Coat of Arms
Republic of Serbia
MINISTRY OF ENVIRONMENTAL PROTECTION
Number: 532-01-1890/2012-03
Date: September 24, 2018
22-26 Nemanjina St.
Belgrade

Pursuant to Article 11 of the Law of Biocidal Products ("RS Official Gazette", No. 36/09, 88/10, 92/11 and 25/15), Article 6, paragraph 1, item 11 of the Law of Chemicals ("RS Official Gazette" No. 36/09, 88/10, 92/11, 93/12 and 25/15), Article 17 of the Law of Amendments to the Law of Chemicals ("RS Official Gazette", No. 93/12), Article 5a, paragraph 1 of the Law of Ministries ("RS Official Gazette", no. 44/14, 14 / l5, 54/15. 96/15 – as amended and 62/17), independent Article 13, para. 2 and 6. of the Law of Amendments to the Law of Ministries ("RS Official Gazette", No. 62/17), Article 23, paragraph 2 of the Law of Public Administration ("RS Official Gazette", No. 79/05, 101/07 , 95/10, 99/14, 30/18 – as amended 47/18), Article 79, paragraph 3 and Article 141, paragraph 2 of the Law of General Administrative Procedure ("RS Official Gazette", No. 18/16), deciding upon the request of company EKO AQUA SYSTEM d.o.o. from Kruševac, 35 Pana Đukića St., for extension of the deadline for submitting the application for issuance of marketing authorization for biocidal product determined by the decision of the Chemicals Agency No. 532-01-1890/2012-03 of 24 September 2012 including the biocidal product in the Temporary List of Biocidal Products for submission of technical dossier, Aleksandar Vesić, Assistant Minister of Environmental Protection, authorized by the Minister's decision number: 021-01-5/4/2017-09 dated 11 December 2017, issues the following

DECISION

1. Approving the request of company EKO AQUA SYSTEM d.o.o. from Kruševac, 35 Pana Đukića St., for extension of the deadline for submitting the application for issuance of marketing authorization for biocidal product determined by the decision of the Chemicals Agency No. 532-01-1890/2012-03 of 24 September 2012 including the biocidal product in the Temporary List of Biocidal Products for submission of technical dossier
   Hereby EKO AQUA SYSTEM d.o.o. from Kruševac, 35 Pana Đukića is obliged to submit the application for the issuance of marketing authorization for biocidal product DEZANOL efikasna dezinfekcija within five years from the day of adopting this decision.
2. This decision amends item 5 of the decision of the Chemicals Agency No. 532-01-1890/2012-03 of September 24, 2012.

Explanation

By the decision of the Chemicals Agency No. 532-01-1890/2012-03 of September 24, 2012, at the request of EKO AQUA SYSTEM d.o.o. from Kruševac, biocidal product DEZANOL efikasna dezinfekcija, classified as Disinfectants and other biocidal products used in households and in public healthcare facilities (not intended for direct application to humans or animals) (PT 2); Biocidal products veterinary hygiene purposes (PT 3); Disinfectants used for surfaces that may enter into contact with food and feed area (RT 4), manufactured by EKO AQUA SYSTEM d.o.o., Srbija, with active substance chlorine (manufacturer: EKO AQUA SYSTEM d.o.o., Srbija) (produced by the reaction of sodium hypochlorite and hypochlorous acid), has been included in the Temporary List of

Biocidal Products for submission of technical dossier (item 1 of the wording); that it must be marketed as a general use disinfectant or professional use disinfectant and used for disinfection of air, surfaces and equipment in households, public healthcare facilities and other public or industrial facilities; surfaces and equipment in facilities where animals are kept, as well as vehicles for their transport; surfaces and equipment that may enter into contact with food and feed, as per Instructions for Use (item 2 of the wording); that the holder of decision including the biocidal product in the Temporary List of Biocidal Products for submission of technical dossier shall abide by the requirements for marketing the biocidal product, mark the biocidal product, and state in the MSDS the methods and conditions for use set in this decision (item 3 of the wording); and that the holder shall keep a record of the marketed quantities of the biocidal product, the total turnover of the biocidal product realized in the previous year, the quantities in stock and the quantities of the biocidal product withdrawn from the market, as well as the persons to whom the biocidal product was sold or assigned (item 4 of the wording). As per item 5 of the wording, the holder of the decision shall submit to the Chemicals Agency an application for the issuance of marketing authorization for biocidal product referred to in item 1 of the decision within six years from the date of issuing the decision. As per item 6 of the wording, it has been determined that if the decision holder fails to act upon the obligations referred to in item 2, 3 and 5 of the decision, a decision shall be made terminating the decision including the biocidal product in the Temporary List of Biocidal Products for submission of technical dossier.

On April 24, 2018, company EKO AQUA SYSTEM d.o.o. from Kruševac submitted a request for extension of the deadline for submitting the application for issuance of marketing authorization for biocidal product set out in the decision including the biocidal product in the Temporary List of Biocidal Products for submission of technical dossier. In its request, it stated that the active substance was active chlorine included in the Program for inclusion in Annex I, Annex Ia or Annex Ib (Review Program) for bnocidal product types RT 2, RT 3 and RT 4.

Having considered the reason stated in the request for extension of the deadline for submitting the application for issuance of marketing authorization for biocidal product, the Ministry of Environmental Protection found it justified, having in mind that Article 7, paragraph 2 and 3 of the Law of Biocidal Products prescribes that Temporary List of Biocidal Products for submission of technical dossier contains technical data of biocidal products, as well as deadlines for the submission of technical specifications for the biocidal product. The deadlines in the Temporary List of Biocidal Products for submission of technical dossier are determined by the Minister on the basis of the fact whether the active substance contained in the biocidal product is included in the list referred to in Article 4 of the Law (Annex I-List of active substances, Annex 1a-List of active substances contained in biocidal product presenting a low risk and Annex 1b-List of basic substances published in the Official Journal of the European Union) or in the Program for inclusion in Annex I, Annex Ia or Annex Ib, or depending on the amount of marketed biocidal product, the properties of the biocidal product affecting human health and the environment, or the lack of data about these properties, the type of biocidal product and the existence of an assessment of the active substance and other substances contained in the biocidal product. In the procedure of considering the request for extension of the deadline for submitting the application for issuance of marketing authorization, this body determined that the active substance chlorine has been included in the Program for inclusion in Annex I-List of active substances, Annex 1a-List of active substances contained in biocidal product presenting a low risk, for the given types of biocidal product, and that there are justified reasons for the extension of the deadline set by the decision number 532-01-1890/2012-03 of September 24, 2012, given that there is no legal presumption that the active substance contained in the biocidal product for which the application for issuance of marketing authorization is submitted is included in Annex I-List of active substances, Annex 1a-List of active substances contained in biocidal product

presenting a low risk, for the given types of biocidal product, so based on the authorization referred to in Article 79, paragraph 3 of the Law of General Administrative Procedure, the Minister has decided as given in the wording of the decision.

Legal remedy:

This decision may be appealed against to the Government within 15 days from the day when this decision was received. The appeal shall be submitted to the Ministry of Environmental Protection.

ASSISTANT MINISTER
Aleksandar Vesić
Signature illegible
[ROUND STAMP: REPUBLIC OF SERBIA,
Ministry of Environmental Protection,
BELGRADE]

Deliver to:
- EKO AQUA SYSTEM d.o.o, Kruševac
- Archives

HEALTH INSTITUTE seated in Ostrava
Partyzánské náměstí 2633/7
Moravská Ostrava, 702 00 Ostrava
Clinical Laboratory Center

Examples of viruses the neutralization of which has been declared by demonstrating the effect of a disinfectant on enveloped viruses according to ČSN EN 14476

Corononaviridae - viruses that cause SARS, MERS, COVID - 19

Filoviridae - Ebola virus, Marburg
Flaviviridae - tick-borne encephalitis virus, yellow fever virus, West Nile fever virus
Hepatitis B Virus (HBV)
Hepatitis C Virus (HCV)
Hepatitis Delta Virus (HDV)
Herpesviridae - HSV-1, HSV-2, VZV, EBV, CMV
Paramyxoviridae – measles, mumps virus
Poxviridae - smallpox virus
Influenza virus
Human immunodeficiency virus (HIV)
Human T-cell lymphotropic virus (HTLV)
Rabies virus
Rubella virus

Prepared by: MA Ludmila Porubova
Guarantor: effect testing laboratory

Literature:
1. ČSN EN 14476 + A2: 2020 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (Phase 2 / Step 1)
2. Miroslav Votava et al.: Medical microbiology special. Neptun, Brno 2003

HEALTH INSTITUTE seated in Ostrava
Clinical Laboratory Center
Pracoviště 1 - Ostrava
Virucidal Testing Laboratory
Partyzánské náměstí 2633/7Moravská Ostrava, 702 00 Ostrava
Company Reg. No.: 71009396
VAT Reg. No.: CZ71009396

