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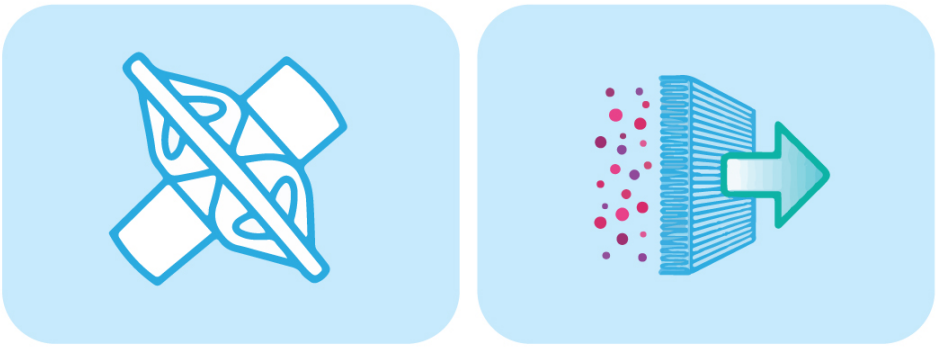
Vitalograph Cross Contamination Report for Bacterial Viral Filters



Vitalograph Cross Contamination Report for Bacterial Viral Filters

Abstract

The Vitalograph Bacterial Viral Filter (BVF) has been designed for use in pulmonary function testing to reduce the risk of cross-contamination and patient infection during testing.

Unlike barrier filters which trap expectorated matter whilst allowing viruses and bacteria to pass through, the Vitalograph BVF uses electrostatically charged material to trap expectorated matter plus bacteria and viruses. This creates a very effective protection against cross-contamination.

In addition to the hygiene benefits of using BVF, they also support productivity in pulmonary function testing by reducing the time spent cleaning and decontaminating the test equipment as the BVF is for single patient use only.

The manufacturers of the BVF barrier material, H&V (Hollingsworth and Vose) Technostat® 150 claim a BFE (Bacterial Filter Efficiency) of 99.99978% and VFE (Viral Filter Efficiency) of 99.99935%. The cross-contamination efficiency, i.e. serial patient usage of the test equipment, each with a new filter, would evidently be much higher.

Cross-contamination refers to the process by which bacteria or other micro-organisms are unintentionally transferred from one object to another, with harmful effects. The BVF prevents cross-contamination by means of reducing the amount of bioburden passing through the filter and then again back through a second filter to a level that is not detectable, based on the methods and materials used here.

This report summarises the work completed to verify that the Vitalograph BVF is effective in preventing cross-contamination. The Vitalograph BVF is a single patient use device and is to be used for pulmonary function testing only. The report confirms this by means of predicted calculations based on the filters manufactures specification, calculated using independent test lab results and demonstrated in another independent laboratory using a different protocol.

This report concludes that the Vitalograph BVF is very effective in the prevention of cross contamination. The verification acceptance criteria for the report was met as all results generated for cross-contamination efficiency against bioburden for both theoretical and practical results were higher than 99.9999%.

Verification Acceptance Criteria

The verification acceptance criteria for the report is that all results generated for cross-contamination efficiency against tested bioburden for both theoretical and practical results are equal to or higher than 99.9999%.

Verification Units

The verifications units for the 4 different tests are as follows:

Predicted Cross-Contamination Prevention – Technostat® Specification Data
N/A – This test was completed using the Specification Data supplied by the Manufacturer of the filter material H&V (Appendix 1).

