HAMILTON ROMANIA

RAPORT DE INCERCARE NR. 1071/23/ROBCH/Z1

Inlocuieste Raportul de Incercare Nr. 1071/23/ROBCH din 02.03.2023

Client	Numărul eEantionului:
ECOCHIM-GRUP SRL STRADA PETRICANI 21/3 2059 CHIŞINĂU	1071/23/ROBCH Descriere obiect de incercat *(conform cu declaratia Clientului)* Dezinfectant Universal "Bio-Dez" Lot: - Data fabricatie: 13.12.2022 Data expirarii: 13.12.2025 Data prelevarii: 13.12.2021 Cantitate prelevata:500 ml Responsabil prelevare: Cristinov Alexandr Ora receptiei probei: 08:00 Temperatura receptie proba: 15°C Sample condition with no objections
Data primirii obiectului de incercat: 11.01.2023	
Data finalizarii incercarii: 02.03.2023	Comanda din 11.01.2023
Data eliberarii raportului: 02.08.2023	Probele au fost prelevate si livrate de catre Client.

Parametrii de testare	Metoda de testare	Unitate de masura	Rezultat
# * Fungicidal activity in medical area. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area. Test method and requirements (phase 2, step 1).	EN 13624:2014	-	Test method performed by the subcontractor; the results are taken in full from the test report No D/23/ B0019 , issued by the subcontractor. The results of the analysis are listed starting with enclosure No1 to report of analysis.

Digitally signed by Poiata Cristian
Date: 2024.10.12 16:48:30 EEST
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

Responsabil incercare: Mariana Ilinca, Sef Laborator Microbiologie
Validat de: Mariana Ilinca, Sef Laborator Microbiologie
Autorizat de: Alina-Roxana Mihai, General Manager *(Aprobat cu semnatura electronica)*

Laborator: Bucuresti 041914, sos. Berceni nr.8

* Metoda de testare acreditata # Test efectuat de catre subcontractor
ø Incercari neacreditate

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Autorizatie A.N.S.V.S.A. nr. 115 din 18.06.2018

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 1071/23/ROBCH/Z1

A) IDENTYFICATION OF THE SAMPLE	
Name of the product/Details about the product	**Dezinfectant Universal "Bio-Dez"** Manufacturer(supplier): Ecochim-Grup Condition of use: Instrument disinfection, surface disinfection without mechanical action textile disinfection
Solvent of the product recommended by the manufacturer	Not indicated
Active(S) substance (S) and its concentration(s)	Ethyl alcool 72-76%, CAS 64-17-5 and CE 200-578-6 Benzalkonium chloride 0.024-0.029%, CAS 68424-85-1 and CE 270-325-2 Methylthionibium chloride 0.00024%, CAS 61-73-4 and 200-515-2.
Concentrations requested for the assay	80% and 97%.
B) TEST METHOD	
Performed in accredited subcontracted partner laboratory: Scope of Accreditation Nr. 648/LE1286 Report no.: D/23/B0019 and D/23/B0413- Quantitative evaluation assay of fungicidal activity in Medicine (phase 2, step1), with the product "Dezinfectant Universal "Bio-Dez"". (EN 13624 : 2022 Standard)	EN 13624: 2022. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of the fungicidal or yeasticidal activity in the medical area. Test method and requirements (phase 2, step 1).
Testing method	EN 13624: 2022
Method	Dilution-neutralization
Neutralizer	Tryptone 5 g/L, yeast extract, 2.5 g/L, dextrose 10g/L, sodium thioglycolate 1g/L, sodium thiosulfate 1g/L, sodium bisulfide 2.5 g/L, soya lecithin 7 g/L, polysorbate-80 5 g/L, glycine 1 g/L, l-histidine 1 g/L and saponin 30 g/L.
C) INFORMATION ABOUT SAMPLE RECEPTION	
Date of reception of the sample	2023/01/13
Date of reception of order with test conditions	2023/01/16.
Date of reception of order with test conditions (test at 97%)	2023/06/14
Aspect of the received product	Blue liquid in plastic package
D) EXPERIMENTAL CONDITIONS	
Assay period	2023/02/08 to 2023/02/17 (test at 80%). 2023/07/05 to 2023/07/15 (test at 97%).
Solvent of the product used in the assay	Sterile distilled water (test at 80%). Not applicable (test at 97%).
Product concentrations for the assay	First test: 80%, 50% and 0.1% Second test: 97%.
Aspect of the dilutions of the product	97%, 80% and 50% blue liquid; 0.1% transparent liquid.
Contact time	90 seconds
Assay temperature	+20°C ± 1°C
Interfering substance	Bovine serum albumin 3 g/L plus erythrocytes 3 mL/L.
Stability of the mixture (interfering substance and product diluted in sterile distilled water)	Formation of flocs at 97% concentration and stable at 80%, 50% and 0,1%.
Temperature of incubation	+30°C ± 1°C
Identification of the origin of viral stains and number of passes	-*Aspergillus brasiliensis* (CECT 2574 = ATCC 16404). − *Candida albicans* (CECT 1394 = ATCC 10231).

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Date: 02.08.2023
Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 1071/23/ROBCH/Z1

Results of the assay

- Assay of validation ..... See tables 1, 2, 4 and 5.
- Evaluation of fungicidal activity ..... See tables 3 and 6.
- Number of replicates per assay organism ..... 1.

Special remarks

- All controls and validation were between the basic limits.
- For a valid test, at least one concentration must show a log reduction equal or higher than 4 log.
- Formation of flocs has been observed when mixed the product at 97% with the interference substance and the inoculum.
- No precipitate formed during the test procedure (test mixtures at 80%, 50 % and 0.1% were homogeneous).

Conclusion

The product **Dezinfectant Universal "Bio-Dez"**, batch 1071/23//ROBCH, at 80% **does not show fungicidal activity**, after 90 seconds at 20ºC ±1ºC, under **dirty conditions** (bovine serum albumin 3 g/L and erythrocytes 3 mL/L), because it does not show activity against *Aspergillus brasiliensis* (CECT 2574 = ATCC 16404) although it shows activity against *Candida albicans* (CECT 1394 = ATCC 10231), when tested as required by the **EN 13624: 2022 Standard**.

The product **Dezinfectant Universal "Bio-Dez"**, batch 1071/23//ROBCH, at 80% **shows yeasticidal activity**, after 90 seconds at 20ºC ±1ºC, under **dirty conditions** (bovine serum albumin 3 g/L and erythrocytes 3 mL/L), for the strain *Candida albicans* (CECT 1394 = ATCC 10231), when tested as required by the **EN 13624: 2022 Standard**.

The product **Dezinfectant Universal "Bio-Dez''**, batch 1071/23/ROBCH, at 97% concentration requested by the client, **shows fungicidal activity**, after 90 seconds at 20ºC ±1ºC, under dirty conditions (bovine serum albumin 3 g/L plus erythrocytes 3ml/L), against the reference strains *Aspergillus brasiliensis* (CECT 2574 = ATCC 16404) and *Candida albicans* (CECT 1394 = ATCC 10231), when tested as required by the **EN 13624: 2022 Standard**.

Note: The results obtained correspond to the sample received in the laboratory.

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 1071/23/ROBCH/Z1

Results of the assay with *Aspergillus brasiliensis* (CECT 2574 = ATCC 16404).
First test at 80%.

Seeding: Pour plates. No. of plates: 4/mL.

Table 1.-Validation and controls.

Suspension of validation (Nv_0)			Control of experimental conditions (A)			Control of the neutralizer (B)			Validation of the method (C) Sample concentration: 80%		
Vc_1	72	X=	Vc_1	68	X=	Vc_1	61	X=	Vc_1	63	X=
Vc_2	78	75	Vc_2	66	67	Vc_2	65	63	Vc_2	69	66
$30 \leq X$ of $Nv_0 \leq$ 160? Yes			X of A is ≥0.5x X if Nv_0? Yes			X of B es ≥ 0.0005 Nv_B? Yes			X of C is ≥0.5x X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 79 Vc_2: 76			X= 77.5 $30 \leq X$ de $Nv_B/1000 \leq$ 160? Yes					

Table 2.-Suspension of the assay.

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-5}	291	297	$Xwm = 2.94 \times 10^7$ $\lg N = 7.47$ $N_0 = N/10$
	10^{-6}	29	30	$\lg N_0 = 6.47$ $6.17 \leq \lg N_0 \leq 6.70$?; Yes

Table 3.- Results of the activity test with the sample.

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=6.47)	Time of contact (seconds)
Pure (80 %)	Na^0	>660	>660	4.05	2.42	90
	Na^{-1}	115	108			
50 %	Na^0	>660	>660	>4.82	<1.65	90
	Na^{-1}	>660	>660			
0.1 %	Na^0	>660	>660	>4.82	<1.65	90
	Na^{-1}	>660	>660			

Explanations:
Vc = number per mL (one or two plates); Xwm = ponderated mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2); R(reduction): ($\lg R = \lg N_0 - \lg Na$).

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Date: 02.08.2023
Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 1071/23/ROBCH/Z1

Counts per plate:

(N) 10^{-5} = 70 + 73 + 74 + 74; 71 + 75 + 76 + 75;
10^{-6} = 6 + 7 + 8 + 8; 8 + 7 + 7 + 8;

Product:

Pure (80%) → Na^{-1} 28 + 27 + 31 + 30; 27 + 28 + 26 + 27;

50% → Na^{-1} = >165 + >165 + >165 + >165; >165 + >165 + >165 + >165;

0.1% → Na^{-1} = >165 + >165 + >165 + >165; >165 + >165 + >165 + >165;

A = 17+16+18+17; 17+16+16+17;
B = 15+16+15+15; 16+15+16+18;
C = 15+16+16+16; 17+18+16+18;
N_{V0} = 18+17+19+18; 19+20+20+19;
N_{VB} = 19+20+20+20; 19+19+19+19;

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 1071/23/ROBCH/Z1

Results of the assay with *Aspergillus brasiliensis* (CECT 2574 = ATCC 16404).
Second test at 97%.

Seeding: plate pouring; No. of plates: 4/mL.

Table 1.-Validation and controls.

Suspension of validation (Nv_0)			Control of experimental (A)			Control of the neutralizer (B)			Validation of the method (C) Sample concentration: 97%		
Vc_1	101	X =	Vc_1	80	X =	Vc_1	85	X =	Vc_1	80	X =
Vc_2	100	100.5	Vc_2	82	81	Vc_2	82	83.5	Vc_2	82	81
$30 \leq X$ of $Nv_0 \leq 160$? Yes			X of A is ≥0.5x X if Nv_0? Yes			X of B es ≥ 0.0005 Nv_B? Yes			X of C is ≥0.5x X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 102 Vc_2: 102			X = 102 $30 \leq X$ de $Nv_B/1000 \leq 160$? Yes					

Table 2.-Suspension of the assay.

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-6}	199	200	$Xwm = 1.99 \times 10^8$ $\lg N = 8.30$ $N_0 = N/10$ $\lg N_0 = 7.30$
	10^{-7}	19	20	$7.17 \leq \lg N_0 \leq 7.70$?; Yes

Table 3.- Results of the activity test with the sample.

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.30)	Time of contact (seconds)
97%	Na^0	<14	<14	<2.15	>5.15	90
	Na^{-1}	<14	<14			
	Na^{-2}	<14	<14			

Explanations:
Vc = number per mL (one or two plates); Xwm = ponderated mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2); R(reduction): ($\lg R = \lg N_0 - \lg Na$).

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 1071/23/ROBCH/Z1

Counts per plate:

(*N*) 10^{-6} = 48 + 52 + 50 + 49; 49 + 50 + 51 + 50;
10^{-7} = 4 + 5 + 5 + 5; 4 + 5 + 6 + 5.

Sample:

97% = Na^0 = 0 + 0 + 0 + 0; 0 + 0 + 0 + 0;

A = 19 + 20 + 22 + 19; 21 + 20 + 22 + 19;
B = 24 + 20 + 21 + 20; 19 + 21 + 22 + 20;
C = 20 + 21 + 20 + 19; 20 + 22 + 20 + 20;
N_{V0} = 28 + 26 + 24 + 23; 25 + 24 + 26 + 25;
N_{VB} = 27 + 24 + 26 + 25; 25 + 24 +27 + 26.

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 1071/23/ROBCH/Z1

Results of the assay with *Candida albicans* (CECT 1394 = ATCC 10231).
First test at 80%.

Seeding: Pour plates. No. of plates: 1 /mL.

Table 4.-Validation and controls.

Suspension of validation (Nv_0)			Control of experimental conditions (A)			Control of the neutralizer (B)			Validation of the method (C) Sample concentration: 80%		
Vc_1	96	X=	Vc_1	83	X=	Vc_1	87	X=	Vc_1	82	X=
Vc_2	100	98	Vc_2	89	86	Vc_2	100	93.5	Vc_2	85	83.5
$30 \leq X$ of $Nv_0 \leq$ 160? Yes			X of A is ≥0.5x X if Nv_0? Yes			X of B es ≥ 0.0005 Nv_B? Yes			X of C is ≥0.5x X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 85 Vc_2: 87			X = 86 $30 \leq X$ de $Nv_B/1000 \leq$ 160? Yes					

Table 5. -Suspension of the assay.