TEST REPORT no. 12/2020/SVU

Quantitative suspension test for the evaluation of virucidal activity of a disinfectant
Test method and requirements (phase 2 / level 1) as per ČSN EN 14476 + A2: 2020

Test required by:
AQUASYSTEM, s.r.o.
Hradská 76
821 07 Bratislava

Order number: not specified
Reference number: ZU/07625/2020

Identification of disinfectant - sample:

Product name:	DEZANOL
Lot number:	030321
Expiration date:	2021-03-03
Manufacturer:	AQUASYSTEM, s.r.o.
Storage conditions:	5–30 °C
Product dilution recommended by the manufacturer:	for direct use
Product appearance:	clear colorless liquid
Active substance(s) and its concentration(s):	active chlorine: a mixture of hypochlorous acid and hypochlorite sodium <0.06%, chlorine dioxide, sodium chloride
Product identification:	hygienic surface wiping and disinfection in healthcare
Product delivery date:	March 18, 2020
Test date:	March 18 – April 4, 2020

Test results - more details attached to the protocol:

According to ČSN EN 14476 + A2: 2020 tested preparation DEZANOL, no. 030321, intended for hygienic medical surface wiping and disinfection, undiluted, reduced the virus titer by 4.667 ± 0.356 lg order of size in 1 min and by 5,000 ± 0.000 lg order of size in 5 min, at 20 °C ± 1 °C, under conditions of higher contamination (bovine serum albumin + bovine serum erythrocytes) by virus titration on a monolayer cell culture in a microtiter plate for the reference Vaccinia virus, Modified Vaccinia Ankara strain, i.e. showed a virucidal effect against Vaccinia virus by 4 lg more than required.*

** Compliance is based on a 95% probability of covering expanded uncertainty.*

Conclusion and interpretation:

According to ČSN EN 14476 + A2: 2020 tested disinfectant DEZANOL, no. 030321, undiluted, showed virucidal activity on enveloped viruses under conditions of higher contamination after only 1 min of exposure.

In Ostrava, on April 4, 2020 Approved by: MA Ludmila Porubová

Responsible for testing

Attachment to Protocol No. 12/2020/SVU

Sample identification:	
Product name:	DEZANOL
Lot number:	030321
Expiration date:	2021-03-03
Manufacturer:	AQUASYSTEM, s.r.o.
Storage conditions:	5–30 °C
Product dilution:	for direct use
Product appearance:	clear colorless liquid
Active substance(s) and its concentration(s):	active chlorine: a mixture of hypochlorous acid and hypochlorite sodium <0.06%, chlorine dioxide, sodium chloride
Experimental conditions:	Quantitative suspension test for the evaluation of virucidal activity of a disinfectant according to ČSN EN 14476 + A2: 2020 (SOP No. 1901) / AKTN / AKTN: standard update
Test date:	March 18 - April 1, 2020
Dilution:	For direct use
Tested product concentration:	100% (actually tested concentration 80%)
Other tested concentrations:	50%, 10%
Product appearance:	clear colorless liquid

Contact period:	1 min, 5 min
Test temperature:	20 °C ± 1 °C
Interfering substance:	higher contamination conditions - 3.0 g / l bovine serum albumin + 3.0 erythrocytes
Stability and appearance of the mixture during procedure:	turbidity after mixing with the virus suspension and the interfering substance
Incubation temperature:	37 °C ± 1 °C
Filtration method:	MicroSpin columns
Virus strain identification:	Vaccinia virus, modified Vaccinia virus Ankara strain (ATCC), 4th pass, EMEM + 2% FBS
Cell line:	BHK-21 cells (ATCC), 41st - 44th pass, DMEM + 10% FBS
Activity stopping procedure:	the virucidal activity of the product is suppressed by transferring the sample to an ice diluent
Titration method:	virus titration on monolayer cell culture in microtiter plate
Reference substance:	Formaldehyde (Sigma-Aldrich, no. MKCH0868)
Titer values calculated according to:	Spaerman - Kärber method

Test details:
1. Preparation of tissue cultures for testing
2. Virus infectivity test
3. Virus titration under conditions
4. Cytotoxic effect of the product
5. Reference virus inactivation test
6. Virus inactivation test with product
7. Virus susceptibility test

Table 1: Test results of DEZANOL for Vaccinia virus, modified Vaccinia virus Ankara - higher contamination

PRODUCT	Product concentration	Conditions of activity	Level of cytotoxicity	log10 TCID50/ ml per … min				Reduction factor (10log10 TCID50/ml in min)	
				1	5	30	60	1	5
DEZANOL	100%*	3 g/l BSA + erytrocyty	1.5	1.833 ± 0.178	1.500± 0.000	n.d.	n.d.	4.667±0.356	5.000±0.000
DEZANOL	50%	3 g/l BSA + erytrocyty	1.5	2.167± 0.178	n.d.	n.d.	n.d.	4.333±0.356	n.d.
DEZANOL	10%	3 g/l BSA + erytrocyty	1.5	5.500± 0.000	n.d.	n.d.	n.d.	1.000±0.000	n.d.
Virus validation	n.a.	3 g/l BSA + erytrocyty	n.a.	6.500± 0.000	n.d.	n.d.	n.d.		
				5	15	30	60	5	15
Formaldehyde - column	0.7% (m/V)	PBS	3.5	≤3.500± 0.000	≤3.500 ± 0.000	n.d.	n.d.	3.000±0.000	3.000±0.000
Virus validation - column	n.a.	PBS	n.a.	6.500± 0.000	n.d.	n.d.	n.d.		

* The product cannot be tested in a concentrated state, as it is diluted by adding stress conditions and virus suspension. The product was tested at a final concentration of 80%.

Prepared by: MA Ludmila Porubová

PRODUCT	Product concentration	Conditions of activity	Exposure period	Dilution (log 10)						
				-1	-2	-3	-4	-5	-6	-7
DEZANOL	100%*	3 g/l BSA + eritrociti	1 min	010100	000000	000000	000000	000000	000000	000000
DEZANOL	100%*	3 g/l BSA + eritrociti	5 min	000000	000000	000000	000000	000000	000000	000000
DEZANOL	50%	3 g/l BSA + eritrociti	1 min	001111	000000	000000	000000	000000	000000	000000
DEZANOL	10%	3 g/l BSA + eritrociti	1 min	444444	444444	444444	323321	000000	000000	000000
DEZANOL cytotoxicity	100%*	3 g/l BSA + eritrociti	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
DEZANOL cytotoxicity	50%	3 g/l BSA + eritrociti	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
DEZANOL cytotoxicity	10%	3 g/l BSA + eritrociti	n.a.	000000	000000	000000	n.d.	n.d.	n.d.	n.d.
Virus validation	n.a.	3 g/l BSA + eritrociti	1 min	444444	444444	444444	444444	323322	000000	000000
Cytotoxicity Formaldehyde - column	0.7% (m/V)	PBS	n.a.	CT	CT	000000	n.d.	n.d.	n.d.	n.d.
Formaldehyde - column	0 .7% (m/V)	PBS	5 min	CT	CT	000000	000000	000000	000000	000000
			15 min	CT	CT	000000	000000	000000	000000	000000
Virus validation - column	n.a.	PBS	5 min	444444	444444	444444	444444	232231	000000	000000

* The product cannot be tested in a concentrated state, as it is diluted by adding stress conditions and virus suspension. The product was tested at a final concentration of 80%.

1 to 4 viruses are present (1 = 25% CPE, 4 = 100% CPE)

0 virus is not present / without cytotoxicity

n.a. not applicable

n.d. not tested

CT Cytotoxic effect

CPE Cytopathic effect

Prepared by: MA Ludmila Porubová

END OF PROTOCOL

Ministry of Economy of the Slovak Republic
Department of Biocides
Mlynské nivy 44 a
827 15 Bratislava

AQUASYSTEM, s.r.o.
Hradská 76
Bratislava
Slovak Republic

Your number / date	Our number	Edited / link	Bratislava
	14598 / 2020-3052-26858	Škultétyová	11 May 2020

Subject
Decision on approval pursuant to Art. 55 of EU Regulation 528/2012 - AQUASYSTEM, s.r.o. (Dezanol) – bio / 1294 / D / 20 / CCHLP

Please find attached the official electronic document for the biocidal product Dezanol.

