Customer Name	Gabriel Scientific Ltd., Paramount Court, Corrig Road, Sandyford, Dublin 18.
Contact	David Woolfson
Test Requested	Microbial Strikethough test
Sample Description	Pneumapure™ filter and Bioshield fabric with SafeWeld™ Seams
Number of Samples	10
Date of Receipt	24th September 2012
Project Number	ASCR092029
Report Date	21st March 2013

Contents

Introduction:

Components of the SleepAngel™ pillow were tested for their ability to act as an effective barrier to bacteria, fungi and viruses as part of client claim validation. The SleepAngel™ pillow is purported to act as barrier which prevents the passage of microbes and allergens into the pillow, thus preventing the bedding material acting as a reservoir for allergens and pathogenic microorganisms. This barrier effect is claimed to enhance the removal of contaminants from the surface of the pillow during cleaning.

The aim of this report is to demonstrate the barrier efficacy of the following components of the SleepAngel™ pillow in comparison to a positive control and a negative control fabric:

1) Pneumapure™ filter material, called 'Filter Test Sample' in this report

2) BioShield™ Fabric with SafeWeld™ seams, called 'Seam Test Sample'

The positive control fabric was selected on the basis of it allowing maximum passage of the micro-organism under test into the collection buffer contained in the petri plate of the BioStage. Likewise negative control fabric was selected on the basis of it preventing passage of micro-organisms into the collection buffer of the petri plate.

The six microorganisms listed below were selected based on their ability to act as pathogens in immunocompromised individuals and/or cause allergic sensitisation.

Bacteria

Methicillin Resistant *Staphylococcus aureus* (MRSA) (ref. 1)

Pseudomonas aeruginosa (ref. 2)

Fungi

Candida albicans (ref. 3)

Aspergillus niger (a fungal pathogen but intended as a surrogate for more harmful filamentous fungi) (ref.4)

Viruses

Influenza Type A (ref 5)

Adenovirus 5 (ref 5)

The report is divided into two parts, Part 1 details the methods and results for the fungi and bacteria and Part 2 describes the methods and results for the viruses.

An overall discussion and conclusion is included at the end of the report.

Part 1: Fungi and Bacteria:

Part 1 Materials and Methods

All work was performed under aseptic conditions. Culture media and dilution buffers were autoclaved at 121°C for 15 minutes. BSA solution was filtered prior to use through a 0.22 µm pore filter. Test materials were cut to dimensions of approximately 15 x 15 cm and were sterilised at a temperature of 105°C for 30 minutes.

1 The vacuum pump and BioStage impaction apparatus was checked prior to use. The BioStage apparatus was sterilised with 70% alcohol prior to use and allowed to dry in laminar cabinet.

1. The flow rate on the pump was ascertained using a rotameter and was adjusted to 28.5 litres per minute (lpm).
2. For bacterial work an overnight cell culture was grown in Tryptic Soy Broth and adjusted to a target concentration of 5 x 10^7 cfu/ml in BSA (3 g/l) in order to simulate "dirty" conditions.
3. A sterile petri dish was placed into the lower part of the chamber. An aliquot of PBS (20 ml) was placed into the petri-dish
4. The control/test material was placed into the upper chamber of the BioStage apparatus and was gently clamped into position using the lid. Once secured, a seal was formed using the clamping springs connected to the lower portion of the apparatus.
5. The bacteria or fungi of interest were aliquoted on the surface of the test material through the spout and the pump was switched on for 5 minutes.
6. During operation care was taken not to move the apparatus in order to insure no loss of collecting buffer from the lower part of the chamber.
7. After 5 minutes of vacuum being applied to the test material the upper part of the BioStage apparatus was carefully removed and the PBS from the lower part of the chamber was transferred to a sterile tube.
8. The recovered samples were subsequently held on ice for no greater than 2 hours and were analysed for the presence of viable colony forming units.
9. Microbial recovery in the PBS collecting buffer was ascertained by serially diluting the recovered buffer in sterile PBS by a factor of ten. The neat and diluted samples were aliquoted on an appropriate agar. The aliquot was then set in appropriate growth agar (Potato Dextrose Agar, YM agar, Tryptic Soy Agar and Mannitol Salt Agar for *A.*

niger, *C. albicans*, *P. aeruginosa* and Methicillin Resistant *Staphylococcus aureus* respectively).