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-5}	>330	>330	$Xwm = 3.65 \times 10^7$ $\lg N = 7.56$ $N_0 = N/10$ $\lg N_0 = 6.56$ $6.17 \leq \lg N_0 \leq 6.70$? Yes
	10^{-6}	38	35	

Table 6.-Results of the activity assays with the sample.

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=6.56)	Time of contact (seconds)
Pure (80 %)	Na^0	<14	<14	<2.15	>4.41	90
	Na^{-1}	<14	<14			
50 %	Na^0	>330	>330	3.94	2.62	90
	Na^{-1}	88	87			
0.1 %	Na^0	>330	>330	>4.52	<2.04	90
	Na^{-1}	>330	>330			

Explanations:
Vc = number per mL (one or two plates); Xwm = ponderated mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2); R (reduction): ($\lg R = \lg N_0 - \lg Na$).

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Date: 02.08.2023
Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 1071/23/ROBCH/Z1

Results of the assay with *Candida albicans* (CECT 1394 = ATCC 10231).
Second test at 97%.

Seeding: plate pouring; No. of plates: 1/mL.

Table 4.-Validation and controls.

Suspension of validation (Nv_0)			Control of experimental conditions (A)			Control of the neutralizer (B)			Validation of the method (C) 97%		
Vc_1	89	X = 87	Vc_1	80	X= 79.5	Vc_1	81	X= 82	Vc_1	80	X= 78
Vc_2	85		Vc_2	79		Vc_2	83		Vc_2	76	
30 ≤ X of Nv_0 ≤ 160? Yes			X of A is ≥ 0,5 x X of Nv_0? Yes			X of B is ≥ 0,5 x X of Nv_0, or 0.0005 Nv_B? Yes			X of C is ≥ 0.5 x X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 87 Vc_2: 89			X = 88 30 ≤ x de Nv_B/1000 ≤ 160? Yes					

Table 5. -Suspension of the assay.

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-6}	>330	>330	$Xwm = 4.95 \times 10^8$ lg N = 8.69 $N_0 = N/10$
	10^{-7}	51	48	lg N_0 = 7.69 7.17 ≤ lg N_0 ≤ 7.70? Yes

Table 6.-Results of the activity assays with the sample.

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.69)	Time of contact (seconds)
97%	Na^0	<14	<14	<2.15	>5.54	90
	Na^{-1}	<14	<14			
	Na^{-2}	<14	<14			

Explanations:
Vc = number per mL (one or two plates); Xwm = ponderated mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2); R (reduction): (lg R = lg N_0 – lg Na).

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Date: 02.08.2023
Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

RAPORT DE INCERCARE NR. 60363/23/ROBCH

Client **ECOCHIM-GRUP SRL** OR. UNGHENI, STR. NAȚIONALĂ 119 - REPUBLICA MOLDOVA	Numărul eșantionului: **60363/23/ROBCH** Descriere obiect de incercat *(conform cu declaratia Clientului)* **Dezinfectant Universal "Bio-Dez"** **Lot: -** **Data fabricatiei: 05.08.2023** **Data expirare: 05.08.2026** **Data receptiei probei: 23.08.2023** **Cantitate prelevata:500 ml** **Responsabil prelevare: Cristinov Alexandr** **Ora receptiei probei: 12:30** **Temperatura receptie proba: 17°C** **Sample condition with no objections** **Comanda din 24.08.2023** Probele au fost prelevate si livrate de catre Client.
Data primirii obiectului de incercat: **24.08.2023**	
Data inceperii incercarii: **30.08.2023**	
Data finalizarii incercarii: **22.11.2023**	
Data eliberarii raportului: **22.11.2023**	

Parametrii de testare	Metoda de testare	Unitate de masura	Rezultat
# * Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants. Test methods and requirements (phase 2, step 1) [1)]	EN 14348: 2005	-	Rezultatul incercarii se regaseste in raportul de incercare nr. D/23/B0672 primit de la subcontractor si anexat acestui raport.

[1)] Incercare efectuata de catre subcontractor cu Certificat de acreditare Nr. 648/LE1286.

Responsabil incercare: Mariana Ilinca, Sef Laborator Microbiologie
Validat de: Mariana Ilinca, Sef Laborator Microbiologie
Autorizat de: Manager general Mihai Alina-Roxana *(Aprobat cu semnatura electronica)*

* Metoda de testare acreditata # Test efectuat de catre subcontractor
ø Incercari neacreditate

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Instituto Valenciano de Microbiología

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Nº648 / LE1286

Test with the certificate of GLPs
(Good Laboratory Practices)
No. 2/21-C.VAL. General Directorate of
Pharmacy and Medical Devices of the Health
Department of the Valencian Region. Spain

Mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants (phase 2, step 1), with the product "Dezinfectant Universal "Bio-Dez"". (EN 14348: 2005 Standard)

Report
Registration No.: D/23/B0672.

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

2. **Client identification** ……... J.S. HAMILTON ROMANIA SRL.
   Address ……... SOS BERCENI, NR 8, SECTOR 4 BUCURESTI, ROMANIA, 041914.

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

- Product name ……..... **Dezinfectant Universal "Bio-Dez".**
- Batch number ……... 60363/23/ROBCH.
- Expiration date ……. 05.08.2026.
- Manufacturer/Supplier …….. SRL "Ecochim-Grup".
- Keeping conditions …… Not indicated.
- Condition for use ……..….. Hygienic handrub, Instrument disinfection, surface disinfection.
- Diluent recommended by the manufacturer ... Not indicated.
- Active compound/s and its concentration/s ... Ethyl alcool 72-76%, CAS 64-17-5 and CE 200-578-6 Benzalkonium chloride 0.024-0.029%, CAS 68424-85-1 and CE 270-325-2 Methylthionibium chloride 0.00024%, CAS 61-73-4 and 200-515-2.
- Concentrations requested for the assay ……. 80%

IVAMI is not responsible for client-supplied information. This information **is not covered** by the ENAC accreditation.

4. Information about sample reception

- Date of reception of the sample ……. 2023/08/31.
- Date of reception of order with test conditions …… 2023/08/25.
- Aspect of the received samples…… Purple transparent liquid in plastic package.

5. Method of assay and its validation (EN 14348: 2005 Standard)

- Method used ……. Dilution-neutralization.
- Neutralizer ……..……... Tryptone 5 g/L, yeast extract 2.5 g/L, dextrose 10 g/L, sodium thioglycolate 1 g/L, sodium thiosulfate 1g/L, sodium bisulphite 2.5 g/L, soya lecithin 7 g/L, polisorbate-80 5 g/L, glycine 1 g/L, l-histidine 1 g/L and saponin 30 g/L.

6. Experimental conditions

- Assay period (including prior preparation of the strains) ……. 2023/09/28 to 2023/11/09.
- Solvent of the product used in the assay ... Sterile distilled water.
- Product concentrations for the assay ……. 80%, 50% and 0.1%.
- Aspect of the dilutions of the product ….. 80% purple transparent liquid;
  50% blue transparent liquid;
  0.1% transparent liquid.
- Contact time …….. 60 seconds.
- Assay temperature ……. 20ºC ± 1ºC.
- Interfering substance ……. Bovine serum albumin 3 g/L and erythrocytes 3 mL/L.
- Stability of the mixture (interfering substance and product diluted in sterile distilled water) ……... Stable.
- Temperature of incubation …… 36ºC ± 1ºC.
- Identification of the strains used ..……

  – *Mycobacterium avium* (ATCC 15769).
  – *Mycobacterium terrae* (CECT 3028 = ATCC 15755).

7. Results of the assay

- Control and validation assays ……. See tables 1, 2, 4 and 5.
- Evaluation of mycobactericidal activity See tables 3 and 6.
- Number of replicates for each assay microorganism ……. 1.

8. Special remarks

- All controls and validation were between the basic limits.
- For a valid test, at least one concentration must show a reduction lower than 4 log and at least one concentration must show a reduction equal or higher than 4 log.
- The highest concentration that can be assayed in the test (80%) is due to the mixtures to perform the assay.

9. Conclusion

The product **Dezinfectant Universal "Bio-Dez",** batch 60363/23/ROBCH, when it is pure (80%), concentration requested by the client, **shows mycobactericidal activity** after 60 seconds, at 20ºC ± 1ºC, under dirty conditions (bovine serum albumin 3 g/L and erythrocytes 3 mL/L), against the strains *Mycobacterium avium* (ATCC 15769) and *Mycobacterium terrae* (CECT 3028 = ATCC 15755), when tested as required by **EN 14348: 2005 Standard**.

Note: The results obtained correspond to the sample received in the laboratory.

Use of the ENAC mark: The ENAC "*mark*" can only be used by the holder of the accreditation. Its use in packaging, installations, shop windows, advertising or other documentation format other than that issued by the accredited entity (IVAMI) is not allowed.

Bétera (Valencia), November 9, 2023.

MIGUEL FRANCO,
CLAUDIA (FIRMA)

Signed: Claudia Miguel.
Responsible Technician

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.

MONTOYA VIECO, ELENA (FIRMA)

Signed. Elena Montoya.
Responsible for the Laboratory Area
(Study Director)

ESTEBAN BERMUDEZ, ENCARNACION PILAR (FIRMA)

Signed. Encarnación Esteban.
Technical Director
(Quality Assurance Director)

Reference

- **EN 14348: 2005**. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants. Test methods and requirements (phase 2, step 1).

Table 1.-Assay with *Mycobacterium avium* (ATCC 15769): Validation and controls.

Suspension of validation (Nv_0)			**Control of experimental conditions (*A*)**			**Neutralizer (*B*)**			**Validation of the method (*C*) with sample concentration: 80%**		
Vc_1	97	X = 100.5	Vc_1	98	X = 96	Vc_1	92	X = 95	Vc_1	93	X = 91
Vc_2	104		Vc_2	94		Vc_2	98		Vc_2	89	
30 ≤ X of Nv_0 ≤ 160? Yes			X of A is ≥ 0.5 x X of Nv_0? Yes			X of B is ≥ 0.5 x X of Nv_0? Yes			X of C is ≥ 0.5 x X of Nv_0? Yes		

Table 2.- Assay with *Mycobacterium avium* (ATCC 15769): Suspension of the assay.

Suspension of the assay (N y N_0)	N	Vc_1	Vc_2	
	10^{-7}	391	405	Xwm = 4.00 x 10^9= lg = 9.60 N_0 = N/10 = lg = 8.60 8.17 ≤ N_0 ≤ 8.70? Yes
	10^{-8}	44	41	

Table 3.- Assay with *Mycobacterium avium* (ATCC 15769).

Concentrations of the sample (%)	**Dilutions**	Vc_1	Vc_2	**Lg Na = lg (X x 10 o Xwm x 10)**	**LgR (lgN_0 = 8.60)**	**Time of contact (sec)**
80 %	10^0	<14	<14	< 2.15	> 6.45	60
	10^{-1}	<14	<14			
	10^{-2}	<14	<14			
	10^{-3}	<14	<14			
50 %	10^0	<14	<14	< 2.15	> 6.45	60
	10^{-1}	<14	<14			
	10^{-2}	<14	<14			
	10^{-3}	<14	<14			
0.1 %	10^0	>660	>660	>6.82	<1.78	60
	10^{-1}	>660	>660			
	10^{-2}	>660	>660			
	10^{-3}	>660	>660			

Observations:

N 10^{-7}: 198 + 193; 202 + 203;
10^{-8}: 20 + 24; 19 + 22;
Na 80% 10^0: 0 + 0; 0 + 0;
50% 10^0: 0 + 0; 0 + 0;
0.1% 10^{-3}: >330 + >330; >330 + >330;

***Nv*₀**: 50 + 47; 55 + 49;
A: 46 + 52; 49 + 45;
B: 45 + 47; 49 + 49;
C: 48 + 45; 42 + 47;

Table 4.-Assay with *Mycobacterium terrae* (CECT 3028 = ATCC 15755): Validation and controls.

Suspension of validation (Nv_0)			**Control of experimental conditions (*A*)**			**Neutralizer (*B*)**			**Validation of the method (*C*) with sample concentration: 80%**		
Vc_1	79	X =	Vc_1	66	X = 68	Vc_1	75	X = 77	Vc_1	67	X =
Vc_2	82	80.5	Vc_2	70		Vc_2	79		Vc_2	72	69.5
30 ≤ X of Nv_0 ≤ 160? Yes			X of A is ≥ 0.5 x X of Nv_0? Yes			X of B is ≥ 0.5 x X of Nv_0? Yes			X of C is ≥ 0.5 x X of Nv_0? Yes		

Table 5.- Assay with *Mycobacterium terrae* (CECT 3028 = ATCC 15755): Suspension of the assay.

Suspension of the assay (N y N_0)	N	Vc_1	Vc_2	
	10^{-7}	295	275	Xwm = 2.86 x 10^9= lg = 9.46 N_0 = N/10 = lg = 8.46 8.17 ≤ N_0 ≤ 8.70? Yes
	10^{-8}	32	29	

Table 6.- Assay with *Mycobacterium terrae* (CECT 3028 = ATCC 15755).

