Ryerson University

Faculty of Science

SG24 LLC – SkinGuard24 BZK Formula

TECHNICAL REPORT:

ISO 22196:2011 Measurement of antibacterial activity on plastics and other non-porous surfaces "Modified Large droplet Inoculation Method."

Antimicrobial Activity against *Staphylococcus aureus* on ***VITRO-SKIN®***

SG24 LLC, c/o Joe Raich P O Box 406, Bolingbroke, GA 31004

Analysis performed by

Alexander Cashera* & Dr. Lukasz Porosa

*Certified Microbiologist, KHN 305

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DEPARTMENT OF CHEMISTRY & BIOLOGY
350 Victoria Street, Toronto, ON, Canada M5B 2K3 Tel: 416.979.5000 x 4471 Fax: 416.979.5277 E-mail: alexander.caschera@ryerson.ca

1.Materials Submitted for Testing:

Test Substrates: Hydrated VITRO-SKIN® (21 x 1.0 x 1.0 in.).

Treatments:
1. 3 x control - untreated
2. 3 x treated 1 – BZK (0.13%): 0 hour residual protection
3. 3 x treated 2 – BZK (0.13%) + SG90C (0.33%): 0 hour residual protection
4. 3 x treated 1 – BZK (0.13%): 6 hour residual protection
5. 3 x treated 2 – BZK (0.13%) + SG90C (0.33%): 24 hour residual protection
6. 3 x treated 1 – BZK (0.13%): 6 hour residual protection
7. 3 x treated 2 – BZK (0.13%) + SG90C (0.33%): 24 hour residual protection

Test Organism: *Staphylococcus aureus*

VITRO-SKIN© swatches (21 x 1.0 x 1.0 in.) were hydrated by Dr. Lukasz Porosa overnight (24 hrs) using the recommended glycerol/water (15:85%, ~ 300 mL) humidifier provided with the VITRO-SKIN starter kit prior to ISO 22196 testing.

VITRO-SKIN© is an advanced testing substrate that effectively mimics the surface properties of human skin. It has been formulated to have topography, pH, critical surface tension and ionic strength that is similar to human skin.

VITRO-SKIN© is currently used by over 155 leading companies worldwide and has been referenced in numerous scientific presentations and patents. It has been successfully applied in a broad range of in vitro methods including the measurement of SPF and UVA protection factors, evaluation of the water resistance of prototype sunscreen formulations, rapid assessment of the performance of sunless tanning formulations, evaluation of the performance of adhesive bandages, assessment of prototype and emollient spreading. Testing done on VITRO-SKIN is generally more reproducible than that performed on human skin due to the consistent topography and wetting properties across each sheet. VITRO-SKIN with N-19 topography is optimized to mimic human back skin. It is a synthetic (non-biological) product.

2.Significance and Use:

Large-droplet inoculation method

Many pathogens are able to remain viable during extended periods of desiccation on surfaces at the solid-air interface. Long-term survival of pathogens in the inanimate environment poses a significant risk for infection transmission and cross-contamination in high-risk environments such as hospital rooms or food-processing plants.

The large-droplet inoculation method was developed to simulate the deposition of bacterial species onto exposed surfaces and to determine the ability of these cells to

survive desiccation and or induce biofilm formation. In this experiment ***VITRO-SKIN*** was sanitized with a common BZK (0.13%) antiseptic foam hand sanitizer with and without SG90C (0.33%) to assess the residual kill of the protective cationic polymer right after product application/drying and after various time points post application (6 and 24 hour residual sanitizer protection).

3.Preparation of Bacterial Inoculum.

Challenge culture of Staphylococcus aureus – Strain (ATCC #4330) was grown in 5-10 mL10% tryptic soy broth for 24 hours on a shaking incubator at 37 C. and washed twice by centrifugation to replace the growth media with sterile water (2 x 2 mL tubes washed with 2 x 1 mL water) and diluted 1:1 (total 4 mL) to give to a target range of 1.96 $x10^5$ cfu/mL after plating on agar.