10. The poured plates were allowed to cool and solidify and were incubated at 37°C for 24 hours for the enumeration of both bacterial species and at 25°C, and 30°C for 48 hours for in order to visualise *A. niger* and *C. albicans* respectively.
11. Calculation of the recovery of colony forming units (CFU) in the collecting buffer (PBS) following challenge of the controls and test material with the microbes of interest with airflow was performed as follows,

*** Colony Count x (1/volume plated) x (Dilution factor) x total volume**.

- Colony Count is the average number of CFU obtained from duplicate analysis.
- The volume plated refers to the volume of each sample aliquoted on the petri-dish.
- The dilution factor is the level of dilution from the neat extract that the cfu count was ascertained from.
- The total volume refers to the volume of collecting buffer (PBS) used to collect microbes that passed through the material (in the case of this experiment the volume was 20 ml).

12. The percentage microbial permeability of the positive control was calculated as follows.

    (Average total CFU recovered/working concentration) x 100

Part 1 Results

Pseudomonas aeruginosa

Sample description	Dilution	Volume plated	Colony Count	Average Count	Concentration Recovered *
Working Stock	1 x 10^{0}	100 µl	TNTC	43	4.3 x 10^{7} cfu/ml
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		TNTC		
	1 x 10^{-4}		TNTC		
	1 x 10^{-5}		**46, 41**		
	1 x 10^{-6}		4, 6		
Positive Control 1	1 x 10^{0}	200 µl	TNTC	35	3.5 x 10^{7} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		TNTC		
	1 x 10^{-4}		**28, 38**		
Positive Control 2	1 x 10^{0}	200 µl	TNTC	22	2.2 x 10^{7} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		TNTC		
	1 x 10^{-4}		**21, 23**		
Positive Control 3	1 x 10^{0}	200 µl	TNTC	27	2.7 x 10^{7} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		TNTC		
	1 x 10^{-4}		31, 23		
Negative Control 1	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		
Negative Control 2	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		
Negative Control 3	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		

Sample description	Dilution	Volume plated	Colony Count	Average Count	Concentration Recovered *
Filter Test Sample 1	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Filter Test Sample 2	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Filter Test Sample 3	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 1	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 2	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 3	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		

*** Colony Count x (1/volume plated) x (Dilution factor) x total volume**.

For the purpose of ascertaining the working stock concentration the total volume was 1ml, as 1 ml was applied to the surface. For test samples the total volume was 20ml, all of which was recovered.

Working stock = 4.3×10^{7} cfu

Average positive control recovery = 2.8×10^{7} cfu, negative control recovery = 0 cfu

filter test sample recovery = 0 cfu, seam test sample = 0 cfu

Recovery of *P. aeruginosa*:

= (Average total CFU recovered/working concentration) x 100

Positive control

= $(2.8 \times 10^{7} / 4.3 \times 10^{7}) \times 100$

= 65.11%

Negative control

= $(0 / 4.3 \times 10^{7}) \times 100$

= 0%

Filter Test Sample

= $(0 / 4.3 \times 10^{7}) \times 100$

= 0%

Seam Test Sample

= $(0 / 4.3 \times 10^{7}) \times 100$

= 0%

It was found that the negative control, filter material and seam which also incorporated the bioshield encasement did not show any evidence of microbial penetration with all analysis demonstrating 0% recovery values following *P. aeruginosa* challenge

Methicillin Resistant *Staphylococcus aureus (MRSA)*

Sample description	Dilution	Volume plated	Colony Count	Average Count	Concentration Recovered *
Working Stock	1 x 10^{0}	100 µl	TNTC	93	9.35 x 10^{7} cfu/ml
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		TNTC		
	1 x 10^{-4}		TNTC		
	1 x 10^{-5}		**99, 88**		
	1 x 10^{-6}		7, 12		
Positive Control 1	1 x 10^{0}	200 µl	TNTC	123	1.23 x 10^{7} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		**130, 117**		
	1 x 10^{-4}		8, 16		
Positive Control 2	1 x 10^{0}	200 µl	TNTC	137	1.37 x 10^{7} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		**155, 119**		
	1 x 10^{-4}		9, 14		
Positive Control 3	1 x 10^{0}	200 µl	TNTC	157	1.57 x 10^{7} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		**148, 167**		
	1 x 10^{-4}		26, 14		
Negative Control 1	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		
Negative Control 2	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		
Negative Control 3	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		

Sample description	Dilution	Volume plated	Colony Count	Average Count	Concentration Recovered *
Filter Test Sample 1	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Filter Test Sample 2	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Filter Test Sample 3	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 1	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 2	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 3	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		

*** Colony Count x (1/volume plated) x (Dilution factor) x total volume**.

For the purpose of ascertaining the working stock concentration the total volume was 1ml, as 1 ml was applied to the surface. For test samples the total volume was 20ml, all of which was recovered.

