# CERTIFICATE

# AND

# EFFECTIVENESS ASSESSMENT REPORT

# OF THE VIRUCIDAL ACTIVITY OF:

**STEAM GENERATORS EV International + detergent product EV $H_2O_2$ SILVER BIO, 30% solution**

**STEAM GENERATORS EV International + detergent product EV $H_2O_2$ SILVER SPRAY ready to use**

**Based on the test UNI EN ISO 16777: 2018**

| | |
|---|---|
| CLIENT: | **E V International Ltd.**<br>Kostur 14 A<br>1618 Sofia, Bulgaria |
| DATE OF RECEIPT: | 22/06/2020 |
| ANALYSIS LAUNCH DATE: | 10/07/2020 |
| ANALYSIS COMPLETION DATE | 31/07/2020 |

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**Laboratory for Chemical and Microbiological Analysis SANA srl 17 Lecce St., 03100 Frosinone**
**tel.: 0775-210531**

**1. FOREWORD**

**2. DESCRIPTION OF THE ACTIVITIES**

**3. TERMS AND DEFINITIONS**

**4. VIRUCIDAL ACTION (PHASE 2, STEP 2)**

**5. TEST REPORT**

**6. CONCLUSIONS**

## 1) FOREWORD

This report was prepared and performed by the laboratory Sana srl, which is a holder of ACCREDIA certificate No. 0747 under UNI EN ISO 17025:2018, and is intended to evaluate the **VIRUCIDAL PERFORMANCE** of E V International's steam generators plus detergent referred to as EV $H_2O_2$ Silver Bio, 30% solution and EV $H_2O_2$ Silver Spray ready to use.

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## 2) DESCRIPTION OF THE ACTIVITIES

This validation protocol was made to validate the **VIRUCIDAL PERFORMANCE** of the products described above.
The tests that were performed were in compliance with the referenced norms as stated in the table below:

| Action | Virus | Applicable standard | % application | Conditions | | Contact time |
|---|---|---|---|---|---|---|
| | | | | Dirty 3g | Clean 0.3 g | |
| Virucidal | **Human Coronavirus Strain 229E ATCC VR-740 / VR -19** | EN ISO 16777:2018 | 30% | yes | yes | **30 sec** |
| Virucidal | **Human Coronavirus Strain 229E ATCC VR-740 / VR -19** | EN ISO 16777:2018 | Ready to use | yes | yes | **30 sec** |

## 3) TERMS AND DEFINITIONS

- **Product** (chemical disinfection and/or antiseptic): chemical agent or formula used as a disinfectant or antiseptic.
- **Virucidal performance**: the capability of the product to reduce at least by **log4** the virus count "on the surface" under standard experimental conditions
- **Cleaning conditions:** conditions of the surfaces subjected to the cleaning procedure, on which there are minimal levels of organic or inorganic substances.

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- **Dirty conditions**: conditions characterized by a considerable degree of contamination, including with organic or inorganic compounds on the surfaces subject to detergent or antiseptic treatment.

- **Virus deactivation:** reducing the virulence of the virus by applying the analyzed product.

- **Interference compounds:** protein solutions and erythrocytes added to the viral suspension before adding the test product to demonstrate the effect of the proteins and erythrocytes on the **virucidal** performance of the tested product.

- **Plaque-forming units (PFU):** the number of virus particles capable of forming plaque per unit of volume (ml).

- **ID50**: infecting dosage 50 % of the viral suspension or diluting the viral suspension which induces in the cellular cultures a 50 % cytopathic effect (CPE).

- **Virus count:** equivalent of the infection virus per unit of volume present in the cellular structure after lysis

## 4) VIRUCIDAL PERFORMANCE (PHASE 2, STEP 2)

**The study is intended to demonstrate and evaluate the effectiveness of a particular product on the human coronavirus, strain 229E ATCC VR-740 / VR -19 virus.**

Procedural phases include:

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- Preparation of the sample viral suspension and the cellular line
- Testing procedure
- End-result assessment

## MATERIALS AND REAGENTS

- Basis for the culture: EMEM for cellular structures
- Cellular structures: human fibroblasts ATCC CCL-171
- Organisms to test: **Human coronavirus, strain 229E ATCC®VR-740 / VR -19**
- Neutral red (solution 1: 1000)
- Foetal calf serum (FCS)
- Trichloroacetic acid (10% solution)
- Hard water
- Interfering compound (bovine serum albumin and sheep's blood)

## Preparing the test virus suspension in accordance with EN ISO 16777: 2018

The viral suspension is multiplied in a suitable cellular line to produce a large viral count. Cellular waste is separated by centrifugal force. The preparation process results in the so called **„viral treatment suspension"**. The count of the testing suspension must be at least 10 by the power of 8 TCID50 / ml. In any case, the count must be high enough to allow a **log4 count reduction to take place.**

**Testing procedure based on customers indicators.**

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**1) Test with EV International's steam generators + EV $H_2O_2$ Silver Bio at 30%**

- Plug the unit into the power grid and switch it on.
- Wait until it heats up.
- Attach the SANIX tool/accessory to the flexible steam hose.
- Fill up the SANIX bottle with the EV $H_2O_2$ Silver Bio (30% solution) and water. Shake well before use.
- Spray the steam + the EV $H_2O_2$ Silver Bio from a distance of 20 to 30 cm for 4 to 5 seconds on the sample surface 10 x 10 cm, then wait for 30 seconds.