Attachment:
bio_1575_O_20_RM_Dezanol

RNDr. Ján Čepček, PhD
Director, Center for Chemicals and Preparations

Informative note - This document has been created electronically

Phone	E-mail	Website	IČO
	maria.skultetyova@mhsr.sk	www.mhsr.sk	00686832

MINISTRY OF ECONOMY OF THE SLOVAK REPUBLIC
Mlynské nivy 44 / a, 827 15 Bratislava 212

Bratislava, May 11, 2020
Our no: 14598 / 2020-3052-26858
Ref. no: bio/1575/O/20/RM

DECISION

Ministry of Economy of the Slovak Republic as the competent authority as per paragraph 5 of Law no. 71/1967 Coll. of Administrative Procedure (Code of Administrative Procedure) as amended (hereinafter "competent authority" or "Law of Administrative Procedure") and Article 3 letter a) and Article 4 letter e) of Law no. 319/2013 Coll. of the competence of state administration bodies for marketing biocidal products and their use and amending certain laws (Law of Biocides) as amended (hereinafter "Law of Biocides") in connection with proposal number 22347/2020 proposed by AQUASYSTEM, sro, Hradska 76, 821 07 Bratislava, file number 521/2020 of 20 April 2020 on issuing authorization pursuant to Art. 55 par. 1 of EU Regulation 528/2012 (hereinafter "Biocides Regulation") for the biocidal product Dezanol, which is registered in the Register of Biocidal Products Available on the Market in Slovakia, under the registration number bio/854/D/14/CCHLP for biocidal products product type 2, 3 and 4, issues the following

AUTHORIZATION

for marketing and / or use of a biocidal product

Commercial name of the biocidal product:	Dezanol							
Type of standard (PT):	01							
User category:	commercial							
	professional							
Active substance: Active chlorine from hypochlorous acid					EČ no. 232-232-5		CAS no. 7790-92-3	
Authorization number: bio/1294/D/20/CCHLP								
Authorization valid to: November 7, 2020								

Geographical area of application: Slovak Republic

for restricted and controlled use under the supervision of the competent authority;

and imposes the following obligations on the holder of this authorization:

1. the authorization holder shall ensure that the efficacy of the biocidal product indicated on the label of the approved biocidal product is within the range of the micro-organisms tested and the concentrations for which that biocidal product tests have shown its efficacy;
2. the authorization holder shall ensure the classification, packaging and labeling of the biocidal product in accordance with Article 69 of the Biocides Regulation and in accordance with the relevant legislation mentioned therein;
3. the authorization holder shall ensure the preparation of the safety data sheet in accordance with Art. 70 of the Biocides Regulation and in accordance with the relevant legislation mentioned therein;
4. the authorization holder shall ensure that the advertising of the biocidal product complies with Article 72 of the Biocides Regulation;
5. the authorization holder shall keep written records of at least the following information: name and address of the subject to which the biocidal product has been made available as per the authorization; the number of units of biocidal products made available to the subject; the date when the biocidal products were made available to the subject; active e-mail and telephone contact of the subject to whom the biocidal product was available as per this authorization;
6. the authorization holder, at the request of the competent authority or control bodies in accordance with the Law of Biocides, shall submit written records of the kept data referred to in the previous item within 24 hours of the request;
7. the authorization holder shall inform all subjects to whom the biocidal product was made available as per this authorization that the use of existing stocks of the biocidal product delivered as per this authorization may not continue after the expiry of this authorization or the deadline provided by the European Commission executive document in accordance with Art. 55 par. 1 of the Biocides Regulation;
8. after the expiration of the validity of this authorization or after the expiration of the period provided by the executive document of the European Commission, the authorization holder shall immediately ensure the extension of the measure as per this authorization issued by the European Commission in accordance with Art. 55 par. 1 of the Biocides Regulation or withdrawal of existing stocks of the biocidal product from the market in the Slovak Republic;
9. Obligations referred to in items 4 to 8 shall also apply to the distributors of this authorization holder.

Explanation:

The Applicant, AQUASYSTEM, s.r.o., Hradska 76, 821 07 Bratislava, on 20 April 2020 submitted an application to the Ministry of Economy of the Slovak Republic (hereinafter “administrative body” or “MH SR”) pursuant to paragraph 4 e) of the Law of Biocides, a proposal for issuing authorization in accordance with Art. 55 par. 1 of the Biocides Regulation.

The proposal for the authorization for the distribution of the biocidal product was submitted due to the increased demand for disinfectants, which exceeds the current supply of approved and available products on the market in Slovakia. Sufficient disinfectants must be provided due to the threat to public health, the COVID-19 pandemic. The lack of hand sanitizers is an acute problem, so it is necessary to continue with the application of Art. 55 of the Biocides Regulations.

An evaluation of the active substance as per Article 90 (1) 2 of the Biocides Regulation is ongoing.

According to Art. 17 of the Biocides Regulation, biocidal products shall not be marketed or used on the market unless authorized in accordance with this Regulation.

According to Art. 55 par. and by way of derogation from Articles 17 and 19 of the Biocides Regulation, the competent authority may, for a period not exceeding 180 days, authorize the marketing or use of a biocidal product which does not comply with the authorization conditions laid down in the Biocides Regulation for restricted and controlled use under the supervision of the competent authority if such a measure is necessary due to the risk to public health, which cannot be supressed by other means.

In connection with the development of the COVID-19 epidemiological situation caused by a new coronavirus called SARS-CoV-2, it is necessary to provide sufficient disinfectants listed in the Main Group 1 of Annex V to the Biocides Regulation on the market in Slovakia.

Based on the above, the administrative body made the decision as stated in the text of this decision.

Legal remedy:

According to Article61 para. 1 of Law no. 71/1967 Coll. of administrative procedure (Code of Administrative Procedure), amended by this decision, an appeal may be filed to the Ministry of Economy of the Slovak Republic, Mlinske nivy 44 / a, 827 15 Bratislava 212 within 15 days from the date of delivery of this decision.

This decision is subject to court review under the conditions set out in § 177 et seq. Of Law no. 162/2015 Coll. Code of Administrative Court Procedure.

RNDr. Ján Čepček, PhD
Director
Center for Chemicals and Preparations

Deliver to: **AQUASYSTEM, s.r.o., Hradská 76, 821 07 Bratislava**

Electronic signatures

Record registration number: 26858/2020
Subject: Decision on authorization referred to in Art. 55 of Regulation EC 528/2012 - AQUASYSTEM, s.r.o. (Dezanol) bio/1294/D/20/CCHLP

Initials	Date / Time	Name	Position	Org. formation	Function	Representation	Representing	Note
Schválené	11/05/2020 1:34 pm	Čepček Ján, RNDr., PhD	Head	3050	Department Director	No		

HEALTH INSTITUTE seated in Ostrava
Clinical Laboratory Center
Pracoviště 1 - Ostrava
Virucidal Testing Laboratory
Partyzánské náměstí 2633/7Moravská Ostrava, 702 00 Ostrava
Company Reg. No.: 71009396
VAT Reg. No.: CZ71009396

TEST REPORT no. 25/DP/19

Quantitative suspension test for the evaluation of bactericidal activity in healthcare sector - phase 2 / level 1

Test required by:
AQUASYSTEM, s.r.o.

Hradská 76
821 07 Bratislava

Order number:
Ordered on: 18/11/2019

Reference number: ZU/07625/2020

Identification of disinfectant - sample:

Product name:	DEZANOL
Lot number:	281020
Expiration date:	28/10/2020
Manufacturer:	AQUASYSTEM, s.r.o.
Storage conditions:	5–30 °C
Product dilution recommended by the manufacturer:	undiluted
Product appearance:	clear liquid
Active substance(s) and its concentration(s):	active chlorine (<0.06%)
Auxiliary agent and its concentration:	
Product identification:	surface disinfection
Product delivery date:	October 29, 2019

Test date: November 20, 2019, December 9, 2019

Results (more details attached to the protocol):

Dezanol intended for surface disinfection was tested in accordance with ČSN EN 13727 + A2 on test organisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

The required concentration was 100%, contact time 5 minutes, in conditions of higher contamination.

The reduced presence of *Staphylococcus aureus CCM 4516* was found at a concentration of 100%> 5.09 lg, at 50% 5.09 lg and at 0.5% < 2.72 lg.
The mean reduction (R) in logarithmic sequences with the test organism *Staphylococcus aureus CCM 4516* was at a concentration of 100% (V / V) R> 2.75 ± 0.013 lg *.

The reduction of *Pseudomonas aeruginosa CCM 7930* was found at a concentration of 100%> 5.19 lg, at 50%> 5.19 lg and at 0.5% <2.82 lg.
The mean reduction (R) in logarithmic sequences with the test organism *Pseudomonas aeruginosa CCM 7930* was at a concentration of 100% (V / V) R> 2.73 ± 0.032 lg *.

All controls and validations were within basic limits. At least one product concentration (0.5%) showed a decrease below 5 lg.
* reproducibility standard deviation

Conclusion:
Product Dezanol 281020 series proved bactericidal activity according to the standard ČSN EN 13727 + A2 in conditions of higher contamination (bovine albumin 0.3 g / l + sheep erythrocytes) and contact time of 5 minutes at a concentration of 100% and 50%.

The mean reduction (R) in logarithmic sequences in the test organism *Staphylococcus aureus CCM 4516* was 0.5% (V / V) R> 2.75 ± 0.013 lg * for a concentration of 100% (V / V).