Concentrations of the sample (%)	**Dilutions**	Vc_1	Vc_2	**Lg Na = lg (X x 10 o Xwm x 10)**	**LgR (lgN_0 = 8.46)**	**Time of contact (sec)**
80 %	10^0	<14	<14	<2.15	>6.31	60
	10^{-1}	<14	<14			
	10^{-2}	<14	<14			
	10^{-3}	<14	<14			
50 %	10^0	<14	<14	<2.15	>6.31	60
	10^{-1}	<14	<14			
	10^{-2}	<14	<14			
	10^{-3}	<14	<14			
0.1%	10^0	>660	>660	>6.82	<1.64	60
	10^{-1}	>660	>660			
	10^{-2}	>660	>660			
	10^{-3}	>660	>660			

Observations:

N 10^{-7}: 150 + 145; 130 + 145;
10^{-8}: 17 + 15; 14 + 15;

Na 80% 10^0: 0 + 0; 0 + 0;
50% 10^0: 0 + 0; 0 + 0;
0.1% 10^{-3}: >330 + >330; >330 + >330;

***Nv*$_0$**: 40 + 39; 40 + 42;
A: 32 + 34; 34 + 36;
B: 39 + 36; 40 + 39;
C: 36 + 31; 35 + 37;

Explanations:
***Vc*:** Counts per mL.
Xwm*:** weighted mean of ***X.
***X*:** Values of Vc_1 and Vc_2 (1. + 2. duplicates); ***R*:** reduction (LgR = lgN_0 – lgNa).

REPORT OF ANALYSIS No. 60362/23/ROBCH

Client **ECOCHIM-GRUP SRL** OR. UNGHENI, STR. NAȚIONALĂ 119 - REPUBLICA MOLDOVA	Sample number: **60362/23/ROBCH** Sample description *(according to declaration of Client)* **Dezinfectant Universal "Bio-Dez"** **Lot: -** **Data fabricatiei: 05.08.2023** **Data expirare: 05.08.2026** **Data receptiei probei: 23.08.2023** **Cantitate prelevata:500 ml** **Responsabil prelevare: Cristinov Alexandr** **Ora receptiei probei: 12:30** **Temperatura receptie proba: 17°C** **Sample condition with no objections**
Sample received: **24.08.2023** Tests performed: **30.08.2023** Tests completed: **30.10.2023** Report dated: **30.10.2023**	**Order of 24.08.2023** Sampling and delivery were carried out by client.

Test	Method	Unit	Result
# * Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of virucidal activity in the medical area	UNE-EN 14476:2014 + A2:2019	-	Test method performed by the subcontractor; the results are taken in full from the test report No D/23/ V0259, issued by the subcontractor. The results of the analysis are listed starting with enclosure No1 to report of analysis.

Test responsible: Mariana Ilinca, Manager of Microbiological Laboratory
Validated by: Mariana Ilinca, Manager of Microbiological Laboratory
Authorized by: Alina-Roxana Mihai, General Manager *(Approved with qualified electronic signature)*

Laboratory: Bucuresti 041914, sos. Berceni nr.8
Responsabilitatea esantionarii/ prelevarii probelor apartine solicitantului. Rezultatele se refera numai la obiectul supus incercarii. Daca nu se specifica altfel, incertitudinea de masurare a fost estimata pentru coeficientul K= 2 si nivel de incredere 95%. Fara aprobarea scrisa a laboratorului acest raport de incercare nu poate fi reprodus decat integral. Responsabilitatea S.C. J.S. HAMILTON ROMANIA S.R.L. se refera exclusiv la rezultatele si declaratiile prezentate in raportul original. Opiniile si interpretarile continute in prezentul raport de incercare nu sunt acoperite de acreditarea RENAR. Pentru detalii suplimentare va rugam sa solicitati Certificatul de Acreditare la adresa de email bucharest@hamilton.com.pl

* Test method accredited # Test performed by external provider
ø Non accredited methods

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A.N.S.V.S.A. Authorization no 115 from 18.06.2018

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

A) IDENTYFICATION OF THE SAMPLE	
Name of the product/Details about the product	**DEZINFECTANT UNIVERSAL "BIO-DEZ"** Manufacturer(supplier): Ecochim-Grup Condition of use: Instrument disinfection, surface disinfection, hygienic handrub.
Active(s) Substance(s) and its concentration(s)	Ethyl alcool 72-76%, CAS 64-17-5 and CE 200-578-6, Benzalkonium chloride 0.024-0.029%, CAS 68424-85-1 and CE 270-325-2, Methylthionibium chloride 0.00024%, CAS 61-73-4 and 200-515-2.
Concentration ordered for the assay	80
B) TEST METHOD	
Performed in accredited subcontracted partner laboratory: Scope of Accreditation Nr. 648/LE1286 Report D/23/V0259. Quantitative suspension test for the evaluation of virucidal activity in the medical area (phase 2, step 1), against Poliovirus type 1, Adenovirus type 5 and Murine Norovirus with the product "Dezinfectant Universal "Bio-Dez" (EN 14476: 2013 + A2: 2019 Standard)	EN 14476: 2013 + A2: 2019 Standard. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of virucidal activity in the medical area. Test method and requirements (phase 2/step1).
Testing method	Procedure DESIN-1078 (EN 14476: 2013 + A2: 2019 Standard).
C) INFORMATION ABOUT SAMPLE RECEPTION	
Date of reception of the sample	2023/08/30
Date of reception of order with test conditions	2023/09/04.
Aspect of the received product	Blue transparent liquid in plastic container with identification label.
D) EXPERIMENTAL CONDITIONS	
Assay period	2023/09/06 to 2023/09/21.
Assay temperature	37°C ± 1°C
Titration method	TCID50 (Tissue Culture Infective Dose 50%).
Product concentrations for the assay	80%, 50% and 0.1%
Contact time	60 seconds
Contact temperature	20°C ± 1°C
Procedure to stop product cytotoxicity	Molecular sieving (< 4 columns).
Procedure to stop product activity	Cooling with ice
Solvent of the product used in the assay	Sterile distilled water
Aspect of the dilutions of the product	Transparent
Stability of the mixture (interfering substance and product diluted in sterile hard water/distilled water)	Stable
Interfering substance	Dirty conditions in the presence of bovine serum albumin 3 g/L and erythrocytes 3 mL/L.
Identification of the origin of viral stains and number of passes	Poliovirus type 1 (ATCC VR-192) aliquot: 2023/03/23 passage 2. Adenovirus type 5 (ATCC VR-5) aliquot: 2022/06/10 passage 2. Murine Norovirus (strain S99 Berlin) aliquot: 2022/06/22 passage 2.
Cell lines (name, origin, number of passes)	Vero, ref: FTVE, working aliquot 4, passages 18 21, 22 and 25. Raw 264.7, Public Health England, working aliquot 4, passages 18, 21 and 25

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Validation of assay results

Poliovirus type 1 (ATCC VR-192)

Titre of the viral suspension for the virus control (at the requested test time)
- Dirty conditions ..... log $10^{-7.50}$

Cytotoxicity level (80%) ..... log $10^{-0.50}$

Maximum level of virus inactivation detectable (difference between the titre of the viral suspension and the cytotoxicity level):
- Dirty conditions ..... log $10^{-7.00}$

Adenovirus type 5 (ATCC VR-5)

Titre of the viral suspension for the virus control (at the requested test time)
- Dirty conditions ..... log $10^{-6.82}$

Cytotoxicity level (80%) ..... log $10^{-0.50}$

Maximum level of virus inactivation detectable (difference between the titre of the viral suspension and the cytotoxicity level):
- Dirty conditions ..... log $10^{-6.32}$

Murine Norovirus (strain S99 Berlin)

Titre of the viral suspension for the virus control (at the requested test time)
- Dirty conditions ..... log $10^{-8.32}$

Cytotoxicity level (80%) ..... log $10^{-0.50}$

Maximum level of virus inactivation detectable (difference between the titre of the viral suspension and the cytotoxicity level):
- Dirty conditions ..... log $10^{-7.82}$

Reference test (formaldehyde 1.4%)

Cytotoxicity level of formaldehyde 0.7% ..... log $10^{-0.50}$

Viral quantification in the reference test (formaldehyde) after 60 minutes and with Poliovirus type 1 ..... log $10^{-3.08}$

Viral quantification in the reference test (formaldehyde) after 60 minutes and with Adenovirus type 5 ..... log $10^{-2.75}$

Viral quantification in the reference test (formaldehyde) after 60 minutes and with Murine Norovirus ..... log $10^{-4.99}$

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Confidence interval

Titre of virus with 95% confidence interval with Poliovirus type 1 (at the requested test time)
- Dirty conditions ..... $\log 10^{-7.50 \pm 0.37}$

Titre of virus with 95% confidence interval with Adenovirus type 5 (at the requested test time)
- Dirty conditions ..... $\log 10^{-6.82 \pm 0.41}$

Titre of virus with 95% confidence interval with Murine Norovirus (at the requested test time)
- Dirty conditions ..... $\log 10^{-8.32 \pm 0.42}$

Reduction with the confidence interval of 95% ..... See tables 1, 3 and 5.

Sensitivity of cells to virus

- Viral quantification of Poliovirus type 1 with cells not treated by the test solution with the test sample ..... $\log 10^{-8.23}$
- Viral quantification of Poliovirus type 1 with cells treated by the test solution with the test sample ..... $\log 10^{-7.65}$

- Viral quantification of Adenovirus type 5 with cells not treated by the test solution with the test sample ..... $\log 10^{-7.41}$
- Viral quantification of Adenovirus type 5 with cells treated by the test solution with the test sample ..... $\log 10^{-6.74}$

- Viral quantification of Murine Norovirus with cells not treated by the test solution with the test sample ..... $\log 10^{-8.66}$
  Viral quantification of Murine Norovirus with cells treated by the test solution with the test sample ..... $\log 10^{-7.99}$

  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 suppression activity

- Viral quantification of Poliovirus type 1 after 30 minutes on bath ice without exposing the virus to the test sample ..... $\log 10^{-7.41}$
- Viral quantification of Poliovirus type 1 exposing the virus to the test sample and incubated 30 minutes on ice bath ..... $\log 10^{-6.99}$

- Viral quantification of Adenovirus type 5 after 30 minutes on bath ice without exposing the virus to the test sample ..... $\log 10^{-7.08}$
- Viral quantification of Adenovirus type 5 exposing the virus to the test sample and incubated 30 minutes on ice bath ..... $\log 10^{-6.83}$

- Viral quantification of Murine Norovirus after 30 minutes on bath ice without exposing the virus to the test sample ..... $\log 10^{-8.25}$
- Viral quantification of Murine Norovirus exposing the virus to the test sample and incubated 30 minutes on ice bath ..... $\log 10^{-7.74}$

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

Special remarks

- All controls and validation were between the basic limits.
- To be accepted the assay, at least one concentration of the product must show a log reduction equal or higher than 4 log, and at least one concentration must show a log reduction lower than 4 log.

9. Assay results

9.1 Description of the results under the requested test conditions

Virus of assay	Test concentrations, reduction obtained with the confidence interval of 95 % and virucidal activity		
	80%	50%	0.1%
Poliovirus type 1	$\geq 7.00 \pm 0.37$ $TCID_{50}$ Shows	2.84 ± 0.56 $TCID_{50}$ Does not show	0.01 ± 0.49 $TCID_{50}$ Does not show
Adenovirus type 5	$\geq 6.32 \pm 0.41$ $TCID_{50}$ Shows	5.07 ± 0.48 $TCID_{50}$ Shows	0.07 ± 0.48 $TCID_{50}$ Does not show
Murine Norovirus	$\geq 7.82 \pm 0.42$ $TCID_{50}$ Shows	5.58 ± 0.56 $TCID_{50}$ Shows	0.08 ± 0.54 $TCID_{50}$ Does not show

Virucidal activity exists when the titre of virus shows a reduction ≥ 4 log.
$TCID_{50}$: Tissue Culture Infectious Dose 50%.

9.2 Tables of results and graphics

See tables 1 to 6 and figures 1 to 3.

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Conclusion

The product **"Dezinfectant Universal "Bio-Dez"**, batch 60362/23/ROBCH, at **80%** concentration, under dirty conditions (bovine serum albumin 3 g/L and erythrocytes 3 mL/L), requested by the client and during 60 seconds of contact time and 20°C of temperature, **shows** activity against Poliovirus type 1, Adenovirus type 5 and Murine Norovirus, when the activity is assayed according with the **EN 14476: 2013 + A2: 2019 Standard.**

Therefore, the disinfectant tested **shows general virucidal activity** at **80%** concentration, when the activity is assayed according with the **EN 14476: 2013 + A2: 2019 Standard**.

Note 1: The results obtained correspond to the sample 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 indicated".

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Table 1. Results of activity of the test sample with Poliovirus type 1 (ATCC VR-192) under test conditions requested by the client.

