Modderfontein
Laboratory Services (Pty) Ltd
Reg. No. 1966/001741/07

P.O. Box 27
Modderfontein
1645
Tel: +27 (011) 606-2274
Fax: +27 (011) 606-2375

FINAL REPORT

CUSTOMER	Vinnic South Africa PTY LTD
ADDRESS	521 Time Square, Park Road, Table View
REPORT TO	Stefan Pretorius
REPORT DATE	21/07/2020
WORK INSTRUCTION	Face Mask Particle Filtration, Bacterial Filtration Efficacy, 7 Day Disinfection Study

1. DESCRIPTION OF STUDY

SUMMARY

In this study four components of mask specifications were evaluated:

- *Liquid resistance (aerosol droplets/synthetic blood),*
- *Resistance of fine solid particles, Bacterial Filtration Efficacy (BFE) and*
- *Viral Filtration Efficiency (VFE).*
- ***A 7-Day study was also conducted to determine whether the masks disinfect themselves and can therefore be safely re-used.***

This was evaluated as follows:

- *Testing for airborne microbes on the inside and outside of the mask and by filtering known concentrations of nebulized S.aureus and E.coli cultures through the mask.*
- *The virus filtration efficacy was carried out with an isolated wild-type E.coli phage which infects E.coli ATCC 25922.*
- *A plaque assay was used to determine the percentage removal of infectious bacteriophage particles after filtration through the AOP-KF face mask.*

INTRODUCTION:

Bacterial and viral diseases are spread through both airborne and blood borne sources within environments containing pathogens. Personal Protective Equipment (PPE), such as face masks can minimize the transmission of disease.

The transfer of micr organisms can be reduced by the physical barrier between the infection source and the healthy individual. Additionally, a face mask can reduce the spread of microbes by filtering the exhaled breath from an infected person, thereby decreasing the concentration of pathogens that a healthy individuals in the vicinity may be exposed to.

Normal activities such as sneezing, coughing, crying, shouting, breathing and speaking may release oral, dermal and nasopharyngeal bacteria that may cause infections. Research has shown there are numerous ways in which bacteria can become airborne and that the microorganisms shed by patients or healthcare workers are the most significant contaminating agents (Skinner and Sutton, 2001).

Studies also indicate that the fit of the mask, the correct way of positioning the mask, movement of the wearer, length of facial hair and voice level all have a direct effect on its filtration efficiency (Koch, 1996; McLure et al., 2000; McLure et al., 1998).

Bacterial Filtration Efficiency (BFE) both in vivo and in vitro is a widely accepted method for evaluating face masks (Davis, 1991). The bacteria penetrating the face mask and passing through are collected and cultured to determine the number of Colony Forming Units (CFU), with each CFU representing a microbe that has passed through the mask and remains infective (able to grow).

The in vitro test uses positive and negative controls to determine the initial number of bacteria. The challenge bacteria (S.aureus or E.coli) are contained in a mist which is produced by aerosolizing the bacteria in 0.1% Peptone Water.

The masks are placed directly over a sterilized filtration sampling system. The aerosol contains droplets which simulate the expulsion of droplets containing microorganisms from an infected person.

S.aureus is a gram-positive cocci that is irregular in shape and often found in clusters of cells. Various diseases including impetigo, toxic shock syndrome, food poisoning and pneumonia are attributed to the presence of S.aureus. The average coccus is about 0.5-1.0 µm in diameter. E.coli is a gram-negative, rod shaped bacteria and averages 1.1 to 1.5um in width by 2.0 to 6.0µm in length. E.coli is one of the leading causes of urinary tract infections.

2. METHODS AND MATERIALS

THREE COMPONENTS OF MASK SPECIFICATIONS WERE EVALUATED

1) Liquid Resistance,
2) Solid Resistance and
3) Microbial Filtration Efficiency (Bacterial and Viral).

AOP-KF face masks were received for testing, each sample was sealed in an individual plastic cover.
The face mask properties are stipulated in Table 1 below.
The masks were pre-conditioned at 37°C and 80% humidity for 24 hours before carrying out tests.

