



THE BOMBAY TEXTILE RESEARCH ASSOCIATION

(Approved Body of the Ministry of Textiles, Govt. of India)
Lal bahadur Shastri Marg, Ghatkopar (West), Mumbai - 400086.
Phones : 022 - 6202 3636 / 6202 3600
Fax : 91-22-2500 0459 Email : btloffice@btraIndia.com / info@btraIndia.com
Website: www.btraIndia.com



BTRA TEST LABORATORIES

TEST REPORT

Report Details : BTL/TR/B2306354/MWO/1/B/2023	ULR No : TC69412300006354	Date : 01-12-2023
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Shree Umiya Rotoplast Container

Plot No. 115, GIDC Vartej,

Bhavnagar

Pincode : 364001

Contact Person : Tarak Patel

THE FOLLOWING SAMPLE(S) WAS/WERE SUBMITTED AND IDENTIFIED BY/ON BEHALF OF THE CUSTOMER AS :

Sample No	: B2306354-1	Product Description	: Inner layer of Plastic Tank
Received on	: 03-10-2023	Total Number of Samples in Order	: 1
Testing Period	: 03.10.2023 To 30.11.2023	Reference	: Dtd.3.10.2023
Despatched on	:	Sample Type	: MWO
Project Name	:	Page	: 1 / 16

TEST RESULTS

Test Material	:	Inner layer of Plastic Tank
Sample Size	:	50 x 50 mm pieces
Date Of Test	:	02/11/2023
Name Of Test	:	Standard practice for determining resistance of synthetic polymeric materials to Fungi [ASTM G:21]
Media Used	:	Nutrient Salt Agar
Test Organism	:	<i>Aspergillus brasiliensis</i> ATCC 9642 <i>Talaromyces pinophilus</i> ATCC 11797 <i>Chaetomium globosum</i> ATCC 6205 <i>Trichoderma virens</i> ATCC 9642 <i>Aureobasidium pullulans</i> ATCC 15233
Incubation Conditions	:	28± 2°C for 28 days

Ashwini Govekar
Microbiology
(Authorised Signatory)



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Results :

Sample	Growth on sample	Rating	Week
B2306354-1 (Inner layer of Plastic Tank)	No growth	0	4

Observed growth on sample	Rating
None	0
Traces of growth (less than 10%)	1
Light growth (10-30%)	2
Medium growth (30-60%)	3
Heavy growth (60% and above)	4

Note : No optical, electronic, or physical test was performed after the end of incubation period.

Conclusion : The sample **B2306354-1 (Inner layer of Plastic Tank)** showed **no growth (Rating 0)** on its surface, when tested as per ASTM G 21- 2015 (RA: 2021) Test Method.

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Despatched on	:	Sample Type	: MWO
Project Name	:	Page	: 3 / 16

TEST RESULTS

Test Material : Inner layer of Plastic Tank
Sample Size : 50mm x 50mm
Date Of Test : 01/11/2023
Name Of Test : Antibacterial Activity on Plastics surfaces ISO 22196(c)
Media Used : Nutrient broth, SCDLP Broth, Plate count agar
Test Organism : *Staphylococcus aureus* ATCC 6538P
Escherichia coli ATCC 8739
Incubation Conditions : 35± 2°C for 24-48 hrs
Procedure : **Diluents:** Phosphate buffer solution, Phosphate buffered physiological saline
Culture density: 2.5 to 10 x 10⁵ CFU/ml
Size of cover film: 40mm x 40 mm
Volume of test inoculum inoculated: 0.4 ml

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Result :

Test organism: <i>Staphylococcus aureus</i> ATCC 6538P			
No. of bacteria from Untreated sample at 0 hour: $N(U_0) = 7.27 \times 10^3$		Log $N(U_0) = 3.86$	
No. of bacteria from Untreated sample at 24 hours: $N(U_t) = 7.73 \times 10^6$		Log $N(U_t) = 4.89$	
Sample Code	No. of bacteria from Treated Sample at 24 hours $N(A_t)$	Log $N(A_t)$	Antimicrobial activity $R = U_t - A_t$
B2306354-1 (Inner layer of Plastic Tank)	36.04	1.56	3.33

