purafil
Filtration Group®

PRODUCT BULLETIN
HEALTH CARE PARTICULATE RESPIRATOR AND SURGICAL APPAREL, KN95

Key Features:
- KN95 rating according to GB2626-2006
- FDA cleared for surgical apparel
- Hydrophobic cover and inner layers
- Adjustable nose clip
- Ear bands designed for comfort

Material Composition:
- Straps – Blended polypropylene and polyethylene
- Nose Clip – Polypropylene and aluminum
- Filter – Polypropylene melt blown
- Dust Layer – Electrostatic fabric
- Coverweb - Polypropylene spunbond
- Inner Layer - Polypropylene spunbond
- Optional Antimicrobial Layer - Puraward Fiber
- Not made with natural rubber latex
- Approximate weight of product: 0.23 oz.

Approvals and Standards:
- KN95 rated particulate respirator
- Meets GB2626-2006 requirements for a minimum 95% filtration efficiency for solid and liquid aerosols that do not contain oil.
- FDA cleared for surgical apparel

Use For:
- Intended to be worn by healthcare personnel during general and plastic surgical procedures.
- Always follow User Instructions and use in manners as indicated

Do Not Use For:
- DO NOT use in industrial settings
- DO NOT use for gases or vapors (i.e. anesthetic gases such as isoflurane or vapors from sterilants such as glutaraldehyde.)
- DO NOT use in any manner not indicated in the User Instructions

Antimicrobial Fibers:
This respirator (FACEMASK-PWD-CS) is available with Puraward fiber (PWF) technology. The PuraWard Fiber is a high efficiency fiber embedded with copper and silver ions that jointly attack bacteria and viruses. PuraWard fiber has been successfully applied to air filters, textiles, and respiratory masks approved by the FDA for their antibacterial and antiviral properties in surgical environments.

Puraward fiber has been tested to remove the following microbials:

Virus	Inhibition Rate
H1N1	99.91%/ 5 minutes contact
H7N9	99.98%/5 minutes contact
SARS	99.58%/5 minutes contact

Bacteria	Killing Rate
S. aureus	99.95%/ 1 hour contact
E. Coli	99.96%/ 1 hour contact
C. albicans	98.90%/ 24 hours contact

Time Use Limitation:

Respirator may be used until damaged, breathing becomes difficult or contaminated with blood or body fluids. Discard after every use when used for surgical procedures. Follow national, state, local, and facility infection control guidance and policies.

Shelf Life and Storage:

- 5 years from the date of manufacture
- Store respirators in the original packaging, away from contaminated areas, dust, sunlight, extreme temperatures, excessive moisture, and damaging chemicals
- Store in temperatures between -22°F (-30°C) and +104°F (+40°C) and not exceeding 80% RH

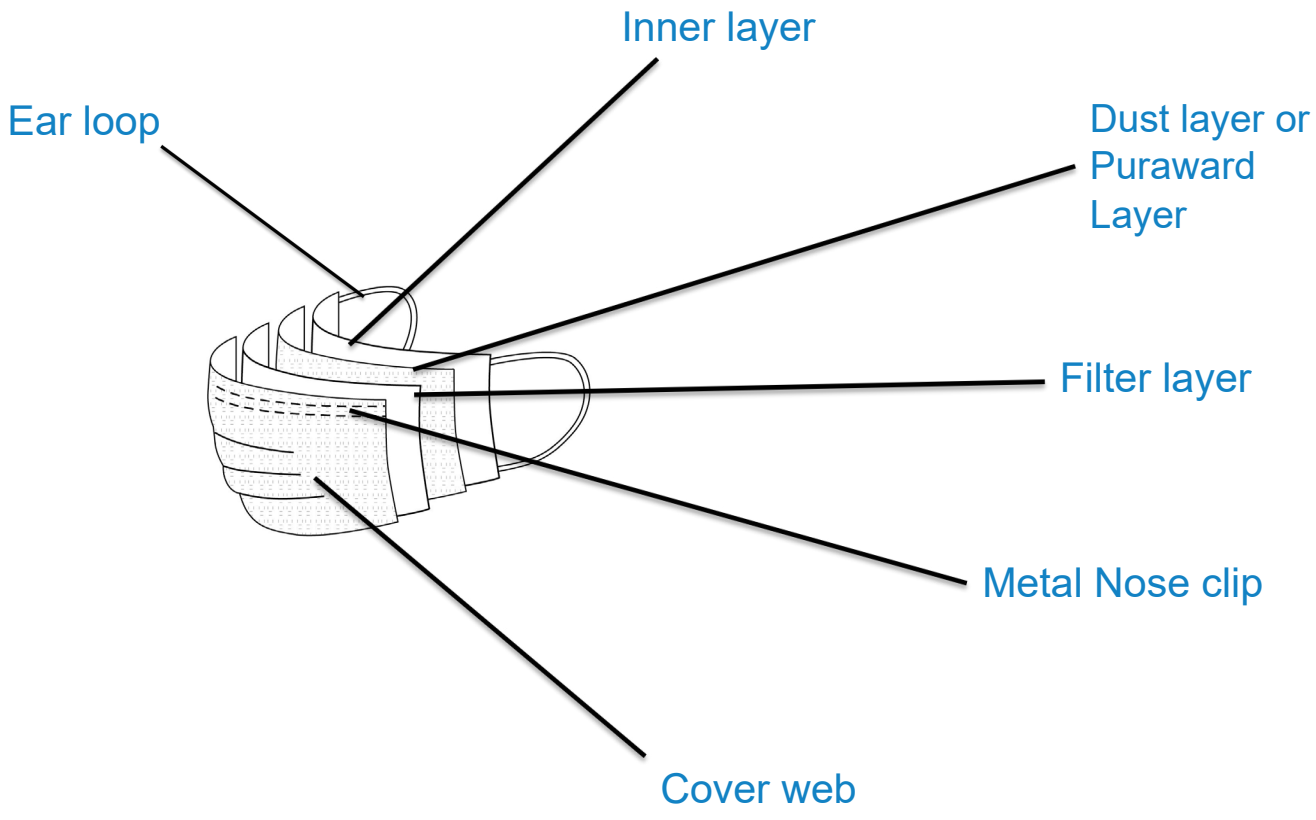
WARNING!

This respirator helps protect against certain particulate contaminants but does not eliminate exposure to or the risk of contracting any disease or infection. Before use, the wearer must read and understand the User Instructions provided as a part of the product packaging. Follow all local regulations. Misuse may result in sickness or death. For correct use, consult supervisor and the User Instructions.

Ordering Information:

Product Code	Description	UPC	Each/ Box	Box/ Case	Case/ Pallet	Pallet / 20' Container	MOQ
FACEMASK-CS	Healthcare Particulate Respirator, KN95	850011212820	50	12	36	10	216,000
FACEMASK-PWD-CS	Healthcare Particulate Respirator with Puraward Fiber, KN95	850011212837	50	12	36	10	216,000

MASK COMPOSITION



purafil Filtration Group®

COMPARISON OF FACEPIECE RESPIRATOR CLASSES

Based on this comparison, it is reasonable to consider China KN95, AS/NZ P2, Korea 1st Class, and Japan DS FFRs as "equivalent" to US NIOSH N95 and European FFP2 respirators, for filtering non-oil-based particles such as those resulting from wildfires, PM 2.5 air pollution, volcanic eruptions, or bioaerosols (e.g. viruses). However, prior to selecting a respirator, users should consult their local respiratory protection regulations and requirements or check with their local public health authorities for selection guidance.

Certification/ Class (Standard)	N95 (NIOSH-42C FR84)	FFP2 (EN 149-2001)	KN95 (GB2626-2006)	P2 (AS/NZ 1716:2012)	Korea 1st Class (KMOEL - 2017-64)	DS (Japan JMHLW-Notification 214, 2018)
Filter performance – (must be ≥ X% efficient)	≥ 95%	≥ 94%	≥ 95%	≥ 94%	≥ 94%	≥ 95%
Test agent	NaCl	NaCl and paraffin oil	NaCl	NaCl	NaCl and paraffin oil	NaCl
Flow rate	85 L/min	95 L/min	85 L/min	95 L/min	95 L/min	85 L/min
Total inward leakage (TIL)* – tested on human subjects each performing exercises	N/A	≤ 8% leakage (arithmetic mean)	≤ 8% leakage (arithmetic mean)	≤ 8% leakage (individual and arithmetic mean)	≤ 8% leakage (arithmetic mean)	Inward Leakage measured and included in User Instructions
Inhalation resistance – max pressure drop	≤ 343 Pa	≤ 70 Pa (at 30 L/min) ≤ 240 Pa (at 95 L/min) ≤ 500 Pa (clogging)	≤ 350 Pa	≤ 70 Pa (at 30 L/min) ≤ 240 Pa (at 95 L/min)	≤ 70 Pa (at 30 L/min) ≤ 240 Pa (at 95 L/min)	≤ 70 Pa (w/valve) ≤ 50 Pa (no valve)
Flow rate	85 L/min	Varied – see above	85 L/min	Varied – see above	Varied – see above	40 L/min
Exhalation resistance - max pressure drop	≤ 245 Pa	≤ 300 Pa	≤ 250 Pa	≤ 120 Pa	≤ 300 Pa	≤ 70 Pa (w/valve) ≤ 50 Pa (no valve)
Flow rate	85 L/min	160 L/min	85 L/min	85 L/min	160 L/min	40 L/min
Exhalation valve leakage requirement	Leak rate ≤ 30 mL/min	N/A	Depressurization to 0 Pa ≥ 20 sec	Leak rate ≤ 30 mL/min	visual inspection after 300 L /min for 30 sec	Depressurization to 0 Pa ≥ 15 sec
Force applied	-245 Pa	N/A	-1180Pa	-250 Pa	N/A	-1,470 Pa
CO_2 clearance requirement	N/A	≤ 1%	≤ 1%	≤ 1%	≤ 1%	≤ 1%

*Japan JMHLW-Notification 214 requires an Inward Leakage test rather than a TIL test.

