

STUDY REPORT 260.2020.102
Version 02

IN VITRO STUDY OF ANTIVIRAL ACTIVITY OF TEXTILE PRODUCTS

Sample NV.355.02

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Product code	NV.355.02
Product name	CARPET 1
Sample receiving	29/10/2020
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1. INTRODUCTION

It is increasing the search for products that offer protection against illness causing agents. According to this demand, agents with antiviral properties are being applied to textile industries with the goal to incorporate functional properties with quality in order to promote health and wellbeing to the population.

The study according to ISO 18184:2019 is the reference in order to evaluate textile products with antiviral activity. It is evaluated whether the contact with the product reduces the number of viral particles.

This study was conducted with a virus from the coronavirus family and it is a member of SARS-CoV-2 family. The coronavirus is a virus with a RNA genome that belongs to the family Coronaviridae. The subfamily Orthocoronaviridae has 4 genus: alpha, beta, gamma and deltacoronavirus. This study was conducted with an alphacoronavirus (CCov – VR 809) as a viral particle that represents a good model in order to study virus.

2. PURPOSE

Evaluate if the sample reduces the alphacoronavirus viral viability following the methodology present at ISO 18184:2019.

3. STUDY RELEVANCE

The experimental conditions used herein are accepted and in accordance with methodologies currently used by ISO 18184:2019.

4. SAMPLE DESCRIPTION

Sample information	Product code	Storage temperature
CARPET 1, White	NV.355.02	Room temperature

5. METHODOLOGY

5.1 Cell culture

It was used VERO cells cultured with DMEM (Dulbecco's Modified Eagle's Medium) with addition of supplements in an incubator at 37°C and 5% of CO₂. At cell passage 5 after thawing, cells were plated in appropriate plates in order to establish a monolayer.

5.2 Virus culture

It was used the alphacoronavirus (CCoV – VR809) previously titred according to the following calculation. This virus was selected since it belongs to the SARS-CoV-2. For this assay, the virus titre was 10^{5.5}.

5.3 Calculation of the virus titre according to TCID₅₀:

$$Y = X \times 10^a$$

$$a = \sum p - 0,5$$

x= base dilution – it was used base 10

Calculation of virus stock:

Calculation of p value according to number of replicates with 7 dilutions with base of 10.

$$a = \sum 7.0 - 1.5 ; a = 5.5$$

Virus titre used in the assay: 10^{5.5}

5.4 Sample preparation

Sample NV.355.02

- Sample conditions in the assay: Pieces of fabric following the dimensions 20mm x 20 mm (±2 mm). Samples were autoclaved according to ISO 18184:2019.

5.5 Control groups preparation

- Cell control group: culture media supplemented;
- Viral control group: culture media supplemented;

5.6 Cytotoxic analysis

The cell control group and the group NV.355.02 were incubated with supplemented culture media in the same conditions as in the viral assay in order to establish if the samples could induce a cytotoxic effect in the cell culture. The culture media was added to the cell monolayer at a 1:1 and at a 1:10 dilution in duplicate to each group. After 24 hours, it was evaluated the cell culture and images were taken.

5.7 Antiviral activity analysis

Viral aliquot was diluted in cell culture media and the fragments of fabric from group NV.355.02 were embedded in closed tubes for 5, 30 and 120 minutes in sterile work conditions. After the times periods, one aliquot was added to a new tube in order to end the contact with the samples. The viral control group was executed in the same way, but it had no contact with any sample. It was then added the work solutions to the cell monolayer in quadruplicated to each group in order to evaluate viral multiplication.

5.8 Results analysis

The cell culture with the viral particles was evaluated according to the cytopathic effect (CPE) caused by the virus. It is compared the cell control group with the viral control group and the sample's group NV.355.02 in order to evaluate the viral replication. The result is evaluated with the TCID₅₀ method with logarithm dilution series. The result is expressed as logarithm reduction of viral particles and calculated the difference of viral particles between the viral titre and the group NV.355.02

6. RESULTS

6.1 Cytotoxic analysis

In order to evaluate the viral titre, it is needed to evaluate cell cytotoxic from the samples. Therefore, it is evaluated the culture monolayer and the cell morphology. It was observed a confluent monolayer with morphology establishment in all groups at a 1:10 dilution.

6.2 Antiviral analysis activity

The virus evaluated causes cell morphology alterations that are called cytopathic effect (CPE). Through CPE, it is possible to analyze and quantify the viral multiplication in each group. It is executed the viral titre identification to each group in order to calculate the antiviral activity value. The results in viral logarithm

reduction and percentage observed in the group NV.355.02 is presented at Table1:

Product	Contact time (minutes)	Log reduction	Percentage
CARPET 1, White	5	4.0	99.99%
	30	4.0	99.99%
	120	5.0	99.999%

Table 1: Results of viral titre at the contact time evaluated. It is shown the log reduction and the viral reduction percentage.

The reduction of 5.0 logarithms in the viral titre by the group NV.355.02 after 120 minutes of contact demonstrated that it has antiviral activity and that is related to a 99.999% reduction of viral particles.

7. CONCLUSION

According to the results obtained herein, it can be concluded that:

- The sample NV.355.02, CARPET 1, White, reduced 4.0 logarithms after 5 minutes and this is related to a 99.99% reduction of viral particles;
- The sample NV.355.02, CARPET 1, White, reduced 4.0 logarithms after 30 minutes and this is related to a 99.99% reduction of viral particles;
- The sample NV.355.02, CARPET 1, White, reduced 5.0 logarithms after 120 minutes and this is related to a 99.999% reduction of viral particles;

8. FINAL CONCLUSION

In the study entitled “**IN VITRO STUDY OF ANTIVIRAL ACTIVITY OF TEXTILE PRODUCTS**” regarding the product **CARPET 1, White**, code **NV.355.02**, sent by the company **TIT İNOVASYON VE TEKNOLOJİ A.Ş** it is observed that:

The product CARPET 1, White, code NV.355.02, has antiviral activity.

This report is exclusively intended to the **National Agency of Sanitary Surveillance of the Ministry of Health** and to the internal use **TIT İNOVASYON VE TEKNOLOJİ A.Ş**. No information of this report can be disclosed in any communication vehicle without the written authorization of the author. The only part that can be presented is the results of this study report for communication purposes.

9. NOTES

The results described herein are regarding the sample evaluated in the conditions and concentrations evaluated in this study.

The results demonstrated herein are exclusively of *in vitro* studies.

10. REFERENCES

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11. APPROVAL

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CONFERENCE