

## **An Evaluation of Safety and Efficacy of the Crosstex<sup>®</sup> Face Mask treated with BIOSAFE<sup>®</sup> Antimicrobial Agent.**

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### **INTRODUCTION**

Crosstex surgical masks are designed to reduce infection in a wide variety of clinical and other settings. They provide a defensive barrier against pathogenic microorganisms. Masks are designed to filter varying sizes of organisms, thereby trapping them and not allowing them to pass through the mask.<sup>3</sup> Once captured, pathogenic microorganisms can continue to live within the fibers of the mask for days.<sup>2</sup> This technical paper discusses the effect of a new antimicrobial technology upon pathogenic microorganisms trapped on the filter media of a Crosstex mask.

### **MATERIALS & METHODS**

BIOSAFE is an antimicrobial agent that covalently bonds to a wide variety of surfaces, including textiles used in the fabrication of protective face masks. Through a proprietary application process, once bound to the surface of a mask, BIOSAFE produces a non-leaching and non-migrating protective coating. The BIOSAFE agent was uniformly applied to Crosstex masks to determine safety characteristics as well as the bactericidal efficacy of those masks when introduced to various potentially harmful microorganisms.

Test methods are described as follows:

#### **Safety Testing of BIOSAFE Treated Masks**

The following tests have been performed by highly accredited independent laboratories to demonstrate that the safety of the BIOSAFE treated masks meets the standards of the International Organization for Standardization (ISO):

#### ISO Skin Irritation Study

##### Method

The test article, BIOSAFE treated face mask, was evaluated for primary skin irritation in accordance with the guidelines of the International Organization for Standardization (ISO 10993). Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Delayed- Type Hypersensitivity. Two 25 mm x 25 mm sections of the test article and control article were topically applied to the skin of each of three rabbits and left in place for 24 hours. The sites were graded for erythema and edema at 1, 24, 48, and 72 hours after removal of the single sample application.

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Under the conditions of this study, very slight erythema and no edema were observed on the skin of the rabbits. The Primary Irritation Index for the test article was calculated to be 0.0. The response of the test article was categorized as negligible.

#### Cytotoxicity Study Using the ISO Agarose Overlay

##### Method

An in vitro biocompatibility study, based on the requirements of the International Organization for Standardization (ISO 10993-5), was conducted on the test article, BIOSAFE treated face mask, to determine the potential for cytotoxicity. Triplicate wells were dosed with a 1 cm x 1 cm portion of the test article. Triplicate wells were dosed with a 1 cm length of high density polyethylene as a negative control. Triplicate wells were dosed with a 1 cm x 1 cm portion of latex, as a positive control. Each was placed on an agarose surface directly overlaying a confluent monolayer of L-929 mouse fibroblast cells. After incubating at 37 °C in 5% CO<sub>2</sub> for 24 hours, the cell culture was examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any).

The culture was then examined microscopically (100X) to verify any decolorized zones and to determine cell morphology in proximity to the articles. Under the conditions of this study, the test article showed evidence of causing slight cell lysis or toxicity.

The test article had a Grade of 0 which meets the ISO requirements of not more than Grade 2 (mild reactivity). The negative control and the positive control performed as anticipated.

#### ISO Closed Patch Sensitization Study

##### Method

A study was conducted in the guinea pig to evaluate the potential for delayed dermal contact sensitization of BIOSAFE treated face mask. The study was conducted based on the requirements of the International Organization for Standardization (ISO 10993): Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Delayed-Type Hypersensitivity.

The test article was occlusively patched for 6 to 8 hours to the intact skin of ten guinea pigs, three times a week, for a total of nine induction treatments over a 3-week period. The control article was similarly patched to five guinea pigs. Following a recovery period, the ten test and five control animals received a challenge patch of the test article and the control article. All sites were observed for evidence of dermal reactions at 24 and 48 hours after patch removal.

Under the conditions of this study, the test article showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

#### Inhalation Toxicity Study

##### Method

This study was designed to comply with the standards set forth by EPA Health Effects Test

Guidelines, OPPTS 870.1300, final guideline, August 1998 to determine the health effects which may arise from short term exposure of the Crosstex treated mask by inhalation.