Purafil, Inc.
2654 Weaver Way
Doraville, GA 30340, USA

PuraWard Testing Results

Purafil has tested its proprietary anti-viral / anti-bacterial filters with independent laboratories and collected the results in the following pages.

Testing for E. Coli was completed by Sciessent in Massachusetts and a 99.96% kill rate was demonstrated.

Testing for 2013 Influenza A (H7N9) was completed by MicroBac in Virginia and a 3.76 log10 reduction was demonstrated. This is equivalent to 99.98% inactivation rate. (Note that because viruses are not alive they cannot be killed, but can be inactivated.)

Enclosed are the detailed results which demonstrate that Purafil PuraWard materials are suitable for protecting people and processes from the damaging effects of bacteria and viruses.

Tim Bryarly

Product Manager

TO: Jacki Traynor

FROM: Linda Patenaude

DATE: October 20, 2015

RE: Request for Sciessent LLC Laboratory Test Method

Jacki,

I received your email today requesting an official copy of the Sciessent test method that was used to perform assay number TXT1-15-576 for your customer.

The document attached, titled “LAB-TM-203: AATCC100 Test Method” is the internal document used by Sciessent to perform AATCC100 at this facility. This document is confidential.

Also, attached is a copy of the test report TXT1-15-576 for your customer.

If you or your customer have any questions pertaining to this test method, do not hesitate to contact me.

Respectfully,

Linda Patenaude

Certificate of Performance

Purafil

Assay Number:	TXT1-15-576	Indicate Lab Trial, Mill Trial or N/A	N/A
Test Articles:	Textiles	Test Organism:	E. coli
Sample Size:	~2" x 2"	ATCC#:	25922
Start Date:	8/6/2015	End Date:	8/7/2015
Test Method	AATCC100 (LAB-TM-203)	Rev#	0

Sample Identification	Organism Count (CFU/ml) Zero Contact Time	1 Hour Contact Time	Percent Reduction**
Assay (+)	3.6×10^5	2.8×10^5	22.22%
Assay (-)	<100*	<100*	N/A
2015-07-13-PBF-Xply Sciessent ID# 150714-1B, Rep 1		<100*	99.96%
2015-07-13-PBF-Xply Sciessent ID# 150714-1B, Rep 2		<100*	99.96%
2015-07-13-PBF-Xply Sciessent ID# 150714-1B, Rep 3		<100*	99.96%

Testing was performed in accordance with standard operating procedures of Sciessent LLC.

Notes: * *≤100 = Limits of detection of assay.*
** *Percent reduction calculated using: Assay + T1 hour.*

Prepared By: Mary Doan *Date:* 8/11/15

Reviewed By: Linda Pateraude *Date:* 8/11/15

The Agion® Antimicrobial is presently registered by the United States Environmental Protection Agency as a preservative and bacteriostatic agent for use in treated articles under 40 CFR 152.25a. This technical data is provided to substantiate the efficacy of the antimicrobial compound. However, the data are not intended to support or endorse public health claims for treated articles.

1. Purpose

The purpose of this procedure is to provide instructions for the inoculation, extraction, dilution, and evaluation of the antibacterial activity of test articles assayed by AATCC 100 test method.

2. Scope

This procedure applies to all test articles and controls for antibacterial assessment submitted to the Microbiology laboratory or an outside testing laboratory for the purpose of determining the efficacy of the Agion® antimicrobial per AATCC100.

3. Reference Documents

3.1 LAB-SOP-201: Receipt, Inventory of AATCC Organisms
3.2 LAB-SOP-001 Receipt of Laboratory Samples and Sample Submission
3.3 LAB-SOP-002: Generation of Certificate of Performance
3.4 AATCC TM 100-1999: AATC Test Method 100-1999: Antibacterial Finishes on Textile Materials

4 Attachments

4.1 Attachment #1: Modified AATCC 100 Test Method

5 Equipment/Reagent

5.1 Equipment List

5.1.1 Bio-Safety Cabinet
5.1.2 Various temperature incubators
5.1.3 Sterile Corning cell culture flasks, vented
5.1.4 Sterile 17 x 100 mm test tubes
5.1.5 Various size sterile pipettes and sterile pipette tips
5.1.6 Automated pipettors
5.1.7 Vortex mixers
5.1.8 Sterile Inoculum spreaders
5.1.9 Spectrophotometer
5.1.10 Spectrophotometer cuvettes

5.2 Reagents

5.2.1 Sterile filtered 70% Isopropyl Alcohol
5.2.2 Sterile Butterfield's Phosphate Buffer
5.2.3 Liquid and solid media to support growth of bacteria
5.2.4 TAT Broth or Neutralizing solution

6 Safety Precautions

6.1 All employees performing this procedure must wear appropriate laboratory clothing. (i.e. gloves, safety glasses, no open toed shoes).
6.2 Tape all media plates with adhesive tape and dispose them into the red bio-hazardous containers in the Microbiology laboratory.

Decontaminate all liquid suspensions containing live ATCC organisms with Bleach. Allow the mixture to sit for approximately 30 minutes. Discard all liquid into the Microbiology sinks then dispose of any disposable containers into the red bio-hazardous containers. If any glassware is to be reused, place the decontaminated glassware in the glass prep area for cleaning and sterilization.

7 Procedure

NOTE All samples submitted for microbiology testing must be submitted per LAB-SOP-001: Receipt of Laboratory Samples and Sample Submission. All documentation of this procedure is to be recorded on Attachment #1 of this procedure.

NOTE Preparation of ATCC organisms must be completed approximately 18-24 hours prior to the execution of this procedure. Preparations of ATCC organisms are to be prepared per SOP: LAB-SOP-201: Receipt, Inventory of AATCC Organisms. Yeast like organisms may require a longer incubation time than 24 hours. Refer to the supplier's directions for these organisms and timeframes of incubation.

7.1 Sample Preparation

NOTE If samples have been prepared by submitter, verify that the amount of samples that have been received corresponds with the test request form. If discrepancies occur, notify the Laboratory Manager or Study Director immediately.

NOTE: Preliminary Sample Evaluation of Treated and Untreated Samples:
Cut (1) 2" x 2" sample. Inoculate with 1ml of Butterfield's buffer. Observe test article for spillage from article. If spillage is observed add another 2" x 2" sample. The number of samples will vary depending on the fiber type. Record the number of samples used on worksheet.

7.1.1 Refer to table I and II for sample preparation. Specific details of sample preparation are to be given on the sample submission form per LAB-SOP-001: Receipt of Laboratory Samples and Sample Submission.

Table I: Sample Preparation

Treated Samples	Prepare the appropriate number of 2" x 2" sample for each treated article for T post incubation testing. Place sample into a 75 cm^2 cell culture .
Untreated Samples	Prepare a minimum of two (2) samples for each untreated for T_0 and T post incubation testing. Place sample into a 75 cm^2 cell culture

Table II: Assay positive and negative sample preparation

Assay positive Control	The Assay positive control is a flask containing Sterile Butterfield's buffer with inoculum. There is no test article included. This control is used to verify the viability of the ATCC organism and is used for calculations. No sample preparation required.
Assay negative Control	The Assay negative control is a flask containing Sterile Butterfields buffer. This control is used to evaluate aseptic technique and sterility of reagents.

7.2 Equipment and Reagent Preparation

7.2.1 Flask Preparation

7.2.1.1 Sterile corning cell culture flasks. Prepare the appropriate number of sterile flasks as indicated in Table III prior to performing any inoculation steps of this assay.