Assay	Concen-tration	Interfering substance	Cytoto-xicity level	$\log_{10}$ TCID$_{50}$ after…				Reduction with the confidence interval of 95 %
				0 min	60 sec	30 min	60 min	
Test sample	80%	3 g/L BSA + 3 mL/L erythrocytes	0.50	-	0.50	-	-	≥ 7.00 ± 0.37
	50%		0.50	-	4.66	-	-	2.84 ± 0.56
	0.1%		0.50		7.49			0.01 ± 0.49
Virus control	NA	3 g/L BSA + 3 mL/L erythrocytes	NA	7.58	7.50	-	-	NA
Formaldehyde	0.7% (w:v)	NA	0.50	NR	NR	4.99	3.08	NA
Virus control formaldehyde	0.7% (w:v)	NA	NA	7.58	NR	NR	7.32	NA

Control of sensitivity of cells to virus (difference between decimal logarithm of titre using treated and untreated cells) ..... $\log10^{-0.58}$

Control of the effectiveness of the disinfectant suppression activity (difference between decimal logarithm of titre without exposing the virus to the sample and of the test suspension)..... $\log10^{-0.42}$

NA: not applicable; NR: not realized.
Times recommended by Standard for surfaces: maximum 5 or 60 minutes.
Times recommended by Standard for instruments: maximum 60 minutes.
Times recommended by Standard 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.

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Table 2. Results of the activity of the test sample, with Poliovirus type 1 (ATCC VR-192) (Assay of titration with 12 wells), under test conditions requested by the client.

Assay	Concen-tration	Interfering substance	Time of contact (sec/min)	Dilutions (log10)[a,b]									
				1	2	3	4	5	6	7	8	9	10
Test sample	80%	3 g/L BSA + 3 mL/L erythrocytes	60 sec	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR	NR
	50%		60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	3240 0403 4432	0002 3010 0020	0200 0000 0000	0000 0000 0000	NR	NR	NR
	0.1%		60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3241 0344 3032	0000 0200 0001	0000 0000 0000	NR
Cytotoxicity	80%	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	0000 0000 0000	NR	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	4444 4444 4444	4444 4444 4444	3244 3021 3044	0000 0220 0003	0000 0000 0000	NR
			60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3204 4310 0233	0010 0000 2020	0000 0000 0000	NR
Formal-dehyde	0.7% (w:v)	NA	30 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0030 2004 4030	0000 0020 0000	0000 0000 0000	NR	NR	NR
			60 min	4444 4444 4444	4444 4444 4444	0302 2304 0002	0000 0000 0100	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR	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	0000 0000 0000	NR	NR	NR
Virus control formal-dehyde	0.7% (w:v)	NA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0321 0044 4342	1000 2000 3020	0000 0000 0000	0000 0000 0000
			60 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0321 0404 4303	0000 2000 0020	0000 0000 0000	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	CCCC CCCC CCCC	CCCC CCCC CCCC	CCC0 CCCC CCCC	0CCC 0CC0 C0CC	0000 C00C 0000	NR
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC CC00 C0CC	C000 C0CC 0C00	0000 0000 C000	NR
Effectiveness control of the disinfectant suppression activity	NA	3 g/L BSA + 3 mL/L erythrocytes	Without sample	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC 0CCC CC00	000C 0000 00C0	0000 0000 0000	NR
			With sample	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CC0C C0CC	C0C0 0CCC 0C0C	0000 C000 0000	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 Standard 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;

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Date: 27.10.2023
Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Table 3. Results of activity of the test sample with Adenovirus type 5 (ATCC VR-5), under test conditions requested by the client.

Assay	Concen-tration	Interfering substance	Cytoto-xicity level	log_{10} $TCID_{50}$ after…				Reduction with the confidence interval of 95 %
				0 min	60 sec	30 min	60 Min	
Test sample	80%	3 g/L BSA + 3 mL/L erythrocytes	0.50	-	0.50	-	-	≥ 6.32 ± 0.41
	50%		0.50	-	1.75	-	-	5.07 ± 0.48
	0.1%		0.50	-	6.75	-	-	0.07 ± 0.48
Virus control	NA	3 g/L BSA + 3 mL/L erythrocytes	NA	6.91	6.82	-	-	NA
Formaldehyde	0.7% (w:v)	NA	0.50	NR	NR	3.49	2.75	NA
Virus control formaldehyde	0.7% (w:v)	NA	NA	7.16	NR	NR	6.98	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 suppression activity (difference between decimal logarithm of titre without exposing the virus to the sample and of the test suspension)..... $log10^{-0.25}$

NA: not applicable; NR: not realized.
Times recommended by Standard for surfaces: maximum 5 or 60 minutes.
Times recommended by Standard for instruments: maximum 60 minutes.
Times recommended by Standard 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.

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Table 4. Results of the activity of the test sample, with Adenovirus type 5 (ATCC VR-5) (Assay of titration with 12 wells), under test conditions requested by the client.

Assay	Concen-tration	Interfering substance	Time of contact (sec/min)	Dilutions (log10)[a,b]									
				1	2	3	4	5	6	7	8	9	10
Test sample	80%	3 g/L BSA + 3 mL/L erythrocytes	60 sec	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR	NR
	50%		60 sec	4433 2113 4443	0010 3002 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR	NR
	0.1%		60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4433 1234 4433	0003 1000 2000	0000 0000 0000	NR	NR
Cytotoxicity	80%	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	0000 0000 0000	NR	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	4444 4444 4444	3244 4302 3042	2004 0423 0001	0100 0000 0000	NR	NR
			60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3201 4443 3440	0023 0102 0200	0000 0000 0001	NR	NR
Formal-dehyde	0.7% (w:v)	NA	30 min	4444 4444 4444	4444 4444 4444	3402 0303 2033	0001 2000 1000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR	NR
			60 min	4444 4444 4444	3334 2444 3442	0202 0010 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR	NR
Control of formal-dehyde 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	0000 0000 0000	NR	NR	NR
Virus control formal-dehyde	0.7% (w:v)	NA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0402 3030 4040	0000 0010 0020	0000 0000 0000	0000 0000 0000
			60 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4344 4340 2433	4020 0200 3200	0000 0010 0010	0000 0000 0000	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	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC CCC0 CC0C	0000 0C00 000C	NR	NR
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC C0CC CC0C	0C00 CC00 0C00	0000 000C 0000	NR	NR
Effectiveness control of the disinfectant suppression activity	NA	3 g/L BSA + 3 mL/L erythrocytes	Without sample	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC C0C0 CCCC	C0C0 CC0C 00C0	000C C000 0C00	NR	NR
			With sample	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	00CC 0C00 00C0	0000 0000 0000	NR	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 Standard 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.

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Table 5. Results of activity of the test sample, with Murine Norovirus, strain S99 Berlin, under test conditions requested by the client.

Assay	Concen-tration	Interfering substance	Cytoto-xicity level	log_{10} $TCID_{50}$ after…				Reduction with the confidence interval of 95 %
				0 min	60 sec	30 min	60 min	
Test sample	80%	3 g/L BSA + 3 mL/L erythrocytes	0.50	-	0.50	-	-	≥ 7.82 ± 0.42
	50%		0.50	-	2.74	-	-	5.58 ± 0.56
	0.1%		0.50	-	8.24	-	-	0.08 ± 0.54
Virus control	NA	3 g/L BSA + 3 mL/L erythrocytes	NA	8.41	8.32	-	-	NA
Formaldehyde	0.7% (w:v)	NA	0.50	NR	NR	5.74	4.99	NA
Virus control formaldehyde	0.7% (w:v)	NA	NA	8.50	NR	NR	8.32	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 suppression activity (difference between decimal logarithm of titre without exposing the virus to the sample and of the test suspension)..... $log10^{-0.43}$

NA: not applicable; NR: not realized.
Times recommended by Standard for surfaces: maximum 5 or 60 minutes.
Times recommended by Standard for instruments: maximum 60 minutes.
Times recommended by Standard 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.

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Table 6. Results of the activity of the test sample, with Murine Norovirus strain S99 Berlin (Assay of titration with 12 wells), under test conditions requested by the client.

Assay	Concen-tration	Interfering substance	Time of contact (sec/min)	Dilutions (log10)[a,b]									
				1	2	3	4	5	6	7	8	9	10
Test sample	80%	3 g/L BSA + 3 mL/L erythrocytes	60 sec	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR	NR
	50%		60 sec	4444 4444 4444	3244 3020 2244	0020 0122 0030	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR	NR	NR
	0.1%		60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0302 2010 3212	0000 0000 0002	NR
Cytotoxicity	80%	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	0000 0000 0000	NR	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	4444 4444 4444	4444 4444 4444	4444 4444 4444	0323 0404 3302	0020 0000 0210	NR
			60 sec	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3244 3321 2013	3213 0030 2203	0002 0200 0010	NR
Formal-dehyde	0.7% (w:v)	NA	30 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3344 0334 4444	0200 0202 0100	0000 0000 0000	NR	NR	NR
			60 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0030 4020 2200	0000 0100 0000	0000 0000 0000	NR	NR	NR
Control of formal-dehyde 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	0000 0000 0000	NR	NR	NR
Virus control formal-dehyde	0.7% (w:v)	NA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3042 4040 4433	0000 1000 0102	0000 0000 0000
			60 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4244 3330	2002 0302 2434	0000 0120 0100	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	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC CCCC 0CCC	CC00 000C 00C0	NR
			Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CC0C CCC0 C0CC	C0CC 00CC C00C	0000 0C00 0C00	NR
Effectiveness control of the disinfectant suppression activity	NA	3 g/L BSA + 3 mL/L erythrocytes	Without sample	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC 00C0 0C0C	C000 00C0 C000	NR
			With sample	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC C0CC C0C0	0000 CC00 C0C0	0C00 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 Standard 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.

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Date: 27.10.2023
Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Figure 1. Results of the activity of the test sample under test conditions requested by the client with Poliovirus type 1 (ATCC VR-192).

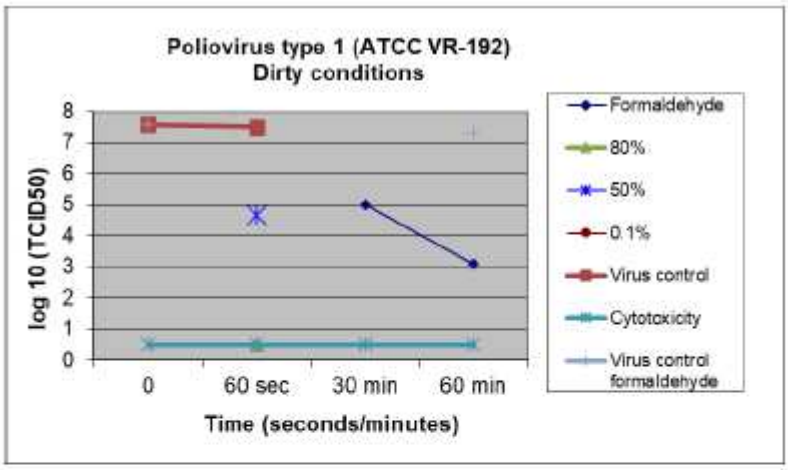
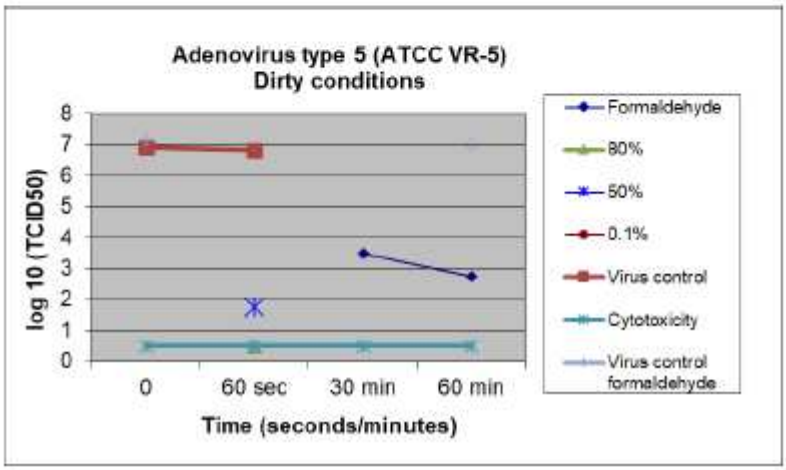
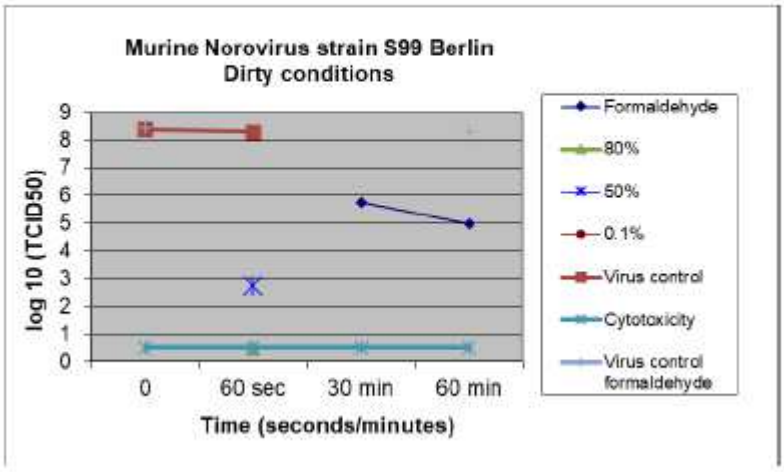


Figure 2. Results of the activity of the test sample under test conditions requested by the client with Adenovirus type 5 (ATCC VR-5).



ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 60362/23/ROBCH

Figure 3. Results of the activity of the test sample under test conditions requested by the client with Murine Norovirus strain S99 Berlin.



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Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

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Nº648 / LE1286

Test with the certificate of GLPs
(Good Laboratory Practices)
No. 2/23-C.VAL. General Directorate of
Pharmacy and Medical Devices of the Health
Department of the Valencian Region. Spain

Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action using wipes in the medical area (4-field test) (phase 2, step 2), with the product "Wipes "Bio-Dez"". (EN 16615: 2015 Standard).

Report

Registration No.: D/24/B0274.

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

2. **Client identification** ……. ECOCHIM-GRUP S.R.L.
   Address ……..……..... Republic of Moldova,
   Ungheni, str. Nationala 119
   Ungheni, MD-3603.

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

- Product name ..…….... **Wipes "Bio-Dez".**
- Batch number ..……..... Not indicated.
- Expiration date ..…….…….... 2027/04/23.
- Manufacturer /supplier ……..……... ECOCHIM-GRUP S.R.L.
- Storing conditions ..……..……..... Not indicated.
- Diluent recommended by the manufacturer ……... Not indicated.
- Conditions of use …… Surface, handrub.
- Active(s) Substance(s) and its concentration (s) ..……... Ethyl alcool 72-76%, CAS 64-17-5 and CE 200- 578-6, Benzalkonium chloride 0.024-0.029%, CAS 68424-85-1 and CE 270-325-2.
- Concentrations ordered for the assay ..... Ready for use.

IVAMI is not responsible for client-supplied information. This information is **not covered** by the ENAC accreditation.

4. Information about sample reception

- Date of reception of the sample ……. 2024/05/02.
- Date of reception of order with test conditions …….. 2024/05/02.
- Aspect of the received sample ……... Impregnated white wipes in plastic packaging.

5. Method of assay and its validation (EN 16615: 2015 Standard)

- Method ……..... Dilution-neutralization.
- Neutralizer ……..……..... Tryptone 5 g/L, yeast extract 2.5 g/L, dextrose 10 g/L, sodium thioglycolate 1 g/L, sodium thiosulfate 1 g/L, Sodium bisulphite 2.5 g/L, soya lecithin 7 g/L, polysorbate-80 5 g/L, glycine 1 g/L, l-histidine 1 g/L and saponin 30 g/L.

6. Experimental conditions

- Assay period (including prior preparation of the strains) ...…… 2024/06/23 to 2024/06/29.
- Diluent of the product used in the assay . Sterile distilled water.
- Product concentrations for the assay ….. 100% and 0.1%.
- Aspect of the dilutions of the product … Transparent liquid.
- Contact time ……... 60 seconds.
- Assay temperature ..……. +20ºC ± 1ºC.
- Interfering substance ..……. Bovine serum albumin 3 g/L plus erythrocytes 3 mL/L.
- Stability of the mixture (product diluted in sterile distilled water) ….……. Stable.
- Temperature of incubation …….. +36ºC ± 1ºC for bacteria. +30ºC ± 1ºC for yeast.
- Identification of the strains used:
  - *Pseudomonas aeruginosa* (CECT 116 = ATCC 15442).
  - *Staphylococcus aureus* (CECT 239 = ATCC 6538).
  - *Enterococcus hirae* (CECT 4081 = ATCC 10541).
  - *Candida albicans* (CECT 1394 = ATCC 10231).

7. Results of the assay

- Validation and controls ……. See tables 1, 8, 15 and 22.
- Suspension of assay and drying control …..….. See tables 2, 9, 16 and 23.
- Drying control (***Dc*$_0$**) ……. See tables 3, 10, 17 and 24.
- Drying controls (***Dct***) …… See tables 4, 11, 18 and 25.
- Field of assay 1 (reduction) ……..... See tables 5, 12, 19 and 26.
- Fields of assay 2 to 4 (CFU/25 cm^2) ……..... See tables 6, 13, 20 and 27.
- ***Nw*** fields of assay 2 to 4 (CFU/25 cm^2) ……... See tables 7, 14, 21 and 28.
- Number of replicates by assay organism …..... 1.

8. Special remarks

- For bactericidal activity, the sample must reach a reduction ≥ 5 log in the field of assay No.1 and must show ≤ de 50 CFU/25 cm^2 in the fields of assay No. 2 to 4, for *Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus hirae.*
- For yeasticidal activity, the sample must reach a reduction ≥ 4 log in the field of assay No.1 and must show ≤ de 50 CFU/25 cm^2 in the fields of assay No. 2 to 4, for *Candida albicans.*

9. Conclusion

The product **Wipes "Bio-Dez"** batch not indicated, when it is pure (100%), concentration required by client, **does not show bactericidal activity** after 60 seconds at 20ºC ± 1ºC, under dirty conditions (bovine serum albumin 3 g/L plus erythrocytes 3 mL/L), because it does not show activity against *Staphylococcus aureus* (CECT 239 = ATCC 6538) and *Enterococcus hirae* (CECT 4081= ATCC 10541) although it shows activity against the reference strain *Pseudomonas aeruginosa* (CECT 116 = ATCC 15442), when assayed according to the **EN 16615: 2015 Standard.**

The product **Wipes "Bio-Dez"** batch not indicated, when it is pure (100%), concentration required by client, **shows yeasticidal activity** after 60 seconds at 20ºC ± 1ºC, under dirty conditions (bovine serum albumin 3 g/L plus erythrocytes 3 mL/L), against the reference strain *Candida albicans* (CECT 1394 = ATCC 10231), when assayed according to the **EN 16615: 2015 Standard.**

Note: The results obtained correspond to the sample received in the laboratory.

Use of the ENAC mark: The ENAC "*mark*" can only be used by the holder of the accreditation. Its use in packaging, installations, shop windows, advertising or other documentation format other than that issued by the accredited entity (IVAMI) is not allowed.

Bétera (Valencia), July 4, 2024.

IBORRA CHISVERT,
ANA (FIRMA)

Signed. Ana Iborra.
Technician responsible

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.

MONTOYA VIECO, ELENA (FIRMA)

Signed. Elena Montoya.
Responsible for the Laboratory Area
(Study Director)

ESTEBAN BERMUDEZ, ENCARNACION PILAR (FIRMA)

Signed. Encarnación Esteban.
Technical Director
(Quality Assurance Director)

Reference

- **EN 16615: 2015**. Chemical disinfectants and antiseptics. Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test). Test method and requirements (phase 2, step 2).

Result of the assay (assay of the bacterial suspension): *Staphylococcus aureus* (CECT 239 = ATCC 6538).
Seeding: Pour plate; Number of plates: 1/mL; Drying time: 19 minutes.

Table 1.-Validation and controls

Suspension of validation (Nv_0)			**Control of the neutralizer (B)**			**Validation of the method (C) Conc. of the sample: 100%**		
Vc_1	48	X = 51	Vc_1	49	X = 47.5	Vc_1	40	X = 42.5
Vc_2	54		Vc_2	46		Vc_2	45	
30 ≤ x Nv_0 ≤ 160? Yes			X of B ≥ 0.5 x Nv_0? Yes			X of C ≥ 0.5 x Nv_0? Yes		

Table 2.- Suspension of assay and control of dryness

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	**10^{-7}**	170	181	Xwm = 1.78 x 10^9 lg$N$ = 9.25 N_0 = N/22.22; lg N_0 = 7.90 7.88 ≤ lg N_0 ≤ 8.40? Yes
	10^{-8}	21	19	

Table 3.- Control of dryness (D_{C0})

	T_0	Vc_1	Vc_2	
Control of dryness (D_{C0})	**10^{-5}**	39	36	Xwm = 3.75 x 10^6 x 5= 1.88 x 10^7 lgDc_0 = 7.27
	10^{-6}	<14	<14	6.88 ≤ lg Dc_0 ≤ 8.40? Yes

Table 4.- Controls of dryness (D_{Ct})

	T_0	Vc_1	Vc_2	
Control of dryness (Dct)	**10^{-5}**	16	18	Xwm = 1.70 x 10^6 x 5 = 8.50 x 10^6 lgDct = 6.93
	10^{-6}	<14	<14	6.88 ≤ lg Dct ≤ 8.40? Yes

Table 5.- Field of assay 1 (reduction)

Concentration of the sample	Dilution step	Vc_1	Vc_2	*Na* (= *X* o *Xwm* x 5)	lg *Na*	lg *R* (lg*Dct*-lg*Na*)	Time of contact (sec)
100%	10^0	>330	>330	>16500	>4.22	<2.71	60
	10^{-1}	>330	>330				
0.1%	10^0	>330	>330	>16500	>4.22	<2.71	60
	10^{-1}	>330	>330				

Table 6.- Fields of assay 2 to 4 (CFU/25 cm^2)

Concentration of the sample	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NaT2\text{ to }4}$ (= *X* or *Xwm* x 5) CFU/25cm^2	Time of contact (sec)
100%	10^0	>330; >330	>330; >330	>330; >330	5375	60
	10^{-1}	125; 132	106; 98	90; 94		
0.1%	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		

Table 7.- *Nw* fields of assay 2 to 4 (CFU/25 cm^2)

Nw	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NWT2\text{ to }4}$ (= *X* or *Xwm* x 5) CFU/25cm^2	Time of contact (sec)
Sterile distilled water + 0.1% polysorbate-80	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		

Wipe + water: initial weight of wipe = 18.74 g; final weight of wipe = 17.22 g.
Wipe with sample at 100% concentration: Initial weight = 2.09 g; final weight = 1.43 g.
Wipe with sample at 0.1% concentration: Initial weight = 19.12 g; final weight = 18.03 g.

Result of the assay (assay of the bacterial suspension): *Pseudomonas aeruginosa (*CECT 116 = ATCC 15442*).*
Seeding: Pour plate; Number of plates: 1/mL; Drying time: 18 minutes.

Table 8.-Validation and controls

Suspension of validation (Nv_0)			**Control of the neutralizer (B)**			**Validation of the method (C) Conc. of the sample: 100%**		
Vc_1	45	$X = 47.5$	Vc_1	43	$X = 42$	Vc_1	40	$X = 42$
Vc_2	50		Vc_2	41		Vc_2	44	
$30 \leq x\ Nv_0 \leq 160$? Yes			X of $B \geq 0.5 \times Nv_0$? Yes			X of $C \geq 0.5 \times Nv_0$? Yes		

Table 9.- Suspension of assay and control of dryness

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-7}	168	179	$Xwm = 1.74 \times 10^9$ $\lg N = 9.24$ $N_0 = N/22.22$; $\lg N_0 = 7.89$ $7.88 \leq \lg N_0 \leq 8.40$? Yes
	10^{-8}	19	17	

Table 10.- Control of dryness (D_{C0})

	T_0	Vc_1	Vc_2	
Control of dryness (D_{C0})	10^{-5}	20	22	$Xwm = 2.10 \times 10^6 \times 5 = 1.05 \times 10^7$ $\lg Dc_0 = 7.02$ $6.88 \leq \lg Dc_0 \leq 8.40$? Yes
	10^{-6}	<14	<14	

Table 11.- Controls of dryness (D_{Ct})

	T_0	Vc_1	Vc_2	
Control of dryness (Dct)	10^{-5}	16	15	$Xwm = 1.55 \times 10^6 \times 5 = 7.75 \times 10^6$ $\lg Dct = 6.89$ $6.88 \leq \lg Dct \leq 8.40$? Yes
	10^{-6}	<14	<14	

Table 12.- Field of assay 1 (reduction)

Concentration of the sample	Dilution step	Vc_1	Vc_2	***Na*** (= ***X*** o ***Xwm*** x 5)	lg ***Na***	lg ***R*** (lg***Dct***-lg***Na***)	Time of contact (sec)
100%	10^0	16	15	77.5	1.89	5.00	60
	10^{-1}	<14	<14				
0.1%	10^0	>330	>330	>16500	>4.22	<2.67	60
	10^{-1}	>330	>330				

Table 13.- Fields of assay 2 to 4 (CFU/25 cm^2)

Concentration of the sample	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NaT2\text{ to }4}$ (= ***X*** or ***Xwm*** x 5) CFU/25cm^2	Time of contact (sec)
100%	10^0	0; 0	0; 0	0; 0	0	60
	10^{-1}	0; 0	0; 0	0; 0		
0.1%	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		

Table 14.- *Nw* fields of assay 2 to 4 (CFU/25 cm^2)

Nw	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NWT2\text{ to }4}$ (= ***X*** or ***Xwm*** x 5) CFU/25cm^2	Time of contact (sec)
Sterile distilled water + 0.1% polysorbate-80	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		

Wipe + water: initial weight of wipe = 18.26 g; final weight of wipe = 17.45 g.
Wipe with sample at 100% concentration: Initial weight = 2.55 g; final weight = 1.69 g.
Wipe with sample at 0.1% concentration: Initial weight = 19.02 g; final weight = 18.09 g.

Result of the assay (assay of the bacterial suspension): *Enterococcus hirae* (CECT 4081 = ATCC 10541).
Seeding: Pour plate; Number of plates: 1/mL; Drying time: 20 minutes.