The mean reduction (R) in logarithmic sequences in the test organism *Pseudomonas aeruginosa CCM 7930* was 0.5% (V / V) R> 2.73 ± 0.032 lg *

In Ostrava, on January 14, 2020

Attachment to Protocol no. 1: 25/DP/19

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Staphylococcus aureus CCM 4516*
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	November 20, 2019

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Preparation of the basic suspension

Dilution of basic suspension	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Number of colonies on plate 1	>330	>330	>330	>330	>330	>330	>330	180	11
Number of colonies on plate 2	>330	>330	>330	>330	>330	>330	>330	174	11

Validation and control:

Validated suspension Nvo (Nv)				Experimental condition control (A)				Neutralizing agent control (B)				Validation method (C) product concentration: undiluted			
Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2
168	132	168	132	156	125	156	125	182	153	182	153	155	113	155	113
Arithmetic mean Vc1+Vc2: $\bar{x}$= 150				Arithmetic mean Vc1+Vc2 $\bar{x}$=140.5				Arithmetic mean Vc1+ Vc2 $\bar{x}$=167.5				Arithmetic mean Vc1+ Vc2 $\bar{x}$=134			
Is 30 ≤ $\bar{x}$of Nvo ≤ 160 ? yes - no				Is $\bar{x}$od A ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of B ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of C ≥ 0.5 x $\bar{x}$ of Nvo ? yes - no			
Suspension validation NVB (suspension validation for control B)								110	102	110	102				
								Arithmetic mean Vc1+ Vc2 $\bar{x}$=106 Is 30 ≤ $\bar{x}$z NVB /1000 ≤ 160 ? yes - no							

Test suspension

Test suspension N	Dilution	Number per plate		Vc1	Vc2	$$\text{Mean t } x_{wm} = \frac{\text{C (sum of value Vc)}}{(n1 + 0.1\ n2)\times10^{-7}} = \frac{382}{(2+0.2)\times10^{-7}}$$ x_{wm} = sum of value (Vc) 382: $2.2\times10^{7} = 1.74\times10^{9}$
	10^{-7}	180	174	180	174	lg N = 9.24
	10^{-8}	11	11	<14	<14	Is 9.17 ≤ lg N ≤ 9.70 ? yes - no
Test suspension No = (N/100)	Dilution	Number per plate		Vc1	Vc2	$$\text{Mean t } \bar{x}_{wm} = \frac{\text{C (sum of value Vc)}}{(n1 + 0.1\ n2)\times10^{-7}} = \frac{382}{(2+0.2)\times10^{-7}}$$ x_{wm}= sum of value (Vc) 597: $2.2\times10^{7} = 1.74\times10^{9}$
	10^{-7}	180	174	180	174	lg N = 7.24
	10^{-8}	11	11	<14	<14	Is 7.17 ≤ lg N ≤ 7.70 ? yes - no

Test

Product concentration (%)	Dilution	Number per plate		Vc1	Vc2	Na = mean $\bar{x}$ or weight average xwm $\bar{x}$ 10	lg Na = lg ($\bar{x}$ or xwm^{-}) x 10	lg R = lg N0 - lgNa lg No = 7.43	Period of exposure (min)
97	10^{0}	0	0	<14	<14	<140	<2.15	>5.09	5
	10^{-1}	0	0	<14	<14				
50	10^{0}	7	10	<14	<14	<140	<2.15	>5.09	5
	10^{-1}	2	1	<14	<14				
	10^{0}	>330	>330	>330	>330	>33000	>4.52	<2.72	5
	10^{-1}	>330	>330	>330	>330				

Legend:

Vc = number per ml (one or more plates), k = average of Vc1 and Vc2 (1st and 2nd duplicate determinations);
Na = number of surviving cells per ml in test suspension at the end of the exposure period;
N = test suspension; No = N / 100 = number of cells per ml in test mixtures in 0 exposure period;
Nvo = Nv / 10 = number of cells per ml of suspension for validation in 0 exposure period;
Nvb = number of cells per ml of validation suspension for control B (neutralizing agent);
x_{wm} = average weight x; R = reduction (lg R = lg N0 - lg Na).

Calculations: At an exposure period of 5 minutes, the reduction refers to *Staphylococcus aureus CCM 4516* in logarithmic sequences:

For 97%: lg R = lg No - lg Na = 7.43 - 2.15 = > 5.09
For 50%: lg R = lg No - lg Na = 7.43 - 4.40 = > 5.09
For 0.5%: lg R = lg No - lg Na = 7.43 - 5.52 = <2.72

No part of this report may be reproduced in any form without the written approval of the testing laboratory. The test results refer only to the received test sample. Clinical Laboratory Center - Trial Laboratory no. 1554 ČIA accredited as per ČSN EN ISO / IEC 17025: 2018. A list of accredited methods is available at www.zuova.cz. The sample was tested in accordance with SOP no. 3037

Attachment to Protocol no. 2: 25/DP/19

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Pseudomonas aeruginosa* CCM 7930
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	November 20, 2019

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Preparation of the basic suspension

Dilution of basic suspension	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-8}	10^{-9}
Number of colonies on plate 1	>330	>330	>330	>330	>330	>330	>330	224	22
Number of colonies on plate 2	>330	>330	>330	>330	>330	>330	>330	220	11

Validation and control:

Validated suspension Nvo (Nv)				Experimental condition control (A)				Neutralizing agent control (B)				Validation method (C) product concentration: undiluted			
Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2
168	139	168	139	182	175	182	175	150	118	150	118	109	119	109	119
Arithmetic mean Vc1+Vc2: $\bar{x}$= 153.5				Arithmetic mean Vc1+Vc2 $\bar{x}$=178				Arithmetic mean Vc1+ Vc2 $\bar{x}$=134				Arithmetic mean Vc1+ Vc2 $\bar{x}$=14			
Is 30 ≤ $\bar{x}$od Nvo ≤ 160 ? yes - no				Is $\bar{x}$od A ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of B ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of C ≥ 0.5 x $\bar{x}$ of Nvo ? yes - no			
								162	149	162	149				
								Arithmetic mean Vc1+ Vc2 $\bar{x}$=155.5 Is 30 ≤ $\bar{x}$z NVB /1000 ≤ 160 ? yes - no							

Test suspension

Test suspension N	Dilution	Number per plate		Vc1	Vc2	C (sum of value Vc) 382 Mean t x_{wm} = ---------- = ---------- (n1 + 0.1 n2)x10^{-7} (2+0.2)x10^{-7} x_{wm} = sum of value (Vc) 383: 2.2x10^{7} = 2.2x10^{9}
	10^{-7}	224	220	224	220	lg N = 9.24
	10^{-8}	22	11	22	<14	Is 9.17 ≤ lg N ≤ 9.70 ? yes - no
Test suspension No = (N/100)	Dilution	Number per plate		Vc1	Vc2	C (sum of value Vc) 480 Average weight t x_{wm} $\bar{}$= ---------- = ---------- (n1 + 0.1 n2)x10^{-7} (2+0.2)x10^{-7} x_{wm}= sum of value (Vc) 480: 2.2x10^{7} = 2.18x10^{9}
	10^{-7}	224	220	224	220	lg N = 7.34
	10^{-8}	22	11	22	<14	Is 7,17 ≤ lg N ≤ 7.70 ? yes - no

Test

Product	Dilution	Number per plate	Vc1	Vc2	Na =	lg Na =	lg R =	Period of

concentration (%)						mean $\bar{x}$ or weight average xwm $\bar{x}$ 10	lg ($\bar{x}$ or xwm^{-}) x 10	lg N0 - lgNa lg No = 7.43	exposure (min)
97	10^{0}	4	4	<14	<14	<140	<2.15	>5.19	5
	10^{-1}	0	0	<14	<14				
50	10^{0}	2	5	<14	<14	<140	<2.15	>5.19	5
	10^{-1}	1	1	<14	<14				
0.5	10^{0}	>330	>330	>330	>330	>33000	>4.52	<2.82	5
	10^{-1}	>330	>330	>330	>330				

Legend:

Vc = number per ml (one or more plates), k = average of Vc1 and Vc2 (1st and 2nd duplicate determinations);
Na = number of surviving cells per ml in test suspension at the end of the exposure period;
N = test suspension; No = N / 100 = number of cells per ml in test mixtures in 0 exposure period;
Nvo = Nv / 10 = number of cells per ml of suspension for validation in 0 exposure period;
Nvb = number of cells per ml of validation suspension for control B (neutralizing agent);
x_{wm} = average weight x; R = reduction ($\lg R = \lg N_0 - \lg Na$).

