
# An investigation into the removal of challenge fluid from a membrane by steam sterilization following aerosol integrity testing using the Valairdata 4.

Product Support - Technical Information - New Developments

Parker

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# Contents

# Parker Bioscience Filtration

## Setting the standard

Parker brings 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 scales to production systems, validation support on-site technical support, design and manufacturing of housings and filtration products.

### Committed to quality

Quality is of paramount importance to Parker. As such we have been certified to ISO9001, providing a quality management system that covers the entire organization including R & D, production, warehousing, materials management and customer support. In addition, our manufacturing facilities operate to the principles of cGMP.

Our manufacturing facilities are also certified to ISO14001 Environmental Management Standard and ISO13485 Medical Device requirements.

### Validation and product certification

To certify that Parker products meet the required regulatory and quality standards of the industries that we supply, all filters are supplied with a certificate of conformance. These certificates are linked to validation documents for both prefilter and sterilizing grade membrane filter products that define methodologies and data appropriate to each filter type. This information typically includes:

- Technical specifications.
- Biological safety testing information; USP <88> Class VI - 121C Plastics and equivalents.
- Extractable testing including 21CFR211.72 and 210.3(b), 6 for fibre release.
- Purified water filtration quality including TOC, bacterial endotoxins, conductivity and particle release.
- Chemical compatibility guidance information.
- Thermal stability.
- Correlation of an appropriate non-destructive integrity test to a defined bacterial challenge.
- EC Food contact safety specification.

### Validation support services

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

# 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 Valairdata system. Aerosol of a given droplet size is generated from Purity™ FG WO 15 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 Valairdata test. Following exposure to aerosol, the test membranes were steam sterilized 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 sterilization of membranes exposed to challenge fluid showed minimal carry over of challenge fluid into the downstream condensate samples. This indicates that during steam sterilization 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 Valairdata 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 sterilization. This suggested that there was no significant retention of aerosol on the membrane following steam sterilization. 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 sterilization procedures.

# 3. Methods

### 3.1 Test procedure

Tests were conducted using 142 mm 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 Purity™ FG WO 15 oil aerosol challenge fluid by normal extractable hydrocarbons from other cartridge components (e.g. plastic cages, cores, end caps etc).

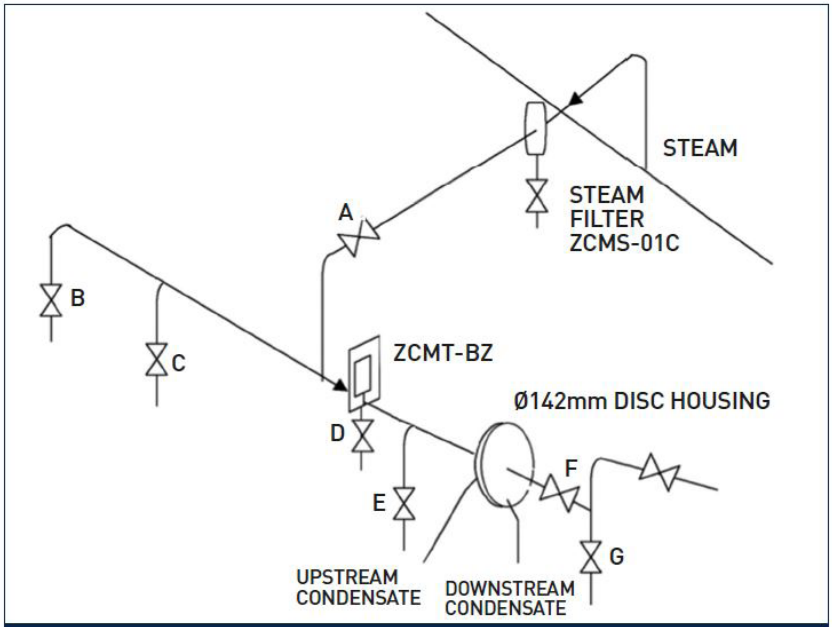


*Figure 1: Test rig for steam sterilization of aerosol loaded 142mm membrane samples*

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 E & 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 E & G was collected in individual Duran bottles previously cleaned with Trichlorofluoroethane. Condensate from the upstream and downstream drains was collected throughout the 30 minutes sterilization cycle.