Table III: Flasks Required for Testing

Sample Description	Instructions
Treated Samples	1 sterile flask for each sample for T post incubation testing
Untreated Samples	1 sterile flask for T_0 and 1 sterile flask for T post incubation testing
Assay + Control	1 sterile flask for T_0 and 1 sterile flask for T post incubation testing
Assay – Control	1 sterile flask for T_0 and 1 sterile flask for T post incubation testing

7.2.1.2 Label each prepared sterile flask with assay number and sample identification. (i.e. Test number = TXT1-XX-001)

TXT1: Stands for AATCC Assay per SOP: LAB-TM-203: AATCC 100 Test Method
XX: Symbol for last 2 digits of current fiscal year (i.e. 2002 designated by 02).
001: Numerical symbol to represent particular lab submission increases by single digit increments

7.2.1.3 Set all flasks aside to be used in Assay Inoculation in step # 7.3 of this procedure.

7.2.2 Media plates and sterile 17 x 100 mm test tubes Preparation

7.2.2.1 Prepare the appropriate number of media plates and 17 x 100mm sterile polystyrene test tubes for each sample as indicated in Table IV prior to the inoculation of samples or control material.

7.2.2.2 Aseptically add 0.9mL of Sterile Butterfield's Phosphate Buffer to each 17 x 100mm test tubes, labeled 10^0-10^4. Put all labeled petri dishes and test tubes aside for use in step 7.1 of this procedure

Table IV: Test tubes and Media required for Testing

Sample Description	# of Tubes	# of Petri Dishes	Instructions
Treated sample Time post incubation	4 each time point	7 each time point	Label 2 media plates with Assay number, sample identification. Label 2 plates with dilution 10^0 (0.5ml) and time point. Label 5 media plates w/ dilution 10^0 –10^4 (0.1ml). Label 5 tubes with Assay number, sample identification, dilution10^0 –10^4
Untreated Sample Time 0 and Time post incubation	4 each time point	7 each time point	Label 2 media plates with Assay number, sample identification. Label 2 plates with dilution 10^0 (0.5ml) and time point. Label 5 media plates w/ dilution 10^0 –10^4 (0.1ml). Label 5 tubes with Assay number, sample identification, dilution10^0 –10^4
Assay + Control Time 0 and T post incubation sample	4 each time point	7 each time point	Label 2 media plates with Assay number, sample identification. Label 2 plates with dilution 10^0 (0.5ml) and time point. Label 5 media plates w/ dilution 10^0 –10^4 (0.1ml). Label 5 tubes with Assay number, sample identification, dilution10^0 –10^4
Assay – Control Time 0 and Time post incubation sample	1 each time point	2 each time point	Label 2 media plates with Assay number, sample identification, dilution 10^0 (0.5ml) and time point.

7.3 Inoculum Preparation

7.3.1 Inoculum Preparation:

7.3.1.1 Preparation of ATCC organisms must be completed approximately 18-24 hours prior to the initiation of this procedure. Preparations of ATCC organisms are to be prepared per: LAB-SOP-001: Receipt, Inventory, Preparation and Maintenance of ATCC organisms. Never use sub-passage #1 for testing. The organism grown in liquid media will be used for the inoculum preparation while the sample cultured onto solid media is used to confirm the purity of the organism preparation.

7.3.1.2 Turn a Spectrophotometer "ON". **The instrument must warm up for 20-30 minutes prior to use. Set at 475nm and optical density mode.**

7.3.1.3 Pour an aliquot of the growth media of the same lot number used to cultivate the ATCC organism into a cleaned dry cuvette to zero the instrument. Example: Organisms grown in Tryptic Soy Broth. For yeast like grow in Sabaroud Dextrose Broth. If organism require any other growth media, zero the instrument with the respective growth media

7.3.1.4 Mix the ATCC organism. If a vortex is used to mix the ATCC organism allow approximately 15 minutes for any air bubbles to dissipate prior to preparing the inoculum; then mix sample by gentle inversion prior to use.

7.3.1.5 Aseptically remove an aliquot of the 18-24 hour ATCC culture and pour into a tube containing sterile growth media.

7.3.1.6 Measure the optical density of the solution prepared in step 7.2.3.4. For S.aureus an optical density of 0.28 +/- 0.02 is approximately a bacterial challenge of 10^8. If the optical density is above 0.3 use the formula V x C = V x C to dilute the preparation and re-measure. If the optical density is below 0.26 add more ATCC organism prepared in step 7.3.1.1. Since bacterial organisms differ in sizes the laboratory must perform some preliminary research to determine what optical density provides an approximate 10^8 concentration of the bacterial challenge.

7.3.1.7 Once the optical density has been diluted to ~10^8, prepare a 1:10 dilution of the organism in fresh growth media then a secondary dilution of 1:100 in Butterfield's buffer. This is approximately a 10^5 bacterial challenge. Different concentrations may be used on some testing, but always maintain a 1% suspension of growth media in the final dilution.

7.3.1.8 This suspension should be used immediately. The liquid suspension may sit on the bench for up to 2 hours if there is any delay in testing. The bottle must be discarded after this time limit.

7.3.1.9 Mix suspension gently by inversion immediately prior to use.

7.4 Assay Inoculation

7.4.1 Prepare all T_0 and T post incubation samples and controls (T_{12}, T_{24}, T_{48}, etc.) per step 7.2 of this procedure.

7.4.2 To all samples and controls inoculate with 1.0 +/- 0.1ml of the appropriate inoculum prepared in step 7.3 of this procedure.

7.4.3 Immediately cap the T post inoculation cell culture flasks. Lay horizontally for the 2" x 2" sample to achieve maximum absorption.

7.4.4 Incubate at 37˚C for 18-24 hours or other times and temperatures as indicated on the Laboratory Submission Form.

7.5 Extraction and Enumeration of T_0 (Time 0) and T post incubation samples bacterial growth

NOTE: Perform the following instructions as soon as possible but no later than 5-10 minutes for all T_0 samples. Perform the following instructions after the appropriate incubation period on the inoculated controls and test samples. Table V indicates the range of time allowed for various T post inoculation times of samples. The time of removal of all T post incubation samples is to be documented.

Table V: Min/Max T post Incubation Times

T (time)	Incubation Time
T 12 hours	±1 hour (s)
T 24, 48, 72	± 2 hours (s)

7.5.1 T_0 and T post incubation testing assay (+) control and untreated samples and treated samples

7.5.1.1 Add 99 ± 1 mL of sterile TAT broth or other neutralizer into the flask with the sample.

7.5.1.2 Cap the flask and vigorously shake the flask for at least 1 minute.

7.5.1.3 Aseptically pour the extraction fluid into the appropriately labeled 17 x 100 mm sterile test tube (capped) labeled 10^0.

7.5.1.4 Prepare ten-fold dilutions of the extraction fluid from 10^0-10^4. Mix each dilution on the vortex prior to removing 0.1mL of the suspension and transfer to the next ten-fold labeled dilution tube. Use a separate sterile pipette tip for each transfer.

7.5.1.5 Upon completion of dilutions, inoculate 0.5ml of the 10^0 solution onto the (2) petri dishes labeled 10^0 (0.5ml). Inoculate 0.1 ml of the 10^0-10^4 onto each respectively identified petri dish. Spread the inoculum around the entire plate with a sterile inoculum spreader.

7.5.1.6 Place all "Time 0" plates into an incubator that supports the growth of the respective ATCC organism for the specified time as indicated in Table VI. Remove the T_0 media plates after incubation and enumerate per step 7.6.

Table VI: Optimum Media & Temperature for growth of ATCC organisms

ATCC Name	Optimum Media	Optimum Temperature	Optimum Inc. Time
Staphylococcus species And Klebsiella pneumoniae	TSA, TSB	35-39°C	Min:18-24 hours Max.: 96 hours

7.5.1.7 Repeat steps 7.5.1.1 thru 7.5.1.6 for all T post incubation flasks at the end of the incubation period.

7.6 Enumeration of T_0 (Time 0) and T-post incubation samples (T_{12}, T_{24}, T_{48}, etc.)

7.6.1 Remove the appropriately labeled petri dishes from the incubator after the completion of incubation. Record the time removed from the incubator.

7.6.2 Enumerate and record all data. If colonies appear to be overlapping or overgrown at approximately 300 CFU's, report as >300 CFU's.

7.6.3 Evaluate each plate for bacterial purity. Indicate if any media plate appears to have more than one type of bacterial organism growing. Document any observations.

7.6.4 Perform assay evaluation and calculations per section 8 of this procedure.

8 Assay Evaluations and Calculation of Results

8.1 Standard Assay Evaluation

8.1.1 Evaluate the ten-fold dilutions of each control and test article to assure that the dilution results are correct. An example of a valid and invalid dilution scheme is given in Table VIII.

Table VIII: Valid and Invalid Dilution schemes

Description	**10^o (0.5 ml)**	**10^0 (0.5 ml)**	**10^1 (0.1 ml)**	**10^2 (0.1 ml)**	**10^3 (0.1 ml)**	**10^4 (0.1 ml)**
Valid Serial Dilution Scenario	>300	>300	>300	188	20	1
Invalid Serial Dilution Scenario	>300	>300	>300	0	40	0

8.1.2 The Assay (+) Control must demonstrate a minimum of 1 x 10^5 CFU's/ml at Time 0. **Reduction may be seen in the Assay (+) Control at T post incubation, but the reduction must not be greater than a 1 log reduction.** If greater than a 1 log reduction is obtained on the T post incubation of the Assay (+) control when compared to the Assay (+) control at T0 then the assay is INVALID and must be rerun. If multiple replicates of the Assay (+) control are requested at time of submission, then the average result of the total number of Assay (+) controls run at Time 0 should agree within 15% of each other. The average of replicates is used in additional calculations.