Calculations: At an exposure period of 5 minutes, the reduction refers to *Staphylococcus aureus CCM 4516* in logarithmic sequences:

For 97%: lg R = lg No - lg Na = 7.24 - 2.15 = > 5.19
For 50%: lg R = lg No - lg Na = 7.24 - 4.47 = > 5.19
For 0.5%: lg R = lg No - lg Na = 7.24 - 5.52 = <2.82

Attachment to Protocol no. 3: 25/DP/19

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name: Dezanol

Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Staphylococcus aureus* CCM 4516
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	December 9, 2019

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Test suspension

Repetition	Dilution	Number on plate		Vc1	Vc2	$N = x_{wm}$ =sum of values(Vc)C : 2,2 x10^7 No = N/100
1 (20/11/2019)	10^{-7}	180	174	180	174	N = 2,71 x 10^9 lg N = 9.24
	10^{-8}	11	11	<14	<14	No = 2,71 x 10^7 lg No = 7.24
2	10^{-7}	191	182	191	182	N = 2,1 x 10^9 lg N = 9.26
	10^{-8}	13	14	<14	<14	No = 2,1 x 10^7 lg No = 7.26
3	10^{-7}	191	194	192	194	N =1,64 x 10^9 lg N = 9.27
	10^{-8}	10	22	<14	22	No = 1,64 x 10^7 lg No = 7.27

4	10^{-7}	214	191	214	191	N = 1,93 x 10^9 lg N = 9.30
	10^{-8}	26	11	26	<14	No = 1,93 x 10^7 lg No = 7.30
5	10^{-7}	188	168	188	168	N = 1,60 x 10^9 lg N = 9.24
	10^{-8}	12	17	<14	17	No = 1,60 x 10^7 lg No = 7.24
6	10^{-7}	206	187	206	187	N = 1,85 x 10^9 lg N = 9.29
	10^{-8}	19	14	19	<14	No = 1,85 x 10^7 lg No =7.29

Testing with limited test organisms:

Č. ponavljanje (za koncentraciju 0.5 %)	Razblaženje	Broj na ploči		Vc1	Vc2	Na = prosek x¯ ili težinski prosek xwm̃x 10	lg Na = lg (x¯ili xwm¯) x 10	lg R = lg N0 - lgNa	Period izloženosti (min)
1 (20.11.2019.)	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
2	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
3	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
4	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
5	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
6	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
Prosečna redukcija:								**>2.75 lg**	
Standardna devijacija:								**± 0.013 lg**	

Attachment to Protocol no. 4: 25/DP/19

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution

Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Pseudomonas aeruginosa* CCM 7930
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	December 9, 2019

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Test suspension :

Repetition	Dilution	Number on plate		Vc1	Vc2	$N = x_{wm}$ =sum of value(Vc)C : 2,2 x10_7 No = N/100
1 (20.11.2019.)	10^{-7}	224	220	224	220	N = 2,71 x 10_9 lg N = 9.34
10^{-8}	22	11	22	<14		No = 2,71 x 10_7 lg No = 7.34
2	10^{-7}	189	127	189	127	N = 2,1 x 10_9 lg N = 9.20
10^{-8}	16	18	16	18		No = 2,1 x 10_7 lg No = 7.20
3	10^{-7}	205	191	205	191	N =1,64 x 10_9 lg N = 9.30
10^{-8}	20	23	20	23		No = 1,64 x 10_7 lg No = 7.30
4	10^{-7}	182	175	182	175	N = 1,93 x 10_9 lg N = 9.25
10^{-8}	10	18	<14	18		No = 1,93 x 10_7 lg No = 7,25
5	10^{-7}	162	149	162	149	N = 1,60 x 10_9 lg N = 9.19
10^{-8}	9	7	<14	<14		No = 1,60 x 10_7 lg No = 7.19

6	10^{-7}	147	179	147	179	N = 1,85 x 10^9 lg N = 9.21
10^{-8}	10	14	<14	14		No = 1,85 x 10^7 lg No = 7,21

Testing with limited tested organisms:

Repetition (for 100 % concentration)	Dilution	Number on plate		Vc1	Vc2	Na = mean $\bar{x}$ or weight avergae $\bar{x}_{wm}$ x 10	lg Na = lg ($\bar{x}$ ili $\bar{x}_{wm}$) x 10	lg R = lg N_0 - lgNa	Period of exposure (min)
1 (20.11.2019.)	10_0	>330	>330	>330	>330	>33000	>4.52	<2.82	5
	10_{-1}	>330	>330	>330	>330				
2	10_0	>330	>330	>330	>330	>33000	>4.52	<2.68	5
	10_{-1}	>330	>330	>330	>330				
3	10_0	>330	>330	>330	>330	>33000	>4.52	<2.78	5
	10_{-1}	>330	>330	>330	>330				
4	10_0	>330	>330	>330	>330	>33000	>4.52	<2.73	5
	10_{-1}	>330	>330	>330	>330				
5	10_0	>330	>330	>330	>330	>33000	>4.52	<2.67	5
	10_{-1}	>330	>330	>330	>330				
6	10_0	>330	>330	>330	>330	>33000	>4.52	<2.69	5
	10_{-1}	>330	>330	>330	>330				
Mean reduction:								**>2.73 lg**	
Standard deviation:								**± 0,032lg**	

End of protocol

HEALTH INSTITUTE seated in Ostrava
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Pracoviště 1 - Ostrava
Virucidal Testing Laboratory
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Company Reg. No.: 71009396
VAT Reg. No.: CZ71009396

TEST REPORT no. 25/DP/19

Quantitative suspension test for the evaluation of bactericidal activity in healthcare sector - phase 2 / level 1

Test required by: AQUASYSTEM, s.r.o.

Order number:
Ordered on: 18/11/2019

Hradská 76
821 07 Bratislava

Reference number: ZU/07625/2020

Identification of disinfectant - sample:

Product name:	DEZANOL
Lot number:	281020
Expiration date:	28/10/2020
Manufacturer:	AQUASYSTEM, s.r.o.
Storage conditions:	5–30 °C
Product dilution recommended by the manufacturer:	undiluted
Product appearance:	clear liquid
Active substance(s) and its concentration(s):	active chlorine (<0.06%)
Auxiliary agent and its concentration:	
Product identification:	surface disinfection
Product delivery date:	October 29, 2019
Test date:	November 20, 2019, December 9, 2019

Results (more details attached to the protocol):

Dezanol intended for surface disinfection was tested in accordance with ČSN EN 13727 + A2 on test organisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

The required concentration was 100%, contact time 5 minutes, in conditions of higher contamination.

The reduced presence of *Staphylococcus aureus CCM 4516* was found at a concentration of 100%> 5.09 lg, at 50% 5.09 lg and at 0.5% <2.72 lg.
The mean reduction (R) in logarithmic sequences with the test organism *Staphylococcus aureus CCM 4516* was at a concentration of 100% (V / V) R> 2.75 ± 0.013 lg *.

The reduction of *Pseudomonas aeruginosa CCM 7930* was found at a concentration of 100%> 5.19 lg, at 50%> 5.19 lg and at 0.5% <2.82 lg.
The mean reduction (R) in logarithmic sequences with the test organism *Pseudomonas aeruginosa CCM 7930* was at a concentration of 100% (V / V) R> 2.73 ± 0.032 lg *.

All controls and validations were within basic limits. At least one product concentration (0.5%) showed a decrease below 5 lg.

* reproducibility standard deviation

Conclusion:
Product Dezanol 281020 series proved bactericidal activity according to the standard ČSN EN 13727 + A2 in conditions of higher contamination (bovine albumin 0.3 g / l + sheep erythrocytes) and contact time of 5 minutes at a concentration of 100% and 50%.

The mean reduction (R) in logarithmic sequences in the test organism *Staphylococcus aureus CCM 4516* was 0.5% (V / V) R> 2.75 ± 0.013 lg * for a concentration of 100% (V / V).