Table 1: Face Mask Description

NUMBER OF MASKS	NAME	DESCRIPTION
32 Face masks each sealed in an individual plastic cover	AOP-KF Anti-Influenza Face Mask	5-layers The inner and outer layers are made of non-woven polypropylene material. Between these two layers is a PET cotton layer, an alkali solid infused solid layer and a polypropylene N95 filter layer. The middle layer contains an AOP-KF patented material which claims to release Chlorine Dioxide at a constant level which is non-toxic to humans and animals. The ClO2 is required for the release of Hydroxyl Radicals (°OH).

The test methods (Table 2) used were adapted from those described for Synthetic Blood Resistance (ASTM F1862-00a) and the Bacterial Filtration Efficiency (ASTM F2101-01), Viral Filtration Efficiency was performed in the same way as a BFE, in this case the challenge organism is an E.coli Coliphage which infects E.coli ATCC 25922.

Table 2: Test Methods and Procedures used to evaluate face mask properties

DESCRIPTION	METHOD NUMBER	TITLE
Synthetic Blood Resistance	ASTM F1862-00a	Standard test method for Resistance of Medical Face Masks to Penetration by Synthetic Blood
Bacterial Filtration Efficiency	ASTM F2101-01	Evaluating the Bacterial Filtration Efficiency (BFE) of medical face mask materials, using a bacterial aerosol of *Staphylococcus aureus.*

LIQUID PENETRATION

- The mask samples were cut into circles with a 5cm diameter and then placed within a 2-stage glass filtration device.
- The desired flow rate of 30L/min was applied to the filtration system which was controlled with an air flow meter.
- The mask was then subjected to the synthetic blood at conditions of 80mmHg and 160mmHg.
- The synthetic blood is prepared with a red dye to aid with visual detection and a thickening agent (Glycerol) to simulate the flow characteristics of blood to ±0.042N/m.
- Inspections and measurements were done visually and by weight. It is a pass/fail test based on the visual detection of synthetic blood penetration through the face mask. Any incidence of synthetic blood penetration constitutes failure.

PARTICLE FILTRATION EFFICIENCY (PFE)

- The particle filtration tests were conducted with a 2-stage filtration system.
- The challenge particles were filtered through the face mask at 30L/min for 15 minutes.
- The control filtration tests without face mask were used to quantify the percentage passing through the AOP-KF face mask.
- These tests were performed with sub-micron particles in the range of 50-100nm; 100-200nm; 200-300nm and 300-1000nm.
- The tests were evaluated visually by microscope observations and quantified by dry weight.

BACTERIAL FILTRATION EFFICIENCY (BFE)

- The Bacterial Filtration Efficiency (BFE) determines the filtration efficiency by comparing the bacterial control counts with the test sample filtration counts.
- The test is conducted using the microorganism Staphylococccus aureus.
- The nebulized aerosol droplets were drawn through a sterilised 2-stage filtration system.
- The concentration of bacteria within an aerosol is determined by conducting the control experiments without the mask filter sample in the test system.
- The challenge controls were maintained at 1000-3000 colony forming units, allowing reporting filtration efficiencies <99.9%.
- The BFE provides a standardised method for testing filtration material.
- The method is reproducible and provides an increased challenge to test filters than would normally be experienced.

GENERAL DESCRIPTION OF TEST ORGANISMS AND MICROBIOLOGICAL MEDIA

a) Apparatus:	Apparatus shall comply with requirements given in SANS 1866-1:2018.
b) Test media:	Culture media for E.coli: Petone Water Buffered (Merck and Plate Count Agar (Merck, Biolab) Culture media for S.aureus: Peptone Water Buffered (Merck, Biolab) and Mannitol Salt Agar (Merck, Biolab)
c) Reference cultures:	E.coli ATCC25922 and S.aureus ATCC6538
d) Phage culture:	E.coli Coliphage (Wild Type Strain)
e) Test organism load:	Culture grown to approximately 105 organisms per millilitre and then diluted to appropriate concentration based on test.
f) Temperature of incubation:	37˚C ± 2 ˚C
g) Exposure time:	1-7 days
h) Period of incubation:	48 hours

VIRAL FILTRATION EFFICIENCY (VFE)

