



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 “ZOTAL-D” against Coronavirus (Based on EN 14476: 2014 + A2: 2019 Guideline) with deviations from the guideline.

Report

Registration No.: D/20/233

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

2. **Client identification** LABORATORIOS ZOTAL, S.L.
Address Carretera Nacional 630, Km. 809
41900- Camas.

3. **Sample identification** (information provided by the customer)
 - Product name..... ZOTAL-D.
 - Batch number..... P001L02F20/02.
 - Expiration date..... February 2025.
 - Manufacturer (supplier)..... LABORATORIOS ZOTAL.
 - Date of manufacturer..... Not indicated.
 - Storing conditions Room temperature.
 - Diluent of the product recommended
by the manufacturer..... Water.
 - Active(s) Substance(s) and its
concentration (s)..... Biphenol-2-ol 4%, Chlorocresol 0.9%-
Glycolic acid 0.1%.
 - Conditions of use..... Surfaces.
 - Concentrations ordered for the assay.... 5%, 2.5% and 1%.

IVAMI is not responsible for customer-supplied information.

4. Information about sample reception.

- Date of reception of order with test conditions 2020/03/23.
- Date of reception of the product..... 2020/03/23.
- Aspect of the received product..... Brown transparent fluid in a metallic commercial container.

5. Testing method

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

6. Experimental conditions

- Assay period..... 2020/03/24 to 2020/04/08.
- Assay temperature..... $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- Titration method TCID₅₀ (Tissue Culture Infective Dose 50%).
- Product concentrations for the assay.... 5%, 2.5% and 1%.
- Contact time..... 30 minutes.
- Contact temperature..... $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- Procedure to stop product cytotoxicity.. Molecular sieving.
- Procedure to stop product activity Cooling with ice.
- Solvent of the product used in the assay..... Sterile hard water.
- Aspect of the dilutions of the product... Transparent.
- Stability of the mixture (interfering substance and product diluted in sterile hard water)..... Stable.
- Interfering substance:
 - Internal control of dirty conditions in the presence of bovine serum albumin 3 g/L.
 - Dirty conditions in the presence of bovine serum albumin 3 g/L plus 3 mL erythrocytes 3 mL/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 16, 18 and 19.

7. Validation of assay results

Coronavirus 229E (ATCC VR-740)

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

- Dirty conditions.....log 10^{-5.91}
 - Internal control of dirty conditions..... log 10^{-5.82}
- Cytotoxicity level (5%).....log 10^{-0.5}

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

- Dirty conditions.....log 10^{-5.41}
- Internal control of dirty conditions.....log 10^{-5.32}

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.....log10^{-2.74}

Confidence interval

Title of virus with 95% confidence interval with Coronavirus 229E (30 minutes)

- Dirty conditionslog 10^{-5.91 ± 0.35}
- Internal control of dirty conditionslog 10^{-5.74± 0.37}

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 “ZOTAL-D” disinfectantlog10^{-5.91}
- Viral quantification of Coronavirus 229E with cells treated with the “ZOTAL-D” disinfectant.....log10^{-5.58}

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 titre of the virus <1log₁₀.

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 “ZOTAL-D” disinfectantlog10^{-5.91}
- Viral quantification of Coronavirus 229E exposing the virus to “ZOTAL-D” disinfectant and incubated 30 minutes on ice bath.....log10^{-5.66}

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.
- No concentration showed a log reduction lower than 4 log.
- One concentration at least showed a log reduction higher than ≥ 4 log.

9. Assay results

9.1 Description

The disinfectant product, “ZOTAL-D”, batch **P001L02F20/02**, under dirty conditions, diluted at 5%, 2.5% and 1% and during 30 minutes of exposure, **shows** virucidal activity against Coronavirus 229E (ATCC VR-740), with a reduction $\geq 5.41 \pm 0.35$ TCID₅₀ for the three concentrations, when the activity is assayed according with the internal procedure DESIN-6255 based on the EN 14476: 2014 + A2: 2019 guideline, with deviations.

9.2 Tables of results and graphics

See tables 1 and 2 and figure 1.