8.1.3 The Assay (-) Control must yield NO growth on both the Time 0 and the Time post incubation samples.).

8.1.4 If any of the above criteria is not met, the laboratory is required to investigate why this laboratory situation occurred, then request additional samples from the submitter for further testing.

8.1.5 Select the result from the lowest dilution that is countable. (This method is typical microbiological lab practice. From Biology of Microorganisms pg 318, "The usual practice , which is most valid statistically, is to count only those plates that have between 30-300 colonies."

8.2 Calculation of CFU/Sample:

8.2.1 If growth on the (2) 10^0 petri dishes is less than 300 CFU's

8.2.2 Add the number of colonies seen on the (2) 10^0 petri dishes. If no growth is observed, use the number 1 in the calculation formula below: Example:

Description	10^0 (0.5 ml)	10^0 (0.5 ml)	10^1 (0.1 ml)	10^2 (0.1 ml)	10^3 (0.1 ml)	10^4 (0.1 ml)
Test Article Results	0	0	0	0	0	0

Formula for CFU's/sample:

CFU's counted X extraction dilution = CFU/sample

≤1 x 100 = ≤1.0 x 10^2

8.2.3 If growth on the (2) 10^0 petri dishes is GREATER than 300 CFU's:

8.2.3.1 Select the lowest dilution exhibiting visually countable colonies. Use the calculation formula below: Example:

Description	10^o (0.5 ml)	10^0 (0.5 ml)	10^1 (0.1 ml)	10^2 (0.1 ml)	10^3 (0.1 ml)	10^4 (0.1 ml)
Test Article	>300	>300	>300	188	20	1

Formula for CFU's/sample:

CFU's counted x reciprocal of dilution X extraction dilution = CFU/sample

188 x 10 x 100 = 1.88 x 10^5

8.3 Percent Reduction Calculation

NOTE Sciessent LLC normally calculates percent reduction using the formula provided in 8.3.2. which is a modification from AATCC 100. If a client requests calculation per AATCC 100, calculate results per 8.3.1 and 8.3.2 report both sets of calculation.

8.3.1 Percent Reduction Using Control T 0 data:

Formula: Percent Reduction = B-A/B * 100

Example: B = 2.0 x 10^5 A = ≤100 Percent Reduction = 99.95%

Where A is the number of recovered bacteria from control or treated sample post incubation (ie 24 hour contact time)

Where B is the number of recovered bacteria from untreated control or treated sample immediately after inoculation (at 0 contact time)

8.3.2 Percent Reduction Using Control T24 data:

Formula:

Percent Reduction = C-A/C* 100

Example: C = 4.0 x 10^6 A = ≤100 Percent Reduction = 99.998%

Where A is the number of recovered bacteria from control or treated sample post incubation (ie 24 hour contact time)

Where C is the number of recovered bacteria from control sample post incubation (ie 24 hour contact time).

8.4 Certificate of Performance and Data Review

8.4.1 The individual performing the assay or designee is responsible for generating a Certificate of Performance per LAB-SOP-002: Generation of a Certificate of Performance.

8.4.2 Submit all data, calculations, and Certificate of Performance to the Laboratory Manager or designee for review.

8.4.3 The reviewer is responsible for verifying all calculations and determining the release criteria on the certificate of performance.

.

Attachment #1: ATCC 100 Test Method Worksheet

Assay Log Number				
Inoculation Performed By			Start Date & Time:	Time into Inc.
T post Incubation Extraction Performed By			Start Date & Time:	
Enumeration Performed By	T0 enumeration By:	Date	T24 enumeration By:	Date

Material Source: _____

Number of 2" x 2" Samples Used for Assay_____

Inoculum:

Inoculum Name & ATCC Number	Lot Number	Expiration Date	Sub-Passage

Inoculum Preparation:

Optical Density @475nm	Dilution Prep Check if 1:10 in TSB then 1:100 in Butterfield's

Supplies:

Name	Lot Number/ Equipment #	Expiration Date or Next Cal Due Date
TSB media		
TSA media		
TAT Broth		
Butterfield's Buffer		
Pipette #		
Pipette #		

Table I: T_0 Enumeration Results **Assay #**_____

Description	10^0 (0.5ml) Plate1/plate 2	10^0 (0.1ml)	10^1 (0.1ml)	10^2 (0.1ml)	10^3 (0.1ml)	10^4 (0.1ml)	Final result per 100ml extraction buffer /fabric sample
Assay + Control							
Assay - Control							

Table II: T_{24} Enumeration Results

Description	10^0 (0.5ml) Plate1/plate 2	10^0 (0.1ml)	10^1 (0.1ml)	10^2 (0.1ml)	10^3 (0.1ml)	10^4 (0.1ml)	Final result per 100ml extraction buffer /fabric sample
Assay + Control							
Assay - Control							

Assay #_____

Calculations

Formula #1: Taken from AATCC: Calculations based upon using T_0 data of untreated article

100 (B-A) / B = R where R = % Reduction, B = Untreated $T_{0,}$ A = Treated T_{24}

Formula #2: Calculations based upon using T24 data of untreated article

100(C-A) / C = R where R = % Reduction C = Untreated T_{24} A = Treated T_{24}

Results:

Description	Percent Reduction Formula#1	Percent Reduction Formula#2
Assay + Control		
Assay - Control		

Discussion and Conclusion:

☐ Authorized for Release ☐ NOT Authorized for Release

Reviewed By: _____Date:_____

REVISION HISTORY

Revision #	Description	Date	DCN#
0	Sciessent New Test Method	7/22/2014	14-007

INDEPENDENT LABORATORY CERTIFICATE OF ANALYSIS

ASSESSMENT OF VIRUCIDAL EFFECTIVENESS OF TREATED FABRIC MATERIAL VIA DIRECT CONTACT– Misting study 2013 Influenza A Virus (H7N9)

Reported to: Purafil
2654 Weaver Way
Doraville, GA 30340, US

Date Tested: 08/20/2015
Project No.: 791-109

Product Tested:
2015-07-13-PBF-XPLY-Active
2015-07-13-PBF-XPLY-No Active

Test Performed:
The test was designed to simulate consumer use and was based on AATCC Test Method 100-2004 with customization for virus testing

Conclusions:
Purafil Sample 2015.07.13-PBF-XPLY-Active is the subject of this study and represents an 18 opsy nonwoven substrate, comprised of Naturion antimicrobial fiber.

The antiviral testing in this study is designed to evaluate the ability of the *Purafil Sample 2015.07.13-PBF-XPLY-Active* to inactivate the 2013 Influenza A (H7N9) virus after 5 minutes of direct contact with the virus. By comparing the viral reductions of the Purafil Active sample to the (1) Liquid Control (no fabric) and the (2) Fabric Control (no-active) the efficacy of the "active" material can be quantified. The results show a 2.70 to 3.76 $\log_{10}$ reduction of the virus after 5 minutes of virus exposure. The 2.70 $\log_{10}$ reduction is the result when the calculation is performed comparing the virus reduction of the active sample to a standard PET control fabric containing "no active". The 3.76 $\log_{10}$ reduction is the result when comparing the active sample to a "liquid" control (no active and no fabric).

Laboratory Qualifications:
Microbac Laboratories, Inc. operates one of the world's most diversified commercial testing and analytical laboratory groups in the environmental, food, pharmaceutical and other testing areas. The MicroBioTest division of Microbac has over 26 years of experience serving microbial and viral testing community and is fully compliant to Good Laboratory Practices (GLP) and ISO 17025.

Reported by: **MicroBioTest**

10/14/2015

S. Steve Zhou, Ph.D. — Date
Director, Virology and Molecular Biology
MicroBioTest, A Division of Microbac Laboratories, Inc.

MicroBioTest, Division of Microbac Laboratories, Inc.
105 Carpenter Drive | Sterling, VA 20164 | 703.925.0100 p | 703.925.9366 f | www.microbac.com

AMENDED FINAL REPORT

(See Study Dates and Facilities Section for Details)

ASSESSMENT OF VIRUCIDAL EFFECTIVENESS OF TREATED FABRIC MATERIAL VIA DIRECT CONTACT–

Misting study

2013 Influenza A Virus (H7N9)

Test Article
2015-07-13-PBF-XPLY-Active
2015-07-13-PBF-XPLY-No Active

Author
Salimatu Lukula, M.S.