Calculated Cross-Contamination Prevention
(New BVF at >in vivo challenge level – see Appendices 2&3)

This test was completed using the following:

- BFE Test: 3 BVFs; part number 28552; LOT number 1829. (Appendix 2)
- VFE Test: 3 BVFs; part number 28552; LOT number 1829. (Appendix 3)

Calculated Cross-Contamination Prevention
(BVF +7 Years at >in vivo challenge level – see Appendices 4&5)

This test was completed using the following:

- BFE Test: 3 BVFs; part number 28362 (supplied in PFT (Pulmonary Function Test) Kit part number 28372); LOT number 1026. (Appendix 4)
- VFE Test: 3 BVFs; part number 28362 (supplied in PFT (Pulmonary Function Test) Kit part number 28372); LOT number 1026. (Appendix 5)

Demonstrated Laboratory Cross-Contamination Prevention
This test was completed using the following:

- BVF-BVF Test: 40 BVFs; part number 28554; LOT Number 1827
- BVF-Alpha Flow Head: 39 BVFs; part number 28554; LOT Number 1827

Assessors

Data supplied by external agencies (Nelson Labs, Hollingsworth & Vose, Professor Colum Dunne)

Calculations completed by Vitalograph Ltd.

Equipment Used

Predicted Cross-Contamination Prevention – Technostat® Specification Data
N/A – This test was completed using the Specification Data supplied by the Manufacturer of the filter material H&V. No equipment was needed for the calculations.

Calculated Cross-Contamination Prevention – Nelson Labs, 2019 (New BVF)
N/A – This test was completed using the test results supplied by Nelson Labs. No equipment was needed for the calculations.

Calculated Cross-Contamination Prevention – Nelson Labs, 2019 (+7 Years)
N/A – This test was completed using the test results supplied by Nelson Labs. No equipment was needed for the calculations.

Laboratory Demonstrated Cross-Contamination Prevention – Professor Colum Dunne, University of Limerick, 2019

The Equipment used for the testing completed to determine the Laboratory Cross-Contamination Prevention can be split into the 2 tests completed:

BVF – BVF

- Transport swabs (101 x 16.5 mm; Ref: 80.625)
- Inoculation spreaders (Ref: 86.1569.005) from Startsted Ltd.
- Pre-set growth media plates namely plate count agar (PCA) and PBS (Phosphate Buffered Saline) Fannin Ltd.
- *Escherichia coli*, also known as *E. coli* (K12).
- 40 x Vitalograph Bacterial Viral Filters (BVFS) Part Number 28554, LOT Number 1827.
- 1 x 3 Litre Syringe: Part Number 36113; Equipment Number T2678.

BVF – Alpha Flow Head

- Transport swabs (101 x 16.5 mm; Ref: 80.625)
- Inoculation spreaders (Ref: 86.1569.005) from Startsted Ltd.
- Pre-set growth media plates namely plate count agar (PCA) and PBS (Phosphate Buffered Saline) Fannin Ltd.
- *Escherichia coli*, also known as *E. coli* (K12).
- 39 x Vitalograph Bacterial Viral Filters (BVFS) Part Number 28554, LOT Number 1827.
- 39 x Alpha Flowheads Part Number 61029 Job Number 14854.
- 1 x Alpha Touch Spirometer: Part Number 65502 Serial Number 26819.
- 1x Silicone Twin–Tubing: Part Number 42172.
- 1 x PowerSAFE – Power lead for Alpha Spirometer: Part Number 41211.
- 1 x Calibrated 3 Litre Syringe: Part Number 36113; Equipment Number T2678.
- 1 x Touch Screen Stylus: Part Number 65813.

Procedure

The Procedure for this test report can be split into 2 subsections. The subsections are as follows:

- The Cross-Contamination Prevention Procedure for the Predicted and Calculated methodologies
- The Laboratory Demonstrated Cross-Contamination Prevention Procedure

The Predicted Cross-Contamination Prevention Procedure was completed on the Technostat® filter Specification Data. The Calculated Cross-Contamination procedure was completed on the Nelson Labs test results from the testing of New BVFs and the Nelson Labs test results from testing +7 year old BVFs.

The calculation was firstly completed using the BFE and VFE values given in the Technostat® filter Specification Data sheet (see Appendix 1). This allowed for the efficiency of the Technostat® filters ability in preventing bacterial and viral cross-contamination to be predicted.