Table 15.-Validation and controls

Suspension of validation (Nv_0)			**Control of the neutralizer (B)**			**Validation of the method (C) Conc. of the sample: 100%**		
Vc_1	49	X = 51.5	Vc_1	45	X = 44.5	Vc_1	40	X = 39.5
Vc_2	54		Vc_2	44		Vc_2	39	
30 ≤ x Nv_0 ≤ 160? Yes			X of B ≥ 0.5 x Nv_0? Yes			X of C ≥ 0.5 x Nv_0? Yes		

Table 16.- Suspension of assay and control of dryness

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	**10^{-7}**	190	201	Xwm = 1.99 x 10^9 lg$N$ = 9.30 N_0 = N/22.22; lg N_0 = 7.95 7.88 ≤ lg N_0 ≤ 8.40? Yes
	10^{-8}	22	24	

Table 17.- Control of dryness (D_{C0})

	T_0	Vc_1	Vc_2	
Control of dryness (D_{C0})	**10^{-5}**	29	31	Xwm = 3.00 x 10^6 x 5= 1.50 x 10^7 lgDc_0 = 7.18 6.88 ≤ lg Dc_0 ≤ 8.40? Yes
	10^{-6}	<14	<14	

Table 18.- Controls of dryness (D_{Ct})

	T_0	Vc_1	Vc_2	
Control of dryness (Dct)	**10^{-5}**	15	17	Xwm = 1.60 x 10^6 x 5 = 8.00 x 10^6 lgDct = 6.90 6.88 ≤ lg Dct ≤ 8.40? Yes
	10^{-6}	<14	<14	

Table 19.- Field of assay 1 (reduction)

Concentration of the sample	Dilution step	Vc_1	Vc_2	*Na* (= *X* o *Xwm* x 5)	lg *Na*	lg *R* (lg*Dct*-lg*Na*)	Time of contact (sec)
100%	10^0	>330	>330	>16500	>4.22	<2.68	60
	10^{-1}	>330	>330				
0.1%	10^0	>330	>330	>16500	>4.22	<2.68	60
	10^{-1}	>330	>330				

Table 20.- Fields of assay 2 to 4 (CFU/25 cm^2)

Concentration of the sample	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NaT2\text{ to }4}$ (= *X* or *Xwm* x 5) CFU/25cm^2	Time of contact (sec)
100%	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		
0.1%	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		

Table 21.- *Nw* fields of assay 2 to 4 (CFU/25 cm^2)

Nw	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NWT2\text{ to }4}$ (= *X* or *Xwm* x 5) CFU/25cm^2	Time of contact (sec)
Sterile distilled water + 0.1% polysorbate-80	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		

Wipe + water: initial weight of wipe = 18.79 g; final weight of wipe = 17.29 g.
Wipe with sample at 100% concentration: Initial weight = 1.95 g; final weight = 1.42 g.
Wipe with sample at 0.1% concentration: Initial weight = 18.91 g; final weight = 17.31 g.

Result of the assay (assay of the yeast suspension): *Candida albicans* (CECT 1394 = ATCC 10231).
Seeding: Pour plate; Number of plates: 1/mL; Drying time: 18 minutes.

Table 22.-Validation and controls

Suspension of validation (Nv_0)			**Control of the neutralizer (B)**			**Validation of the method (C) Conc. of the sample: 100%**		
Vc_1	58	$X = 60$	Vc_1	54	$X = 55$	Vc_1	49	$X = 48$
Vc_2	62		Vc_2	56		Vc_2	47	
$30 \leq x\ Nv_0 \leq 160$? Yes			X of $B \geq 0.5 \times Nv_0$? Yes			X of $C \geq 0.5 \times Nv_0$? Yes		

Table 23.- Suspension of assay and control of dryness

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-6}	220	231	$Xwm = 2.29 \times 10^8$ $\lg N = 8.36$ $N_0 = N/22.22$; $\lg N_0 = 7.01$ $6.88 \leq \lg N_0 \leq 7.40$? Yes
	10^{-7}	25	27	

Table 24.- Control of dryness (D_{C0})

	T_0	Vc_1	Vc_2	
Control of dryness (D_{C0})	10^{-4}	20	22	$Xwm = 2.10 \times 10^5 \times 5 = 1.05 \times 10^6$ $\lg Dc_0 = 6.02$ $5.88 \leq \lg Dc_0 \leq 7.40$? Yes
	10^{-5}	<14	<14	

Table 25.- Controls of dryness (D_{Ct})

	T_0	Vc_1	Vc_2	
Control of dryness (Dct)	10^{-4}	17	18	$Xwm = 1.75 \times 10^5 \times 5 = 8.75 \times 10^5$ $\lg Dct = 5.94$ $5.88 \leq \lg Dct \leq 7.40$? Yes
	10^{-5}	<14	<14	

Table 26.- Field of assay 1 (reduction)

Concentration of the sample	Dilution step	Vc_1	Vc_2	***Na*** (= ***X*** o ***Xwm*** x 5)	lg ***Na***	lg ***R*** (lg***Dct***-lg***Na***)	Time of contact (sec)
100%	10^0	<14	<14	<70	<1.85	>4.09	60
	10^{-1}	<14	<14				
0.1%	10^0	>330	>330	>16500	>4.22	<1.72	60
	10^{-1}	>330	>330				

Table 27.- Fields of assay 2 to 4 (CFU/25 cm^2)

Concentration of the sample	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NaT2\text{ to }4}$ (= ***X*** or ***Xwm*** x 5) CFU/25cm^2	Time of contact (sec)
100%	10^0	0; 0	0; 0	0; 0	0	60
	10^{-1}	0; 0	0; 0	0; 0		
0.1%	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		

Table 28.- *Nw* fields of assay 2 to 4 (CFU/25 cm^2)

Nw	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NWT2\text{ to }4}$ (= ***X*** or ***Xwm*** x 5) CFU/25cm^2	Time of contact (sec)
Sterile distilled water + 0.1% polysorbate-80	10^0	>330; >330	>330; >330	>330; >330	>16500	60
	10^{-1}	>330; >330	>330; >330	>330; >330		

Wipe + water: initial weight of wipe = 19.12 g; final weight of wipe = 17.98 g.
Wipe with sample at 100% concentration: Initial weight = 2.67 g; final weight = 2.06 g.
Wipe with sample at 0.1% concentration: Initial weight = 18.31 g; final weight = 17.91 g.

Explanations:

***N*:** Number of CFL/mL in the bacterial/yeast suspension of the assay.
***N*$_0$:** Number of CFL in the mixture of assay at the beginning of the time of contact (time 0).
Nv*$_0$:** Number of CFU/mL in the mixtures ***B and ***C*** at the beginning of the time of contact (time 0).
***B*:** Number of CFU/mL in the neutralizer control.
C: Number of CFU/mL in the validation method.
***Dco*:** Number of CFL/25 cm^2 in the recovery immediately after drying (time 0).
Dct*:** Number of CFU/25 cm^2 in the recovery after drying and the time of contact ***t.
***Na*:** Number of survivals in the mixture of assay bactericidal /yeasticidal of the Surface of the field 1 (CFU/25 cm^2).
***Nw*:** Number of survivals in the mixture of assay in the control of the surface water (CFU/25 cm^2).
***T*2:** Number of CFU/field of assay 2 (CFU/25 cm^2).
***T*3:** Number of CFU/field of assay 3 (CFU/25 cm^2).
***T*4:** Number of CFU/field of assay 4 (CFU/25 cm^2).
Nw is approximately ≥ 10 CFU/25 cm^2 is in the fields of assay 2 to 4.
$V_{Na\ T2\ a\ T4}$**:** Mean in CFU/25 cm^2 in the fields of assay 2 to 4 in the bactericidal/yeasticidal assay.
$V_{Nw\ T2\ a\ T4}$**:** Mean in CFU/25 cm^2 in the fields of assay 2 to 4 in the control of water of the surface.
Vc = Counts per mL (one or more plates);
Xwm = weighted mean of ***X***.
X = mean of ***Vc*$_1$** and ***Vc*$_2$** (1 + 2 duplicated);
R = reduction (lg ***R*** = lg ***Dct*** – lg ***Na***).

HAMILTON ROMANIA

RAPORT DE INCERCARE NR. 34692/23/ROBCH/Z1

Inlocuieste Raportul de Incercare Nr. 34692/23/ROBCH din 07.11.2023

Client **ECOCHIM-GRUP SRL** OR. UNGHENI, STR. NAȚIONALĂ 119 - REPUBLICA MOLDOVA	Numărul eșantionului: **34692/23/ROBCH** Descriere obiect de incercat *(conform cu declaratia Clientului)* **Dezinfectant Universal "Bio-Dez"** **Lot: -** **Cantitate prelevata:500 ml** **Responsabil prelevare: Cristinov Alexandr** **Ora receptiei probei: 08:00** **Temperatura receptie proba: 15°C** **Sample condition with no objections**
Data primirii obiectului de incercat: **17.05.2023** Data finalizarii incercarii: **07.11.2023** Data eliberarii raportului: **19.01.2024**	**Comanda numar 5331/23/ROBCH din 17.05.2023** Probele au fost prelevate si livrate de catre Client.

Parametrii de testare	Metoda de testare	Unitate de masura	Rezultat
# * Quantitative suspension test for the evaluation of bactericidal activity in medical area [1)]	EN 13727:2012+A2:2015	-	Test efectuat de catre subcontractor.; rezultatele se regasesc integral in raportul Nr D/23/B0419, primit de la subcontractor. Rezultatele analizelor sunt incluse in anexa nr 1 la raportul de analiza.

[1)] Modificare efectuata: se ataseaza raportul de incercare Nr D23/B0840, primit de la subcontractor, raport care include rezultatele incercarii solicitate.

Preluare date de catre: Mariana Ilinca, Sef Laborator Microbiologie
Validat de: Mariana Ilinca, Sef Laborator Microbiologie
Autorizat de: Alina-Roxana Mihai, Manager General *(Aprobat cu semnatura electronica)*

Laborator: Bucuresti 041914, sos. Berceni nr.8

* Metoda de testare acreditata # Test efectuat de catre subcontractor
ø Incercari neacreditate

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Autorizatie A.N.S.V.S.A. nr. 115 din 18.06.2018

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 34692/23/ROBCH

A) IDENTYFICATION OF THE SAMPLE	
Name of the product/Details about the product	**Dezinfectant Universal "Bio-Dez".** Manufacturer(supplier): Ecochim-Grup Condition of use: Instrument disinfection, surface disinfection, Hygienic handrub, surgical handrub.
Active(s) Substance(s) and its concentration(s)	Ethyl alcool 72-76%, CAS 64-17-5 and CE 200-578-6 Benzalkonium chloride 0.024-0.029%, CAS 68424-85-1 and CE 270-325-2 Methylthionibium chloride 0.00024%, CAS 61-73-4 and 200-515-2.
Concentration ordered for the assay	97%, 80%.
B) TEST METHOD	
Performed in accredited subcontracted partner laboratory: Scope of Accreditation Nr. 648/LE1286 Report No.: D/23/B0419– Quantitative evaluation assay of the bactericidal activity in the medical area (phase 2, step 1), with the product "Dezinfectant Universal "Bio-Dez"". (EN 13727: 2012 + A2: 2015 Standard)	EN 13727: 2012 + A2: 2015. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants for instruments used in Medicine. Test method and requirements (phase 2, step 1).
Testing method	EN 13727: 2012 + A2: 2015 Standard
Methods of assay and its validation UNE-EN 13727: 2012 + A2: 2015 Standard	
Method	Dilution-neutralization.
Neutralizer	Tryptone 5 g/L, yeast extract 2.5 g/L, dextrose 10 g/L, sodium thioglycolate 1 g/L, sodium thiosulfate 1 g/L, sodium bisulphite 2.5 g/L, soya lecithin 7 g/L, polysorbate-80 5 g/L, glycine 1 g/L, l-histidine 1 g/L and saponin 30 g/L.
C) INFORMATION ABOUT SAMPLE RECEPTION	
Date of reception of the sample	2023/05/18.
Date of reception of order with test conditions	2023/08/30.
Aspect of the received product	Blue liquid in plastic container.
D) EXPERIMENTAL CONDITIONS	
Assay period	2023/10/11 to 2023/10/15.
Solvent of the product used in the assay	Sterile distilled water.
Product concentrations for the assay	97%, 80%, 50% and 0.1%.
Aspect of the dilutions of the product	97%, 80% and 50% blue liquid; 0.1% transparent liquid..
Contact time	60 seconds.
Assay temperature	20°C ± 1°C
Interfering substance	Bovine serum albumin 3 g/L + erythrocytes 3 mL/L.
Stability of the mixture (interfering substance and product diluted in sterile distilled water)	97% flocs formation; 80%, 50% and 0.1% stable.
Incubation temperature	+36°C ± 1°C
Identification of the strains used:	– *Pseudomonas aeruginosa* CECT-116 (ATCC-15442). – *Staphylococcus aureus* CECT-239 (ATCC-6538). – *Enterococcus hirae* CECT-4081 (ATCC-10541). - *Escherichia coli* K12 (CECT 433 = NCTC 10538).

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Date: 07.11.2023
Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

Results of the assay

-	Assay of validation	See tables 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19, 20, 22 and 23.
-	Evaluation of bactericidal activity	See tables 3, 6, 9, 12, 15, 18, 21 and 24.
-	Number of replicates per assay organism.	1.

