Study Report	Virucidal activity evaluation ISO 18184:2019 . Textiles – Determination of antiviral activity of textiles products
Reference	AV11203799
Identification of the Sponsor	INDEXCITY, LDA. Sede na Rua José António Serrano, Nº 5, 1150-199 Lisboa, Portugal, NIPC: 515 484 210
Sample Identification	REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens Batch: na
Sampling	By the sponsor
Type of sample	Solid, Textile
Date of sample reception	23/11/2020

Covilhã, December 3th 2020

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Num. de Identificação Civil: BI112753469

Study Director

(Ana Palmeira de Oliveira, PhD, Pharmaceutics)

Document history

Version / Addition	Alterations	Date
v_01	First document version	03/12/2020

Proponent and Test Facilities identification

Proponent	Labfit – HPRD: Health Products Research and Development Lda
Address	Edifício UBIMEDICAL, Estrada Municipal 506, 6200-284 Covilhã
Responsible for the quality management system	Lígia Borges, MsC, Chemistry
Study director	Ana Palmeira de Oliveira, PhD, Pharmaceutics
Technicians	Sara Felício, Inês Pinto, Carlos Gaspar

Quality Management System

Labfit's quality management system is assured by periodic external audits inspections to the implemented quality management systems: ISO 9001:2015, ISO 13485:2016, NP 4457:2007 and OECD Good Laboratory Practices (GPL). The last GPL audit took place on 22-24 May 2018 and the last external audits occurred on August 07th 2019.

The results hereby reported reflect the data registered during the study made for the tested sample.

Test Principle

In December 2019, coronavirus disease 19 (COVID-19), caused by SARS-CoV-2 virus, was first identified in Wuhan, China [1]. By January 2020, it was declared a Public Health Emergency of International Concern by the WHO (World Health Organization) and as a pandemic in March 2020 [2,3]. COVID-19 spreads most often before symptom onset or from asymptomatic people through close proximity, via small droplets or aerosols [4]. There is currently no specific vaccine or antiviral treatment, so the best measure is prevention including hand washing, social distancing, wearing a mask, surface disinfection and self-isolating for people with symptoms. Considering this ongoing pandemic, it is clear that there is a need for products with proven virucidal efficacy. This can be achieved by using the ISO 18184:2019, which specified test methods for determining the antiviral activity of textile products against specific viruses. Modified vaccinia virus Ankara (MVA) can be used as a reference virus for all enveloped viruses, meaning that it is a suitable surrogate test virus that can be safely used for testing the virucidal efficacy of products against other enveloped viruses such as SARS-CoV-2 and other coronaviruses [5,6,7]

The test described in ISO 18184:2019 aims to determine the virucidal activity of textiles products that are capable of reducing the number of infectious virus particles that come in contact with the surface of the textile [8]. In the test, tissue pieces of both control (without treatment) and the product (with treatment) are used. The tissues are sterilized in an autoclave and then inoculated at various points with a virus suspension with a known $TCID_{50}$ and are maintained for a defined contact time (standard is 2 hours but can go up to maximum of 24 h) with the sample pieces. After the contact time, the virus is diluted in ice cold maintenance virus. The dilutions are transferred into wells in microtiter plates with a monolayer of BHK-21 cells. Quantification are done by using the $TCID_{50}$ procedure. After incubation, the titres of infectivity are calculated according to Behrens and Kärber and evaluated. Reduction of virus infectivity is calculated from differences of log_{10} virus titres on tissues where the quantification was done immediately after placing the virus and on both the tissue without antiviral, or with antiviral without inoculating the virus suspension.

Study conditions

Study beginning date	24/11/2020
Test beginning date	24/11/2020
Test conclusion date	01/12/2020
Study conclusion date	03/12/2020
Sample storage during the test	in the package sent by the sponsor at room temperature

Materials

Culture Medium and Reagents

Dulbeco's Modified Eagle's Medium	DMEM, Sigma - Aldrich
Fetal Bovine Serum	FBS, VWR
SCDLP medium	Peptone made of casein, VWR Peptone made of soybean, VWR Sodium chloride, VWR Dipotassium hydrogen phosphate, VWR D-glucose, Laborspirit, Lda Lecithin, VWR Nonionic surfactant, VWR

Virus and cells

The modified vaccinia virus Ankara (MVA) was purchased in ATCC. BHK 21-cells (passage 53). The cells were inspected regularly for morphological analysis. Before inactivation assays, virus had been passaged three times in BHK 21-cells (Baby Hamster Kidney).

Apparatus, glassware and small items of equipment

Analytical balance	Kern, 770-15
Incubator	Binder, APT.lineTM C150E2
Pipettes	P5000, VWR, Model VE5000 P1000, VWR, Model VE1000 P200, Model VE200 P20, Model VE20
Water bath	VWR, VWB 6
Biosafety cabinet, type 2	Telstar
Equipment	usual laboratory equipment

Reference Substances

Negative control	Wells with only culture medium

Experimental conditions

Test temperature	25 °C ± 1 °C
Contact time(s)	24 hours
Temperature of incubation	37 °C ± 1 °C
Virus strain	Modified vaccinia virus Ankara (MVA) (ATCC-1508)

Method

The method used to perform this test complies with ISO 18184:2019 – Textiles – Determination of antiviral activity of textiles products.