Test organism: <i>Escherichia coli</i> ATCC 8739			
No. of bacteria from Untreated sample at 0 hour: $N(U_0) = 5.67 \times 10^3$		Log $N(U_0) = 3.75$	
No. of bacteria from Untreated sample at 24 hours: $N(U_t) = 6.13 \times 10^4$		Log $N(U_t) = 4.79$	
Sample Code	No. of bacteria from Treated Sample at 24 hours $N(A_t)$	Log $N(A_t)$	Antimicrobial activity $R = U_t - A_t$
B2306354-1 (Inner layer of Plastic Tank)	43.13	1.63	3.16

P.S. : The value of Antimicrobial activity (R) obtained by the testing methods of this standard shall not be less than 2.0 for the antimicrobial products.

Conclusion : The Sample **B2306354-1 (Inner layer of Plastic Tank)** showed Antibacterial activity value (R) against different organisms as follows, when tested by ISO 22196 Method.

Staphylococcus aureus ATCC 6538P = **3.33**
Escherichia coli ATCC 8739 = **3.16**

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Despatched on	:	Sample Type	: MWO
Project Name	:	Page	: 5 / 16

TEST RESULTS

Test Material	:	Inner layer of Plastic Tank
Sample Size	:	50mm x 50mm
Date Of Test	:	08/11/2023
Name Of Test	:	ASTM G29:2016 Determining Cyanobacterial Resistance of Polymeric Films
Experimental Conditions	:	Test vessel : 500 ml capacity Sample Size : 25 mms x 65 mms approx. Propagation Medium : BG 11 broth Test algae : Oscillatoria brevis UTEX B 1567 (Blue green algae) Illumination : Cool white fluorescent light of intensity 2000 lux Incubation conditions : Room Temperature / 2 weeks
Procedure	:	1. Treated and untreated specimens are suspended in Test vessel containing homogenized algal cells in Culture medium under static conditions. 2. Test vessels are incubated under 12 hours of light and 12 hours of dark for 2 weeks. 3. Test vessels are reinoculated with fresh algae every 2 to 3 days and the test is completed after 14 days. 4. Visual observations are made for presence of algal growth on the surface of the Test substrate over a period of 2 weeks or whenever untreated films show dense Cyanobacterial growth.

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Results :

Sample Description	Growth of algae	Rating
B2306354 (Inner layer of Plastic Tank)	No Growth	0
Lab Control	Growth	4

Evaluation Criteria : Extent of algal growth upon surface is as follows: -

Growth on specimen	Rating
No Growth	0
Trace of Growth (< 10 %)	1
Light Growth (11 to 30 %)	2
Medium Growth (31 to 60 %)	3
Heavy Growth (>60% to complete coverage)	4

P.S. : This test was subcontracted to a competent laboratory.

Conclusion : Sample **B2306354-1 (Inner layer of Plastic Tank)** showed No growth (Rating 0) when tested ASTM G29.

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Despatched on	:	Sample Type	: MWO
Project Name	:	Page	: 7 / 16

TEST RESULTS

Test Material	: Inner layer of Plastic Tank. Sample surface (50 mm x 50 mm); Pre-sterilized by ETO
Control Sample	: LDPE film (40 mm x 40 mm); Pre-sterilized by ETO
Date Of Test	: 08/11/2023
Date of completion	: 28/11/2023
Name Of Test	: ISO 21702:2019; Measurement of Antiviral activity on plastics and other non-porous surfaces and coating materials
Virus strains and host cells	: Test Virus: Influenza A virus (H1N1): A/Hong Kong/8/68; ATCC VR-1679 Host Cell: MDCK cell ATCC CCL-34; Passage No.: Cells from PN 76
Virus inoculum volume	: 0.4 ml
Viral titre	: 1.60×10^8 PFU/ ml
Contact Period	: 24 hours
Wash out Medium	: SCDLP
TCID50 method	: 96 Well plate
Medium of Cell culture	: Eagle's minimal essential medium (EMEM). Supplemented with inactivated FBS & antibiotics.
Incubation	: 37^0 C in CO ₂ incubator/ 7 days

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Verification of cytotoxicity on Host cells

1. Sterile Control and Test samples were added with 10 ml wash out solution, SCDLP medium. To ensure that the neutralizer completely washes the specimens, SCDLP broth was pipetted at least four times.
2. 0.1 ml of the washing out solution was inoculated into 6 well plate having monolayer of host cells in duplicates.
3. It was incubated at 34⁰ C for 2-3 days.
4. After incubation, plates were examined visually for cytotoxic effect if any.