Special remarks

- All controls and validation were between the basic limits.
- For a valid test, at least one concentration must show a log reduction lower than 5 log, and at least one concentration must show a log reduction equal or higher than 5 log.
- Flocs formation is observed during the test procedure at 97%.
- The client requests the complete test including the concentration of 80% as the maximum concentration. It also requests the modified method (97%) additionally.

Conclusion

The product **Dezinfectant Universal "Bio-Dez"**, batch 34692/23/ROBCH, when it is pure (97%) (modified method) and 80%, concentrations requested by the client, **shows bactericidal activity** after 60 seconds at 20°C ± 1°C, under dirty conditions (bovine serum albumin 3 g/L and erythrocytes 3 mL/L), for the reference strains *Pseudomonas aeruginosa* (CECT 116 = ATCC 15442), *Staphylococcus aureus* (CECT 239 = ATCC 6538), *Enterococcus hirae* (CECT 4081 = ATCC 10541) and *Escherichia coli* K12 (CECT 433 = NCTC 10538), when tested according to **EN 13727: 2012 + A2: 2015 Standard.**

Note: The results obtained correspond to the sample received in the laboratory.

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 34692/23/ROBCH

Results of the assay (Bactericidal suspension) with *Pseudomonas aeruginosa* (CECT 116 = ATCC 15442).

Seeding: Pour plates. No. of plates: 1 /mL.

Table 1.-Validation and controls

Suspension of validation (*Nv0*)			**Control of experimental conditions (*A*)**			**Control of the neutralization (*B*)**			**Validation of the method (*C*) Sample concentration: 80%**		
Vc1	44	*X*= 42	*Vc1*	36	*X*= 35	*Vc1*	38	*X*=	*Vc1*	32	*X*= 33
Vc2	40		*Vc2*	34		*Vc2*	37	37.5	*Vc2*	34	
30 ≤ *X* of *Nv0* ≤ 160? Yes			*X* of *A* is ≥ 0.5 *X* of *Nv0*? Yes			*X* of *B* is ≥ 0.5 *X* of *Nv0*? Yes			*X* of *C* is ≥ 0.5 *X* of *Nv0*? Yes		
Suspension of validation (*NvB*)			*Vc1*: 40 *Vc2*: 39			*X* = 39.5 30 ≤ x of *NvB*/1000 ≤ 160? Yes					

Table 2.-Suspension of the assay

Suspension of assay (*N* and *N0*)	*N*	*Vc1*	*Vc2*	*Xwm* = 1.68 x 10^8, lg *N* = 8.22 *N0* = *N*/10; lg *N0* = 7.22 7.17 ≤ lg *N0* ≤ 7.70? Yes
	10^{-6}	164	171	
	10^{-7}	18	16	

Table 3.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	*Vc1*	*Vc2*	Lg *Na* = lg (*X* x 10 o *Xwm* x 10)	Lg *R* (Lg *N0*=7.22)	Time of contact (sec)
80%	Na^0	<14	<14	<2.15	>5.07	60
	Na^{-1}	<14	<14			
50%	Na^0	<14	<14	<2.15	>5.07	60
	Na^{-1}	<14	<14			
0.1%	Na^0	>330	>330	>4.52	<2.70	60
	Na^{-1}	>330	>330			

Explanations:
Vc = number per mL (one or two plates); *Xwm* = weighted mean of *X*.
X = mean of *Vc1* and *Vc2* (duplicate of 1 + 2);
Logarithmic reduction *R*: (lg *R* = lg *N0* – lg *Na*).

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Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 34692/23/ROBCH

Results of the assay (Bactericidal suspension) with *Pseudomonas aeruginosa* (CECT 116 = ATCC 15442) following the modified method (97%).

Seeding: Pour plates. No. of plates: 1 /mL.

Table 4.-Validation and controls

Suspension of validation (Nv_0)			**Control of experimental conditions (A)**			**Control of the neutralization (B)**			**Validation of the method (C) Sample concentration: 97%**		
Vc_1	42	X= 41	Vc_1	37	X= 36	Vc_1	31	X= 32	Vc_1	30	X= 31
Vc_2	40		Vc_2	35		Vc_2	33		Vc_2	32	
$30 \leq X$ of $Nv_0 \leq 160$? Yes			X of A is ≥ 0.5 X of Nv_0? Yes			X of B is ≥ 0.5 X of Nv_0? Yes			X of C is ≥ 0.5 X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 43 Vc_2: 45			X = 44 $30 \leq$ x of $Nv_B/1000 \leq 160$? Yes					

Table 5.-Suspension of the assay

Suspension of assay (N and N_0)	N	Vc_1	Vc_2	$Xwm = 1.86 \times 10^9$, $\lg N = 9.27$ $N_0 = N/100$; $\lg N_0 = 7.27$ $7.17 \leq \lg N_0 \leq 7.70$? Yes
	10^{-7}	189	183	
	10^{-8}	20	18	

Table 6.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.27)	Time of contact (sec)
97%	Na^0	<14	<14	<2.15	>5.12	60
	Na^{-1}	<14	<14			

Explanations:
Vc = number per mL (one or two plates); Xwm = weighted mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2);
Logarithmic reduction R: ($\lg R = \lg N_0 - \lg Na$).

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 34692/23/ROBCH

Results of the assay (Bactericidal suspension) with *Staphylococcus aureus* (CECT 239 = ATCC 6538).

Seeding: Pour plates. No. of plates: 1 /mL.

Table 7.-Validation and controls

Suspension of validation (*Nv*0)			**Control of experimental conditions (*A*)**			**Control of the neutralization (*B*)**			**Validation of the method (*C*) Sample concentration: 80%**		
Vc_1	48	X= 49	Vc_1	47	X= 46	Vc_1	48	X= 46	Vc_1	40	X= 42
Vc_2	50		Vc_2	45		Vc_2	44		Vc_2	44	
30 ≤ X of Nv_0 ≤ 160? Yes			X of A is ≥ 0.5 X of Nv_0? Yes			X of B is ≥ 0.5 X of Nv_0? Yes			X of C is ≥ 0.5 X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 52 Vc_2: 56			X = 54 30 ≤ x of Nv_B/1000 ≤ 160? Yes					

Table 8.-Suspension of the assay

Suspension of assay (N and N_0)	N	Vc_1	Vc_2	Xwm = 1.84 x 10^8, lg N = 8.27 N_0 = N/10; lg N_0 = 7.27 7.17 ≤ lg N_0 ≤ 7.70? Yes
	10^{-6}	177	189	
	10^{-7}	20	19	

Table 9.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.27)	Time of contact (sec)
80%	Na^0	<14	<14	<2.15	>5.12	60
	Na^{-1}	<14	<14			
50%	Na^0	<14	<14	<2.15	>5.12	60
	Na^{-1}	<14	<14			
0.1%	Na^0	>330	>330	>4.52	<2.75	60
	Na^{-1}	>330	>330			

Explanations:
Vc = number per mL (one or two plates); Xwm = weighted mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2);
Logarithmic reduction R: (lg R = lg N_0 – lg Na).

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Results of the assay (Bactericidal suspension) with *Staphylococcus aureus* (CECT 239 = ATCC 6538) following the modified method (97%).
Seeding: Pour plates. No. of plates: 1 /mL.

Table 10.-Validation and controls

Suspension of validation (Nv_0)			**Control of experimental conditions (A)**			**Control of the neutralization (B)**			**Validation of the method (C) Sample concentration: 97%**		
Vc_1	51	X= 52	Vc_1	46	X= 48	Vc_1	50	X= 52	Vc_1	39	X= 40
Vc_2	53		Vc_2	50		Vc_2	54		Vc_2	41	
30 ≤ X of Nv_0 ≤ 160? Yes			X of A is ≥ 0.5 X of Nv_0? Yes			X of B is ≥ 0.5 X of Nv_0? Yes			X of C is ≥ 0.5 X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 56 Vc_2: 58			X = 57 30 ≤ x of Nv_B/1000 ≤ 160? Yes					

Table 11.-Suspension of the assay

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-7}	190	201	Xwm = 1.97 x 10^9, lg N = 9.29 N_0 = N/100; lg N_0 = 7.29 7.17 ≤ lg N_0 ≤ 7.70? Yes
	10^{-8}	22	20	

Table 12.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.29)	Time of contact (sec)
97%	Na^0	<14	<14	<2.15	>5.14	60
	Na^{-1}	<14	<14			

Explanations:
Vc = number per mL (one or two plates); Xwm = weighted mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2);
Logarithmic reduction R: (lg R = lg N_0 – lg Na).

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Date: 07.11.2023
Authorized by: Mariana Ilinca, Manager of Microbiological laboratory
Aprobat de: Mihai Alina-Roxana, General manager (Approved with qualified electronic signature)

ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 34692/23/ROBCH

Results of the assay (Bactericidal suspension) with *Enterococcus hirae* (CECT 4081 = ATCC 10541).

Seeding: Pour plates. No. of plates: 1 /mL.

Table 13.-Validation and controls

Suspension of validation (*Nv0*)			**Control of experimental conditions (*A*)**			**Control of the neutralization (*B*)**			**Validation of the method (*C*) Sample concentration: 80%**		
Vc_1	47	X= 49	Vc_1	40	X=	Vc_1	47	X=	Vc_1	45	X= 44
Vc_2	51		Vc_2	44	42	Vc_2	42	44.5	Vc_2	43	
$30 \leq X$ of $Nv_0 \leq 160$? Yes			X of A is ≥ 0.5 X of Nv_0? Yes			X of B is ≥ 0.5 X of Nv_0? Yes			X of C is ≥ 0.5 X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 50 Vc_2: 52			X= 51 $30 \leq$ x of $Nv_B/1000 \leq 160$? Yes					

Table 14.-Suspension of the assay

Suspension of assay (N and N_0)	N	Vc_1	Vc_2	$Xwm = 1.88 \times 10^8$, $\lg N = 8.27$ $N_0 = N/10$; $\lg N_0 = 7.27$ $7.17 \leq \lg N_0 \leq 7.70$? Yes
	10^{-6}	181	192	
	10^{-7}	20	21	

Table 15.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.27)	Time of contact (sec)
80%	Na^0	<14	<14	<2.15	>5.12	60
	Na^{-1}	<14	<14			
50%	Na^0	<14	<14	<2.15	>5.12	60
	Na^{-1}	<14	<14			
0.1%	Na^0	>330	>330	>4.52	<2.75	60
	Na^{-1}	>330	>330			

Explanations:
Vc = number per mL (one or two plates); Xwm = weighted mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2);
Logarithmic reduction R: ($\lg R = \lg N_0 - \lg Na$).

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 34692/23/ROBCH

Results of the assay (Bactericidal suspension) with *Enterococcus hirae* (CECT 4081 = ATCC 10541), following the modified method (97%).

Seeding: Pour plates. No. of plates: 1 /mL.

Table 16.-Validation and controls

Suspension of validation (Nv_0)			**Control of experimental conditions (A)**			**Control of the neutralization (B)**			**Validation of the method (C) Sample concentration: 97%**		
Vc_1	46	X= 47	Vc_1	40	X= 42	Vc_1	42	X= 44	Vc_1	37	X= 36
Vc_2	48		Vc_2	44		Vc_2	46		Vc_2	35	
$30 \leq X$ of $Nv_0 \leq 160$? Yes			X of A is ≥ 0.5 X of Nv_0? Yes			X of B is ≥ 0.5 X of Nv_0? Yes			X of C is ≥ 0.5 X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 51 Vc_2: 53			X= 52 $30 \leq$ x of $Nv_B/1000 \leq 160$? Yes					

Table 17.-Suspension of the assay

Suspension of assay (N and N_0)	N	Vc_1	Vc_2	$Xwm = 1.84 \times 10^9$, $\lg N = 9.27$ $N_0 = N/100$; $\lg N_0 = 7.27$ $7.17 \leq \lg N_0 \leq 7.70$? Yes
	10^{-7}	179	188	
	10^{-8}	20	18	

Table 18.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.27)	Time of contact (sec)
97%	Na^0	<14	<14	<2.15	>5.12	60
	Na^{-1}	<14	<14			

Explanations:
Vc = number per mL (one or two plates); Xwm = weighted mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2);
Logarithmic reduction R: ($\lg R = \lg N_0 - \lg Na$).

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 34692/23/ROBCH

Results of the assay (Bactericidal suspension) with *Escherichia coli* K12 (CECT 433 = NCTC 10538).

Seeding: Pour plates. No. of plates: 1 /mL.