- The Viral Filtration Efficiency is carried out according to the same procedure as the BFE, except that the challenge organism is an *E.coli* Coliphage (Wild Type).
- The Challenge Control concentrations are maintained at 1000-3000 Plaque Forming Units (PFU). This allows filtration efficiencies to be reported up to >99.9%.
- The phage sample is nebulized and then applied across a sterilized filtration chamber.
- The virus particles that pass through are collected into a sterile medium and then tested against the host bacteria *E.coli* ATCC 25922, using the extensively used plaque assay.
- The plaque assay provides an accurate representation of the infectious viral particles which are able to penetrate through the mask and then infect the host bacteria. The coliphage is used as an indicator virus in this instance. Working with an Influenza virus or Covid-19 would however require higher biosafety levels.

3. RESULTS AND DISCUSSION

3.1: MOISTURE RESISTANCE

Table 3: Moisture Resistance and Synthetic Blood Resistance

SYNTHETIC BLOOD PENETRATION (% PASSING)	
80mmHg	160mmHg
0	0
Pass	*Pass*

The synthetic blood was unable to pass through the AOP-KF face mask at 80mmHg or 160mmHg.

The face mask therefore passes the synthetic blood test.

3.2 PARTICLE FILTRATION STUDY

Particles of different sizes were filtered through the mask within a 30cm³ chamber. The particles that pass through were measured by weight or counted microscopically.

Table 4 : Percentage Particle Removal by the AOP-KF face mask over a period of 8 minutes

TIME (min)	DIAMETER (nm)	% PARTICLE REMOVAL
8	50 - 100	99.99
8	100 - 200	99.99
8	200 - 300	99.99
8	300 - 1000	99.99

3.3 7-DAY DISINFECTION STUDY

- A 7-day study was conducted to determine whether the masks were able to disinfect themselves from known pathogens such as S.aureus and E.coli.
- The masks were also swabbed for natural airborne microorganisms which may over time colonise the mask surface.

Table 5 : Colony Forming Units present on face masks after use for 8 hours a day for 7 days

DAY	AOP-KF (CFU)/25CM²	FFP1 (CFU)/ 25CM²	3-LAYER CLOTH MASK (CFU)/ 25CM²
1	<1	5	10
Test 1 – 24 Hours No Colony Forming Units were detected on the AOP Mask. On the FFP1 Mask and the 3 ply Face Mask there was clear evidence that Colonies of Bacteria and Virus have formed within the first 24 hours, being 5 and 10 colonies.			
2	<1	8	18
Test 2 – 48 Hours No Colony Forming Units were detected on the AOP Mask. On the FFP1 Mask and the 3 ply Face Mask there was clear evidence that Colonies of Bacteria and Virus have grown and a healthy colony of Bacteria and Viruses were growing and multiplying, bringing the levels of CFU to 8 on the FFP1 and 18 on the 3 Layer Cloth Mask.			
3	<1	10	22
Test 3 – 72 Hours No Colony Forming Units were detected on the AOP Mask. On the FFP1 Mask and the 3 ply Face Mask there was clear evidence that Colonies of Bacteria and Virus have grown and a healthy colony of Bacteria and Viruses were growing and multiplying, bringing the levels of CFU to 10 on the FFP1 and 22 on the 3 Layer Cloth Mask			
4	<1	15	27
Test 4 – 96 Hours No Colony Forming Units were detected on the AOP Mask. On the FFP1 Mask and the 3 ply Face Mask there was clear evidence that Colonies of Bacteria and Virus have grown and a healthy colony of Bacteria and Viruses were growing and multiplying, bringing the levels of CFU to 15 on the FFP1 and 27 on the 3 Layer Cloth Mask			
5	<1	29	55
Test 5 – 120 Hours No Colony Forming Units were detected on the AOP Mask. On the FFP1 Mask and the 3 ply Face Mask there was clear evidence that Colonies of Bacteria and Virus have grown and a healthy colony of Bacteria and Viruses were growing and multiplying, bringing the levels of CFU to 29 on the FFP1 and 55 on the 3 Layer Cloth Mask			
6	<1	58	69
Test 6 – 144 Hours No Colony Forming Units were detected on the AOP Mask. On the FFP1 Mask and the 3 ply Face Mask there was clear evidence that Colonies of Bacteria and Virus have grown and a healthy colony of Bacteria and Viruses were growing and multiplying, bringing the levels of CFU to 58 on the FFP1 and 69 on the 3 Layer Cloth Mask			
7	<1	91	124
Test 7 – 168 Hours No Colony Forming Units were detected on the AOP Mask. On the FFP1 Mask and the 3 ply Face Mask there was clear evidence that Colonies of Bacteria and Virus have grown and a healthy colony of Bacteria and Viruses were growing and multiplying, bringing the levels of CFU to 91 on the FFP1 and 124 on the 3 Layer Cloth Mask			