Observations :

Sample Description	Observations
B2306354 (Inner layer of Plastic Tank)	Non-Cytotoxic

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Verification of cytotoxicity on: 1. Sterile Control and Test samples were added with 10 ml wash out solution, SCDLP Host cells medium. To ensure that the neutralizer completely washes the specimens, SCDLP broth was pipetted at least four times.

2. 0.1 ml of the washing out solution was inoculated into 6 well plate having monolayer of host cells in duplicates.

3. It was incubated at 34⁰C for 2-3 days.

4. After incubation, plates were examined visually for cytotoxic effect if any.

Verification of cytotoxicity by: 1. Sterile Control and Test samples were added with 10 ml wash out solution, SCDLP cell sensitivity to virus and the medium. To ensure that the neutralizer completely washes the specimens, SCDLP inactivation of antiviral activity broth was pipetted at least four times.

2. The washing out solution was inoculated with virus suspension and incubated at 25⁰C for 30 minutes.

3. Infective titre of this solution was determined by TCID50 Method.

4. Log of TCID50/ ml of Control LDPE film - Log of TCID50/ ml of Test LDPE film less than or equal to 0.5.

Observations :

Log of TCID50/ ml of Control LDPE film	Log of TCID50/ ml of Test Sample	Log of TCID50/ ml of Control LDPE film - Log of TCID50/ ml of Test Sample	Acceptable Criteria
3.5	3.26	0.24	less than or equal to 0.5

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Test Procedure

1. 0.4 ml of the test inoculum was added onto the test surface. It was covered with LDPE film to ensure that the test inoculum makes contact with test surface and spreads to the edges.
2. After the specimen was inoculated and the cover film applied, It was closed with the lid of the Petri dish and incubated at $35 \pm 1^\circ\text{C}$ and a relative humidity of not less than 90 % for 5 minutes.
3. Immediately after inoculation, the three Control sample were terminated using SCDLP medium to retrieve the virus.
4. After the contact time of 24 hours remaining Control and Test samples were terminated using SCDLP medium to retrieve the virus.
5. Infectivity titer of virus recovered from the sample was determined by TCID₅₀ method

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Results :

Virus	Contact Duration	Group	Logarithm of Infectivity titre of virus (IgTCID ₅₀ /cm ²)	Average titre Infectivity of virus (IgTCID ₅₀ /cm ²)
Influenza virus suspension: (1.70 × 10 ⁸ PFU/ml)	0 hours	Control (U ₀)	6.74	6.51
			6.40	
			6.40	
	24 hours	Control (U _t)	6.40	5.96
			6.07	
			5.40	
	24 hours	B2306354 [Inner layer of Plastic Tank] (A _t)	3.74	3.74
			3.40	
			4.07	
Antiviral activity R= U _t - A _t (24 hours contact)			-	2.22 (99.39%)

Where

R is the Antiviral activity

U₀ is the average of common logarithm from three control/ untreated specimen immediately after inoculation

U_t is the average of common logarithm from three control/ untreated specimen after 24 hours

A_t is the average of common logarithm from three Treated specimen after 24 hours

P.S. : This test was subcontracted to a competent laboratory.

