Instituto Valenciano de Microbiología

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Test with the certificate of GLPs (Good Laboratory Practices) No. 2/19-C.VAL. General Directorate of Pharmacy and Medical Devices of the Health Department of the Valencian Region. Spain

Virucidal test with the product "BIOSAN STERIDET / STERIDET" against Coronavirus 229E (EN 14476: 2014 + A2: 2019 Guideline)

Report
Registration No.: D/20/178

1. **Laboratory identification** ……. Instituto Valenciano de Microbiología.

2. **Client identification …**……..…… KEMPER SRL.
   Address ……..…… Via Ponderosso ncm
   Dello (BS) 25020 Italy

3. **Sample identification** (information provided by the customer)

- Product name……. BIOSAN STERIDET / STERIDET.
- Batch number……. 0263.
- Expiration date……... February 17, 2023.
- Manufacturer (supplier)……. KEMPER SRL.
- Date of manufacturer……. Not indicated.
- Storing conditions ……. Room temperature.
- Active(s) Substance(s) and its concentration (s)…… Pentapotassium bis (peroxymonosulphate) bis (sulphate) with sodium chlorine.
- Conditions of use……... Surfaces.
- Concentrations ordered for the assay…. 2%.

IVAMI is not responsible for customer-supplied information.

4. **Information about sample reception.**

- Date of reception of order with test conditions ...…….…….. 2020/02/21.
- Date of reception of the product……. 2020/03/02.
- Aspect of the received product……... Pink powder in commercial plastic container.

5. Testing method

Procedure **DESIN-6225** (EN 14476: 2014 + A2: 2019 guideline).

6. Experimental conditions

- Assay period…….. 2020/03/03 to 2020/03/16.
- Assay temperature……. 37ºC ± 1ºC.
- Titration method …….. $TCID_{50}$ (Tissue Culture Infective Dose 50%).
- Product concentrations for the assay…. 5%, 2% and 0.01%.
- Contact time……... 30 minutes.
- Contact temperature……... 25ºC ± 2ºC.
- Procedure to stop product cytotoxicity.. Molecular sieving.
- Procedure to stop product activity Cooling with ice.
- Solvent of the product used in the assay…….. Hard water.
- Aspect of the dilutions of the product… Transparents.
- Stability of the mixture (interfering substance and product diluted in sterile distilled water/hard water)……. Stable.
- Interfering substance:
    - Clean conditions in the presence of bovine serum albumin 0.3 g/L.
- Identification of the origin of viral strains and number of passes…….. Coronavirus 229E (ATCC VR-740) aliquot: 2019/03/04 passage 2.
- Cell lines (name, origin, number of passes)……..... MRC-5 ref. FTMR, working aliquot 3, passages 9, 10 and 12.

7. Validation of assay results

Coronavirus 229E (ATCC VR-740)

Titre of the viral suspension for the virus control (30 minutes):

- Clean conditions.....log $10^{-5.73}$

Cytotoxicity level (80%).....…….....log $10^{-0.5}$

Maximun level of virus inactivation detectable (difference between the titre of the viral suspension and the cytotoxicity level):

- Clean conditions……...…...log $10^{-5.23}$

Reference test (formaldehyde 1.4%)

Cytotoxicity level of formaldehyde 0.7%……..... log $10^{-0.5}$

Viral quantification in the reference test (formaldehyde) after 15 minutes and with Coronavirus 229E.....…….....log$10^{-2.49}$

Confidence interval

Title of virus with 95% confidence interval with Coronavirus 229E (5 minutes)

- Clean conditions ……log $10^{-5.73 \pm 0.47}$

Reduction with the confidence interval of 95 % ……See table 1.

Sensitivity of cells to virus

- Viral quantification of Coronavirus 229E with cells not treated with "BIOSAN STERIDET / STERIDET" disinfectant …….....log$10^{-5.99}$
- Viral quantification of Coronavirus 229E with cells treated with the "BIOSAN STERIDET / STERIDET" disinfectant……..log$10^{-5.32}$

Note: only can be used to determine the infectivity of cells, those dilutions which: a) show a low degree of cellular destruction (< 25% of cell monolayer) and b) produce a reduction of the title of the virus <1$\log_{10}$.