The mean reduction (R) in logarithmic sequences in the test organism *Pseudomonas aeruginosa CCM 7930* was 0.5% (V / V) R> 2.73 ± 0.032 lg *

In Ostrava, on January 14, 2020

Approved by: MUDr. Linda Stryjová

Attachment to Protocol no. 1: 25/DP/19

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Staphylococcus aureus CCM 4516*
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	November 20, 2019

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Preparation of the basic suspension

Dilution of basic suspension	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Number of colonies on plate 1	>330	>330	>330	>330	>330	>330	>330	180	11
Number of colonies on plate 2	>330	>330	>330	>330	>330	>330	>330	174	11

Validation and control:

Validated suspension Nvo (Nv)				Experimental condition control (A)				Neutralizing agent control (B)				Validation method (C) product concentration: undiluted			
Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2
130	104	130	104	130	142	130	142	94	120	94	120	141	150	141	150
Arithmetic mean Vc1+Vc2: $\bar{x}$= 117				Arithmetic mean Vc1+Vc2 $\bar{x}$=136				Arithmetic mean Vc1+ Vc2 $\bar{x}$=107				Arithmetic mean Vc1+ Vc2 $\bar{x}$=145.5			
Is 30 ≤ $\bar{x}$of Nvo ≤ 160 ? yes - no				Is $\bar{x}$od A ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of B ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of C ≥ 0.5 x $\bar{x}$ of Nvo ? yes - no			
Suspension validation NVB (suspension validation for control B)								110	102	110	102				
								Arithmetic mean Vc1+ Vc2 $\bar{x}$=14.5 Is 30 ≤ $\bar{x}$z N_{VB} /1000 ≤ 160 ? yes - no							

Test suspension

Test suspension N	Dilution	Number per plate		Vc1	Vc2	$\text{Mean t } x_{wm} = \frac{\text{C (sum of value Vc)}}{(n1 + 0.1\ n2)x10^{-7}} = \frac{382}{(2+0.2)x10^{-7}}$
						x_{wm} = sum of value (Vc) 382: $2.2x10^7 = 1.74x10^9$
	10^{-7}	180	174	180	174	lg N = 9.24
	10^{-8}	11	11	<14	<14	Is 9.17 ≤ lg N ≤ 9.70 ? yes - no

Test suspension No = (N/100)	Dilution	Number per plate		Vc1	Vc2	C (sum of value Vc) 382 Mean t x_{wm}^{-}= ---------- = ---------- (n1 + 0.1 n2)x10^{-7} (2+0.2)x10^{-7} x_{wm}= sum of value (Vc) 597: 2.2x10^{7} = 1.74x10^{9}
	10^{-7}	180	174	180	174	lg N = 7.24
	10^{-8}	11	11	<14	<14	Is 7.17 ≤ lg N ≤ 7.70 ? yes - no

Test

Product concentration (%)	Dilution	Number per plate		Vc1	Vc2	Na = mean x^{-} or weight average xwm x^{-} 10	lg Na = lg (x^{-} or xwm^{-}) x 10	lg R = lg N0 - lgNa lg No = 7.43	Period of exposure (min)
97	10^{0}	0	0	<14	<14	<140	<2.15	>5.09	5
	10^{-1}	0	0	<14	<14				
50	10^{0}	4	1	<14	<14	<140	<2.15	>5.09	5
	10^{-1}	0	0	<14	<14				
0.5	10^{0}	>330	>330	>330	>330	>33000	>4.52	<2.72	5
	10^{-1}	>330	>330	>330	>330				

Legend:

Vc = number per ml (one or more plates), k = average of Vc1 and Vc2 (1st and 2nd duplicate determinations);
Na = number of surviving cells per ml in test suspension at the end of the exposure period;
N = test suspension; No = N / 100 = number of cells per ml in test mixtures in 0 exposure period;
Nvo = Nv / 10 = number of cells per ml of suspension for validation in 0 exposure period;
Nvb = number of cells per ml of validation suspension for control B (neutralizing agent);
x_{wm} = average weight x; R = reduction (lg R = lg N0 - lg Na).

Calculations: At an exposure period of 5 minutes, the reduction refers to *Staphylococcus aureus CCM 4516* in logarithmic sequences:

For 97%: lg R = lg No - lg Na = 7.43 - 2.15 = > 5.09
For 50%: lg R = lg No - lg Na = 7.43 - 4.40 = > 5.09
For 0.5%: lg R = lg No - lg Na = 7.43 - 5.52 = <2.72

Attachment to Protocol no. 2: 25/DP/19

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Pseudomonas aeruginosa* CCM 7930
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	November 20, 2019

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Preparation of the basic suspension

Dilution of basic suspension	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-8}	10^{-9}
Number of colonies on plate	>330	>330	>330	>330	>330	>330	>330	224	22

1									
Number of colonies on plate 2	>330	>330	>330	>330	>330	>330	>330	220	11

Validation and control:

Validated suspension Nvo (Nv)				Experimental condition control (A)				Neutralizing agent control (B)				Validation method (C) product concentration: undiluted			
Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2
124	115	124	115	152	172	152	172	134	125	134	125	119	105	119	105
Arithmetic mean Vc1+Vc2: $\bar{x}$= 153.5				Arithmetic mean Vc1+Vc2 $\bar{x}$=178				Arithmetic mean Vc1+ Vc2 $\bar{x}$=134				Arithmetic mean Vc1+ Vc2 $\bar{x}$=14			
Is 30 ≤ $\bar{x}$of Nvo ≤ 160 ? yes - no				Is $\bar{x}$od A ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of B ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of C ≥ 0.5 x $\bar{x}$ of Nvo ? yes - no			
								118	121	118	121				
								Arithmetic mean Vc1+ Vc2 $\bar{x}$=119.5 Is 30 ≤ $\bar{x}$z NVB /1000 ≤ 160 ? yes - no							

Test suspension

Test suspension N	Dilution	Number per plate		Vc1	Vc2	$$\text{Mean t } x_{wm} = \frac{\text{C (sum of value Vc)}}{(n1 + 0.1\ n2)\times10^{-7}} = \frac{382}{(2+0.2)\times10^{-7}}$$ x_{wm} = sum of value (Vc) 383: $2.2\times10^{7} = 2.2\times10^{9}$
	10^{-7}	224	220	224	220	lg N = 9.24
	10^{-8}	22	11	22	<14	Is 9.17 ≤ lg N ≤ 9.70 ? yes - no
Test suspension No = (N/100)	Dilution	Number per plate		Vc1	Vc2	$$\text{Average weight t } x_{wm}^{-} = \frac{\text{C (sum of value Vc)}}{(n1 + 0.1\ n2)\times10^{-7}} = \frac{480}{(2+0.2)\times10^{-7}}$$ x_{wm}= sum of value (Vc) 480: $2.2\times10^{7} = 2.18\times10^{9}$
	10^{-7}	224	220	224	220	lg N = 7.34
	10^{-8}	22	11	22	<14	Is 7.17 ≤ lg N ≤ 7.70 ? yes - no

Test

Product concentration (%)	Dilution	Number per plate		Vc1	Vc2	Na = mean x^- or weight average xwm x^- 10	lg Na = lg (x^- or xwm^-) x 10	lg R = lg N0 - lgNa lg No = 7.43	Period of exposure (min)
97	10^0	0	0	<14	<14	<140	<2.15	>5.19	5
	10^{-1}	0	0	<14	<14				
50	10^0	1	0	<14	<14	<140	<2.15	>5.19	5
	10^{-1}	0	0	<14	<14				
0.5	10^0	>330	>330	>330	>330	>33000	>4.52	<2.82	5
	10^{-1}	>330	>330	>330	>330				

Legend:

Vc = number per ml (one or more plates), k = average of Vc1 and Vc2 (1st and 2nd duplicate determinations);
Na = number of surviving cells per ml in test suspension at the end of the exposure period;
N = test suspension; No = N / 100 = number of cells per ml in test mixtures in 0 exposure period;
Nvo = Nv / 10 = number of cells per ml of suspension for validation in 0 exposure period;
Nvb = number of cells per ml of validation suspension for control B (neutralizing agent);
x_{wm} = average weight x; R = reduction ($\lg R = \lg N_0 - \lg Na$).

Calculations: At an exposure period of 5 minutes, the reduction refers to *Staphylococcus aureus CCM 4516* in logarithmic sequences:

For 97%: lg R = lg No - lg Na = 7.24 - 2.15 = > 5.19
For 50%: lg R = lg No - lg Na = 7.24 - 4.47 = > 5.19
For 0.5%: lg R = lg No - lg Na = 7.24 - 5.52 = <2.82

Attachment to Protocol no. 3: 6/DP/20

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Staphylococcus aureus* CCM 4516
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	December 9, 2019

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Test suspension

Repetition	Dilution	Number on plate		Vc1	Vc2	N = x_{wm}^{-} =sum of values(Vc)C : 2,2 x10_7 No = N/100
1 (20/11/2019)	10^{-7}	180	174	180	174	N = 2,71 x 10_9 lg N = 9.24
	10^{-8}	11	11	<14	<14	No = 2,71 x 10_7 lg No = 7.24
2	10^{-7}	191	182	191	182	N = 2,1 x 10_9 lg N = 9.26
	10^{-8}	13	14	<14	<14	No = 2,1 x 10_7 lg No = 7.26
3	10^{-7}	191	194	192	194	N =1,64 x 10_9 lg N = 9.27
	10^{-8}	10	22	<14	22	No = 1,64 x 10_7 lg No = 7.27
4	10^{-7}	214	191	214	191	N = 1,93 x 10_9 lg N = 9.30
	10^{-8}	26	11	26	<14	No = 1,93 x 10_7 lg No = 7.30
5	10^{-7}	188	168	188	168	N = 1,60 x 10_9 lg N = 9.24
	10^{-8}	12	17	<14	17	No = 1,60 x 10_7 lg No = 7.24
6	10^{-7}	206	187	206	187	N = 1,85 x 10_9 lg N = 9.29
	10^{-8}	19	14	19	<14	No = 1,85 x 10_7 lg No =7.29

