
product support

Investigation into the Removal of Challenge Fluid from a Membrane by Steam Sterilisation following Aerosol Integrity Testing using the VALAIRDATA

technical information • product support • new developments



domnick hunter - Process Filtration

domnick hunter - Setting the Standard

domnick hunter bring extensive experience through our scientists, engineers and sales representatives to the process of offering specific filtration systems to meet the needs of your production process. Support services are available covering a wide range of activities including scale up advice from laboratory through pilot scale to production systems, validation support, design and manufacturing of custom housings and filtration products and on-site technical support.

Committed to quality

Quality is of paramount importance to **domnick hunter**. As such **domnick hunter** has been certified to ISO 9001 since 1987, providing a quality management system that covers the entire organisation from R&D, production, warehousing, materials management and customer support. In addition, our manufacturing facilities operate to the principles of cGMP.

This commitment is underlined by our registration to ISO 14001 during 2001 and our move to ISO9001:2000.

Validation and product certification

To certify that **domnick hunter** products meet the required regulatory and quality standards of the industries we supply, all filters are supplied with a certificate of conformance. These certificates are linked to validation guides for both pre-filter and sterilising grade membrane filter cartridges that define methodologies and data appropriate to each filter type. This information typically includes:

- technical specifications
- biological safety testing including Current USP<88> Class VI - 121°C Plastics
- extractable testing including 21 CFR 211.72 and 210.3(b), (6) for fibre releasing filters
- effluent quality including TOC, bacterial endotoxins, water conductivity and particle release
- chemical compatibility information
- thermal stability
- correlation of a non destructive integrity test to a defined bacterial challenge

Where appropriate these data are included in the **domnick hunter** Drug Master File – DMF 7564 at the U.S. Food and Drug Administration (FDA).

Validation support services

domnick hunter has extensive laboratory facilities and trained personnel capable of providing a range of validation support services to support manufacturers meet their requirements for process validation relating to the use of filtration products.

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1. Summary

A detailed study was completed to investigate the efficiency of steam sterilization for the removal of aerosol challenge fluid from membranes following integrity testing using the VALA/RDATA system. Aerosol of a given droplet size is generated from Shell Ondina oil.

The study was undertaken using an extreme test condition where the test membranes (PTFE) were subjected to a concentration of aerosol that was 20 times higher than that normally used for a VALA/RDATA test. Following exposure to aerosol, the test membranes were steam sterilised for 30 minutes at 121°C (250°F) and residual aerosol detected using Infrared spectroscopy. In all cases, no residual aerosol could be detected on the membranes following steam sterilization. The sensitivity of the test was 2mg/ml of target analyte.

In addition, analysis of condensate samples collected from upstream and downstream drains during steam sterilisation of membranes exposed to challenge fluid showed minimal carry over of challenge fluid into the down stream condensate samples. This indicates that during steam sterilisation the majority of challenge fluid is removed from PTFE membranes via the upstream drain and vent.

2. Introduction

The principle of aerosol testing sterile air cartridges has been well received in industrial applications as it has many practical advantages over liquid based integrity test methods. However, one key concern has been raised concerning the potential retention of aerosol on the membrane following integrity testing.

During the development of the VALA/RDATA integrity test system, studies were completed to show that there was no increase in the differential pressure at the rated flow of the cartridge following repeated integrity testing and steam sterilisation. This suggested that there was no significant retention of aerosol on the membrane following steam sterilisation. These studies have been supplemented by the quantitative studies reported here to determine the actual concentration of challenge fluid, if any, which remains on the membrane following steam sterilisation procedures.

3. Methods

3.1 Test Procedure

Tests were conducted using 142mm discs of PTFE test membranes installed in a stainless steel disc holder. The test set up is shown in Figure 1. Membranes were used in preference to complete filter cartridges to avoid possible masking of residual Shell Ondina EL aerosol challenge fluid by normal extractable hydrocarbons from other cartridge components (e.g. plastic cages, cores, end caps etc).