Control of the effectivity of the disinfectant detection activity

- Viral quantification of Coronavirus 229E after 30 minutes on bath ice without exposing the virus to the "BIOSAN STERIDET / STERIDET" disinfectant ……..log$10^{-5.57}$
- Viral quantification of Coronavirus 229E exposing the virus to "BIOSAN STERIDET / STERIDET" disinfectant and incubated 30 minutes on ice bath……...log$10^{-5.16}$

Note: The difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension should be ≤0.5

8. Special remarks

All controls and validation were between the basic limits.
One concentration at least showed a log reduction less than 4 log.
One concentration at least showed a log reduction higher than ≥4 log.

9. Assay results

9.1 Description

The disinfectant product**, "BIOSAN STERIDET / STERIDET",** batch **0263,** under clean conditions, diluted at 5% and 2% and during 30 minutes of exposure, **shows** virucidal activity against Coronavirus 229E (ATCC VR-740), with a reduction ≥ 5.23 ± 0.47 $TCID_{50}$ when the activity is assayed according with the internal procedure DESIN-6255 based on the EN 14476: 2014 + A2: 2019 guideline.

The disinfectant product**, "BIOSAN STERIDET / STERIDET",** batch **0263,** under clean conditions, diluted at 0.01% and during 30 minutes of exposure, **does not show** virucidal activity against Coronavirus 229E (ATCC VR-740), with a reduction 0.32 ± 0.61 $TCID_{50}$, when the activity is assayed according with the internal procedure DESIN-6255 based on the EN 14476: 2014 + A2: 2019 guideline.

9.2 Tables of results and graphics

See tables 1 and 2 and figure 1.

10. Conclusion

The disinfectant product **"BIOSAN STERIDET / STERIDET",** batch **0263,** under clean conditions (bovine serum albumin 0.3 g/L), diluted at 2% and during 30 minutes of exposure, **shows** virucidal activity against Coronavirus 229E (ATCC VR-740), when the activity is assayed according with the internal procedure DESIN-6255 based on the EN 14476: 2014 + A2: 2019 guideline.

Tests performed, only with Coronavirus strain 229E, does not allow to conclude that the product tested shows a general virucidal activity, but only that it shows activity against Coronaviruses.

Note 1: The results obtained correspond to the product received in this laboratory.
Note 2: The information that depend on the information received from the client and are not facilitated by the same one, shown as "not provided".

Bétera (Valencia), March 18, 2020

Signed. Noelia Ros
Responsible Technician
(Investigator)

Quality Assurance Review:

The assay development and the results obtained have been supervised by the Director of the study.

The Quality Assurance Director has inspected the development of the assay, proving that has been realized following the proper procedure and using the adequate media, materials and reagents, following as well the Good Laboratory Practices (GLPs) and the final report contains the primary data obtained.

Signed. Ruth Novella	Signed. Encarnación Esteban
Responsible for the Laboratory Area	Technical Director
(Study Director)	(Quality Assurance Director)

Reference:

- EN 14476: 2014 + A2: 2019 Guideline. Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine. Test method and requirements (Phase 2/Step 1).

Table 1. Results of activity of the product "**BIOSAN STERIDET / STERIDET",** batch **0263** with Coronavirus 229E (ATCC VR-740) under clean conditions.