Preparation of test virus suspension

For preparation of test virus suspension, BHK-21 were cultivated with DMEM supplemented with 10% of FBS. Cells were infected with a multiplicity of infection of 0.1 in the presence of 2% FBS. After cells showed cytopathic effects, they were subjected to a procedure of freeze/thaw followed by a centrifugation to sediment cell debris. After aliquoting, test virus suspension was stored at -80 °C.

Preparation of Control Fabric

An untreated Control Fabric was provided by the study Sponsor. Pieces of the Control Fabric were cut to dimensions measuring 20 mm by 20 mm and weighed to a mass of 0.40 g ± 0.05 g. The Control Fabric cuttings were loaded into nine tubes of 50 mL, covered with foil and autoclaved for 20 minutes at 121 °C (103 kPa).

Preparation of Test Fabric

The Test Fabric was provided by the study Sponsor. Pieces of the Control Fabric were cut to dimensions measuring 20 mm by 20 mm and weighed to a mass of 0.40 g ± 0.05 g. The Control Fabric cuttings were loaded into six tubes of 50 mL, covered with foil and autoclaved for 20 minutes at 121 °C (103 kPa).

Test Procedure

Six Control Fabric and three test Fabric were each inoculated with 0.2 mL of evenly spread viral stock. Three Control Fabric and three Test Fabric were incubated at 20 °C for the defined contact time. After the time, the triplicate Control and Test Fabric were neutralized to assess the viral titer by adding 20 mL of SCDLP medium to the vial containers. The tubes were then vortexed five times for five seconds each to wash out the viruses from the fabric pieces. Three other Control fabric were immediately neutralized to assess the viral titer upon inoculation (time zero) by

adding 20 mL of SCDLP medium to the vial containers. The tubes were then vortexed five times for five seconds each to wash out the viruses from the fabric pieces.

Verification of cytotoxicity by cell sensitivity to virus and the inactivation of antiviral activity

To the additional triplicate Control and Test Fabric that did not contain viral inoculum were added 20 mL of SCDLP medium each. The tubes were then vortexed five times for five seconds each and 5 mL of solution were taken into new tubes for all vial containers. The virus was then added and the tubes were incubated for 30 minutes at 25 °C.

Infectivity Assay

Control and Test suspensions were diluted ten-fold in ice cold maintenance medium or in SCDLP medium for the cytotoxicity controls. Before use, each well was washed three times with 0.1 mL of maintenance medium. Plating was made by transferring 0.1 mL of each dilution into eight wells of a microtitre plate with a monolayer of BHK-21 cells (6-7 x 10^3 cells per well), beginning with the highest dilution. Furthermore, a cell control (only addition of culture medium) was incorporated. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after five days.

Verification of virucidal activity

Log_{10} and reductions of the mean viral inoculum on the Test Fabrics after the incubation time were calculated relative to the mean viral titer of the Control Fabric at the same time using the method of Behrens and Kärber. Log_{10} and reductions were also determined for the Control Fabric after incubation relative to the titer yielded from Control Fabrics at time zero to verify stability of the viral inoculum over the course of testing.

The antiviral efficacy – Antiviral performance of the products (according to ISO 18184:2019 Annex F) may be evaluated according to the following table:

Table 1: Antiviral performance standard according to ISO 18184:2019

Item	Antiviral performance standard	Standard
Tested textile product	3.0 > Mv ≥ 2.0	Good effect
	Mv ≥ 3.0	Excellent effect

Test results and Discussion

The obtained results are shown in Table 2 to Table 8.

The following criteria are mentioned in ISO 18184:2019 (section 14.3) and should be fulfilled:

a) The virus infective titre of inoculated concentration for the test shall be > 10^7 $TCID_{50}$/mL (MVA titre was $10^{17.5}$).
b) The difference of the logarithmic $TCID_{50}$/mL of Control Fabric minus the logarithmic $TCID_{50}$/mL of Test Fabric in the cytotoxicity and sensitivity control was 0.00 for a contact time of 24 hours (must be ≤ 0.5).
c) The logarithmic reduction of infective titre of Control Fabric was 1.94 (must be <2.0 after 24 hours)

Furthermore, according to ISO 18184:2019, the antiviral activity value (Mv) is calculated by the difference between the logarithm average of 3 infectivity titre value of the Control Fabric at time zero minus the logarithm average of 3 infectivity titre value of the Test Fabric after exposure time. For the fabric to be considered as having a good effect according to the standard, the Mv must be situated between or equal to 2.0 and 3.0. If the Mv is equal to or greater than 3.0, the fabric is considered to have an excellent effect.

