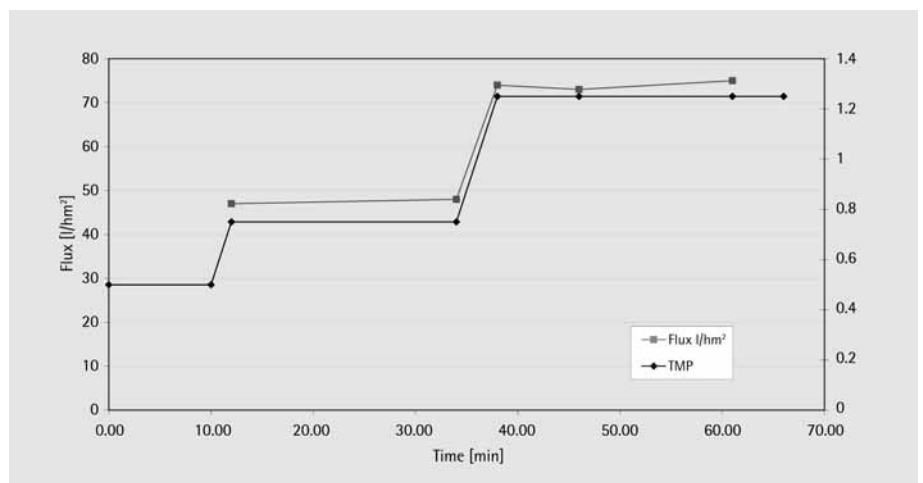
4.1.3. Clarification of toxin

To avoid a secondary layer on the membrane it was decided to work with low transmembrane pressures. The clarification started with an inlet pressure of 0.5 bar, which was increased slowly up to 1 bar during the filtration. Permeate flow rates were determined at various time intervals throughout the process. Flux rates were calculated and are presented in a table. Samples of permeate were taken at various times throughout the clarification step. Lateral-Flow (LF) assays (**LF assays** to detect specific nucleic acids) were performed on these samples and the results are presented in a table.

Tetanus clarification



Flow rate performance through the entire MF process



Flow rate performance through the entire UF process

4.1.4. Results

The total filtration time was 2 hours.

After first hour LF was measured with 30 in permeate. This indicates a secondary layer on the membrane (Caused by too high inlet pressure at the beginning, 2 bar). This layer could be easily removed by closing the permeate valve for 2 minutes.

By avoiding too high inlet pressures we estimate a filtration time of 1 hour.

At the end of filtration the total batch was LF 40, this means 100% recovery of toxin.

Permeate was visually clear, the optical quality is much better than after sheet filtration.

5.1. Concentration of Toxin (UF)

5.1.1. Set up of Ultrafiltration Membrane

The cassette was changed to a Hydrosart® Ultrafiltration membrane (cat no. 3021445906E--SG, lot no. 00080146).

Cut off of the membrane is 30.000, membrane area 0.6 m². After the completion of conditioning the system the clarified toxin (permeate of trial 1) was brought into the system. The inlet pressure at the beginning was 1 bar. Permeate flux was constant. To optimize flux rate transmembrane pressure was increased by us during filtration. At the end we achieved a constant flux on a higher level of 75 l/hm². The flux was independent from the concentration of toxin.

The clarified toxin pool had a volume of approx 45 liter which was concentrated to a final volume of approx. 8 liter.

Recovery of toxin

LF of the concentrate was measured.

The total recovery from the tetanus culture to the end of the concentration was 100%.

6. Cleaning efficiency

Product was flushed out by 0.9% NaCl (Clarification) or permeate (Concentration).

The system was cleaned by recirculating 5 liter of 1 M NaOH at room temperature for 30 min. The cleaning was sufficient, decrease of water flux could not be detected.

7. Summary

Microfiltration | Clarification step

Average flux rate:	50 l/hm ²
Processing volume:	200 l
Processing time:	2 hours

Based on the results of the test a membrane area of 2.5 m² (4 cassettes, 2.8 m²) are recommended for the clarification step to run 200 liter within approx. 2 working hours.

Ultrafiltration | Concentration

Average flux rate:	75 l/hm ²
Processing volume:	200 l
End volume concentration:	5 l
Estimated diafiltration volume:	5 × 5 l
Processing time:	2 hours

Based on the results of the test a membrane area of 1.5 m² (3 cassettes, 1.8 m²) are recommended for the toxin concentration step to run 200 liter within approx. 2 working hours.

In the trial the concentration was limited to factor 5.5. If this factor is increased, the processing time might be longer. This can be overcome by increasing the filtration area.

This investigation underline the superior performance of Hydrosart® ultrafilter membranes in:

- ... Excellent product recovery (approximately 100%).
- ... Superior flow rates (compared with Millipore trial).
- ... Excellent cleanability of the membrane.
- ... No fouling tendencies of the membrane.

Note:

Flow rates (and therefore a reduction in the required membrane surface area) can be improved by optimizing the operating parameters prior investigation runs. Optimization procedures are performed under constant concentration and constant temperature conditions. Various crossflow rates combined with various TMP's supports the definition of optimum processing point.

Sales and Service Contacts

For further contacts, visit 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

Sartorius Stedim Systems GmbH
Schwarzenberger Weg 73-79
34212 Melsungen

Phone +49.5661.71.3400
Fax +49.5661.71.3702

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 10-12,
Planta 4, Oficina 121
Polígono Industrial de Fuencarral
28050 Madrid

Phone +34.91.3586102
Fax +34.91.3588804

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