4.Preparation of the Test Specimen:

Prior to application of hand sanitizer a sheet of ***VITRO-SKIN*** was cut into a total of 21 1 inch by 1 inch squares (3 x 7 samples) using high-quality paper cutters or shears and hydrated for 24 hrs inside a closed, controlled-humidity plastic chamber provided with the ***VITRO-SKIN*** starter-kit. The humidity in the chamber was regulated by a solution of 85% water / 15% glycerine (350g / 52 g), placed in the bottom of the chamber. *The substrate was placed above the liquid on a shelf or tray*. This step insures reproducible hydration of the ***VITRO-SKIN*** prior to product application. Nitrile gloves were worn while handling the sheets.

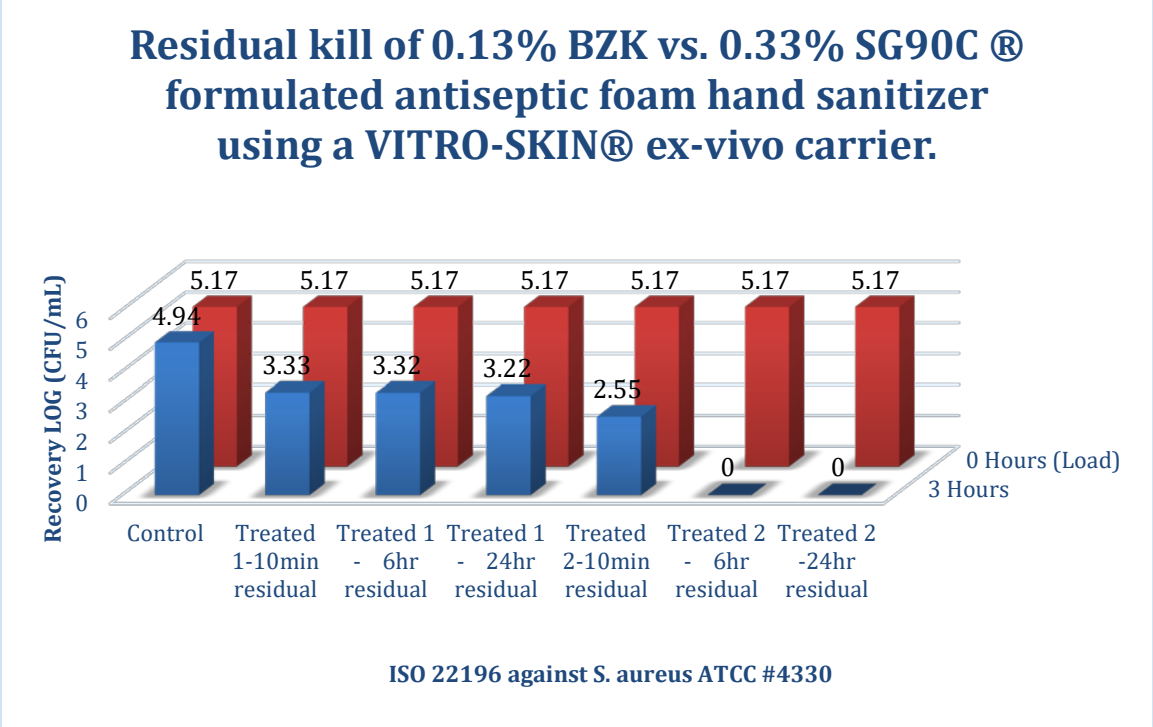
All samples were sanitized with 0.5 mL 70% (v/v) ethanol by delivering 10 x 0.05mL mini drops which was rubbed with a sterile nitrile glove until dry. BZK (0.13%) antiseptic foam hand sanitizer was delivering onto the skin by means of 10 x 0.05mL mini drops totalling 0.5 mL with a 1 mL syringe. Sterile nitrile gloves were used to massage and spread the product across 1 inch x 1 inch VITRO-SKIN squares and rubbed for 30 seconds until completely dry. The above product application was repeated with the BZK + SG90C product. Two samples in triplicate were inoculated immediately after 10 minutes of drying and the remaining two samples in triplicate were inoculated after 6 and 24 hours of leave on residual.