Performing Laboratory
MicroBioTest
Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
791-109

Sponsor
Purafil
2654 Weaver Way
Doraville, GA 30340, US

TABLE OF CONTENTS

COMPLIANCE STATEMENT

This study meets the requirements for 21 CFR § 58 with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Salimatu Lukula, Cory Chiossone, Semhar Fanuel

Study Director: MicroBioTest

Salimatu Lukula 10/15/2015

Salimatu Lukula, M.S. Date

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: ASSESSMENT OF VIRUCIDAL EFFECTIVENESS OF TREATED FABRIC MATERIAL VIA DIRECT CONTACT - Misting study Influenza A Virus (H7N9)

The Quality Assurance Unit of MicroBioTest has inspected the Project Number 791-109 in compliance with current Good Laboratory Practice regulations, (21 CFR § 58).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

PHASE INSPECTED	DATE OF INSPECTION	DATE REPORTED TO STUDY DIRECTOR	DATE REPORTED TO MANAGEMENT
Protocol	08/19/15	08/20/15	08/20/15
In Process	08/20/15	08/20/15	08/20/15
Final Report	09/08/15	09/08/15	09/08/15
Amended Final Report	10/05/15	10/05/15	10/05/15

Jeanne M. Anderegg 10-15-15

Jeanne M. Anderegg Date
Quality Assurance Manager

TEST SUMMARY

TITLE: ASSESSMENT OF VIRUCIDAL EFFECTIVENESS OF TREATED FABRIC MATERIAL VIA DIRECT CONTACT - Misting study Influenza A Virus (H7N9)

STUDY DESIGN: This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (See Appendix).

TEST MATERIALS SUPPLIED BY THE SPONSOR OF THE STUDY:

1. 2015-07-13-PBF-XPLY-Active, received at MicroBioTest 08/10/15, assigned DS No. F554

2. 2015-07-13-PBF-XPLY- No Active, received at MicroBioTest 08/10/15, assigned DS No. F555

SPONSOR: Purafil
2654 Weaver Way
Doraville, GA 30340, US

TEST CONDITIONS

Challenge virus:

2013 Influenza A Virus (H7N9), strain: A/Anhui/1/2013; CDC

Host:

Madin-Darby canine kidney (MDCK) cells; ATCC CCL-34

Organic load:

Not required

Active ingredient in test product:

Ag-Cu Zeolite

Neutralizer / Recovery / Extraction medium:

1X Minimum Essential Medium (MEM) + 1% Fetal Bovine Serum + 1% $NaHCO_3$ + 1% HEPES +10 µg/mL Gentamicin + 2.5 µg/mL Amphotericin B + 1 mM EDTA

Dilution medium:

1X MEM + 3.0 µg/mL Trypsin

Virus suspension medium:

0.1X MEM

Contact time:

5 minutes

Contact temperature:

Ambient temperature (20°C actual)

Application:

As applicable, virus (or dilution medium) was misted onto a 2 x 2 inch area of pre-cut (approximately 2.5 x 2.5 inch) test and control fabrics using a Nalgene Aerosol Spray Bottle (Fisher Cat. # 15-232-8; Nalgene Cat. # 2430-0200) from a distance of 3 – 6 inches for two pumps, one second per pump.

TEST CONDITIONS (continued)

Incubation time:

4-6 days (Actual: 5 days)

Incubation temperature:

36±2°C with 5±1% CO_2

Media and reagents:

1X Minimum Essential Medium (MEM) + 1% Fetal Bovine Serum + 1% $NaHCO_3$ + 1% HEPES + 10 µg/mL Gentamicin + 2.5 µg/mL Amphotericin B + 1 mM EDTA

1X MEM + 3.0 µg/mL Trypsin

0.1X MEM

Phosphate Buffered Saline

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164. Testing was laboratory initiated on 08/20/15 and was concluded on 08/25/15. The study director signed the protocol on 08/20/15. The study completion date is the date the study director signed the final report.

This amended final report replaces the final report issued on 09/16/2015. This amended final report was issued to change the sponsor name, address and typographical error on page 8, 9, and 10.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

CALCULATION OF TITER

The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d\sum p_i$$

where:

m = the logarithm of the dilution at which half the wells are infected relative to the test volume
x_k = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i

The values were converted to $TCID_{50}$/mL using a sample inoculum of 1.0 mL.

RESULTS

Results are presented in Tables 1 – 4.

The Theoretical Load was determined in the following manner:

Theoretical Load (Log_{10} $TCID_{50}$) = Virus Stock Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Average Volume of challenge virus per run (mL)]

The Viral Load was determined in the following manner:

Viral Load (Log_{10} $TCID_{50}$) = Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume (mL)]

The Log_{10} Reduction Factor (LRF) was calculated in the following manner:

Log_{10} Reduction Factor = Initial Viral Load (Log_{10} $TCID_{50}$) – Output Viral Load (Log_{10} $TCID_{50}$)

The Mean Viral Log_{10} Reduction from n replicates was determined as follows:

Mean Viral Log_{10} Reduction = (LRF_1 + LRF_2 +……+LRF_n) / n

Note: The LRF's was anti-logged prior to performing calculations

RESULTS (continued)

Table 1
Titer Results

Sample	Replicate	Contact time	Titer ($Log_{10}TCID_{50}/mL$)	Volume (mL)	Viral Load ($Log_{10}TCID_{50}$)
Virus Stock Titer control		NA	6.75	-	-
Volume application evaluation			average volume of challenge: 0.42 mL per run		
Theoretical load [a]					6.37
Cell viability/media sterility control			no virus detected, cells viable; media sterile		
Liquid Control (No fabric)	Rep. 1	5 minutes	4.75	40	6.35
	Rep. 2		4.25	40	5.85
	Rep. 3		4.50	40	6.10
					6.15
2015-07-13-PBF-XPLY-No Active	Rep. 1	5 minutes	3.25	40	4.85
	Rep. 2		3.25	40	4.85
	Rep. 3		3.75	40	5.35
					5.09
2015-07-13-PBF-XPLY- Active	Rep. 1	5 minutes	1.00	40	2.60
	Rep. 2		0.62	40	2.22
	Rep. 3		0.62	40	2.22
					2.39

[a] The theoretical load is determined based on the Virus Stock Titer control and average volume of virus challenged per run.

RESULTS (continued)

Table 2
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls
2015-07-13-PBF-XPLY-Active

Dilution of the Test Agent/Neutralizer Mixture	Neutralizer Effectiveness Control	Cytotoxicity Control
Undilute	cytotoxicity observed; viral CPE could not be evaluated	cytotoxicity observed
10^-1	virus detected in all inoculated wells	no cytotoxicity observed
10^-2	virus detected in all inoculated wells	no cytotoxicity observed

Table 3
Viral Reduction - based on Liquid (No fabric) Control

Test Article	Contact time	Input Load* ($Log_{10}TCID_{50}$)	Output Load* ($Log_{10}TCID_{50}$)	Log_{10} Reduction
2015-07-13-PBF-XPLY- Active	5 minutes	6.15	2.39	3.76

* Results represent the average of three replicates.

Table 4
Viral Reduction - based on 2015-07-13-PBF-XPLY- No Active

Test Article	Contact time	Input Load* ($Log_{10}TCID_{50}$)	Output Load* ($Log_{10}TCID_{50}$)	Log_{10} Reduction
2015-07-13-PBF-XPLY- Active	5 minutes	5.09	2.39	2.70

* Results represent the average of three replicates.

CONCLUSIONS

Purafil's 2015-07-13-PBF-XPLY-Active was evaluated for the ability to inactivate 2013 Influenza A Virus (H7N9), A/Anhui/1/2013. MicroBioTest personnel performed the inactivation procedure using 2013 Influenza A Virus (H7N9), A/Anhui/1/2013 independently to spike the fabric material. Samples were titrated by the 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) endpoint assay using MDCK cells.

Tables 3 and 4 report the individual Log_{10} virus Reduction Factor(s) for the 2015-07-13-PBF-XPLY-Active based on the Liquid (No fabric) control and 2015-07-13-PBF-XPLY-No Active respectively. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

APPENDIX

MicroBioTest Protocol

ASSESSMENT OF VIRUCIDAL EFFECTIVENESS OF TREATED FABRIC MATERIAL VIA DIRECT CONTACT –

Misting study

2013 Influenza A Virus (H7N9)

Testing Facility
MicroBioTest
Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

MicroBioTest Protocol: 791.1.08.04.15

MicroBioTest Project: 791-109

OBJECTIVE:

This test is designed to evaluate virucidal effectiveness of a treated fabric sample material via direct contact with virus. It determines the potential of the test fabric sample, which is impregnated with antimicrobial agents, to inactivate influenza virus on direct contact using multiple strains of influenza viruses. The test is designed to simulate consumer use and is based on AATCC Test Method 100-2004 with customization for virus testing.

TESTING CONDITIONS:

One treated sample (active) and one non-treated control sample (non-active), one lot each, will be evaluated in this study. In addition, a liquid control in the absence of any fabric sample will be performed using the same level of viral challenge to serve as a control for the input virus load. The test and control fabric sample will be challenged with the test virus using a misting procedure and held for an exposure (contact) time specified by the sponsor. After completion of the exposure period, surviving virus will be extracted using an appropriate medium, serially diluted and inoculated onto host cells to determine the amount of the infectious virus. One contact time will be tested for the test and control fabric samples at three replicates (N=3). The 2013 Influenza A Virus (H7N9) will be tested in this protocol.