Table 19.-Validation and controls

Suspension of validation (Nv_0)			**Control of experimental conditions (A)**			**Control of the neutralization (B)**			**Validation of the method (C) Sample concentration: 80%**		
Vc_1	47	X= 46	Vc_1	40	X=	Vc_1	39	X= 38	Vc_1	41	X= 42
Vc_2	45		Vc_2	39	39.5	Vc_2	37		Vc_2	43	
30 ≤ X of Nv_0 ≤ 160? Yes			X of A is ≥ 0.5 X of Nv_0? Yes			X of B is ≥ 0.5 X of Nv_0? Yes			X of C is ≥ 0.5 X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 45 Vc_2: 41			X= 43 30 ≤ x of Nv_B/1000 ≤ 160? Yes					

Table 20.-Suspension of the assay

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-6}	168	175	Xwm = 1.72 x 10^8, lg N = 8.24 N_0 = N/10; lg N_0 = 7.24 7.17 ≤ lg N_0 ≤ 7.70? Yes
	10^{-7}	19	17	

Table 21.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.24)	Time of contact (sec)
80%	Na^0	<14	<14	<2.15	>5.09	60
	Na^{-1}	<14	<14			
50%	Na^0	<14	<14	<2.15	>5.09	60
	Na^{-1}	<14	<14			
0.1%	Na^0	>330	>330	>4.52	<2.72	60
	Na^{-1}	>330	>330			

Explanations:
Vc = number per mL (one or two plates); Xwm = weighted mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2);
Logarithmic reduction R: (lg R = lg N_0 – lg Na).

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ENCLOSORE NO. 1 SUBCONTRACTED TESTS TO REPORT OF ANALYSIS NO 34692/23/ROBCH

Results of the assay (Bactericidal suspension) with *Escherichia coli* K12 (CECT 433 = NCTC 10538), following the modified method (97%).

Seeding: Pour plates. No. of plates: 1 /mL.

Table 22.-Validation and controls

Suspension of validation (Nv_0)			**Control of experimental conditions (A)**			**Control of the neutralization (B)**			**Validation of the method (C) Sample concentration: 97%**		
Vc_1	42	X= 43	Vc_1	36	X= 37.5	Vc_1	33	X= 34.5	Vc_1	37	X= 36
Vc_2	44		Vc_2	39		Vc_2	36		Vc_2	35	
$30 \leq X$ of $Nv_0 \leq 160$? Yes			X of A is ≥ 0.5 X of Nv_0? Yes			X of B is ≥ 0.5 X of Nv_0? Yes			X of C is ≥ 0.5 X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 40 Vc_2: 39			X = 39.5 $30 \leq$ x of $Nv_B/1000 \leq 160$? Yes					

Table 23.-Suspension of the assay

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-7}	180	165	$Xwm = 1.74 \times 10^9$, $\lg N = 9.24$ $N_0 = N/100$; $\lg N_0 = 7.24$ $7.17 \leq \lg N_0 \leq 7.70$? Yes
	10^{-8}	19	18	

Table 24.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.24)	Time of contact (sec)
97%	Na^0	<14	<14	<2.15	>5.09	60
	Na^{-1}	<14	<14			

Explanations:
Vc = number per mL (one or two plates); Xwm = weighted mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2);
Logarithmic reduction R: ($\lg R = \lg N_0 - \lg Na$).

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Nº648 / LE1286

Test with the certificate of GLPs
(Good Laboratory Practices)
No. 2/23-C.VAL. General Directorate of
Pharmacy and Medical Devices of the Health
Department of the Valencian Region. Spain

Quantitative evaluation assay of the bactericidal activity in the medical area (phase 2, step 1), with the product "Dezinfectant Universal "Bio-Dez"" with deviations from the standard. (EN 13727: 2012 + A2: 2015 Standard)

Report
Registration No.: D/23/B0840.

1. Laboratory identification …….……	Instituto Valenciano de Microbiología.
2. Client identification ……..……..…….....	J.S. HAMILTON ROMANIA SRL.
Address ……..……..	Sos Berceni, Nr 8, sector 4, Bucuresti. Romania.

3. Sample identification (information provided by the client)

• Product name ……..……....	**Dezinfectant Universal "Bio-Dez".**
• Batch number ……..……....	34692/23/ROBCH.
• Expiration date ……..……	Not indicated.
• Manufacturer /supplier ……..….	SRL " Ecochim-Grup"
• Store conditions ……..	Not indicated.
• Conditions of use …….....	Instrument disinfection, surface disinfection, Hygienic handrub, surgical handrub.
• Diluent of the product recommended by the manufacturer …….....	Not indicated.
• Active(s) Substance(s) and its concentration (s) …….....	Ethyl alcool 72-76%, CAS 64-17-5 and CE 200-578-6, Benzalkonium chloride 0.024-0.029%, CAS: 68424-85-1 and CE 270-325-2 Methylthonibium chloride 0.00024%, CAS 61-73-4 and 200-515-2.
• Concentrations ordered for the assay ….....	Ready to use.

IVAMI is not responsible for client-supplied information. This information **is not covered by** the ENAC accreditation.

4. Information about sample reception

- Date of reception of the sample ……. 2023/05/18.
- Date of reception of order with test conditions ...…….…….. 2023/11/24.
- Aspect of the received sample……... Blue transparent liquid received in plastic container.

5. Method of assay and its validation (EN 13727: 2012 + A2: 2015 Standard)

- Method used ……... Dilution-neutralization.
- Neutralizer ……..……..... Tryptone 5 g/L, yeast extract 2.5 g/L, dextrose 10 g/L, sodium thioglycolate 1 g/L, sodium thiosulfate 1 g/L, sodium bisulphite 2.5 g/L, soya lecithin 7 g/L, polysorbate-80 5 g/L, glycine 1 g/L, l-histidine 1 g/L and saponin 30 g/L.

6. Experimental conditions

- Assay period (including prior preparation of the strains)……..... 2023/12/13 to 2023/12/17.
- Solvent of the product used in the assay ... Sterile distilled water.
- Product concentrations for the assay …..... Pure (80%), 50% and 0.1%.
- Aspect of the dilutions of the product …… Pure (80%), and 50% bluish transparent liquid; 0.1% transparent liquid.
- Contact time ……..... 60 seconds.
- Assay temperature ……..... 20ºC ± 1ºC.
- Interfering substance …….. Bovine serum albumin 3 g/L + erythrocytes 3mL/L.
- Stability of the mixture (interfering substance and product diluted in sterile distilled water) ……..... Stable.
- Incubation temperature ……. +36ºC ± 1ºC.
- Identification of the strains used:

  – *Staphylococcus aureus* MRSA (ATCC 33592).
  – *Enterococcus faecium* (ATCC 6057 = CECT 8108).

7. Results of the assay

- Assay of validation ……..... See tables 1, 2, 4 and 5.
- Evaluation of bactericidal activity …..…... See tables 3 and 6.
- Number of replicates per assay organism. 1.

8. Special remarks

- All controls and validation were between the basic limits.
- For a valid test, at least one concentration must show a log reduction lower than 5 log, and at least one concentration must show a log reduction equal or higher than 5 log.
- No precipitation was observed during the assay procedure (the mixtures were homogenous).
- The highest concentration that can be assayed in the test (80%) is due to the mixtures to perform the assay.

9. Conclusion

The product **Dezinfectant Universal "Bio-Dez",** batch 34692/23/ROBCH, when it is pure (80%), concentration requested by the client, **shows bactericidal activity** after 60 seconds at 20ºC ± 1ºC, under dirty conditions (bovine serum albumin 3 g/L and erythrocytes 3mL/L), for the reference strains *Staphylococcus aureus* MRSA (ATCC 33592) and *Enterococcus faecium* (ATCC 6057 = CECT 8108), when tested according to **EN 13727: 2012 + A2: 2015 Standard, with deviations from the standard due to not testing all mandatory microorganisms.**

With the results obtained with the product **Dezinfectant Universal "Bio-Dez",** batch 34692/23/ROBCH, **it cannot be concluded** that the product has general bactericidal activity, but only that it has activity against *Staphylococcus aureus* MRSA and *Enterococcus faecium.*

Note: The results obtained correspond to the sample received in the laboratory.

Use of the ENAC mark: The ENAC "*mark*" can only be used by the holder of the accreditation. Its use in packaging, installations, shop windows, advertising or other documentation format other than that issued by the accredited entity (IVAMI) is not allowed.

Bétera (Valencia), January 4, 2024.

GARCIA DE LOMAS
LATIN, JAIME (FIRMA)

Signed. Jaime García de Lomas.
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 the Good Laboratory Practices (GLPs) as well and the final report contains the primary data obtained.

MONTOYA VIECO, ELENA (FIRMA)

Signed. Elena Montoya.
Responsible for the Laboratory Area
(Study Director)

TEMPRANO LOPEZ ANA - 47374856P

Signed. Encarnación Esteban.
Technical Director
(Quality Assurance Director)
Signed by delegation by Ana Temprano

Reference

- **EN 13727: 2012 + A2: 2015.** Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants for instruments used in Medicine. Test method and requirements (phase 2, step 1).

Results of the assay (Bacterial suspension) with *Staphylococcus aureus* MRSA (ATCC 33592).

Method: Dilution-neutralization; Seeding: Pour plate; No. of plates: 1/mL.

Table 1.-Validation and controls

Suspension of validation (Nv_0)			**Control of experimental conditions (A)**			**Control of the neutralization (B)**			**Validation of the method (C) Sample concentration: Pure (80%)**		
Vc_1	83	X= 87	Vc_1	71	X= 77	Vc_1	89	X=	Vc_1	77	X=
Vc_2	91		Vc_2	83		Vc_2	100	94.5	Vc_2	80	78.5
30 ≤ X of Nv_0 ≤ 160? Yes			X of A is ≥ 0.5 X of Nv_0? Yes			X of B is ≥ 0.5 X of Nv_0 or 0.0005 Nv_B? Yes			X of C is ≥ 0.5 X of Nv_0? Yes		
Suspension of validation (Nv_B)			Vc_1: 100 Vc_2: 112			X = 106 30 ≤ X of Nv_B/1000 ≤ 160? Yes					

Table 2.-Suspension of the assay

	N	Vc_1	Vc_2	
Suspension of assay (N and N_0)	10^{-6}	>330	>330	Xwm = 2.70 x 10^8, lg N = 8.43 N_0 = N/10; lg N_0 = 7.43 7.17 ≤ lg N_0 ≤ 7.70? Yes
	10^{-7}	24	30	

Table 3.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	Vc_1	Vc_2	Lg Na = lg (X x 10 o Xwm x 10)	Lg R (Lg N_0=7.43)	Time of contact (sec)
Pure (80%)	Na^0	<14	<14	<2.15	>5.28	60
	Na^{-1}	<14	<14			
50%	Na^0	<14	<14	<2.15	>5.28	60
	Na^{-1}	<14	<14			
0.1 %	Na^0	>330	>330	>4.52	<2.91	60
	Na^{-1}	>330	>330			

Explanations:
Vc = number per mL (one or two plates); Xwm = weighted mean of X.
X = mean of Vc_1 and Vc_2 (duplicate of 1 + 2);
Logarithmic reduction R: (lg R = lg N_0 – lg Na).

Results of the assay (Bacterial suspension) with *Enterococcus faecium* (ATCC 6057 = CECT 8108).

Method: Dilution-neutralization; Seeding: Pour plate; No. of plates: 1/mL.

Table 4.-Validation and controls

Suspension of validation (*Nv*$_0$)			**Control of experimental conditions (*A*)**			**Control of the neutralization (*B*)**			**Validation of the method (*C*) Sample concentration: Pure (80%)**		
Vc$_1$	58	*X*= 60	*Vc*$_1$	57	*X*=	*Vc*$_1$	54	*X*= 58	*Vc*$_1$	50	*X*=
Vc$_2$	62		*Vc*$_2$	56	56.5	*Vc*$_2$	62		*Vc*$_2$	57	53.5
30 ≤ *X* of *Nv*$_0$ ≤ 160? Yes			*X* of *A* is ≥ 0.5 *X* of *Nv*$_0$? Yes			*X* of *B* is ≥ 0.5 *X* of *Nv*$_0$ or 0.0005 *Nv*$_B$? Yes			*X* of *C* is ≥ 0.5 *X* of *Nv*$_0$? Yes		
Suspension of validation (*Nv*$_B$)			*Vc*$_1$: 68 *Vc*$_2$: 64			*X* = 66 30 ≤ *X* of *Nv*$_B$/1000 ≤ 160? Yes					

Table 5.-Suspension of the assay

	N	*Vc*$_1$	*Vc*$_2$	
Suspension of assay (*N* and *N*$_0$)	10^{-6}	>330	>330	*Xwm* = 2.45 x 10^8, lg *N* = 8.39 *N*$_0$ = *N*/10; lg *N*$_0$ = 7.39 7.17 ≤ lg *N*$_0$ ≤ 7.70? Yes
	10^{-7}	26	23	

Table 6.-Results of the activity assays with the sample

Concentrations of the sample (%)	Dilutions steps	*Vc*$_1$	*Vc*$_2$	Lg *Na* = lg (*X* x 10 o *Xwm* x 10)	Lg *R* (Lg *N*$_0$=7.39)	Time of contact (sec)
Pure (80%)	Na^0	<14	<14	<2.15	>5.24	60
	Na^{-1}	<14	<14			
50%	Na^0	<14	<14	<2.15	>5.24	60
	Na^{-1}	<14	<14			
0.1 %	Na^0	>330	>330	>4.52	<2.87	60
	Na^{-1}	>330	>330			

Explanations:
Vc = number per mL (one or two plates); *Xwm* = weighted mean of *X*.
X = mean of *Vc*$_1$ and *Vc*$_2$ (duplicate of 1 + 2);
Logarithmic reduction ***R*:** (lg ***R*** = lg *N*$_0$ – lg *Na*