10. Conclusion

The disinfectant product “ZOTAL-D”, batch **P001L02F20/02**, under dirty conditions (bovine serum albumin 3 g/L plus 3 mL erythrocytes 3 mL/L), diluted at 5%, 2.5% and 1%, requested by the customer, 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, with deviations from the guideline since a non-active concentration has not been tested.

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), April 14, 2020.

Signed. Miguel Ángel Fernández
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
Responsible for the Laboratory Area
(Study Director)

Signed. Encarnación Esteban
Technical 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 “ZOTAL-D”, batch P001L02F20/02 with Coronavirus 229E (ATCC VR-740) under dirty conditions.

Product	Concentration*	Interfering substance	Cytotoxicity level	log ₁₀ TCID ₅₀ after.....				Reduction with the confidence interval of 95 % after 30 minutes
				0 min	5 min	15 min	30 min	
ZOTAL-D	5%	3 g/L BSA + 3 mL/L erythrocytes	0.5	-	-	-	0.5	≥5.41 ± 0.35
	2.5%		0.5	-	-	-	0.5	≥5.41 ± 0.35
	1%		0.5	-	-	-	0.5	≥5.41 ± 0.35
ZOTAL-D	5%	3 g/L BSA	0.5	-	-	-	0.5	≥5.24 ± 0.37
	2.5%		0.5	-	-	-	0.5	≥5.24 ± 0.37
	1%		0.5	-	-	-	0.5	≥5.24 ± 0.37
Formaldehyde	0.7% (w:v)	NA	0.5	NR	3.82	2.74	NR	NA
Virus control	NA	3 g/L BSA + 3 mL/L erythrocytes	NA	5.99	-	-	5.91	NA
Virus control	NA	3 g/L BSA	NA	6.00	-	-	5.82	NA
Virus control Formaldehyde	0.7% (w:v)	NA	NA	5.91	NR	5.83	NR	NA
<p>Control of sensitivity of cells to virus (difference between decimal logarithm of titre using treated and untreated cells)log10^{-0.33}</p> <p>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^{-0.25}</p>								
<p>NA: not applicable; NR: not realized</p> <p>Times recommended by Guideline for surfaces: maximum 5 or 60 minutes</p> <p>Times recommended by Guideline for instruments: maximum 60 minutes</p> <p>Times recommended by Guideline for Hygienic treatment of hands by friction and hygienic handwashing: between 30 or 120 seconds</p> <p>PBS: phosphate buffered saline; BSA: bovine serum albumin.</p> <p>Virucidal activity exists when the titre of virus shows a reduction ≥4 log.</p> <p>*: see Special remarks to understand the values of these concentrations.</p>								

Table 2. Results of the activity of the product “ZOTAL-D”, batch P001L02F20/02, with Coronavirus 229E (ATCC VR-740) (Assay of titration with 12 wells), under dirty conditions.

Product	Concentration *	Interfering substance	Time of contact (min)	Dilutions (log10) ^{a,b}							
				1	2	3	4	5	6	7	8
ZOTAL-D	5%	3 g/L BSA + 3 mL/L erythrocytes	30	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	2.5%		30	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	1%		30	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
ZOTAL-D	5%	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
	2.5%		30	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
	1%		30	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
Cytotoxicity	5%	3 g/L BSA + 3 mL/L erythrocytes	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR
Virus control	NA	3 g/L BSA + 3 mL/L erythrocytes	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3022 3322 3223	1000 0212 0111	0000 0000 0000	NR
			30	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2333 2333 3220	0102 1100 1200	0000 0000 0000	NR
Virus control	NA	3 g/L BSA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3234 4432 4443	2022 3001 0200	0000 0000 0000	NR
			30	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2332 3303 3223	0011 1000 0200	0000 0000 0000	NR
Formaldehyde	0.7 (w/v)	NA	5	4444 4444 4444	4444 4444 4444	2330 0233 3222	0000 0020 1110	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
			15	4444 4444 4444	2020 0232 2322	0000 0010 2110	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
Control of formaldehyde cytotoxicity	0.7 (w/v)	3 g/L BSA + 3 mL/L erythrocytes	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	2332 0232 3322	0100 0021 1012	0000 0000 0000	NR
			15	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2322 0302 3222	0212 0002 0110	0000 0000 0000	NR