Product	Concen-tration*	Interfering substance	Cytoto-xicity level	log_{10} $TCID_{50}$ after…..				Reduction with the confidence interval of 95 % after 30 minutes
				0 min	5 min	15 min	30 min	
BIOSAN STERIDET / STERIDET	5%	0.3 g/L BSA	0.5	-	-	-	0.50	≥ 5.23 ± 0.47
	2%		0.5	-	-	-	0.50	≥ 5.23 ± 0.47
	0.01%		0.5	-	-	-	5.41	0.32 ± 0.61
Virus control	NA	0.3 g/L BSA	NA	6.00	NR	NR	5.73	NA
Formaldehyde	0.7% (w:v)	NA	0.5	-	2.99	2.49	-	NA
Virus control Formaldehyde	0.7% (w:v)	NA	0.5	6.16	-	-	5.99	NA

Control of sensitivity of cells to virus (difference between decimal logarithm of titre using treated and untreated cells) …….…….$log10^{-0.67}$
Control of the effectiveness of the disinfectant detection activity (difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension)……$log10^{-0.41}$

NA: not applicable; NR: not realized
Times recommended by Guideline for surfaces: maximum 5 or 60 minutes
Times recommended by Guideline for instruments: maximum 60 minutes
Times recommended by Guideline for Hygienic treatment of hands by friction and hygienic handwashing: between 30 or 120 seconds
PBS: phosphate buffered saline; BSA: bovine serum albumin.
Virucidal activity exists when the titre of virus shows a reduction ≥4 log.
*: see Special remarks to understand the values of these concentrations.

Table 2. Results of the activity of the product "**BIOSAN STERIDET / STERIDET",** batch **0263,** with Coronavirus 229E (ATCC VR-740) (Assay of titration with 12 wells), under clean conditions.

Product	Concen-tration *	Interfering substance	Time of contact (min)	Dilutions (log10)[a,b]							
				1	2	3	4	5	6	7	8
BIOSAN STERIDET / STERIDET	5%	0.3 g/L BSA	30	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	2%		30	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	0.01%		30	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0230 2044 3202	0000 0200 2010	0000 0000 0000	NR
Cytotoxicity	5%	0.3 g/L BSA	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR
Virus control	NA	0.3 g/L BSA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2302 3404 4330	0020 0240 0332	0003 2000 0002	0000 0000 0000
			30	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0230 2023 4340	2003 3200 0002	0020 0002 0000	0000 0000 0000
Formaldehyde	0.7 (w/v)	NA	5	4444 4444 4444	3230 2440 3322	0002 2023 3002	1020 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
			15	4444 4444 4444	0023 0343 0432	2003 2000 2000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
Control of formaldehyde cytotoxicity	0.7 (w/v)	0.3 g/L BSA	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR
Virus control formaldehyde	0.7 (w/v)	NA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0344 3223 4242	2030 2023 2000	0002 0010 0020	0000 0000 0000
			15	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2302 2343 2002	0220 2003 2202	0002 0000 0020	0000 0000 0000
Sensitivity control of cells to virus	NA	NA	Cells not treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CC0C CCCC 0CCC	00C0 0C00 CCC0	0C00 C000 C000	0000 0000 0000
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CC0C CCCC CCCC	0CC0 CCCC CC0C	000C 0000 00C0	0000 0000 0000	0000 0000 0000
Effectiveness control of the disinfectant detection activity	NA	0.3 g/L BSA	Without product	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0C0C C00C CC0C	00C0 00C0 0CC0	000C C000 0000	0000 0000 0000
			With product	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	C0CC CCCC CC0C	0CC0 0CCC 0CC0	000C C000 0C00	0000 0000 0000	0000 0000 0000

a): 1 to 4, virus present and grade of cytophatic effect in 12 units of cellular culture, or grade of cellular lesions in the cytotoxicity assay.

C = cytopathic effect with presence of virus (in this case and according to guideline does not take into account the degree of cytopathic effect only, the presence or absence of the same).

0 = no virus present or absence of cellular lesions in the cytotoxicity assay; NA: not applicable; NR: not realized; BSA: Bovine serum albumin; PBS: phosphate buffered saline.

sec: seconds; min: minutes.

*: see Special remarks to understand the values of these concentrations.

Figure 1. Results of the activity of the product **BIOSAN STERIDET / STERIDET",** batch **0263**, at 5%, 2% and 0.01% concentration under clean conditions with Coronavirus 229E (ATCC VR-740).