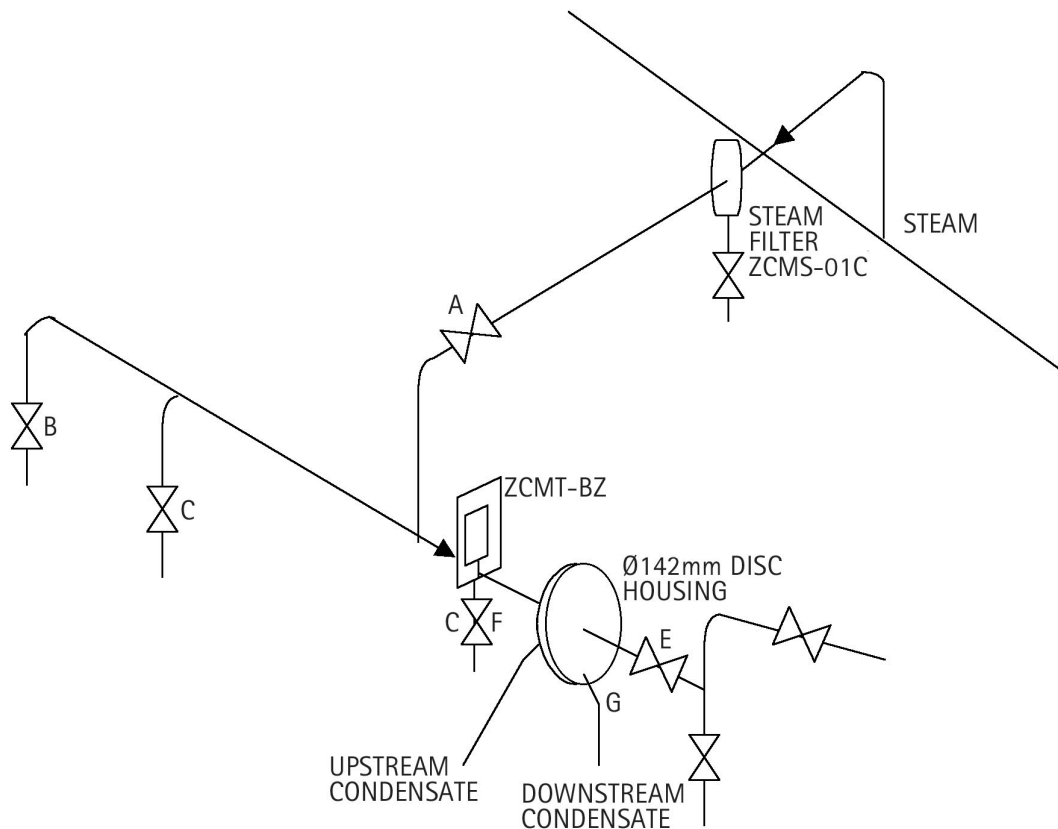


Figure 1 : Test rig for steam sterilisation of aerosol loaded 142mm membrane samples

The following test procedure was followed for each membrane evaluation;

- 3.1.1 A new PTFE membrane was installed in the 142mm disc holder.
- 3.1.2 All valves closed.
- 3.1.3 Crack open Valves F & G and fully open Valves C & D.
- 3.1.4 Open steam Valve A.
- 3.1.5 When condensate flow ceases from drains C & D, and is replaced by free steam flow, close Valves C & D to a cracked position.
- 3.1.6 As pressure increases to 1.1 barg, the condensate issuing from drains F & G was collected in individual Duran bottles previously cleaned with Trichlorofluoroethane. Condensate from the upstream and downstream drains was collected throughout the 30 minutes sterilisation cycle.
- 3.1.7 Following cooling of the test rig, the \varnothing 142mm disc holder was removed from the rig and integrity tested using the VALA/RDATA. The disc was tested such that it was subjected to a concentration of challenge fluid 20 times higher than that normally seen in a standard integrity test.
- 3.1.8 Following integrity testing, the 142mm-disc housing was re-installed into the test rig and steam sterilised in accordance with the sequence described in sections 3.1.2 to 3.1.6.
- 3.1.9 Following steam sterilisation, the disc holder was allowed to cool to ambient. The test membrane was then carefully removed from the holder using tweezers and placed in a petri dish prior to analysis using a laser spectrophotometer.

This procedure was repeated on a number of sample discs. Between each test the test rig was steamed empty for 30 minutes at 121°C (250°F) to ensure no cross over contamination between samples.

3.2. Analysis of Oil Content

A Mattson Laser spectrophotometer was used to determine the amount of oil in both the collected condensate and that which remained on the membrane following steam sterilisation. The Shell Ondina EL gives a characteristic absorbance at specific wavelengths, which can then be used to quantify the oil content. Before analysing the samples a background solvent analysis was undertaken to determine a value for 100% transmission in the infrared.

3.2.1 Determination of residual challenge fluid on test membranes following steam sterilisation

To determine the residual challenge fluid on membranes following steam sterilisation, test membranes were placed in individual glass Duran bottles previously cleaned with Trichlorotrifluoroethane. 25ml of solvent was added to each Duran bottle and these were agitated for 1 minute to ensure that all traces of challenge fluid were transferred to the solvent.

Approximately 10ml of the solvent was added to a quartz glass sample cell (4cm-path length), which was placed in the spectrophotometer for analysis.

3.2.2 Determination of challenge fluid in upstream and downstream condensate samples

50ml of condensate was transferred to a stoppered glass funnel. 25ml of solvent was added to the condensate and agitated for 1 minute to ensure all traces of challenge fluid were extracted from the condensate into the solvent.

3.2.3 The mixture was then allowed to separate into two discrete layers (approximately 1 minute). 15ml of the upper solvent layer was then transferred into a quartz glass sample cell, which was placed in the spectrophotometer for analysis.

4.0 Results and discussion

The levels of residual challenge fluid on non-steamed and steamed PTFE membrane samples are shown in Table 1. It can be seen that any residual challenge fluid on the steamed samples was below detectable limits (<2mg/ml) of solvent. In contrast the non-steamed samples showed an average extractable level of 722mg. This demonstrates that steam sterilisation is extremely effective at removing residual challenge fluid from tested filter cartridges.