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Project Name	:	Page	: 12 / 16

TEST RESULTS

Test Material	: Inner layer of Plastic Tank. Sample surface (50 mm x 50 mm); Pre-sterilized by ETO
Control Sample	: LDPE film (40 mm x 40 mm); Pre-sterilized by ETO
Date Of Test	: 08/11/2023
Date of completion	: 28/11/2023
Name Of Test	: ISO 21702:2019; Measurement of Antiviral activity on plastics and other non-porous surfaces and coating materials
Virus strains and host cells	: Test Virus: Human Coronavirus HCoV-229E (Surrogate of SARS-CoV-2) Host Cell: MRC-5 cell line (ATCC CCL-171); Passage No.: Cells from PN 17
Virus inoculum volume	: 0.4 ml
Viral titre	: 1.60×10^8 PFU/ ml
Contact Period	: 24 hours
Wash out Medium	: SCDLP
TCID50 method	: 96 Well plate
Medium of Cell culture	: Eagle's minimal essential medium (EMEM). Supplemented with inactivated FBS & antibiotics.
Incubation	: 37^0 C in CO ₂ incubator/ 7 days

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Verification of cytotoxicity on Host cells

1. Sterile Control and Test samples were added with 10 ml wash out solution, SCDLP medium. To ensure that the neutralizer completely washes the specimens, SCDLP broth was pipetted at least four times.
2. 0.1 ml of the washing out solution was inoculated into 6 well plate having monolayer of host cells in duplicates.
3. It was incubated at 34⁰ C for 2-3 days.
4. After incubation, plates were examined visually for cytotoxic effect if any.

Observations :

Sample Description	Observations
B2306354 (Inner layer of Plastic Tank)	Non-Cytotoxic

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2. 0.1 ml of the washing out solution was inoculated into 6 well plate having monolayer of host cells in duplicates.

3. It was incubated at 34⁰C for 2-3 days.

4. After incubation, plates were examined visually for cytotoxic effect if any.

Verification of cytotoxicity by: 1. Sterile Control and Test samples were added with 10 ml wash out solution, SCDLP cell sensitivity to virus and the medium. To ensure that the neutralizer completely washes the specimens, SCDLP inactivation of antiviral activity broth was pipetted at least four times.

2. The washing out solution was inoculated with virus suspension and incubated at 25⁰C for 30 minutes.

3. Infective titre of this solution was determined by TCID50 Method.

4. Log of TCID50/ ml of Control LDPE film - Log of TCID50/ ml of Test LDPE film less than or equal to 0.5.

Observations :

Log of TCID50/ ml of Control LDPE film	Log of TCID50/ ml of Test Sample	Log of TCID50/ ml of Control LDPE film - Log of TCID50/ ml of Test Sample	Acceptable Criteria
3.40	3.16	0.24	less than or equal to 0.5

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Test Procedure

1. 0.4 ml of the test inoculum was added onto the test surface. It was covered with LDPE film to ensure that the test inoculum makes contact with test surface and spreads to the edges.
2. After the specimen was inoculated and the cover film applied, It was closed with the lid of the Petri dish and incubated at $35 \pm 1^{\circ}\text{C}$ and a relative humidity of not less than 90 % for 5 minutes.
3. Immediately after inoculation, the three Control sample were terminated using SCDLP medium to retrieve the virus.
4. After the contact time of 24 hours remaining Control and Test samples were terminated using SCDLP medium to retrieve the virus.
5. Infectivity titer of virus recovered from the sample was determined by TCID₅₀ method

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Results :

Virus	Contact Duration	Group	Logarithm of Infectivity titre of virus ($\lg TCID_{50}/cm^2$)	Average titre Infectivity of virus ($\lg TCID_{50}/cm^2$)
Human Coronavirus suspension: (1.90×10^8 PFU/ml)	0 hours	Control (U_0)	6.40	6.29
			6.07	
			6.40	
	24 hours	Control (U_t)	6.07	5.85
			6.07	
			5.40	
	24 hours	B2306354 [Inner layer of Plastic Tank] (A_t)	3.07	2.96
			3.07	
			2.74	
Antiviral activity $R = U_t - A_t$ (24 hours contact)			-	2.89 (99.87%)

Where

R is the Antiviral activity

U_0 is the average of common logarithm from three control/ untreated specimen immediately after inoculation

U_t is the average of common logarithm from three control/ untreated specimen after 24 hours

A_t is the average of common logarithm from three Treated specimen after 24 hours

P.S. : This test was subcontracted to a competent laboratory.

-- End of the Report --

Ashwini Govekar
 Microbiology
 (Authorised Signatory)