- The AOP-KF face masks were attached to a personal breathing pump apparatus to simulate a person wearing the mask for 8 hours per day.
- Each day the mask was swabbed inside and outside to detect any living microbes on the mask surface, the results are presented below in table 6.

Table 6: Colony Forming Units (CFU) present on interior and exterior of AOP-KF face mask over a period of 7 days.

DAY	AOP-KF Inside Mask (EXHALED)- CFU/ 25CM²	AOP-KF Outside Mask (INHALED)- CFU/ 25CM²
1	<1	<1
2	<1	<1
3	<1	<1
4	<1	<1
5	<1	<1
6	<1	<1
7	<1	<1

3.4 BACTERIAL FILTRATION EFFICIENCY (BFE)

- In the Bacterial Filtration Efficiency test, each organism was suspended in a 0.1% Peptone water solution which was then nebulized and dispersed over the surface of the mask.
- Microbes that pass through will grow on an agar plate which allows for the enumeration of bacteria.
- The filtration efficiency percentages were calculated using the equation provided in the test method: 100(C-T)/C = % BFE
- Where C = average plate count for test control and T= plate count for the test sample that has passed through the face mask filter.

Table 7: AOP-KF Face Mask Bacterial Filtration Efficiency – Mean and (Standard Deviation)

DAY	E.COLI - % BFE	S.AUREUS - % BFE
1	99.98 (±0.4)	99.98 (±0.49)
2	99.98 (±0.4)	99.98 (±0.4)
3	99.96 (±0.4)	99.96 (±0.49)
4	99.96 (±0.4)	99.94 (±0.49)
5	99.95 (0.75)	99.80 (±0.75)
6	99.95 (±0.4)	99.69 (±1.02)
7	99.92 (±0.8)	99.40 (±2.04)

- The AOP-KF face mask significantly reduced the bacterial load that was able to pass through the mask over a 7-day period.

- There is a small decrease in %BFE after 4 days, but still over 99,9% for E.coli and S.aureus over a seven day period.

3.5 VIRAL FILTRATION EFFICIENCY (VFE)

Table 8: Viral Filtration Efficiency (%VFE) determined by plaque assay with E.coli coliphage (Wild Type)

DAY	% VFE
1	99.91 (±1)
4	99.835 (±1)
7	99.28 (±0.5)

4. CONCLUSION

THE AOP-KF FACE MASK WAS EVALUATED ACCORDING TO KNOWN ASTM TEST METHODS.

- The AOP-KF mask passed the synthetic blood test at 160mmHg.

- The solid Particle Filtration Efficiency (PFE) was then evaluated with particles within the sub-micron size range, all tests passed with >99% filtration of solid particles.

- The Bacterial Filtration Efficiency (BFE) test was then carried out with E.coli ATCC25922 and S.aureus ATCC6538, and AOP-KF face masks were found to have a %BFE of >99% over a 7-day period.

- The masks were also tested for ongoing passive disinfection by testing for bacteria present on the surface of the mask. No growth was observed on the inside or outside of the face mask over a 7-day period.

- The Viral Filtration Efficiency (VFE) was conducted with an E.coli coliphage (wild type) and the host bacteria E.coli ATCC 25922.

- Virus infectivity and quantification was performed with the double overlay plaque assay.

- Results indicate that the AOP-KF facemask was able to remove >99% of the infective E.coli Coliphage virus particles continuously over a 7-day period.

5. REFERENCES

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