Table 2: Summary of results with REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens and MVA

Product	Contact time	Antiviral efficacy value, Mv	Standard Effect
REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens	24 hours	2.3	Good Effect

If one applies the following formula[9] commonly used to quantify the antiviral effect in percentage, a result of **99.49** % viral reduction is obtained.

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

Where:

P is the percent reduction

L is the log reduction

Table 3: Raw data for REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens (Control Fabric) tested against MVA (8 wells)

Product	Sample ID	Contact Time (hours)	Dilutions (log_{10})											
			0	1	2	3	4	5	6	7	8	9	10	control
REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens	Control Fabric	0	3333	3333	1111	1111	1111	0000	0000	0000	0000	0000	0000	0000
			3333	3333	1111	1111	0101	0000	0000	0000	0000	0000	0000	0000
			3333	3333	3333	1111	1111	1110	0000	0000	0000	0000	0000	0000
			3333	3333	3333	1111	1111	0101	0000	0000	0000	0000	0000	0000
			3333	3333	1111	1111	1111	1110	0000	0000	0000	0000	0000	0000
			3333	3333	1111	1111	1111	1101	0000	0000	0000	0000	0000	0000
		24	3333	2222	1111	1111	0000	0000	0000	0000	0000	0000	0000	0000
			3333	2222	1111	1111	0000	0000	0000	0000	0000	0000	0000	0000
			3333	1111	1111	0000	0000	0000	0000	0000	0000	0000	0000	0000
			3333	1111	1111	0000	0000	0000	0000	0000	0000	0000	0000	0000
			3333	1111	1111	0000	0000	0000	0000	0000	0000	0000	0000	0000
			3333	1111	1111	0000	0000	0000	0000	0000	0000	0000	0000	0000

n.a. = not applicable
n.d. not done

0 = no virus present; t= cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units)

Table 4: Raw data for REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens (Test Fabric) tested against MVA (8 wells)

Product	Sample ID	Contact Time (hours)	Dilutions (log_{10})											
			0	1	2	3	4	5	6	7	8	9	10	control
REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens	Test Fabric	24	4444	4444	2222	1010	0000	0000	0000	0000	0000	0000	0000	0000
			4444	4444	2222	1101	0000	0000	0000	0000	0000	0000	0000	0000
			4444	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
			4444	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
			3333	2222	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000
			3333	2222	2202	1000	0000	0000	0000	0000	0000	0000	0000	0000

n.a. = not applicable
n.d. not done

0 = no virus present; t= cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units)

Table 5: Raw data for REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens cytotoxicity controls tested against MVA (8 wells)

Product	Sample ID	Dilutions (log_{10})											
		0	1	2	3	4	5	6	7	8	9	10	control
REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens	Control Fabric	3333	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	3333	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	3333	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
	Test Fabric	3333	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	2222	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	1111	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
		3333	1111	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000

n.a. = not applicable
n.d. not done

0 = no virus present; t= cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units)

Table 6: ISO 18184:2019 Evaluation of REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens against MVA

Test Virus	Contact Time	Sample ID	Virus Titer ($TCID_{50}$/mL)	Mean Virus Titer ($TCID_{50}$/mL)
MVA	Time zero	Control Fabric	1,78E+05	1,10E+06
			1,33E+06	
			1,78E+06	
	24 hours	Control Fabric	3,16E+04	1,26E+04
			3,16E+03	
			3,16E+03	
	24 hours	Test Fabric	1,33E+04	5,60E+03
			3,16E+02	
			3,16E+03	

Table 7: ISO 18184:2019 Evaluation of cytotoxicity controls of REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens against MVA

Test Virus	Contact Time	Sample ID	Toxicity Titer ($TCID_{50}$/mL)	Mean Toxicity Titer ($TCID_{50}$/mL)
MVA	24 hours	Control Fabric	3,16E+02	3,16E+02
			3,16E+02	
			3,16E+02	
		Test Fabric	3,16E+02	3,16E+02
			3,16E+02	
			3,16E+02	

n.a. – not applicable

Table 8: Determination of virus titre at 20 °C

Virus titration	Dilutions (log_{10})											
	1	2	3	4	5	6	7	8	9	10	11	control
MVA	4444	4444	3333	3333	3333	3333	3333	3333	3333	3333	3333	0000
	4444	4444	3333	3333	3333	3333	3333	3333	3333	3333	3333	0000

n.a. = not applicable
n.d. not done

0 = no virus present; t= cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units)

Conclusion

The textile **REF 8535 com tratamento após 50 lavagens + REF 8535 sem tratamento após 50 lavagens** demonstrated **good effect** against MVA after an exposure time of **24 hours.**

Study records storage and general data storage

All study records (study plans, raw data, spreadsheet and reports) will be archived and kept in Labfit premises.

The samples in study for the Microbiological Quality assay will be kept in Labfit premises for a 2-month period after the assay report is sent to the sponsor.

Concerning the remaining assays, the sample storage period in Labfit premises is of 6 months after the assay report is submitted to the sponsor.

References

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