Testing with limited test organisms:

Repetition (for 0.5 % concentration)	Dilution	Number on plate		Vc1	Vc2	Na = prosek x^{-} ili težinski prosek $xw\tilde{m}x$ 10	lg Na = lg (x^{-}ili xwm^{-}) x 10	lg R = lg N0 - lgNa	Period of exposure (min)
1 (20.11.2019.)	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
2	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
3	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
4	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
5	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
6	100	>330	>330	>330	>330	>33000	>4.52	<2.72	5
10-1	>330	>330	>330	>330					
Prosečna redukcija:								**>2.75 lg**	
Standardna devijacija:								**± 0.013 lg**	

Attachment to Protocol no. 4: 6/DP/20

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100%, 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Pseudomonas aeruginosa* CCM 7930
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	December 9, 2019

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Test suspension :

Repetition	Dilution	Number on plate		Vc1	Vc2	N = x_{wm}^{-} =sum of values(Vc)C : 2,2 x10_7 No = N/100
1 (20.11.2019.)	10^{-7}	224	220	224	220	N = 2,71 x 10_9 lg N = 9.34
10^{-8}	22	11	22	<14		No = 2,71 x 10_7 lg No = 7.34
2	10^{-7}	189	127	189	127	N = 2,1 x 10_9 lg N = 9.20
10^{-8}	16	18	16	18		No = 2,1 x 10_7 lg No = 7.20
3	10^{-7}	205	191	205	191	N =1,64 x 10_9 lg N = 9.30
10^{-8}	20	23	20	23		No = 1,64 x 10_7 lg No = 7.30
4	10^{-7}	182	175	182	175	N = 1,93 x 10_9 lg N = 9.25
10^{-8}	10	18	<14	18		No = 1,93 x 10_7 lg No = 7,25
5	10^{-7}	162	149	162	149	N = 1,60 x 10_9 lg N = 9.19
10^{-8}	9	7	<14	<14		No = 1,60 x 10_7 lg No = 7.19
6	10^{-7}	147	179	147	179	N = 1,85 x 10_9 lg N = 9.21
10^{-8}	10	14	<14	14		No = 1,85 x 10_7 lg No = 7,21

Testing with limited tested organisms:

Repetition (for 100 % concentration)	Dilution	Number on plate		Vc1	Vc2	Na = mean x^{-} or weight average x_{wm}^{-}x 10	lg Na = lg (x^{-}or x_{wm}^{-}) x 10	lg R = lg N_0 - lgNa	Exposure period (min)
1 (20.11.2019.)	10_0	>330	>330	>330	>330	>33000	>4.52	<2.82	5
10^{-1}	>330	>330	>330	>330					
2	10_0	>330	>330	>330	>330	>33000	>4.52	<2.68	5
10^{-1}	>330	>330	>330	>330					
3	10_0	>330	>330	>330	>330	>33000	>4.52	<2.78	5
10^{-1}	>330	>330	>330	>330					
4	10_0	>330	>330	>330	>330	>33000	>4.52	<2.73	5
10^{-1}	>330	>330	>330	>330					
5	10_0	>330	>330	>330	>330	>33000	>4.52	<2.67	5
10^{-1}	>330	>330	>330	>330					
6	10_0	>330	>330	>330	>330	>33000	>4.52	<2.69	5
10^{-1}	>330	>330	>330	>330					
Mean reduction:								**>2.73 lg**	
Standard deviation:								**± 0,032lg**	

End of protocol

No part of this report may be reproduced in any form without the written approval of the testing laboratory. The test results refer only to the received test sample. Clinical Laboratory Center - Trial Laboratory no. 1554 ČIA accredited as per ČSN EN ISO / IEC 17025: 2018. A list of accredited methods is available at www.zuova.cz. The sample was tested in accordance with SOP no. 3037

HEALTH INSTITUTE seated in Ostrava
Clinical Laboratory Center
Pracoviště 1 - Ostrava
Virucidal Testing Laboratory
Partyzánské náměstí 2633/7Moravská Ostrava, 702 00 Ostrava
Company Reg. No.: 71009396
VAT Reg. No.: CZ71009396

TEST REPORT no. 6/DP/20

Quantitative suspension test for the evaluation of bactericidal activity in healthcare sector - phase 2 / level 1

Test required by:
AQUASYSTEM, s.r.o.

Hradská 76
821 07 Bratislava

Order number:
Ordered on: 4/3/2020

Reference number: ZU/06141/2020

Identification of disinfectant - sample:

Product name:	Dezanol
Lot number:	030321
Expiration date:	3/3/2021
Manufacturer:	AQUASYSTEM, s.r.o.
Storage conditions:	5–30 °C
Product dilution recommended by the manufacturer:	undiluted
Product appearance:	clear liquid
Active substance(s) and its concentration(s):	active chlorine (<0.06%)
Auxiliary agent and its concentration:	
Product identification:	hand disinfection
Product delivery date:	5/3/2020
Test date:	16/03/2020, 26/03/2020

Results (more details attached to the protocol):

Dezanol intended for hygienic hand wiping was tested in accordance with ČSN EN 13727 + A2 on test organisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae* a *Escherichia coli.*

The required concentration was 100%, contact time 1 minute, in conditions of lower contamination.

The reduced presence of *Staphylococcus aureus CCM 4516* was found at a concentration of 100%> 5.28 lg, at 50% 3.03 lg and at 0.5% < 1.91 lg.
The mean reduction (R) in logarithmic sequences with the test organism *Staphylococcus aureus CCM 4516* was at a concentration of 100% (V / V) R> 5.14 ± 0.045 lg *.

The reduction of *Pseudomonas aeruginosa CCM 7930* was found at a concentration of 100%> 5.09 lg, at 50%> 2.77 lg and at 0.5% <1.72 lg.
The reduction of *Escherichia coli CCM* 7929 was found at a concentration of 100%> 5.37 lg, at 50%> 5.37 lg and at 0.5% <2 lg.
The reduction of *Enterococcus hirae CCM 4533* was found at a concentration of 100% 5.2 lg, at 50% 3.31 lg and at 0.5% <1.84 lg.

All controls and validations were within basic limits. At least one product concentration (0.5%) showed a decrease below 5 lg.
* reproducibility standard deviation

Conclusion:
Product Dezanol 030321 series proved bactericidal activity according to the standard ČSN EN 13727 + A2 in conditions of lower contamination (bovine albumin 0.3 g / l) and contact time of 1 minutes at a concentration of 100%

The mean reduction (R) in logarithmic sequences in the test organism *Staphylococcus aureus CCM 4516* was R> 5.14 ± 0.045 lg * for a concentration of 100% (V / V).

In Ostrava, on 09/04/2020

Approved by: MUDr. Linda Stryjová

Attachment to Protocol no. 1: 6/DP/20

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Staphylococcus aureus CCM 4516*
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	16/03/2020

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Preparation of the basic suspension

Dilution of basic suspension	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Number of colonies on plate 1	>330	>330	>330	>330	>330	>330	>330	258	36
Number of colonies on plate 2	>330	>330	>330	>330	>330	>330	>330	275	28

Validation and control:

Validated suspension Nvo (Nv)	Experimental condition control (A)	Neutralizing agent control (B)	Validation method (C) product concentration:

																undiluted			
Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2
61	56	61	56	70	67	70	67	94	91	94	91	74	82	74	82
Arithmetic mean Vc1+Vc2: x^{-}= 58.5				Arithmetic mean Vc1+Vc2 x^{-}=68.5				Arithmetic mean Vc1+ Vc2 x^{-}=92.5				Arithmetic mean Vc1+ Vc2 x^{-}=78			
Is 30 ≤ $x^{-}$of Nvo ≤ 160 ? yes - no				Is x^{-}od A ≥ 0.5 x $x^{-}$of Nvo ? yes - no				Is $x^{-}$of B ≥ 0.5 x $x^{-}$of Nvo ? yes - no				Is $x^{-}$of C ≥ 0.5 x x^{-} of Nvo ? yes - no			
Suspension validation NVB (suspension validation for control B)								63	68	63	68				
								Arithmetic mean Vc1+ Vc2 x^{-}=65.5 Is 30 ≤ x^{-}z NVB /1000 ≤ 160 ? yes - no							

Test suspension

Test suspension N	Dilution	Number per plate		Vc1	Vc2	C (sum of value Vc) 597 Mean t x_{wm} = ---------- = ---------- (n1 + 0.1 n2)x10^{-7} (2+0.2)x10^{-7}
						x_{wm} = sum of value (Vc) 382: 2.2x10^{7} = 2.71x10^{9}
	10^{-7}	258	275	258	275	lg N = 9.43
	10^{-8}	36	28	36	28	Is 9.17 ≤ lg N ≤ 9.70 ? yes - no
Test suspension No = (N/100)	Dilution	Number per plate		Vc1	Vc2	C (sum of value Vc) 597 Mean t x_{wm}^{-}= ---------- = ---------- (n1 + 0.1 n2)x10^{-7} (2+0.2)x10^{-7}
						xwm= sum of value (Vc) 597: 2.2x10^{7} = 2.71x10^{9}
	10^{-7}	258	275	258	275	lg N = 7.43
	10^{-8}	36	28	36	28	Is 7.17 ≤ lg N ≤ 7.70 ? yes - no