Vitalograph BVFs were then sent to Nelson Labs to be tested for BFE (see Appendix 2) and VFE (see Appendix 3). The testing was completed on 3 BVFs chosen at random from a batch. The lowest results attained for BFE and VFE was then used to calculate the BVFs' ability in preventing bacterial and viral cross-contamination.

To verify that the Vitalograph BVFs continue to function for the entirety of their shelf life, Vitalograph's BVFs that were over 7 years old were sent to Nelson Labs to be tested.

5 BVFs were chosen at random from a batch and sent, 3 BVFs were subsequently tested for BFE (see Appendix 4) and VFE (see Appendix 5).

The lowest results attained for BFE and VFE was then used to calculate the + 7 years old BVFs ability in preventing bacterial and viral cross-contamination.

The BVFs were also tested by Professor Colum Dunne in the University of Limerick. The testing completed was to assess the Vitalograph BVFs ability to prevent bioburden cross-contamination in a laboratory setting. The parts were initially tested for its ability to prevent bioburden contamination passing through a BVF onto an Alpha Flow Head at determined Flowrates (BVF-Alpha Flow Head assemblies). The testing was then conducted using BVF-BVF assemblies.

The determined flowrates used were separated into 3 sets. The sets were as follows:

- Low - less than 55 L/ min
- Medium - Between 55 L/min and 750 L/min
- High - Higher than 750 L/min

The highest flowrate the Alpha Spirometry device can test is 960 L/min. This flow rate is well in excess of what would be expected for a patient to exhale in a clinical setting. From the NHANES III reference paper, used to calculate predicted values for PEF, the highest flowrate used is for a 206cm, 30 year old Mexican American male at 13.79 L/s (827.4 L/min).

Theoretical Cross-Contamination Prevention Calculation

X = Let X equal the efficiency of the filter.

$1- X$ = This is the amount of bioburden to pass through each of the filters, as X (the efficiency) is subtracted from the total amount of bioburden.

$(1- X) \times (1- X)$ = The amount of bioburden to pass through both the first and second filter is then calculated by multiplying the amount of bioburden to pass through the first filter by the amount of bioburden that will then pass through the second filter.

$1-((1-X)^2) =$ The efficiency of the 2 filters being used in preventing cross-contamination can then be calculated by subtracting the amount of bioburden to pass through both the first and second filter from the total amount of bioburden.

Demonstrated Cross-Contamination Prevention

The Vitalograph BVF was tested for bioburden cross-contamination prevention in a laboratory environment. The efficiency of the filter was tested to calibrated flowrates. This testing was completed to determine whether the Vitalograph BVF protects against cross-contamination at various flowrates. This was completed utilizing Alpha Flow Heads, an Alpha Touch Spirometer and a 3L Syringe to deliver defined volume and flow of air simulating use in practice.

BVF-Alpha Flow Head

The BVF-Alpha Flow Head Methodology was split into 2 sub-sections: Flowrate Generation using the Alpha Touch Spirometer and 3 L Syringe and Microbial Testing.

Flowrate Generation

1. Alpha Touch was calibrated using the Calibrated 3 L Syringe.
2. In the Main Menu FVC was selected.
3. The Temperature was entered in degrees Celsius.
4. The required flowrates were divided into 3 bands: low flowrate (under 30 L/min), medium flowrate (between 55 L/min and 750 L/min) and high flowrate (over 750 L/min).
5. 5 BVFs were to be completed with a high flowrate, 29 BVFs with a medium flowrate and 5 BVFs with a low flowrate.
6. A stroke of the syringe was completed using the required speed/pressure to generate the desired flowrate.
7. The flowrate was displayed under PEF on the right of the display. This value was recorded.