MATERIALS:

A. Test, control and reference substances will be supplied by the sponsor of the study (see last page).

The test agent will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test agent such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures MicroBioTest testing facility management that the test agent has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

8/6/2015

MicroBioTest will retain all unused test agents for a period of one year upon completion of the test, and then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by MicroBioTest, including, but not limited to:

1. Challenge viruses (requested by the sponsor of the study): 2013 Influenza A Virus (H7N9), A/Shanghai/1/2013 (Source: CDC)

2. Host: Madin-Darby canine kidney (MDCK) cells

3. Laboratory equipment and supplies.

4. Media and reagents:

   Media and reagents relevant to the virus-host system and test agent being tested will be documented in the first project sheet and the data pack.

TEST SYSTEM IDENTIFICATION:

All Petri dishes, dilution tube racks, and host-containing apparatus will be labeled with virus identification and project number.

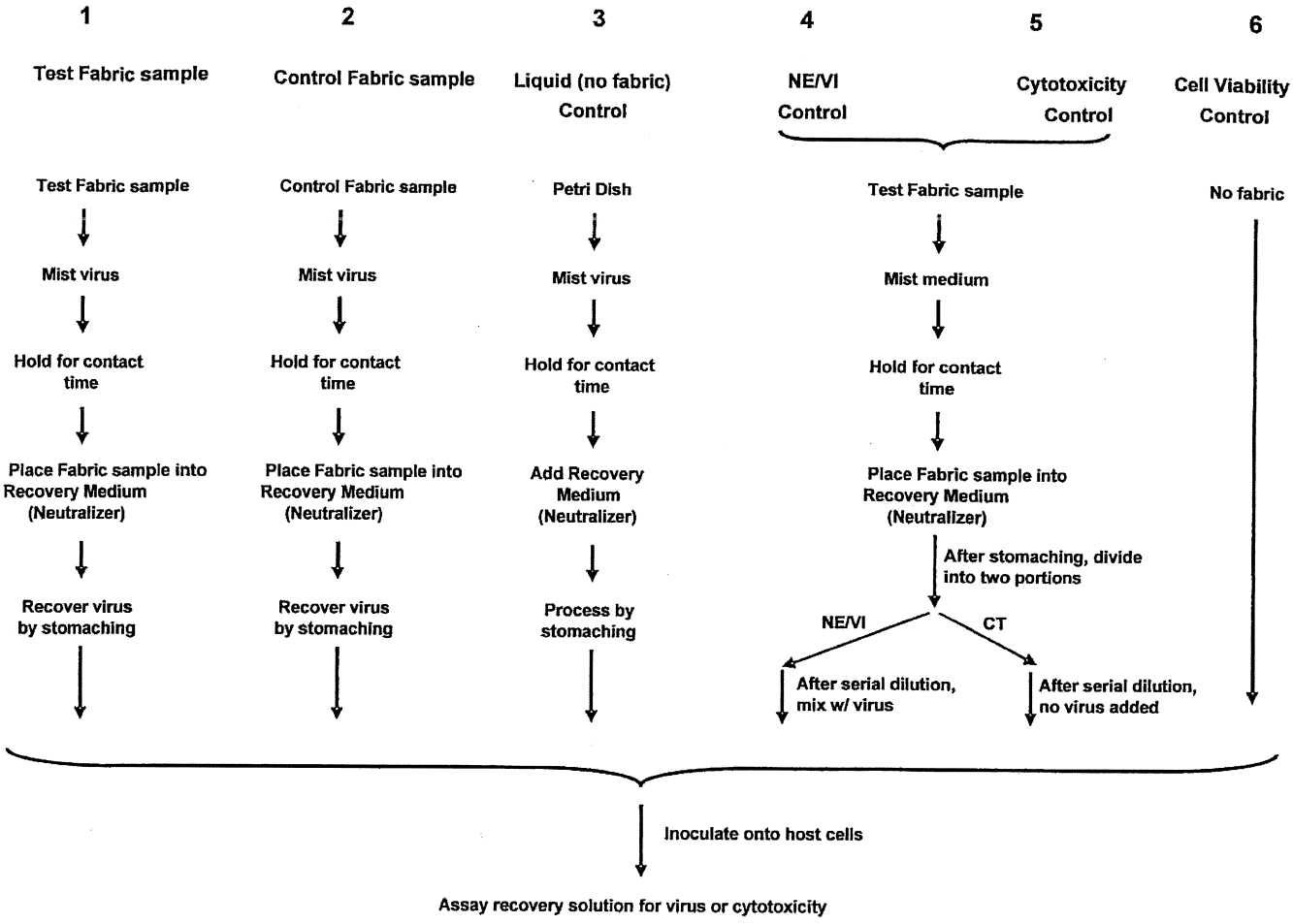
EXPERIMENTAL DESIGN:

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at MicroBioTest SOPs and Logs are referred to in the raw data and are required as part of GLP regulations.

The study flow diagram is summarized in Figure 1, with details described below.

8/10/15

FIGURE 1



NE/VI: Neutralizer Effectiveness/Viral Interference control
CT: Cytotoxicity control

Note: One test fabric sample, one control fabric sample and one liquid (no fabric) control will be tested at one contact time, three replicates against 2013 Influenza A Virus (H7N9). The NE/VI and CT controls will be performed for the test fabric sample only (see Table 1 for details).

8/6/15

A. Inoculum preparation:

Viral stocks are purchased from reputable sources that identify them by scientifically accepted methods and may have been propagated at MicroBioTest. Records are maintained that demonstrate the origin of the virus. The virus stocks are stored at an ultra-low temperature.

Frozen viral stocks will be thawed on the day of the test (fresh stock cultures may be used at the discretion of the Study Director). The titer of virus stock should be sufficiently high (usually at least 6.5-Log_{10}) to ensure a minimum of 4-Log_{10} reduction window.

B. Test material preparation:

Each test and control fabric sample will be cut to approximately 2.5" x 2.5" or 3" x 3" size. After cutting, each fabric sample piece may be stitched around the edges to ensure that all layers stay together throughout the testing. All carriers will be exposed to UV light, under the hood, for a minimum of 30 minutes per side prior to use to reduce the bioburden. No other pre-treatment or pre-conditioning will be performed.

For each viral inoculum application, a sterile barrier containing a 2" x 2" opening will be paced over the test or control fabric sample material to ensure that the inoculum is only applied to the upper (i.e., external) side of the sample, and not the sides or underside of the carriers.

C. Test:

One test (treated) fabric sample and one control fabric sample will be tested at one contact time and in three replicates against 2013 Influenza A Virus (H7N9). In addition, a liquid control using the same level of viral challenge in the absence of any fabric sample will be performed at one contact time and in three replicates (see Section E).

For each run, the challenge virus will be added in the following manner: the inoculum will be misted on using a spray device from a distance of 3" – 6" for two pumps, one second per pump. The volume of inoculum applied should be between 0.3 – 0.5 mL and not exceeding 0.5 mL. The average amount of inoculum

applied will be measured and recorded. After the designated contact time, the carrier will be immediately placed into a sterile stomacher bag containing 40 mL of neutralizer (= recovery medium or extraction medium) and will be designated as the low salt extraction sample. Each sample will be processed in a stomacher for five minutes. Following this process, an aliquot of the extraction sample will be removed and ten-fold serially diluted and inoculated onto the host cells (see below).

D. Infectivity assay:

The residual infectious virus in the test and controls will be detected by viral-induced cytopathic effect (CPE).

Selected dilutions of the neutralized inoculum/test agent mixture will be added to cultured cell monolayers at a minimum of four wells per dilution per sample. The inoculated plates will be incubated at 36±2C in 5±1% CO_2 for 4-6 days. The host cells may be washed with phosphate buffered saline (PBS) twice prior to inoculation. The host cell cultures will be observed and refed, as necessary, during the incubation period. These activities, if applicable, will be recorded. Then the host cells will be examined for presence of infectious virus. The resulting virus-specific cytopathic effects and test article-specific cytotoxic effects will be scored by examining both test and controls. These observations will be recorded.

E. Controls:

1. Liquid (no fabric) Control:

   Three replicates, using a glass petri dish, will be tested for the Liquid (no fabric) control at one contact time (same as for the test fabric sample samples). A sterile glass petri dish will be misted with virus in the same manner as the test carriers and a timer will be started. After the contact time, 40 mL of neutralizer (extraction medium) will be added to the petri dish and mixed thoroughly by pipetting. The recovery solution will then be transferred to a stomacher bag and processed in a stomacher for five minutes as described in the test runs. An aliquot of the extraction sample will be serially ten-fold diluted in dilution medium and inoculated onto the host cells.