**3.1.7** Following cooling of the test rig, the ø142mm disc holder was removed from the rig and integrity tested using the Valairdata. 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 sterilized in accordance with the sequence described in sections 3.1.2 to 3.1.6.

**3.1.9** Following steam sterilization, 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 sterilization. The Purity™ FG WO 15 oil 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 sterilization.
To determine the residual challenge fluid on membranes following steam sterilization, 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. 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 sterilization is extremely effective at removing residual challenge fluid from tested filter cartridges.

| 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 | 782µg |
| M7 | Challened PTFE unsteamed | 25 | 662µg |

*Table 1*

Table 2 shows the results for condensate samples collected upstream and downstream of membranes following steam sterilization. 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 carry over of challenge fluid during steam sterilization is very small.

To put the quantity of challenge fluid used during a test into context, reference can be made to a typical prefiltration 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/m$^3$**
**Class 3 < 1 mg/m$^3$**
**Class 2 < 0.1 mg/m$^3$**
**Class 1 < 0.01 mg/m$^3$**

Taking the cleanest Class 1 system with a max remaining oil content of 0.01mg/m$^3$ a 5" sterile air cartridge (e.g. ZCHT/AT) would process approximately 8000m$^3$ 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.

| Sample ID | Description | Total volume of condensate (ml) | Total volume (ml) | Solvent volume (ml) | Solvent µg/ml | Total µg |
|---|---|---|---|---|---|---|
| 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 | 10.2 | 255 |
| M2 | PTFE unchallenged downstream | 50 | 50 | 25 | 3.30 | 82 |
| M3 | Challenge upstream | 80 | 50 | 25 | 2.87 | 115 |
| M3 | Challenge downstream | 110 | 50 | 25 | 8.04 | 442 |
| M4 | Challenge upstream | 110 | 50 | 25 | 8.35 | 459 |
| M4 | Challenge downstream | 95 | 50 | 25 | 2.46 | 116 |
| M5 | Challenge upstream | 105 | 50 | 25 | 8.1 | 425 |
| M5 | Challenge downstream | 10 | 50 | 25 | 2.13 | 117 |

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

# 5. Appendix 1 - 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 Purity™ FG WO 15 oil is 0.023 and is determined by obtaining traces at various dilutions of oil/solvent beginning with 0.1g/100ml of solvent.

**The formula is as follows:**

$$\text{absorbance} = \ln_{10} \frac{10^3}{I_1 \times 1_2 \times 1_3}$$

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:

$$\frac{\text{absorbance}}{\text{oil coeficient}}$$

this gives a result in µg/ml solvent used to extract the oil from the sample.

$I_0$ = 100%
$I_1$ = 65.3%
$I_2$ = 66.6%
$I_3$ = 43.89%

The absorbance is given by:

$$\frac{\ln_{10}\ (10^3)}{(I_1 \times 1_2 \times 1_3)}$$

$$\text{absorbance} = \ln_{10} \frac{(100^3)}{66.6 \times 65.3 \times 43.89}$$

$$= 0.719$$

The amount of oil in µg/ml is given by:

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

**The sample disc was placed in 25ml of solvent therefore the total amount of oil deposited on the discs was 25 x 31.26 = 781µg.**

# 6. Technical Support Group activities

Parker 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 in-house:

- Filterability testing to optimize filter system design
- Advice on the development of integrity testing, steam sterilization and clean-in-place procedures
- Development of validation protocols
- Troubleshooting
- Facility audits to ensure continued optimization of filter use
- Operator training including filtration theory, validation, filter system design and management

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

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