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Buenos Aires, November 5th, 2020

OBJECTIVE: Evaluation of virucidal activity of XYNTRUS SMELL TEST-MOUTHWASH against herpes simplex virus type 1 (HSV-1).

Company: Brix SRL
Product name: XYNTRUS smell test mouthwash
Sample: XYNTRUS
Lot: 20200901-1000
Elaboration Date:01/09/2020
Expiration date: 09/22

XYNTRUS SMELL TEST-MOUTHWASH COMPOSITION

INCI Nomenclature	% w/v
Active ingredients	
D-Limonene	0,300
Menthol	0,100
Eucalyptus Globulus Leaf oil	0,093
Methyl salicylate	0,060
Cetylpyridinium chloride	0,050

Evaluation

The evaluation of the virucidal capacity of the product was carried out according to **UNE-EN 14476:2014+A2**: Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of virucidal activity in the medical area.

Experimental conditions

Product Concentration in the assays: 97 %: virus and product with strong agitation as mouthwash simulation, for 30 seconds at 25ºC

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MATERIALS

Cells: Vero E-76 (African green monkey kidney cells).
Virus: herpes simplex virus type 1 Strain F (HSV-1).
Infection Medium (IM): E-MEM supplemented with NE AA, 2 mM glutamine, Penicillin 100 IU/ml, streptomycin 100 µg/ml and Fetal bovine serum (FBS) 2 %.
Growth Medium (GM): E-MEM supplemented with NEAA, glutamine 2mM, Penicillin100 IU/ml, streptomycin 100 µg/ml and 10% FBS.
Plaque medium (PM): IM supplemented with Methylcellulose 0.8%.

METHODOLOGY

The virucidal activity of **XYNTRUS** (final concentration 97%) was evaluated against HSV-1 (3.0×10^7 forming units of plaque-PFU) in 15 ml tubes. After mixing the virus and product, the tube was stirred for 30 seconds at 25°C (treated tube).
Simultaneous and independently, a virus control and cytotoxicity control were performed. For the virus control, Xyntrus product was replaced with the same volume of sterile water. For the cytotoxicity control, virus suspension was replaced with the same volume of infection medium. Both control tubes were assayed under identical experimental conditions as treated tubes.
After 30 seconds of treatment, the tubes were incubated at 4°C and the residual viral infectivity in control virus and treated tubes was quantified by viral plaques assay method, in Vero cell monolayers. After 48 hours of incubation at 37°C in humidified atmosphere containing 5% CO2, the cell monolayers were fixed with formaldehyde 10% and stained with violet crystal dye solution. The number of PFU of HSV-1 was counted.
The infectivity reduction percentage (RP) for the product treatment relative to untreated virus control was calculated according to the following formula:

$$RP = 100 - (\text{PFU product treated} \times 100 / \text{number of PFU virus control})$$

$$\Delta \log = (\log_{10} \text{PFU number}_{\text{product}} - \log_{10} \text{number PFU}_{\text{virus control}})$$

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RESULTS

Table1. Virucidal activity of XYNTRUS SMELL TEST-MOUTHWASH against herpes simplex virus type 1 (HSV-1).

HSV-1	Residual viral infectivity PFU/ml/ log_{10} PFU 30''	RP (% / Δ log) 30''
Virus Control	$2,3 \times 10^7$ 7,36	------
XYNTRUS SMELL TEST-MOUTHWASH 97 % v/v	$< 1,0 \times 10^3$ 3,0	>99,9956 >4,36

NOTE: The XYNTRUS SMELL TEST-MOUTHWASH at final concentration 97% was cytotoxic for Vero cells at a 1:100 dilution after 48 h of incubation at 37°C.

CONCLUSIONS

The product XYNTRUS SMELL TEST-MOUTHWASH at a final concentration of 97% after 30 seconds incubation with strong agitation at 25°C, reduced the number of infective viral particles of herpes simplex type 1 (HSV-1) by more than 99.9956% or by more than 4,36 log_{10} in relation to untreated virus control.

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