Working stock = 9.35×10^{7} cfu

Average positive control recovery = 1.39×10^{7} cfu Average negative control recovery = 0 cfu

Average filter test sample recovery = 0 cfu Average seam test sample = 0 cfu

Recovery of MRSA:

= (Average total CFU recovered/working concentration) x 100

Positive control

= $(1.39 \times 10^{7}/9.35 \times 10^{7}) \times 100$

= 65.11%

Negative control

= $(0/9.35 \times 10^{7}) \times 100$

= 0%

Filter Test Sample

= $(0/9.35 \times 10^{7}) \times 100$

= 0%

Seam Test Sample

= $(0/9.35 \times 10^{7}) \times 100$

= 0%

It was found that the negative control, filter material and seam which also incorporated the bioshield encasement did not show any evidence of microbial penetration with all analysis demonstrating 0% recovery values following MRSA challenge

Candida albicans

Sample description	Dilution	Volume plated	Colony Count	Average Count	Concentration Recovered *
Working Stock	1 x 10^{0}	100 µl	TNTC	376	3.76 x 10^{6} cfu/ml
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		**362, 391**		
	1 x 10^{-4}		44, 50		
Positive Control 1	1 x 10^{0}	200 µl	TNTC	103	1.03 x 10^{6} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		**94, 113**		
	1 x 10^{-3}		11, 12		
Positive Control 2	1 x 10^{0}	200 µl	TNTC	125	1.25 x 10^{6} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		**128, 122**		
	1 x 10^{-3}		16, 16		
Positive Control 3	1 x 10^{0}	200 µl	TNTC	130	1.3 x 10^{6} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		**138, 122**		
	1 x 10^{-3}		11, 10		
Negative Control 1	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		
Negative Control 2	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		
Negative Control 3	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		

Sample description	Dilution	Volume plated	Colony Count	Average Count	Concentration Recovered *
Filter Test Sample 1	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Filter Test Sample 2	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Filter Test Sample 3	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 1	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 2	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 3	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		

Colony Count x (1/volume plated) x (Dilution factor) x total volume.

For the purpose of ascertaining the working stock concentration the total volume was 1ml, as 1 ml was applied to the surface. For test samples the total volume was 20ml, all of which was recovered.

Working stock = 3.76×10^{6} cfu

Average positive control recovery = 1.19×10^{6} cfu Average negative control recovery = 0 cfu

Average filter test sample recovery = 0 cfu Average seam test sample = 0 cfu

Recovery of *C. albicans*:

= (Average total CFU recovered/working concentration) x 100

Positive control

= $(1.19 \times 10^{6} / 3.76 \times 10^{6}) \times 100$

= 31.64%

Negative control

= $(0 / 3.76 \times 10^{6}) \times 100$

= 0%

Filter Test Sample

= $(0 / 3.76 \times 10^{6}) \times 100$

= 0%

Seam Test Sample

= $(0 / 3.76 \times 10^{6}) \times 100$

= 0%

It was found that the negative control, filter material and seam which also incorporated the bioshield encasement did not show any evidence of microbial penetration with all analysis demonstrating 0% recovery values following *C. albicans* challenge.

Aspergillus niger

Sample description	Dilution	Volume plated	Colony Count	Average Count	Concentration Recovered *
Working Stock	1 x 10^{0}	100 µl	TNTC	81.5	8.15 x 10^{5} cfu/ml
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		TNTC		
	1 x 10^{-3}		76, 87		
	1 x 10^{-4}		16, 9		
Positive Control 1	1 x 10^{0}	200 µl	TNTC	41	4.1 x 10^{5} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		42, 40		
	1 x 10^{-3}		5, 4		
Positive Control 2	1 x 10^{0}	200 µl	TNTC	38	3.8 x 10^{5} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		37, 39		
	1 x 10^{-3}		2, 3		
Positive Control 3	1 x 10^{0}	200 µl	TNTC	34	3.4 x 10^{5} cfu
	1 x 10^{-1}		TNTC		
	1 x 10^{-2}		28, 40		
	1 x 10^{-3}		4, 3		
Negative Control 1	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		
Negative Control 2	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		
Negative Control 3	1 x 10^{0}	200 µl	0	0	0 cfu
	1 x 10^{-1}		0		