Microbiology Testing

1. For each test, a BVF was placed in-line with an Alpha Flow Head.
2. In a microbiology containment hood, 1.0ml of 1 x 10^6 CFU *E. coli* was introduced by sprayer into the BVF inlet.
3. Using the calibrated 3 L Syringe, air is directed through the inoculated BVF-Alpha Flowhead assembly.
4. The BVF-Alpha Flow Head is disassembled, and separated.
5. Alpha Flow Head Inlets, outlets and internal “insert filter” (Plastic Mesh Filter) were swabbed using diluent PBS (Phosphate Buffered Saline).
6. Each swab was vortexed to free microbial cells, and the suspensions diluted serially before being spread-plated onto the PCA.
7. Incubation was at 37 degrees C, aerobically.
8. Counts were performed at 24 hours.
9. This testing was performed a stipulated above.

BVF-BVF

1. For each test, two BVFs were joined in-line using parafilm to create an airtight seal such that air flowing through the first BVF would not leak before entering the second BVF.
2. In a microbiology containment hood, 1.0ml of 1 x 106 CFU E. coli was introduced by sprayer into the first BVF inlet.
3. Using the 3 L Syringe, air is directed through the inoculated BVF assembly.
4. The joined BVFs are disassembled, and using sterile scissors each BVF inlet, filter and outlet is separated.
5. Plastic inlets and outlets were swabbed using diluent PBS (Phosphate Buffered Saline).
6. Each swab was vortexed to free microbial cells, and the suspensions diluted serially before being spread-plated onto the PCA.

Results

Predicted Cross-Contamination Prevention - Technostat® Specification Data

The Technostat® Specification Data states that the BVF's Technostat® filter should have a BFE of 99.99978% and VFE of 99.99935%. This data allows for the cross-contamination efficiency of the filter to be calculated as follows:

Bacterial Cross-Contamination Prevention

99.99978% =	the % bacterial efficiency of the filter
0.9999978 =	the bacterial efficiency of the filter in decimal
1-0.9999978 =	the amount of bacteria to pass through the first filter
2.2×10^{-6} =	
$(2.2 \times 10^{-6}) \times (2.2 \times 10^{-6})$ =	the amount of bacteria to then pass through the second filter
$1-(4.84 \times 10^{-12})$ =	the bacterial efficiency of both the filters
0.99999999999 =	
99.999999999% =	the % bacterial efficiency of the filters in preventing Cross-Contamination

Viral Cross-Contamination Prevention

99.99935% =	the % viral efficiency of the filter
0.9999935 =	the viral efficiency of the filter in decimal
1-0.9999935 =	the amount of virus to pass through the first filter
6.5×10^{-6} =	

$(6.5 \times 10^{-6}) \times (6.5 \times 10^{-6}) =$ the amount of virus to then pass through the second filter

$1-(4.225 \times 10^{-11}) =$

$0.99999999995 =$ the viral efficiency of both the filters

99.999999995% = the % viral efficiency of the filters in preventing Cross-Contamination

Theoretical Cross-Contamination Prevention – Nelson Labs Test Results (New BVF)

The efficiency of the filter was tested for both BFE and VFE by Nelson Labs (see Appendices 2 and 3 for details). The lowest BFE for the filters tested was 99.962%. The lowest VFE for the filters tested was 99.925%

Bacterial Cross-Contamination Prevention

99.962% = the % bacterial efficiency of the filter

0.99962 = the bacterial efficiency of the filter in decimal

$1 - 0.99962 =$

$3.8 \times 10^{-4} =$ the amount of bacteria to pass through the first filter

$(3.8 \times 10^{-4}) \times (3.8 \times 10^{-4}) =$ the amount of bacteria to pass through the second filter

$1 - (1.444 \times 10^{-7}) =$

$0.9999998556 =$ the bacterial efficiency of both the filters

99.99998556% = the % bacterial efficiency of the filters in preventing Cross-Contamination