5.Testing Procedure:

The untreated and treated VITRO-SKIN® samples in triplicate were each placed into separate sterile petri dishes and 0.05 mL *Staphylococcus aureus* inoculum was placed on each 1.0 by 1.0 inch square for a total of 1.05 mL (21 x 0.05 mL). Samples were left inside the petri dishes and allowed to air-dry in a biological safety cabinet and incubated at 36 ± 1 °C until the droplet of bacteria was visibly dry. Drying typically occurred 3 hours after 0.05 mL inoculations, and surviving cells were enumerated immediately after drying. For enumeration, inoculated coupons were sacrificed in triplicate and placed inside separate 50 mL falcon tubes containing 5mL of a 0.9% saline collection liquid. Saline was used as the recovery liquid instead of nutrient broth in order to stress the cells to stimulate biofilm production and to prevent osmotic shock capable of weakening the membranes. Each coupon was agitated vigorously for 1 minute with a bench-top vortex to transfer cells from the test surface to the collection liquid. Standard agar plate counts after 48-72 hours at 35^{o}C were then performed on serial dilutions (10^0-10^3) of the collection liquid (5 x 10 uL x 2 plates), and colony counts of *Staphylococcus aureus* colonies (20-200) from triplicate treated surfaces were averaged and compared to colony counts from tests of untreated control surfaces carried out in parallel.

6.Evaluation of Results:

Sample ID	LOG (CFU/mL)	Log Reduction	%Log Kill
Control T = 0	**5.17**		
Control T = 3	**4.94**	**0.23**	**41.115634**
Residual BZK = 10 min	**3.33**	**1.61**	**97.545291**
Residual BZK = 6 hr	**3.32**	**1.62**	**97.601167**
Residual BZK = 24 hr	**3.22**	**1.72**	**98.094539**
Residual SGC = 10 min	**2.15**	**2.79**	**99.837819**
Residual SGC = 6 hr	**0.00**	**4.94**	**99.998852**
Residual SGC = 24 hr	**0.00**	**4.94**	**99.998852**



7.Calculation of the "Antibacterial Activity":

This is the difference in the logarithm of the viable cell count found on an antimicrobial-treated product and a control product after inoculation with, and incubation of, the bacteria. The following equation were used:

(a)Log Reduction Calculation

$$\text{Log Reduction} = \log_{10}\left(\frac{A}{B}\right)$$

or,

$$\text{Log Reduction} = \log_{10}(A) - \log_{10}(B)$$

A = the average number of viable bacteria (bacteria/mL) recovered from the control test specimens..
B = the average number of viable bacteria (bacteria/mL) after 3 hr of contact time

(b) Log Reduction to Percent Reduction Calculation

$$P = (1 - 10^{-L})\,x\,100$$

P = % reduction
L = log reduction

(c) Relationship between log reduction and percent reduction.

1 log reduction = 10^1 times less organisms = 90% reduction = ninety
2 log reduction = 10^2 times less organisms = 99% reduction = one hundred
3 log reduction = 10^3 times less organisms = 99.9% reduction = one thousand
4 log reduction = 10^4 times less organisms = 99.99% reduction = ten hundred thousand
5 log reduction = 10^5 times less organisms = 99.999% reduction = one hundred thousand
6 log reduction = 10^6 times less organisms = 99.9999% reduction = one million
7 log reduction = 10^7 times less organisms = 99.99999% reduction = ten million
etc...

8. Antibacterial Activity

Antibacterial Activity	%Kill compared to control	Comment
<1.5	**<96.8**	**poor**
1.5 to 2.0	**96.8-99.0**	**borderline**
2.0 to 3.0	**99.0-99.9**	**good**
>3.0	**>99.9**	**excellent**

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9. Conclusion

Typically, when a product performs with > 3-log (99.9%) or greater it is deemed bacteriostatic by FDA standards. Leave on antimicrobial hand sanitizer formulated with SG90C exhibited excellent antimicrobial performance > log 4 (99.99) reduction of *Staphylococcus aureus* (S.*aureus*) a common skin inhabitant by the large droplet inoculation method (50 uL) on VITRO-SKIN. Long lasting residual antimicrobial activity was demonstrated of up to 24 hours.