2) **Test with EV International's steam generators + EV $H_2O_2$ Silver Spray ready to use**

- Plug the unit into the power grid and switch it on.
- Wait until it heats up.
- Attach the SANIX tool/accessory to the flexible steam hose.
- Fill up the SANIX bottle with the ready to use EV $H_2O_2$ Silver Spray. Shake well before use.
- Spray the steam + the EV $H_2O_2$ Silver Spray from a distance of 20 to 30 cm for 4 to 5 seconds on the sample surface 10 x 10 cm, then wait for 30 seconds.

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## Evaluation of the results

**STEAM GENERATORS of EV International + detergent EV $H_2O_2$ SILVER BIO at 30% solution in clean and dirty conditions**

| Contact time | Virus count (clean) | Virus count (dirty) |
|---|---|---|
| 30 seconds | **n.d** Not detected | **n.d** Not detected |

**n.d = virus count not detected**

**STEAM GENERATORS of E V International + detergent EV $H_2O_2$ SILVER SPRAY ready to use in clean and dirty conditions**

| Contact time | Virus count (clean) | Virus count (dirty) |
|---|---|---|
| 30 seconds | **n.d** Not detected | **n.d** Not detected |

**n.d = virus count not detected**

# TEST REPORT

**UNI EN ISO 16777: 2018 - QUANTITATIVE TESTING OF NON-POROUS SURFACES TO ASSESS - USING NO MECHANICAL INFLUENCE - THE ANTIVIRAL PERFORMANCE OF CHEMICAL DISINFECTANTS USED IN MEDICINE**

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**Laboratory for Chemical and Microbiological Analysis SANA srl 17 Lecce St., 03100 Frosinone**
**tel.: 0775-210531**

## Test method and requisites

Phase 2 Step 2

## Laboratory

The Microbiology and Chemistry Laboratory of SANA srl, 17 Lecce St., 03100 Frosinone, holder of ACCREDIA certificate No. 0747

| | |
|---|---|
| **Formulation** | Steam generated by EV International's steam cleaners + EV $H_2O_2$ Silver Bio at 30% solution |
| **Disinfectant batch** | EV $H_2O_2$ Silver Bio - 20-01184 |
| **Best before** | 05/2022 |
| **Composition:** | Hydrogen peroxide $H_2O_2$ with silver ions + dry steam |

## Manufacturer

**E V International Ltd.**

Kostur 14A

1618 Sofia, Bulgaria

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## Testing conditions

| | |
|---|---|
| Test date: | 10/07/2020- 31/07/2020 |
| Test surface: | 10 x 10 cm |
| Ambient temperature: | 20°C |
| Interference compounds: | Clean conditions: Bovine serum albumin 0.3 %<br>Dirty conditions: Bovine serum albumin and sheep's blood 3 % |
| Concentration: | 30% solution |
| Usage conditions: | Spraying steam from EV International's steam generators + the EV $H_2O_2$ Silver Bio from a distance of 20 to 30 cm for 4 to 5 seconds on the sample surface 10 x 10 cm, then waiting for 30 seconds sec |

## Conclusions

Based on the test data and the criteria of UNI EN ISO 16777: 2018, the application of EV $H_2O_2$ Silver Bio **(**at 30%) with steam from EV International's steam generators under both clean and dirty conditions, triggered a reduction by more than 4 *log* of the viability of the tested organisms after 30 seconds contact time, i.e. virus count was not detected on the test surface.

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# TEST REPORT

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## Testing method and requisites

Phase 2, Step 2

## Laboratory

The Microbiology and Chemistry Laboratory of SANA srl, 17 Lecce St., 03100 Frosinone, holder of ACCREDIA certificate No. 0747

| | |
|---|---|
| Formulation | Steam generated by EV International's steam cleaners + EV $H_2O_2$ Silver Spray ready to use |
| Disinfectant batch | EV $H_2O_2$ Silver Spray 20-01249 |
| Best before | 05/2022 |
| Composition | Hydrogen peroxide $H_2O_2$ with silver ions + dry steam |

## Manufacturer

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## Testing conditions

| | |
|---|---|
| Test date: | 10/07/2020- 31/07/2020 |
| Test surface: | 10 x 10 cm |
| Ambient temperature: | 20°C |
| Interference compounds | Clean conditions: Bovine serum albumin 0.3 %<br>Dirty conditions: Bovine serum albumin and sheep's blood 3 % |
| Concentration: | ready-to-use product |
| Usage conditions: | Spraying steam from EV International's steam generators + the EV $H_2O_2$ Silver Spray from a distance of 20 to 30 cm for 4 to 5 seconds on the sample surface 10 x 10 cm, then waiting for 30 seconds |

## Conclusions

Based on the test data and the criteria of UNI EN ISO 16777: 2018, the application of EV $H_2O_2$ Silver Spray with steam from EV International's steam generators under both clean and dirty conditions, triggered a reduction by more than 4 *log* of the viability of the tested organisms after 30 seconds contact time, i.e. virus count was not detected on the test surface.

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