Viral Cross-Contamination Prevention

99.925% =	the % viral efficiency of the filter
0.99925 =	the viral efficiency of the filter in decimal
1 - 0.99925 =	the amount of virus to pass through the first filter
7.5×10^{-4} =	
$(7.5 \times 10^{-4}) \times (7.5 \times 10^{-4})$ =	the amount of virus to then pass through the second filter
1 - (5.625×10^{-7}) =	the viral efficiency of both the filters
0.9999994375 =	
99.99994375% =	the % viral efficiency of the filters in preventing Cross-Contamination

Theoretical Cross-Contamination Prevention - Nelson Labs Test Results (+7 Years)

The efficiency of a filter that had exceeded its 'Use by Date' was tested for both BFE and VFE by Nelson Labs (see Appendices 4 and 5 for details). The lowest BFE for the filters tested was 99.9948%. The lowest VFE for the filters tested was 99.90%

Bacterial Cross-Contamination Prevention

99.9948% =	the % bacterial efficiency of the filter
0.999948 =	the bacterial efficiency of the filter in decimal
1 - 0.999948 =	the amount of bacteria to pass through the first filter
5.2×10^{-5} =	
$(5.2 \times 10^{-5}) \times (5.2 \times 10^{-5})$ =	the amount of bacteria to pass through the second filter

$1 - (2.704 \times 10^{-9}) =$	
$0.9999999973 =$	the bacterial efficiency of both the filters

99.999999973% =	the % bacterial efficiency of the filters in preventing Cross-Contamination

Viral Cross-Contamination Prevention

99.90% =	the % viral efficiency of the filter
0.9990 =	the viral efficiency of the filter in decimal
$1 - 0.9990 =$	
$1 \times 10^{-3} =$	the amount of virus to pass through the first filter
$(1 \times 10^{-3}) \times (1 \times 10^{-3}) =$	the amount of virus to then pass through the second filter
$1 - (1 \times 10^{-6}) =$	
$0.999999 =$	the viral efficiency of both the filters

99.9999% =	the % viral efficiency of the filters in preventing Cross-Contamination

Demonstrated Cross-Contamination Prevention

The cross-contamination prevention testing completed on the Vitalograph BVFs concluded that no bioburden was detected when a flowrate below 960L/min was used.

BVF-Alpha Flow Head

No bioburden growth was observed from the Alpha Flowheads when tests were completed at low or medium flow rates. Of the tests performed, only two were positive. At flow rates at or above 960L/min, two of the tests demonstrated levels of bioburden in the inlet of the Alpha Flow Head. This flow rate is well in excess of what would be expected for a patient to exhale in a clinical setting. From the NHANES III reference paper, used to calculate

predicted values for PEF, the highest flowrate is for a 206cm, 30 year old Mexican American male at 13.79 L/s (827.4 L/min).

These results indicate that, when used at low, medium or high flow rates up to 960 L/min, any bioburden transfer allowed by the BVF to Alpha Flow Heads was below the level of detection based on the methods and materials used. From this we deduce that the BVF was effective in the prevention of bioburden transfer.

BVF-BVF

The BVF-BVF assembly was a substitute model for BVF-Alpha Flow Head assembly.

No growth was observed from any second BVF in the assemblies.

This indicated that any bioburden transfer allowed by the first BVF in assemblies to the second BVF was below the level of detection based on the methods and materials used.

Conclusion

This report concludes that the Vitalograph BVF is effective in the prevention of cross-contamination. The verification acceptance criteria for the report was met as all results generated for cross-contamination efficiency against bioburden for both theoretical and practical results are equal to or higher than 99.9999%.

As can be seen the laboratory testing results generated corroborates well with the results theoretically calculated for cross-contamination efficiency.

References

NHANES III: Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general US population. Am J Respir Crit Care Med 1999; 159: 179-187.