Five healthy male and five healthy female Wistar Albino rats were exposed to an aerosol atmosphere of the extract of BIOSAFE Treated Crosstex Mask, Lot/Batch# 1 (one) at a concentration of 2.1 mg/L for a period of four hours. Chamber temperature, relative humidity of air entering the chamber, chamber air flow and negative pressure were monitored and recorded. All rats were monitored during the exposure period, one hour after exposure and once daily thereafter for 14 days for toxicity and pharmacological effects. The rats were observed twice daily for mortality.

To determine results body weights were recorded prior to exposure, weekly and at termination. All animals were examined for gross pathology. The Target Concentration was estimated prior to exposure. Actual concentrations were determined gravimetrically during exposure. Particle size analyses revealed an average mass median aerodynamic diameter of 2.20  $\mu\text{m}$  with an average geometric standard deviation of 3.38  $\mu\text{m}$ .

Under the conditions of this study all animals survived the four hour 2.1 mg/L exposure. Coating of the fur with test article extract was noted during the 4 hour exposure. All animals appeared normal one hour after dosing to day 14. Body weight changes were normal. Necropsy results were normal.

Lastly, the  $\text{LC}_{50}$  of extract of BIOSAFE Crosstex Mask, Lot/Batch# 1 (one) is greater than 2.39 mg/L. This indicated that no adverse effects will result from wearing the BIOSAFE Treated Crosstex Mask.

### **Antibacterial Efficacy Testing of BIOSAFE Treated Masks**

#### **Method**

The efficacy of BIOSAFE treated face masks has been tested following the ASTM method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions (E 2149-01). The test organisms were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Salmonella enterica*, Methicillin Resistant *Staphylococcus aureus* (MRSA), and Vancomycin-Resistant *Enterococcus* (VRE).

The materials used in ASTM E 2149-01 are:

1. Large sterile petri dishes
2. Crosstex<sup>®</sup> masks (Non-treated and Treated)
3. Sterile scissors and forceps
4. Sterile 250 ml Erlenmeyer Flasks
5. Appropriate agar plates
6. Sterile Saline Solution (0.85% NaCl) for standard dilutions and rinsing
7. Sterile test tubes

8. Flame for sterilization

9. 100-1,000  $\mu$ l pipettes and pipette tips

The Test method was as follows:

1. Prepare the buffer solution.
  - a. For the **stock solution** (0.25 M  $\text{KH}_2\text{PO}_4$ ): prepare every 6 mo.
  - b.  $34 \pm 1$  g of Potassium dihydrogen phosphate and 500 ml DI water
  - c. Mix thoroughly and pH to  $7.2 \pm 0.1$
  - d. Fill to 1000 ml with DI water
  - e. Store at  $4^\circ\text{C}$
  - f. For the **working solution**: prepare every 2 mo.
  - g. 1 ml of stock solution
  - h. 800 ml of DI water
  - i. Cap and sterilize
2. Grow *S. aureus* cells in 100 ml of sterile TSB for 24-48 hrs in incubator.
  - a. Dilute *S. aureus* in buffer solution until achieve  $1 \times 10^8$  cfu/ml
  - b. Dilute to obtain final concentration of  $1 \times 10^5$  cfu/ml.
  - c. Use for inoculum
3. Cut Masks into  $9\text{in}^2$  pieces
4. Add 50 ml of working dilution (inoculum) to Erlenmeyer flask.
  - a. Cap flask and shake on shaker for 1 min.
  - b. Determine bacterial concentration of flask at this point (time 0)
5. Place treated and non-treated masks into flasks (for each sample a non-treated mask will be run along with it as a control).
6. Place flasks onto shaker for allotted time points
7. Immediately transfer 1 ml from each flask to test tube. Dilute if necessary and plate onto TSA.  
Always plate each in duplicate.
8. Incubate plates for 24-48 hrs in  $37 \pm 2^\circ\text{C}$

As described within this document the masks are shown to be safe and, in lab testing, BIOSAFE treated Crosstex masks have proven effective against:

- Staphylococcus aureus (Staph)
- Methicillin Resistant Staphylococcus aureus (MRSA)
- Vancomycin-Resistant Enterococcus (VRE)

## RESULTS

### Safety:

The BIOSAFE masks passed all of the safety tests.

### Antibacterial Efficacy:

Table 1 shows the percent reductions of Staphylococcus aureus, Methicillin Resistant Staphylococcus aureus, and Vancomycin-Resistant Enterococcus on BIOSAFE treated masks at 5 and 10 minute inoculations (kill times). Influenza A is also shown in this table.

