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(Ministry of Home Affairs, Government of India)

COVID -19 Testing Facility (ICMR APPROVED)

ANALYSIS REPORT

EFFICACY TESTING OF MERINO LAMINATES FOR COVID-19 VIRUS AS PER ISO 21702:2019 (MODIFIED) TEST METHOD

Report No.

NFSU/IFS/AntiViral Testing/03/2020

Date: 21st December 2020

Ref. Letter from Merino Industries Limited **Date: 30-11-2020**

Objective

To evaluate the antiviral activity on the surface of one sample as demonstrated by the ISO 21702:2019 (Modified) test method.

Test Sample Details

Name of the Product:	Merino High Pressure laminates (HPL)
Type of the product:	High Pressure laminates (HPL)
State of Product:	High Pressure laminates (HPL)
Name of Company	Merino Industries Limited

Brief Note on Test Organism and Target Genes

- SARS-CoV-2 is a positive-sense, single-stranded RNA (ssRNA), group IV virus. CoV genomes code for a ORF1a / ORF1ab polyprotein and four structural proteins and the non-structural genes include the RNA dependent RNA polymerase (RdRP) which are widely studied as major drug and detection targets.

Test Procedure Summary

- The test organism was adjusted and diluted to obtain the starting inoculum concentration of Ct value of 24.
- The control was tested in triplicate at Time = 00, 10, 20, 30, 40 and 120 min. The test samples were tested in triplicate at Time = 00, 10, 20, 30, 40 and 120 min.
- Each sample piece was placed in a sterile Petri dish, inoculated and then covered with the sterile plastic in order to spread the inoculum evenly over the sample surface and hold it in place and incubated at 35°C till the sample got dried.
- Then the samples were incubated at 35°C and a relative humidity of at least 90%. At the appropriate time the entire area of the sample piece was swabbed using the sterile wet cotton swab to collect the inoculum from the surface and mixed in 200 µL nuclease free water and treated with Ambion™ RNase A (As per the manufacturer protocol).
- Then the RNAse Treated sample was processed for RNA extraction using MagMAX™ RNA Extraction Kit (Thermo Fisher Scientific) method.
- After RNA extraction, the ICMR-NIV Covid-19 Confirmation kit was used to measure the Ct value of each sample.

- The C_T value of each sample was recorded. The results are found in the "Test Results" section below. These results pertain only to the samples tested. All the samples were run in triplicate.

Test Variables

No.	Variable	Details
1	Sample Submission Date	12-12-2020
2	Sample Testing Date	19-12-2020
3	Report Date	21-12-2020
4	Sample to be tested	6005431 Grade – Treated Merino High-Pressure laminates (HPL)
5	Test Organism	COVID-19 Virus
6	Sample Size	50 mm x 50 mm x 1.0 mm
7	Method of Sterilization / Pre-Cleaning	Cleaning of the laminate sheet with mild detergent followed by final washing with sterile deionised water.
8	Control Sample	6005428 Grade – Untreated Merino High-Pressure laminates (HPL)
9	Dilution Medium Used	Viral Transport Medium
10	Starting Inoculum Concentration	C_T value 24
11	Amount of Inoculum	70 µL
12	Contact Time	00, 10, 20, 30, 40, 120 min
13	Deviations from Standard Test Method	Yes. The method (ISO 21702:2019) was modified for the test organism COVID-19 Virus.

Test Results

The tested material (Treated High Pressure laminates (HPL) was found to be 99.9% efficient to remove Covid-19 virus within 30 min after exposure to Treated Merino High-Pressure Laminates. It is noted that these results pertain only to the samples (Batch No. 6005431) tested only.

No.	Contact Time (Min	Treated Merino High-Pressure laminates (HPL)						Untreated Merino High-Pressure laminates (HPL)					
		**RdRp Gene C_T (Average)	Delta C_T	% Reduction in viral Load	**ORF Gene C_T (Mean of Triplicate)	Delta C_T	% Reduction in viral Load	**RdRp Gene C_T (Average)	Delta C_T	% Reduction in viral Load	**ORF Gene C_T (Average)	Delta C_T	% Reduction in viral Load
1	0	29	1	-	29	1	-	29	1	NS	29	1	NS
2	10	30	2	99	29	1	-	29	1		29	1	
3	20	30	2	99	30	2	99	29	1		29	1	
4	30	31	3	99.9	31	3	99.9	29	1		29	1	
5	40	32	4	99.99	32	4	99.99	29	1		29	1	
6	120	-	-	End Point	-	-	End point	29	1		29	1	
7	Positive Control	28	-	-	28	-	-	28	-	-	28	-	
8	Negative Control	ND	-	-	ND	-	-	ND	-	-	ND	-	

** = Mean of Triplicates, ND=Not Detected, NS=Not Significant

Test Results Interpretation

The value of the antiviral activity was calculated according to the formula listed below and recorded as log reduction.

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

Where,

R : antiviral activity

U_0 : average of logarithm numbers of viable viral particles from untreated control at Time = 0 h

U_t : average of logarithm numbers of viable viral particles from untreated control at Time = t Min

A_t : average of logarithm numbers of viable viral particles from test sample at Time = t Min

According to the standard, an antiviral product is determined to have antiviral effectiveness when the antiviral activity (R) is 2.0 or more.

Percent reductions are determined by comparing the sample after the contact time to the untreated laminate control after the contact. Reporting of percent reduction is not indicated by the test method but is provided by NFSU as additional information.

Percent reduction is translated into log reduction by the following:

90% reduction = 1 log reduction; i.e. Ct 10 increased to Ct 11 is a 1 log reduction

99% reduction = 2 log reduction; i.e. Ct 10 increased to Ct 12 is a 2 log reduction

99.9% reduction = 3 log reduction; i.e. Ct 10 increased to Ct 13 is a 3 log reduction

99.99% reduction = 4 log reduction; i.e. Ct 10 increased to Ct 14 is a 4 log reduction

99.999% reduction = 5 log reduction; i.e. Ct 10 increased to Ct 15 is a 5 log reduction

Analysis Performed and Approved by

22/12/2020

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