Study CDe 19/039 Calibrated Flow Bioburden testing of Vitalograph Alpha Flow Heads and Bacterial Viral Filters (BVF): Colum Dunne. 2019; 1-7

Appendix 1 – Technostat ® Technical Data

Technical Data

Technostat® Filter Media Data Sheet

SUPERIOR
Felt & Filtration, LLC

Technostat® 150 g/m²

Technical Data	Weight	Color	Material Composition
Technostat®	150 g/m²	white	Blended Synthetic Fiber
Scrim	15 g/m²	white	Spunbond Polypropylene (Other Colors available)
Total Media Weight	165 g/m2		
Available Forms	Single or Double Scrimmed Rolls, Sheets, coils or fabricated cut parts (including heat sealed, edge sealed)		

Filtration Performance

NaCl Penetration at 32 LPM*	< 3.00%	*Tested in accordance to TSI8130 NaCl .1 micron particle size*
NaCl Efficiency at 32 LPM*	> 97.00%	*Tested in accordance to TSI8130 NaCl .1 micron particle size*
Pressure Drop at 32 LPM*	< 0.6 mm H2O	*Tested in accordance to TSI8130 NaCl .1 micron particle size*
Air Permeability**	> 190 CFM	*Tested in accordance to Spec ASTM Spec ASTM D737*
BFE Efficiency***	>99.99978	*Tested in accordance to Spec MIL-M-36954C By Nelson Labs*
VFE Efficiency***	>99.99935	*Tested in accordance to Spec MIL-M-36954C By Nelson Labs*

Testing Apparatus / Sample Size:

		*Rig:	TSI 8130 Fiter Particulate Testing Apparatus
		Sample Size:	100 cm²
		Weight Tolerance:	+ \ - 10%
**Rig:	Fazier High Pressure Tester	***Rig:	BFE/VFE Test Chamber
Flow:	0.5" Water Gauge	Challenge Level:	BFE: 4.0 x 10^6 CFU VFE: 1.9 x $10^{5\ PFU}$
		Chuck Size	3.0" Diameter Particle Size: 3.2 micron
		Test Flow:	30 L/min 0.1 m/sec

Test data included on this sheet is intended as guidance and does not constitute a specification. Data may be changed without notice

Conversion Factors:

Air Flow:	32 LPM = 10.5 CFM
Area Weight:	1 g/m2 = 0.0295 oz/y2

Appendix 2 - Nelson Labs BFE

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[redacted]
Vitalograph Ireland Ltd.
Gort Road Business Park
Ennis, Co. Clare,
IRELAND

Bacterial Filtration Efficiency (BFE) at an Increased Challenge Level Final Report

Test Article:	p/n 28552 Lot #1829
Purchase Order:	NS57984046
Study Number:	1138681-S01
Study Received Date:	07 Jan 2019
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0009 Rev 11
Deviation(s):	None

Summary: This test procedure was performed to evaluate the BFE of test articles at an increased challenge level. A suspension of *Staphylococcus aureus*, ATCC #6538, was delivered to the test article at a challenge level of greater than 10^6 colony forming units (CFU). The challenge was aerosolized using a nebulizer and delivered to the test article at a fixed air pressure and flow rate of 30 liters per minute (LPM). The aerosol droplets were generated in a glass aerosol chamber and drawn through the test article into all glass impingers (AGIs) for collection. The challenge was delivered for a one minute interval and sampling through the AGIs was conducted for two minutes to clear the aerosol chamber. The mean particle size (MPS) control was performed at a flow rate of 28.3 LPM using a six-stage, viable particle, Andersen sampler for collection.

This test procedure was modified from Nelson Laboratories, LLC (NL), standard BFE procedure in order to employ a more severe challenge than would be experienced in normal use. This method was adapted from ASTM F2101. All test method acceptance criteria were met. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820.