Sample description	Dilution	Volume plated	Colony Count	Average Count	Concentration Recovered *
Filter Test Sample 1	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Filter Test Sample 2	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Filter Test Sample 3	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 1	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 2	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		
Seam Test Sample 3	1×10^{0}	200 µl	0	0	0 cfu
	1×10^{-1}		0		

*** Colony Count x (1/volume plated) x (Dilution factor) x total volume**.

For the purpose of ascertaining the working stock concentration the total volume was 1ml, as 1 ml was applied to the surface. For test samples the total volume was 20ml, all of which was recovered.

Working stock = 8.15×10^{5} cfu

Average positive control recovery = 3.76×10^{5} cfu Average negative control recovery = 0 cfu

Average filter test sample recovery = 0 cfu Average seam test sample = 0 cfu

Recovery of *A. niger*:

= (Average total CFU recovered/working concentration) x 100

Positive control

= $(3.76 \times 10^{5} / 8.15 \times 10^{5}) \times 100$

= 46.13%

Negative control

= $(0 / 8.15 \times 10^{5}) \times 100$

= 0%

Filter Test Sample

= $(0 / 8.15 \times 10^{5}) \times 100$

= 0%

Seam Test Sample

= $(0 / 8.15 \times 10^{5}) \times 100$

= 0%

It was found that the negative control, filter material and seam which also incorporated the bioshield did not show any evidence of microbial penetration with all analysis demonstrating 0% recovery values following *A. niger* challenge.

Part 2: Viruses:

Part 2 Materials and Methods

All work was performed under aseptic conditions. Sterile culture media, used in propagation of virus host cell lines and in sample diluents, were supplemented with an antibiotic-antimycotic to minimise risk of contamination.

1. Positive and Negative Control fabrics, SleepAngel™ Filter Material and Blue Fabrics were cut to dimensions of approximately 15 x 15 cm and were sterilised at a temperature of 105°C for 30 minutes.
2. The BioStage apparatus and vacuum pump was set up and operated as described in Part 1 Materials and Methods, except that sterile culture media (20ml for Influenza or 10ml for Adenovirus), specific to the test virus, was used to collect any virus passing through the fabric under test. 1ml of the test virus (Adenovirus or Influenza) was placed on the upper side of the test chamber on the test fabric and the vacuum pump was operated for 5min as described previously. The apparatus was sterilised with 70% alcohol between each test sample.
3. The recovered samples were immediately kept on ice until analysis.
4. Virus passage through the various fabrics was determined by performing 10-fold serial dilutions from 10^{-1} to 10^{-9} of the culture media recovered from the Biostage petri plates. The neat and diluted samples were transferred to pre-prepared virus-specific host cell lines and incubated at 37^{0}C with 5% CO_2 until evidence of virus growth was observed, known as cytopathic effects (CPE). The quantity of adenovirus or influenza virus present in the serially diluted culture media was then determined using the Tissue Culture Infectious Dose ($TCID_{50}$) Assay, a standard technique in virology. The average of 3 separate titrations was obtained. The Recovery of virus from the test and control fabrics was calculated by dividing the quantity of virus recovered ($TCID_{50}$) in the samples by the titre of the working virus stock applied.

$TCID_{50}$ is calculated using the Karber Formula:

Karber Formula = LLD + LI (S-0.5)

LLD = reciprocal of the Log of the lowest dilution plated

Li= logarithmic interval S= Sum of the Mortalities (CPE) at each dilution

Part 2 Results

Influenza A

Sample description	Dilution Volume titered	$TCID_{50}$/ml calculated	Average Virus Recovered $TCID_{50}$/ml *
Working Virus Stock	100 µl	1.78×10^{9}	1.78×10^{7}
Positive Control 1	100 µl	5.62×10^{6}	7.08×10^{6}
Positive Control 2	100 µl	1.00×10^{7}	
Positive Control 3	100 µl	5.62×10^{6}	
Negative Control 1	100 µl	0	0
Negative Control 2	100 µl	0	
Negative Control 3	100 µl	0	
Filter Test Sample 1	100 µl		0
Filter Test Sample 2	100 µl	0	
Filter Test Sample 3	100 µl	0	
Seam Test Sample 1	100 µl	0	0
Seam Test Sample 2	100 µl	0	
Seam Test Sample 3	100 µl	0	

***Average Influenza Virus Recovered TCID50/ml**.

For the purpose of ascertaining the working stock concentration, the virus stock was initially diluted 1/5 and then 1 ml was applied to the fabric surface. For test samples a total volume of 20ml was used in the petri plates to collect the virus passing through the fabric, all of which was recovered. Therefore the overall dilution of the virus stock was 1/100.