Sensitivity control of cells to virus	NA	NA	Cells not treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC 0CCC CCCC	0000 0C0C C0C0	0000 0000 0000	NR
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	C0CC CCCC CCCC	0000 CC00 0C00	0000 0000 0000	NR
Effectiveness control of the disinfectant detection activity	NA	3 g/L BSA + 3 mL/L erythrocytes	Without ZOTAL-D	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	C0CC CCCC CCCC	0000 CCCC CC0C	0000 0000 0000	NR
			With ZOTAL-D	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC 0CCC CCCC	00C0 00CC C000	0000 0000 0000	NR

a): 1 to 4, virus present and grade of cytopathic 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 “ZOTAL-D”, batch P001L02F20/02, at 5%, 2.5% and 1% concentration under dirty conditions with Coronavirus 229E (ATCC VR-740).

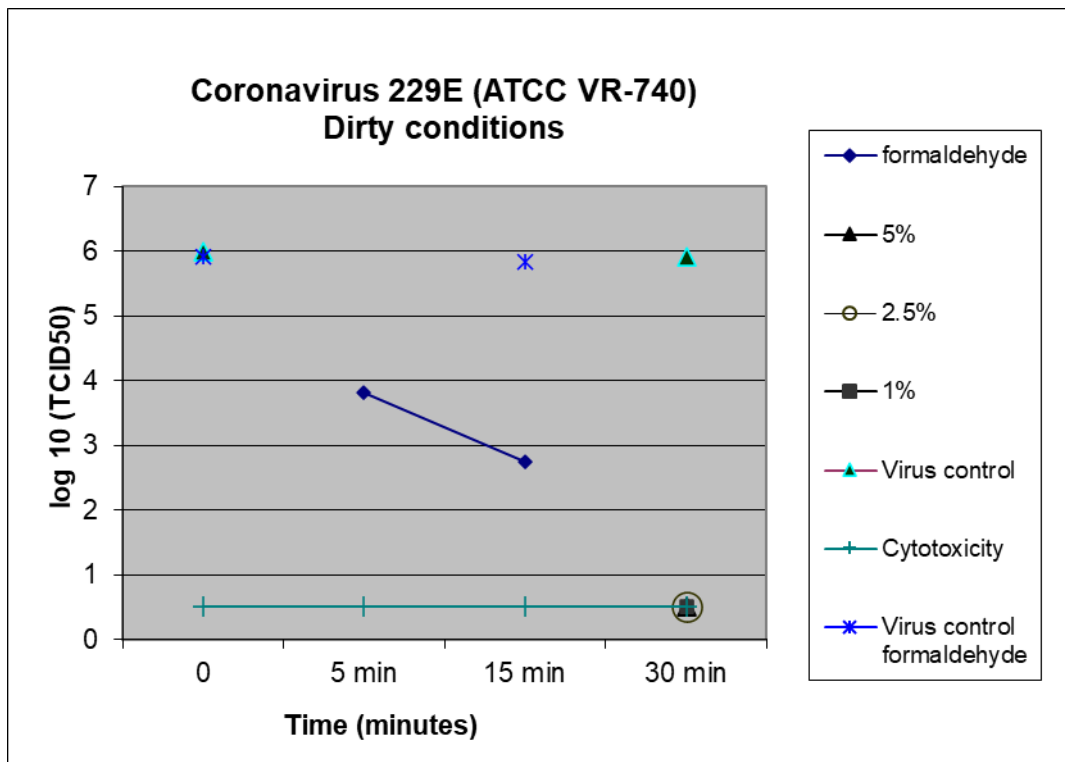


Figure 1.1 Results of the activity of the product “ZOTAL-D”, batch **P001L02F20/02**, at 5%, 2.5% and 1% concentration under internal control of dirty conditions with Coronavirus 229E (ATCC VR-740).