8/6/16

2. Control fabric sample:

Three replicate runs using an untreated control fabric sample containing no active ingredient will be used for the virus recovery control at one contact time (same as for the test fabric sample samples). The carrier will be inoculated in the same manner as the test carriers. After the contact time, the carrier will be placed into a sterile stomacher bag containing 40 mL of neutralizer (extraction medium). The sample will be processed in a stomacher for five minutes. After the stomaching process, the carrier will be immediately removed, and an aliquot of the extraction sample will be serially ten-fold diluted in dilution media. This control will determine the relative loss in virus infectivity resulting from exposure to the fabric sample carrier and neutralization alone.

3. Neutralizer effectiveness/viral interference control (NE/VI):

This control will be included to determine if residual active ingredient is present after neutralization and if the neutralized test agent interferes with virus infectivity. It will be performed for the test fabric sample only (see Table 1 for details) at one contact time and one replicate.

For each run, the test fabric sample carrier will be misted with medium in lieu of virus and held for the same contact period. After the contact time, the carrier will be placed in a sterile stomacher bag containing 40 mL neutralizer (= recovery medium or extraction medium) and stomached for five minutes. Immediately, the carrier will be removed and discarded.

Two aliquots of the extraction sample, one for cytotoxicity control and one for the neutralizer effectiveness/viral interference control, will be transferred to tubes containing dilution medium and serially ten-fold diluted.

For the Neutralizer effectiveness/viral interference control, following serial dilutions, 100 µL of a low titered virus (containing no more than approximately 5,000 infective units of virus) will be added to 4.5 mL of each dilution and held for a period greater than or equal to the contact time. These samples will then be used to inoculate host cells as described for the test procedure and incubated in the same manner as the test.

8/6/15

4. Cytotoxicity control (CT):

This control will evaluate the cytotoxicity of the neutralized extraction sample to the host cells. It will be performed for selected fabric samples only (see Table 1 for details) at one contact time and one replicate.

Selected dilutions of the sample, obtained from the Neutralizer effectiveness/viral interference control run, will be inoculated onto host cells and incubated together with other test and control samples as described for the test procedure. The condition of the host cells will be recorded at the end of the incubation period. These effects must be distinct from virus-specific cytopathic effects, which will be evident in the stock titer and virus recovery control cultures.

5. Volume application evaluation:

A mock inoculum (e.g., medium) will be misted onto a dish using the same techniques employed for the test using three repetitions. Into three independent dishes, the inoculum will be misted. The inoculum will be allowed to settle and the volume in each dish will be measured and reported.

6. Cell viability control:

At least four cells will be inoculated with an appropriate medium during the incubation phase of the study.

This control will demonstrate that the cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the media employed throughout the assay period.

7. Virus Stock Titer control (VST):

An aliquot of the virus inoculum used in the study will be directly serially diluted and inoculated onto the host cells to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

F. Calculation:

The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber or other appropriate methods such as Reed and Muench, Am. J. of Hyg. 1938, 27:493. These analyses will be described in detail in the final report. The test results will be reported as the reduction of the virus titer due to treatment with test agent expressed as log_{10}.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- Viral-induced cytopathic effects (CPE) must be distinguishable from test agent induced cytotoxic effects.
- Virus must be recovered from the neutralizer effectiveness/viral interference controls (not exhibiting cytotoxicity).
- Virus must not be detected in the Cell Viability Control.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at MicroBioTest, 105 Carpenter Drive, Sterling, Virginia 20164.

REPORT FORMAT:

MicroBioTest employs a standard report format for each test design. Each final report will provide the following information:

- Sponsor identification
- Test agent identification
- Type of assay and project number
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable

- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge virus and host used and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the study initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

JT 8/6/15

Table 1
Summary of samples to be assayed

Sample #	fabric sample material	Contact Time	Replicate
1	Active test fabric sample	Contact time	Replicate 1
2			Replicate 2
3			Replicate 3
4	Non-active control fabric sample	Contact time	Replicate 1
5			Replicate 2
6			Replicate 3
7	Liquid (no fabric) control	Contact time	Replicate 1
8			Replicate 2
9			Replicate 3
10	NE/VI Control – Test fabric sample	Contact time	Replicate 1
11	TOX Control – Test fabric sample	Contact time	Replicate 1
12a – 12c	Volume Application Evaluation	NA	Replicate 1 - 3
13	Cell Viability Control	NA	Replicate 1
14	Virus Stock Titer control	NA	Replicate 1

NE/VI control: Neutralizer Effectiveness/Viral Interference control
TOX control: Cytotoxicity control

qt 8/6/15

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor prior to initiation of the study.

A. Name and address: Foss Manufacturing Company, LLC
11 Merrill Industrial Drive
Hampton, NH 03843-5000

B. Active sample name: ☑ 2015-07-13-PBF-XPLY Active
Active ingredient(s): ☑ Ag-Cu Zeolite
Lot Number: ■ To be recorded from package

Non-active sample name: ☑ 2015-07-13- PBF-XPLY No Active
Lot Number: ■ To be recorded from package

Contact time: ■ 5 minutes
Contact temperature: ■ Ambient

C. Organic load in inoculum: ■ Not required

D. MSDS or certificate of analysis: ☑ Provided or ☐ Not provided

REPORT HANDLING AND STUDY CONDUCT:

The sponsor intends to submit this information to: ■ Internal Information
Study conduct: ■ GLP

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: Jacqueline Traynor Date: 8/6/15

Printed Name: Jacqueline Traynor

PROTOCOL APPROVAL BY STUDY DIRECTOR (MicroBioTest):

Study Director Signature: Salimatu Lukula Date: 8/20/15

Printed Name: Salimatu Lukula

Date Issued: 08/20/15 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 791-109

STUDY TITLE: ASSESSMENT OF VIRUCIDAL EFFECTIVENESS OF TREATED FABRIC MATERIAL VIA DIRECT CONTACT – Misting study 2013 Influenza A Virus (H7N9)	**STUDY DIRECTOR:** Salimatu Lukula, M.S. Salimatu Lukula 8/20/15 Signature Date		
TEST MATERIAL(S): 2015-07-13-PBF-XPLY-Active 2015-07-13-PBF-XPLY- No Active	**LOT NO.** NA NA	**DATE RECEIVED:** 08/10/15 08/10/15	**DS NO.** F554 F555
PERFORMING DEPARTMENT(S): Virology and Molecular Biology	**STORAGE CONDITIONS:** Location: D6 ■ Dark ■Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		

PROTECTIVE PRECAUTION REQUIRED: MSDS ■ Yes / ☐ No

PHYSICAL DESCRIPTION: ☐ Solid ☐ Liquid ☐ Aerosol ■ Other: fabric material

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 08/20/15 **TERMINATION DATE:** 08/27/15

CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: Internal Information

SPONSOR: Foss Manufacturing Company, LLC
11 Merrill Industrial Drive
Hampton, NH 03843-5000

CONTACT PERSON: Jacqueline Traynor
Email: JTraynor@fossmfg.com

TEST CONDITIONS :

Challenge organisms:	2013 Influenza A Virus (H7N9), strain: A/Anhui/1/2013; CDC		
Host:	Madin-Darby canine kidney (MDCK) cells, ATCC CCL-34		
Dilution medium:	1X Minimum Essential Medium (MEM) + 3.0 µg/mL Trypsin		
Neutralizer:	1X MEM + 1% Fetal Bovine Serum + 1% $NaHCO_3$ + 1% HEPES + 10 µg/mL Gentamicin + 2.5 µg/mL Amphotericin B + 1mM EDTA		
Virus suspension medium:	0.1X MEM		
Active ingredient(s):	Ag-Cu Zeolite	Organic load:	Not required
Contact time:	5 minutes	Contact temperature:	Ambient temperature
Incubation time:	4-6 days	Incubation temperature:	36±2C with 5±1%CO2

PROTOCOL AMENDMENT(S):

1. Protocol, page 3 states the strain of the challenge virus as A/Shanghai/1/2013. The strain of the challenge virus is A/Anhui/1/2013. This amendment was issued to correct the typographical error in the protocol.

Date Issued: 10/05/15 Project Sheet No.2 Page No. 1 Laboratory Project Identification No. 791-109			
STUDY TITLE: ASSESSMENT OF VIRUCIDAL EFFECTIVENESS OF TREATED FABRIC MATERIAL VIA DIRECT CONTACT – Misting study 2013 Influenza A Virus (H7N9)	**STUDY DIRECTOR:** Salimatu Lukula, M.S. Salimatu Lukula 10/6/15 Signature Date		
TEST MATERIAL(S): 2015-07-13-PBF-XPLY-Active 2015-07-13-PBF-XPLY- No Active	**LOT NO.** NA NA	**DATE RECEIVED:** 08/10/15 08/10/15	**DS NO.** F554 F555
PERFORMING DEPARTMENT(S): Virology and Molecular Biology	**STORAGE CONDITIONS:** Location: D6 ■ Dark ■Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: Internal Information			
SPONSOR: Purafil 2654 Weaver Way, Doraville, GA 30340, US	**CONTACT PERSON: Ash Dhokte** **Global Director Business Development** **Email:** adhokte@purafil.com Office: +1 770825 7348 Mobile: +1 404 578 1379		

PROTOCOL AMENDMENT(S):

2. Protocol and Project sheet no.1 stated the sponsor name and address as Foss Manufacturing Company, LLC; 11 Merrill Industrial Drive Hampton, NH 03843-5000. Per the sponsor, the correct sponsor name and address was Purafil 2654 Weaver Way, Doraville, GA 30340, US. This amendment was issued to correct the Sponsor name and address in the protocol.