Working stock = 1.77×10^7 $TCID_{50}$/ml

Average positive control recovery = 7.08×10^6 $TCID_{50}$/ml

Average negative control recovery = 0 $TCID_{50}$/ml

Average filter test sample recovery = 0 $TCID_{50}$/ml

Average seam test sample = 0 $TCID_{50}$/ml

Recovery of *Influenza A*:

= (Average $TCID_{50}$/ml recovered/working $TCID_{50}$/ml) x 100

Positive control

= $(7.08 \times 10^6 / 1.78 \times 10^7) \times 100$

= 39.77%

Negative control

= $(0 / 1.78 \times 10^7) \times 100$

= 0%

Filter Test Sample

= $(0 / 1.78 \times 10^7) \times 100$

= 0%

Seam Test Sample

= $(0 / 1.78 \times 10^7) \times 100$

= 0%

It was found that the negative control, filter material and seam which also incorporated the bioshield did not show any evidence of microbial penetration with all analysis demonstrating 0% recovery values following *Influenza A* challenge.

Adenovirus

Sample description	Dilution Volume titered	$TCID_{50}$/ml calculated	Average Virus Recovered $TCID_{50}$/ml *
Working Virus Stock	100 µl	3.16×10^8	6.31×10^6
Positive Control 1	100 µl	3.16×10^6	2.70×10^6
Positive Control 2	100 µl	1.77×10^6	
Positive Control 3	100 µl	3.16×10^6	
Negative Control 1	100 µl	0	0
Negative Control 2	100 µl	0	
Negative Control 3	100 µl	0	
Filter Test Sample 1	100 µl		0
Filter Test Sample 2	100 µl	0	
Filter Test Sample 3	100 µl	0	
Seam Test Sample 1	100 µl	0	0
Seam Test Sample 2	100 µl	0	
Seam Test Sample 3	100 µl	0	

***Average Adenovirus Recovered TCID50/ml**.

For the purpose of ascertaining the working stock concentration, the virus stock was initially diluted 1/5 and then 1 ml was applied to the fabric surface. For test samples a total volume of 10ml was used in the petri plates to collect the virus passing through the fabric, all of which was recovered. Therefore the overall dilution of the virus stock was 1/50.

Working stock = 6.31×10^6 $TCID_{50}$/ml

Average positive control recovery = $2.70 \times 10^6 TCID_{50}$/ml

Average negative control recovery = 0 $TCID_{50}$/ml

Average filter test sample recovery = 0 $TCID_{50}$/ml

Average seam test sample = 0 $TCID_{50}$/ml

Recovery of *Adenovirus*:

= (Average $TCID_{50}$/ml recovered/working $TCID_{50}$/ml) x 100

Positive control

= $(2.70 \times 10^6 / 6.31 \times 10^6) \times 100$

= 42.78%

Negative control

= $(0 / 6.31 \times 10^6) \times 100$

= 0%

Filter Test Sample

= $(0 / 6.31 \times 10^6) \times 100$

= 0%

Seam Test Sample

= $(0 / 6.31 \times 10^6) \times 100$

= 0%

It was found that the negative control, filter material and seam which also incorporated the bioshield did not show any evidence of microbial penetration with all analysis demonstrating 0% recovery values following *Adenovirus* challenge.

Discussion

From the data presented here it is clear that the Pneumapure™ filter is a 100% effective barrier against bacterial and fungal penetration as evidenced by the absence of colony forming units (CFU) being detected in the collection buffer after subjecting them to an airflow of 28.5 LPM. The barrier effect was also demonstrated for virus in Part 2 of this report.

Furthermore, the SafeWeld™ seam, incorporated with BioShield™ fabric, also was a 100% effective microbial barrier, as no penetration of the bacteria, fungi or viruses were obtained.

Conclusion

Based on the evidence presented here it is clear that the PneumaPure™ filter and hermetically sealed SafeWeld™ seam, incorporating the BioShield fabric, are highly effective barriers against the bacteria, fungi and viruses that were tested in this report.

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Technical Manager

*** End of Report***

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