Challenge Flow Rate:	30 LPM
Area Tested:	Entire Test Article
Side Tested:	Oval Side
Challenge Level:	4.7×10^6 CFU
MPS:	~3.2 µm
Test Monitor Results:	Acceptable

[redacted] 17 Jan 2019
Study Completion Date

1138681-S01

801-290-7500 | nelsonlabs.com | sales@nelsonlabs.com

ks FRT0009-0001 Rev 11
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Study Number 1138681-S01
Bacterial Filtration Efficiency (BFE) at an Increased Challenge Level Final Report

Results:

Test Article Number	Total CFU Recovered	Filtration Efficiency (%)
1	1.2×10^3	99.975
2	1.8×10^3	99.962
3	5.6×10^2	99.988

The filtration efficiency percentages were calculated using the following equation:

$$\% BFE = \frac{C - T}{C} x\ 100$$

C = Challenge Level
T = Total CFU recovered downstream of the test article

Appendix 3 - Nelson Labs VFE

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Viral Filtration Efficiency (VFE) at an Increased Challenge Level Final Report

Test Article:	p/n 28552 Lot #1829
Purchase Order:	NS57984046
Study Number:	1138680-S01
Study Received Date:	07 Jan 2019
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0010 Rev 12
Deviation(s):	None

Summary: This test procedure was performed to evaluate the VFE of test articles at an increased challenge level. A suspension of ΦX174 bacteriophage was delivered to the test article at a challenge level of greater than 10^7 plaque-forming units (PFU) to determine the filtration efficiency. The challenge was aerosolized using a nebulizer and delivered to the test article at a fixed air pressure and flow rate of 30 liters per minute (LPM). The aerosol droplets were generated in a glass aerosol chamber and drawn through the test article into all glass impingers (AGIs) for collection. The challenge was delivered for a one minute interval and sampling through the AGIs was conducted for two minutes to clear the aerosol chamber. The mean particle size (MPS) control was performed at a flow rate of 28.3 LPM using a six-stage, viable particle, Andersen sampler for collection. The VFE at an Increased Challenge Level test procedure was adapted from ASTM F2101.

This test procedure was modified from Nelson Laboratories, LLC (NL), standard VFE test procedure in order to employ a more severe challenge than would be experienced in normal use. All test method acceptance criteria were met. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820.

Challenge Flow Rate:	30 LPM
Area Tested:	Entire Test Article
Side Tested:	Oval End
Challenge Level:	1.7 x 10^7 PFU
MPS:	~2.9 µm
Test Monitor Results:	Acceptable

[redacted]

17 Jan 2019
Study Completion Date

1138680-S01

Nelson Labs™
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Study Number 1138680-S01
Viral Filtration Efficiency (VFE) at an Increased Challenge Level Final Report

Results:

Test Article Number	Total PFU Recovered	Filtration Efficiency (%)
1	8.9×10^3	99.947
2	1.3×10^4	99.925
3	1.3×10^3	99.9923

The filtration efficiency percentages were calculated using the following equation:

$$\%\,VFE = \frac{C - T}{C} \times 100$$

C = Challenge Level
T = Total PFU recovered downstream of the test article

Appendix 4 - Nelson Labs BFE +7 years

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Bacterial Filtration Efficiency (BFE) at an Increased Challenge Level Final Report

Test Article:	Bacterial Viral Filter (BVF) Part Number 28362, supplied in Pulmonary Function Test (PFT) Kit Part Number 28372, LOT Number 1026
Purchase Order:	NS63104105
Study Number:	1201445-S01.1 Amended
Study Received Date:	11 Jul 2019
Study Completion Date:	22 Jul 2019
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0009 Rev 12
Deviation(s):	None

Summary: This test procedure was performed to evaluate the BFE of test articles at an increased challenge level. A suspension of *Staphylococcus aureus*, ATCC #6538, was delivered to the test article at a challenge level of greater than 10^6 colony forming units (CFU). The challenge was aerosolized using a nebulizer and delivered to the test article at a fixed air pressure and flow rate of 30 liters per minute (LPM). The aerosol droplets were generated in a glass aerosol chamber and drawn through the test article into all glass impingers (AGIs) for collection. The challenge was delivered for a one minute interval and sampling through the AGIs was conducted for two minutes to clear the aerosol chamber. The mean particle size (MPS) control was performed at a flow rate of 28.3 LPM using a six-stage, viable particle, Andersen sampler for collection.