Table 1: Percent Reductions of Microorganisms on BIOSAFE Treated Masks

<i>Kill Time</i>	5 minutes	10 minutes	30 minutes
<i>Staphylococcus aureus</i>	> 99.999%	> 99.999%	NA
MRSA (Methicillin-resistant <i>Staphylococcus aureus</i> )	--	> 99.8%	--
VRE (Vancomycin-resistant <i>Enterococci</i> )	--	99.4%	--
<i>Enterococcus faecalis</i>	> 99.999%	> 99.999%	NA
<i>Salmonella enterica</i>	> 99.99%	> 99.999%	--
<i>Pseudomonas aeruginosa</i>	> 99.999%	> 99.999%	NA
Influenza A*	98.5%	--	99.3%

\* H3N2 used as a surrogate for all Influenza A viruses including H5N1 (avian or bird flu), novel H1N1 (swine flu), and other strains

## DISCUSSION

According to the Centers for Disease Control and Prevention (CDC), as many as 2 million patients nationwide contract bacterial infections in hospitals each year – resulting in 103,000 deaths. Clinical Infections Diseases has reported that each infection cost \$15,275 or \$30.5 billion annually [This figure does not include doctors’ bills, home nursing care, lost time at work, and other non-hospital costs]. Additionally, Infection Control Today estimates that hospital-acquired infections produce an additional \$4.5 billion in annual liability claims.

The use of surgical Crosstex masks or respirators is one component of infection control practices that may reduce transmission between infected and non-infected persons.<sup>3</sup> Crosstex surgical masks are used as a physical barrier to protect employees from hazards such as splashes of large droplets of blood or body fluids. Crosstex masks also prevent contamination by trapping large particles of body fluids that may contain bacteria or viruses when they are expelled by the wearer, thus protecting other people against infection from the person wearing the surgical mask.<sup>3</sup>

Crosstex masks are used for several different purposes, including the following:

- Placed on sick people to limit the spread of infectious respiratory secretions to others.

- Worn by healthcare providers to prevent accidental contamination of patients' wounds by the organisms normally present in mucus and saliva.
- Worn by employees to protect themselves from splashes or sprays of blood or body fluids; they may also have the effect of keeping contaminated fingers/hands away from the mouth and nose.<sup>3</sup>

Because the filter portion of the mask harbors bacteria collected from the nasopharyngeal airway (or from exposure to airborne substances), care must be taken when removing the mask to avoid contamination of the hands.<sup>4</sup> OSHA (Occupational Safety & Health Administration) recommends that people who wear surgical masks should follow specific steps for donning, removing and discarding their mask in order to minimize cross-infection to the mask from contaminated hands or from the mask to the hands or other surfaces. These steps include thoroughly washing or decontaminating hands before and after removal of the mask, and to avoid touching the outside of the mask.<sup>5</sup> If adherence to these protocols is not followed, contamination can occur of the hands or mucous membranes of the wearer or others, possibly resulting in disease transmission.<sup>5</sup> Exposure to airborne substances can result in contamination of the external surface of the respirator or medical mask as well as contamination of the filter material.<sup>1</sup> The testing results described above demonstrate that the BIOSAFE treated mask clearly kills harmful bacteria trapped on the mask.

### CONCLUSIONS

1. The results of biocompatibility and cytotoxicity studies at highly accredited independent laboratories utilizing ISO Standards indicated that BIOSAFE treated Crosstex masks are safe to use.
2. The results of the microbiology testing indicate that BIOSAFE treated Crosstex masks kill or inactivate at least 99% of the harmful bacteria and viruses tested within 10 minutes of contact with the mask.

1 REUSABILITY OF FACEMASKS DURING AN INFLUENZA PANDEMIC FACING THE FLU Committee on the Development of Reusable Facemasks for Use During an Influenza Pandemic Board on Health Sciences Policy INSTITUTE OF MEDICINE OF THE NATIONAL ACADEMIES THE NATIONAL ACADEMIES PRESS Washington, D.C. [www.nap.edu](http://www.nap.edu) 2 Journal of Clinical Microbiology. 2000 February; 38(2): 724–726. 3 Guidance on Preparing Workplaces for an Influenza Pandemic OSHA 3327-02N 2007 4 AORN 2005 5 Interim Guidance on Planning for the Use of Surgical Masks and Respirators in Health Care Settings during an Influenza Pandemic

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