Technical Report

From: Jay Joshi, PhD

Date: 04/15/2020

Subject: Antimicrobial Efficacy of Purashield Air Filtration Unit

Scope

This report is intended to communicate the antimicrobial efficacy of Purafil's Purashield filtration equipment. Viral and bacterial kill rates were assessed on a completed Purashield-500 (CPUM-500) unit. Standardized third-party testing revealed significant airborne microbial reduction in as little as one hour by the Purashield unit in a test space representative of residential and commercial rooms and offices.

Experimental Method

All testing was performed by the Guangdong Detection Center of Microbiology (Guangzhou, CN). Measurements were collected in accordance with the Technical Standard for Disinfection (2002 Ministry of Health P.R. China)-2.1.3.[1] General test conditions specified by the standard are outlined in Table 1 for convenience.

Table 1. Conditions of Purashield Antimicrobial Efficacy Evaluation

Microbial Contaminants	H1N1 Influenza A; *Staphylococcus albus* 8032
Air Circulation?	Yes
Room Volume	1059ft^3 / 30m^3
Duration	1hr
Temperature	Ambient
Relative Humidity	50-70%
Device flowrate	353 CFM / 600 CMH

Two separate tests were conducted using the Influenza A subtype H1N1 virus and *Staphylococcus albus (*also called *Staphylococcus epidermis*). The aerosolized contaminant was introduced into the 30m^3 test chamber and circulated throughout the space for one hour. Initial control and final sampling measurements over three independent trials for each contaminant were used to ascertain CPUM-500 sterilization rates.

Described test results on Purafil SP media were performed through the same methodology. 500g of Purafil SP media was placed in a 1m^3 test chamber, and exposed to the same aerosolized microbial agents over a 2hr measurement period.

Results and Discussion

Overview of Test Conditions

Commonly-used HEPA filtration measurements are based non-biological components, such as DOP/PAO (0.3µm particles) and sodium flame challenge evaluations (0.58µm particles)[2,3] Typical 99.97% removal efficiency claims on 0.3µm particle sizes are derived from uniform, unidirectional flow tests.[3] Conversely, chamber tests like the one implemented here with the Purashield-500 unit also account for natural non-uniformities in air mixing in a realistic end-use environments for air purifiers, which can foster lower measurable particulate removal efficiencies. Differences in the size, shape, and other physical characteristics of aerosolized viruses and bacteria can furthermore generate disparate transport behavior from relatively invariable and inert filtrates. Additionally, HEPA filters themselves do not have the capacity to kill microbial contaminants, creating leakage risk potential over time. This is not the case with antimicrobial media within Purashield, where Puraward and Purafil SP media both enact antimicrobial activity. Accordingly, testing on actual microbial agents in realistic use environments, as performed here with Purashield, provide a more accurate reflection of pathogenic removal efficacy for filtration products.

Over the one hour test period, 20 air exchanges were achieved by the Purashield-500 unit in the 30m^3 test chamber. The large turnover rate demonstrates how the Purashield-500 unit can easily achieve the recommended 9 air exchanges within relatively short time periods in commercial workspaces and residential rooms.

Antimicrobial Efficacy of Purashield Filtration Unit

Laboratory test data for viral and bacterial disinfection efficacy are outlined in Table 2. Measurements reveal average kill rates of **99.22%** against viruses and **98.42%** against bacteria for CPUM-500 over just one hour of operation. Longer operational times would likely enhance sterilzation effects through enhanced filter contact time with airborne contaminants. Results show the capacity of Purashield to significantly and permanently reduce the concentration of airborne pathogens over relatively short operational periods.

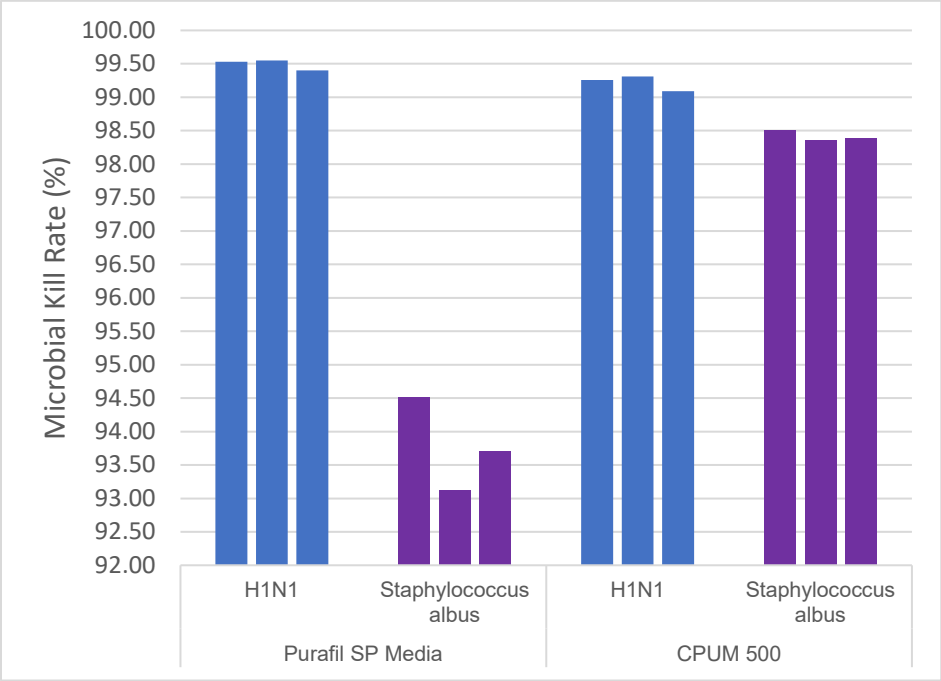
Table 2. Antimicrobial Efficacy Measurements on Purashield (CPUM-500) Unit

Contaminant	Trial (#)	Airborne Microbial Content ($TCID_{50}/m^3$)		Kill Rate (%)
		Initial	After 1hr	
Staphylococcus albus 8032	1	5.7×10^4	5.5×10^2	98.51
	2	5.8×10^4	6.2×10^2	98.35
	3	5.9×10^4	6.4×10^2	98.39
Influenza A subtype H1N1	1	6.11×10^5	1.06×10^3	99.26
	2	7.65×10^5	1.34×10^3	99.31
	3	9.04×10^5	1.80×10^3	99.09

Comparison to Purafil SP Media Testing

Antimicrobial testing on Purafil SP media, one of Purashield's antimicrobial components, was also evaluated seperately. Calculated kill rates of both the CPUM-500 unit and Purafil SP are displayed together in Figure 1 to facilitate comparison.

Figure 1. Antimicrobial Filtration Efficacies for Purafil Products



The significantly higher bacterial reduction of the CPUM-500 unit in comparison to Purafil SP-alone is enacted by combinatorial microbial filtration from Puraward, Purafil SP, and HEPA filtration in the Purashield unit. It is important to note that tests conducted on Purafil SP media alone were performed for twice as long (2hr vs. 1hr) and with a magnitude higher microbial concentration (Intial $TCID_{50}/m^3 \approx 10^6$ vs 10^5) than measurements acquired with Purashield-500. These conditions would enhance contact time in the media-only evaluations relative to described testing for the Purashield unit, and likely account for ~0.1% differences in H1N1 reduction calculations between Purafil SP-only and CPUM-500.

Conclusions

Test data on actual microbial contaminants show Purashield can effectively disinfect spaces with airborne pathogenic contaminants. Measurements using the CPUM-500 unit against H1N1 and *Staphylococcus albus* suggest the Purashield removes >**99.2%** of viruses and >**98.4%** of bacteria within only 1hr of operation. The complete Purashield unit, which utilizes several microbial filtration platforms, generates enhanced bacterial removal and comparable viral filtration in comparison to antimicrobial media alone under impressively half the exposure time and a magnitude lower initial contaminant concentration. As such, Purashield filtration devices enact effective removal capability for airborne microbial contaminants.

References

1) Antibacterial and Cleaning Functions of Household and Similar Electrical Appliances. From *Methods for the Determination of Inhalable Particles in Air in Public Places "Technical Standard for Disinfection*. Ministry of Public Health. 2002 ed. Peoples Republic of China.

2) Meek J.; Milholland D.; Litauszki L. Alternative Methods for HEPA Filter Leak Detection. *Pharm. Eng.* **2011**, 2 (31), 22-32

3) Comparison of High Efficiency Particulate Filter Testing Methods. International Atomic Energy Agency. Vienna, AT. **1985**.