This test procedure was modified from Nelson Laboratories, LLC (NL), standard BFE procedure in order to employ a more severe challenge than would be experienced in normal use. This method was adapted from ASTM F2101. All test method acceptance criteria were met. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820.

Challenge Flow Rate:	30 LPM
Area Tested:	Entire Test Article
Side Tested:	Side with Smiley Face
Challenge Level:	3.2 x 10^6 CFU
MPS:	~3.0 µm
Test Monitor Results:	Acceptable

[redacted]

26 Jul 2019
Amended Report Date

1201445-S01

801-290-7500 | nelsonlabs.com | sales@nelsonlabs.com

lbv FRT0009-0001 Rev 12
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Results:

Test Article Number	Total CFU Recovered	Filtration Efficiency (%)
1	1.7×10^2	99.9948
2	1.6×10^2	99.9951
3	1.2×10^2	99.9963

The filtration efficiency percentages were calculated using the following equation:

$$\% BFE = \frac{C - T}{C} x\ 100$$

C = Challenge Level
T = Total CFU recovered downstream of the test article

Amendment Justification: At the request of the sponsor, the test article was changed from "LOT #11026" to "Bacterial Viral Filter (BVF) Part Number 28362, supplied in Pulmonary Function Test (PFT) Kit Part Number 28372, LOT Number 1026".

Appendix 5 - Nelson Labs VFE +7 years

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Viral Filtration Efficiency (VFE) at an Increased Challenge Level Final Report

Test Article:	Bacterial Viral Filter (BVF) Part #28362, supplied in Pulmonary Function Test (PFT) Kit Part #28372, LOT #1026
Purchase Order:	NS63104105
Study Number:	1201446-S01
Study Received Date:	11 Jul 2019
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0010 Rev 13
Deviation(s):	None

Summary: This test procedure was performed to evaluate the VFE of test articles at an increased challenge level. A suspension of ΦX174 bacteriophage was delivered to the test article at a challenge level of greater than 10^8 plaque-forming units (PFU) to determine the filtration efficiency. The challenge was aerosolized using a nebulizer and delivered to the test article at a fixed air pressure and flow rate of 30 liters per minute (LPM). The aerosol droplets were generated in a glass aerosol chamber and drawn through the test article into all glass impingers (AGIs) for collection. The challenge was delivered for a one minute interval and sampling through the AGIs was conducted for two minutes to clear the aerosol chamber. The mean particle size (MPS) control was performed at a flow rate of 28.3 LPM using a six-stage, viable particle, Andersen sampler for collection. The VFE at an Increased Challenge Level test procedure was adapted from ASTM F2101.

This test procedure was modified from Nelson Laboratories, LLC (NL), standard VFE test procedure in order to employ a more severe challenge than would be experienced in normal use. All test method acceptance criteria were met. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820.

Challenge Flow Rate:	30 LPM
Area Tested:	Entire Test Article
Side Tested:	Side with Smiley Face
Challenge Level:	8.9×10^6 PFU
MPS:	~3.3 µm
Test Monitor Results:	Acceptable

[redacted]

23 Jul 2019
Study Completion Date

1201446-S01

Results:

Test Article Number	Total PFU Recovered	Filtration Efficiency (%)
1[a]	~9.0 x 10^3	~99.90
2	3.2 x 10^3	99.964
3	6.1 x 10^3	99.931

[a] The plate count total for this test article exceeded the countable range of 25-250 PFU/plate. As such, the results are reported as an estimate.

The filtration efficiency percentages were calculated using the following equation:

$$\%\,VFE = \frac{C - T}{C}\,x\,100$$

C = Challenge Level
T = Total PFU recovered downstream of the test article