ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL'EMILIA-ROMAGNA "BRUNO UBERTINI"
(ENTE SANITARIO DI DIRITTO PUBBLICO)
BRESCIA
Sede Legale: Via Bianchi, 9 – 25124 Brescia
Tel.: 03022901 – Fax 0302425251 – E-mail info@izsler.it
C.F. – P.IVA 00284840170
N. REA CCIAA di Brescia 88834

Via Bianchi, 9 – 25124
BRESCIA (Italy)
Tel.: 03022901 – Fax
0302425251 – E-mail
virologia@izsler.it
virologia@cert.izsler.it

VIROLOGY DEPARTMENT

Prot. VS: 25/2020

EXPERIMENTAL ZOOPROPHYLACTIC INSTITUTE OF

LOMBARDY AND EMILIA-ROMAGNA

"BRUNO UBERTINI"

IN VITRO TESTS TO EVALUATE THE VIRUCIDAL ACTIVITY OF 3 MASK-AGB SUBSTANCES AGAINST BETA-CORONAVIRUS

Final Report

Ana Moreno

Laboratory of Specialized Virology, Serology and Electron Microscopy

Experimental Zooprophylactic Institute of Lombardy and Emilia-Romagna

Via Bianchi, 9 — 25124 Brescia, Italy

Phone: +39 030 2290347

Fax: +39 030 2290535

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The following protocol was developed to evaluate the inactivating power of three colloidal silver-based substances (AGB 3K Base) at different concentrations against a beta-coronavirus belonging to the same genus as SARS-CoV-2. Three different contact times were tested for each substance: 10, 30, and 60 minutes. A strain of bovine coronavirus (BCoV) titrated on the HRT18 cell line was used as the beta-coronavirus model. The protocol included the following steps:

a) **Toxicity control of the inactivating substance** (protocols IZSLER no. 178648/2020 and 241415/2020): Three inactivating substances (AGB 3K preparation) were tested, defined as:

1. Substance 1: Mask Extra AGB
2. Substance 2: Mask 100AGB
3. Substance 3: Mask 50AGB

b) **Preparation of a viral suspension** of bovine beta-coronavirus (Bov-CoV strain 9WBL77) with a viral titer of 10^6 $TCID_{50}$/ml using the HRT18 cell line.

c) **Contact of each substance** with the Bov-CoV viral suspension (9WBL77) containing 10^6 $TCID_{50}$/ml for the three set contact times: 10 min, 30 min, 60 min.

d) **Titration of the mixtures** (substance + viral suspension) on 96-well microplates using the HRT18 cell line (protocol IZSLER no. 241549/2020).

e) **Evaluation of the persistence of the inactivating effect** of substance 1 (protocol IZSLER no. 241549/2020).

a – Toxicity control of the inactivating substance

Substances 1, 2, and 3 were applied in two dilutions (TQ and 2,) to HRT-18 cells to assess any cytotoxic effect. No cytotoxic effect was observed in the HRT-18 cells for any of the three tested substances.

b – Titration of the viral suspension used

The Bov-CoV viral suspension used in the in vitro tests was re-titrated during the experiment to confirm its viral titer. The titer was calculated using the Reed and Muench method, with 8 logarithmic dilutions from -1 to -8 (10 replicates per dilution). The viral titer was expressed as tissue culture infectious dose 50 ($TCID_{50}$) per ml of suspension and resulted in 10^6 $TCID_{50}$/ml.

c-d – Preparation of the virus + substance mixture with contact time and subsequent viral titration:

c-d.1 – Substance 1: Mask Extra AGB + Bov-CoV viral suspension

- 10 minutes: no viral growth detected
- 30 minutes: no viral growth detected
- 60 minutes: no viral growth detected

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c-d.2 – Substance 2: Mask 100 AGB + Bov-CoV viral suspension

- 10 minutes: no viral growth detected
- 30 minutes: no viral growth detected
- 60 minutes: no viral growth detected

c-d.3 – Substance 3: Mask 50 AGB + Bov-CoV viral suspension

- 10 minutes: no viral growth detected
- 30 minutes: no viral growth detected
- 60 minutes: no viral growth detected

e – Evaluation of the persistence of the inactivating power of substance 1

The persistence of the inactivating effect of substance 1 (Mask Extra AGB) was evaluated by leaving the substance in a Petri dish in the open air for 48 hours at room temperature and then bringing it into contact with the Bov-CoV suspension for 10 minutes.
The titration of the "48h substance + virus" mixture revealed no viral growth.

Conclusions

All tested substances demonstrated a complete inactivation of the bovine beta-coronavirus suspension containing 10^6 $TCID_{50}$/ml after the three scheduled contact times. Furthermore, the persistence of the inactivating power of substance 1 was confirmed even after 48 hours.

Brescia, 13 August 2020

Ana Moreno

Laboratorio di Virologia e Sierologia

Laboratorio

Laboratory of Specialized Virology and Electron Microscopy