Radiation Shield Technologies, Inc.
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Subject Product: Demron C/ Demron ICE
Project No.: DG20118-RAD01

Purpose

The test article was subjected to Viral Penetration testing as outlined in International Organization for Standardization, ISO16604:2004, *Clothing for protection against contact with blood and body fluids — Determination of resistance of protective clothing materials to penetration by blood-borne pathogens — Test method using Phi-X174 bacteriophage*[1] and American Society for Testing and Materials (ASTM) F1671/F1671M-13, *Standard Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Blood-Borne Pathogens Using Phi-X174 Bacteriophage Penetration as a Test System.*[2] It is important to note that the standards referenced have specific criteria for the sampling plan of products submitted for testing. This approach is controlled and justified by the sponsor. The test results will be compared against the ISO16604 and ASTM F1671 standards,

Testing Results and Discussion

All testing was conducted in accordance with the referenced standards. The results of the testing are outlined in Table 1.

Table 1. Demron C/ Demron ICE Viral Penetration Results

Test	NL Study Number	# Test Articles	Pre-Challenge Concentration (PFU/mL)	Post-Challenge Concentration (PFU/mL)	Assay Titer (PFU/mL)	Visual Penetration	Test Result
ISO16604:2004 Procedure A	1290356-S02	3	3.3×10^8	1.8×10^8	< 1[a]	None Seen	Pass
ISO16604:2004 Procedure C	1290356-S02	3	3.3×10^8	1.8×10^8	< 1[a]	None Seen	Pass
ASTM F1671	1289030-S02	3	2.0×10^8	2.4×10^8	< 1[a]	None Seen	Pass

a) A value of <1 plaque forming unit (PFU)/mL is reported for assay plates showing no plaques.

The ϕX174 bacteriophage is a spherical (icosahedral) non-enveloped virus that infects the bacteria *Escherichia coli C.* ATCC 13706*.* The bacteriophage was selected as the model for the following test methods because it is one of the smallest known viruses (0.027 µm in diameter), is environmentally stable, is non-infectious to humans, and has a high-assay sensitivity.

ISO16604:2004 This test method was performed to evaluate the barrier performance of protective materials which are intended to protect against blood borne pathogen hazards. Test articles were conditioned for a minimum of 24 hours at 21 ± 5°C and 60 ± 10% relative humidity (RH), and then tested for viral penetration using a ϕX174 bacteriophage suspension. At the conclusion of the test,

the observed side of the test article was rinsed with a sterile medium and assayed for the presence of ϕX174bacteriophage. The grey side of the pre-cut material was tested.

Procedure A: Used for selecting critical zone materials and components to limit exposure in situations involving presence of a large amount of blood or body fluids, a direct liquid contact, pressing, and leaning. A retaining screen is not used to support the sample and the following pressure and time sequence was used: 0 kPa for 5 min, followed by 14 kPa for 1 min, followed by 0 kPa for 4 min. All three Demron C/ Demron ICE test articles showed no visual penetration and <1 PFU after plaque assay.

Procedure C: Used for selecting critical zone materials and components of protective apparel, to limit exposure in situations involving the presence of blood or body fluid, and different levels of contact pressures. A retaining screen is not used to support the sample and the following pressure and time sequence was used: 0 kPa for 5 min, followed by 0 kPa for 5 min. All three Demron C/ Demron ICE test articles showed no visual penetration and <1 PFU after plaque assay.

ASTM F1671: This test method was performed to evaluate the barrier performance of protective materials which are intended to protect against blood borne pathogen hazards. Test articles were conditioned for a minimum of 24 hours at 21 ± 5°C and 30-80% relative humidity (RH), and then tested for viral penetration using a ϕX174 bacteriophage suspension. At the conclusion of the test, the observed side of the test article was rinsed with a sterile medium and assayed for the presence of ϕX174 bacteriophage. The grey side of three (3), pre-cut test articles were tested using exposure procedure A (no retaining screen). All three Demron C/ Demron ICE test articles showed no visual penetration and <1 PFU after plaque assay. (ASTM) F1671/F1671M-13 states, “This test method was developed to assess the effectiveness of materials used in protective clothing for protecting the wearer against contact with blood-borne pathogens using a surrogate microbe suspended in a body fluid simulant under conditions of continuous contact.”

Recommendations

It is recommended to assess the biocompatibility of the Demron C/ Demron ICE material at a minimum with cytotoxicity test and a material review to ensure a low risk of sensitization or irritation response from the user of the material.

Additional testing should be performed with replicate samples to achieve a statistically significant result as outlined in ISO 16604:2004 8.10 and ASTM F1671 15.2.2 “When qualifying the integrity of materials, supporting broad product claims, or using the test as a quality control and assurance procedure, the test should be modified for larger data sets with proper statistical design and analysis.” Such analysis is the responsibility of the sponsor.

Conclusion

The discussion and conclusion generated in this report apply only to the Radiation Shield Technologies, Inc. Demron C/ Demron ICE material which has been subjected to the testing reported in **Table 1**. The testing outlined above was conducted in accordance with ISO16604 and ASTM

F1671. The Demron C/ Demron ICE material submitted for testing showed no visual penetration of viral media and no plaque forming units were observed, as outlined in ISO16604 Procedure A, ISO16604 Procedure C, and ASTM F1671. Since there is no lab testing that can be performed against SARS-CoV-2, these passing results of the viral penetration test, using Phi-X174 Bacteriophage, demonstrates effective barrier protection against envelope viruses like SARS-CoV-2 under continuous contact conditions, since the average diameter of a coronavirus, 0.125 µm, is larger than the ϕX174 bacteriophage, 0.027 µm.[3] Any requirements for labeling are the responsibility of the sponsor in order to comply with current laws and regulations.

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References

1. ISO16604:2004, Clothing for protection against contact with blood and body fluids — Determination of resistance of protective clothing materials to penetration by blood-borne pathogens — Test method using Phi-X174 bacteriophage. *International Organization for Standardization* 2004.
2. ASTM F1671/F1671M-13, Standard Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Blood-Borne Pathogens Using Phi-X174 Bacteriophage Penetration as a Test System. *American Society for Testing and Materials* 2013.
3. Goldsmith CS, Tatti KM, Ksiazek TG, et al. Ultrastructural characterization of SARS coronavirus. *Emerg Infect Dis* 2004; **10**(2): 320-6.