Table 1: Residual levels of challenge fluid on steamed and non-steamed PTFE membrane samples

Sample ID	Description	Solvent Volume (ml)	Total Oil Content (ml)
M1	Unchallenged PTFE	25	Not Detectable
M2	Unchallenged PTFE	25	Not Detectable
M3	Challenged PTFE Steamed	25	Not Detectable
M4	Challenged PTFE Steamed	25	Not Detectable
M5	Challenged PTFE Steamed	25	Not Detectable
M6	Challenged PTFE Unsteamed	25	782ug
M7	Challenged PTFE Unsteamed	25	662ug

Table 2 shows the results for condensate samples collected upstream and downstream of membranes following steam sterilisation. Results are presented for membranes exposed to challenge fluid, a non-exposed membrane control sample and an empty disc holder control sample.

These data show that there was a significant level of extractable from both the empty housing and the PTFE control membrane. As expected the upstream samples from integrity tested membrane samples showed an elevated extractable level compared to these controls. However, in all cases the downstream extractable level was similar for the control and integrity tested samples. This indicates that the majority (83%) of aerosol challenge fluid retained on the membrane is removed during steaming through the upstream drain valve. This means that the level of downstream carryover of challenge fluid during steam sterilisation is very small.

To put the quantity of challenge fluid used during a test into context, reference can be made to a typical pre filtration system for a sterile gas supply to a fermenter. Air quality levels with respect to residual oil are defined in ISO 8573.1:2001 (E) as follows:

- Class 4 < 5 mg/m³
- Class 3 < 1 mg/m³
- Class 2 < 0.1 mg/m³
- Class 1 < 0.01 mg/m³

Taking the cleanest Class 1 system with a max remaining oil content of 0.01mg/m³ a 5" sterile air cartridge (e.g. ZCHT/AT) would process approximately 8000m³ of air with a total oil content of 80mg during a typical 7 day fermentation run.

The amount of challenge fluid deposited during an integrity test is therefore negligible.

Table 2: Extractable levels in upstream and downstream condensate samples.

Sample ID	Description	Total Volume of Condensate (ml)	Total Volume (ml)	Solvent Volume (ml)	Solvent ug/ml	Total ug
M1	PTFE Unchallenged Upstream	60	50	25	9.19	275
M1	PTFE Unchallenged Downstream	45	45	25	3.6	90
M2	PTFE Unchallenged Upstream	45	45	25	10.22	255
M2	PTFE Unchallenged Downstream	50	50	25	3.30	82
M3	Challenged Upstream	80	50	25	2.87	115
M3	Challenged Downstream	110	50	25	8.04	442
M4	Challenged Upstream	110	50	25	8.35	459
M4	Challenged Downstream	95	50	25	2.46	116
M5	Challenged Upstream	105	50	25	8.1	425
M5	Challenged Downstream	10	50	25	2.13	117

5. Appendix I - Calculation of Oil Content

The oil content is determined by taking the absorbance level at the three specific wavelengths as a fraction of the 100% transmission and dividing this by the oil coefficient.

The oil coefficient for Shell Ondina is 0.023 and is determined by obtaining traces at various dilutions of oil/solvent beginning with 0.1 grammes/100ml of solvent.

The formula is as follows:

$$\text{absorbance} = \ln_{10} \frac{10^3}{I_1 \times I_2 \times I_3}$$

Where I_0 is the base line absorbance, I_1 , I_2 and I_3 are the absorbance at the specific wavelengths.

The oil content is then $\frac{\text{absorbance}}{\text{oil coefficient}}$

which gives a result in $\mu\text{g/ml}$ of solvent used to extract the oil from the sample.

Example PTFE challenged

From graph M6 PTFE:

$$I_0 = 100\%$$

$$I_1 = 65.3\%$$

$$I_2 = 66.6\%$$

$$I_3 = 43.89\%$$

The absorbance is given by: $\ln_{10} \frac{(10^3)}{(I_1 \times I_2 \times I_3)}$

$$\begin{aligned} \text{absorbance} &= \ln_{10} \frac{(100^3)}{(66.6 \times 65.3 \times 43.89)} \\ &= 0.719 \end{aligned}$$

The amount of oil in $\mu\text{g/ml}$ is given by

$$\frac{\text{absorbance}}{\text{oil coefficient}} = \frac{0.719}{0.023} = 31.26\mu\text{g/ml}$$

The sample disc was placed in 25ml of solvent therefore the total amount of oil deposited on the discs was $25 \times 31.26 = 781\mu\text{g}$.

6. Technical Support Group activities

domnick hunter have a trained team of scientists and engineers available to answer questions regarding the technical capabilities of our products, to assist in the selection and design of appropriate filtration systems and to provide user training programs. The following services can be delivered both on site and through our local **domnick hunter** offices:

- filterability testing to optimise filter system design
- advice on the development of integrity testing, steam sterilisation and clean in place procedures
- development of validation procedures
- troubleshooting
- facility audits to ensure continued optimisation of filter use
- operator training including filtration theory, filter system design and management, validation, etc.

For more information on any of the above support services please contact your local **domnick hunter** representative.

email: tsg@domnickhunter.com
website: www.domnickhunter.com