Test

Product concentration (%)	Dilution	Number per plate	Vc1	Vc2	Na = mean x^{-} or weight average xwm x^{-} 10	lg Na = lg (x^{-} or xwm^{-}) x 10	lg R = lg N0 - lgNa lg No = 7.43	Period of exposure (min)

97	10_0	0	0	<14	<14	<140	<2,15	>5,28	1
	10_{-1}	0	0	<14	<14				
	10_{-2}	0	0	<14	<14				
50	10_0	>330	>330	>330	>330	25 409	4,40	3,03	1
	10_{-1}	208	267	208	267				
	10_{-2}	21	63	21	63				
0,5	10_0	>330	>330	>330	>330	>330 000	>5,52	<1,91	1
	10_{-1}	>330	>330	>330	>330				
	10_{-2}	>330	>330	>330	>330				

Legend:

Vc = number per ml (one or more plates), k = average of Vc1 and Vc2 (1st and 2nd duplicate determinations);
Na = number of surviving cells per ml in test suspension at the end of the exposure period;
N = test suspension; No = N / 100 = number of cells per ml in test mixtures in 0 exposure period;
Nvo = Nv / 10 = number of cells per ml of suspension for validation in 0 exposure period;
Nvb = number of cells per ml of validation suspension for control B (neutralizing agent);
x_{wm} = average weight x; R = reduction (lg R = lg N0 - lg Na).

Calculations: At an exposure period of 5 minutes, the reduction refers to *Staphylococcus aureus CCM 4516* in logarithmic sequences:

For 97%: lg R = lg No - lg Na = 7.43 - 2.15 = > 5.28
For 50%: lg R = lg No - lg Na = 7.43 - 4.40 = > 3.03
For 0.5%: lg R = lg No - lg Na = 7.43 - 5.52 = <1.91

Attachment to Protocol no. 2: 6/DP/20

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution

Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	*Pseudomonas aeruginosa* CCM 7930
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	16/03/2020

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Preparation of the basic suspension

Dilution of basic suspension	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-8}	10^{-9}
Number of colonies on plate 1	>330	>330	>330	>330	>330	>330	>330	184	6
Number of colonies on plate 2	>330	>330	>330	>330	>330	>330	>330	171	3

Validation and control:

Validated suspension Nvo (Nv)				Experimental condition control (A)				Neutralizing agent control (B)				Validation method (C) product concentration: undiluted			
Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2
111	107	111	107	71	77	71	77	83	56	83	56	53	71	53	71
Arithmetic mean Vc1+Vc2: $\bar{x}$= 109				Arithmetic mean Vc1+Vc2 $\bar{x}$=74				Arithmetic mean Vc1+ Vc2 $\bar{x}$=69.5				Arithmetic mean Vc1+ Vc2 $\bar{x}$=62			
Is 30 ≤ $\bar{x}$of Nvo ≤ 160 ? yes - no				Is $\bar{x}$od A ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of B ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of C ≥ 0.5 x $\bar{x}$ of Nvo ? yes - no			
								121	110	121	110				
								Arithmetic mean Vc1+ Vc2 $\bar{x}$=155.5 Is 30 ≤ $\bar{x}$z NVB /1000 ≤ 160 ? yes - no							

Test suspension

Test suspension N	Dilution	Number per plate		Vc1	Vc2	Mean t x_{wm} = C (sum of value Vc) / (n1 + 0.1 n2)x10^{-7} = 383 / (2+0.2)x10^{-7}
						x_{wm} = sum of value (Vc) 383: 2.2x10^{7} = 1.74x10^{9}
	10^{-7}	184	171	184	171	lg N = 9.24
	10^{-8}	6	3	<14	<14	Is 9.17 ≤ lg N ≤ 9.70 ? yes - no
Test suspension No = (N/100)	Dilution	Number per plate		Vc1	Vc2	Average weight t x_{wm}^{-}= C (sum of value Vc) / (n1 + 0.1 n2)x10^{-7} (2+0.2)x10^{-7} = 383
						x_{wm}= sum of value (Vc) 480: 2.2x10^{7} = 1.74x10^{9}
	10^{-7}	184	171	184	171	lg N = 7.24
	10^{-8}	6	3	<14	<14	Is 7,17 ≤ lg N ≤ 7.70 ? yes - no

Test

Product concentration (%)	Dilution	Number per plate		Vc1	Vc2	Na = mean x^{-} or weight average xwm x^{-} 10	lg Na = lg (x^{-} or xwm^{-}) x 10	lg R = lg N0 - lgNa lg No = 7.43	Period of exposure (min)
97	10_0	0	0	<14	<14	<140	<2,15	>5,09	1
	10_{-1}	0	0	<14	<14				
	10_{-2}	0	0	<14	<14				
50	10_0	>330	>330	>330	>330	25 400	4,40	2,77	1
	10_{-1}	>330	>330	>330	>330				
	10_{-2}	>330	>330	>330	>330				
0,5	10_0	>330	>330	>330	>330	>330 000	>5,52	<1,72	1
	10_{-1}	>330	>330	>330	>330				
	10_{-2}	>330	>330	>330	>330				

Legend:

Vc = number per ml (one or more plates), k = average of Vc1 and Vc2 (1st and 2nd duplicate determinations);
Na = number of surviving cells per ml in test suspension at the end of the exposure period;
N = test suspension; No = N / 100 = number of cells per ml in test mixtures in 0 exposure period;
Nvo = Nv / 10 = number of cells per ml of suspension for validation in 0 exposure period;
Nvb = number of cells per ml of validation suspension for control B (neutralizing agent);
x_{wm} = average weight x; R = reduction ($\lg R = \lg N_0 - \lg Na$).

Calculations: At an exposure period of 5 minutes, the reduction refers to *Staphylococcus aureus CCM 4516* in logarithmic sequences:

For 97%: lg R = lg No - lg Na = 7.24 - 2.15 = > 5.09
For 50%: lg R = lg No - lg Na = 7.24 - 4.47 = > 2.77
For 0.5%: lg R = lg No - lg Na = 7.24 - 5.52 = <1.72

Attachment to Protocol no. 3: 6/DP/20

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	***Escherichia coli* CCM 7929**
Incubation temperatures and time:	36 ± 1 °C, 48 h

Test date: 16/03/2020

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Preparation of the basic suspension

Dilution of basic suspension	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Number of colonies on plate 1	>330	>330	>330	>330	>330	>330	>330	>330	46
Number of colonies on plate 2	>330	>330	>330	>330	>330	>330	>330	311	44

Validation and control:

Validated suspension Nvo (Nv)				Experimental condition control (A)				Neutralizing agent control (B)				Validation method (C) product concentration: undiluted			
Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2
47	43	47	43	88	63	88	63	66	89	66	89	71	79	71	79
Arithmetic mean Vc1+Vc2: $\bar{x} = 45$				Arithmetic mean Vc1+Vc2 $\bar{x}=75.5$				Arithmetic mean Vc1+ Vc2 $\bar{x}=77.5$				Arithmetic mean Vc1+ Vc2 $\bar{x}=75$			
Is $30 \leq \bar{x}$ of Nvo ≤ 160 ? yes - no				Is $\bar{x}$ od A $\geq 0.5 \times \bar{x}$ of Nvo ? yes - no				Is $\bar{x}$ of B $\geq 0.5 \times \bar{x}$ of Nvo ? yes - no				Is $\bar{x}$ of C $\geq 0.5 \times \bar{x}$ of Nvo ? yes - no			
								50	59	50	59				
								Arithmetic mean Vc1+ Vc2 $\bar{x}=54.5$ Is $30 \leq \bar{x}$ z NVB /1000 ≤ 160 ? yes - no							

Test

Product concentration (%)	Dilution	Number per plate		Vc1	Vc2	Na = mean $\bar{x}$ or weight average xwm $\bar{x}$ 10	lg Na = lg ($\bar{x}$ or $\bar{xwm}$) x 10	lg R = lg N0 - lgNa lg No = 7.43	Period of exposure (min)
97	10_0	0	0	<14	<14	<140	<2,15	>5,37	1
	10_{-1}	0	0	<14	<14				
	10_{-2}	0	0	<14	<14				
50	10_0	3	11	<14	<14	<140	<2,15	>5,37	1
	10_{-1}	0	1	<14	<14				
	10_{-2}	0	0	<14	<14				
0,5	10_0	>330	>330	>330	>330	>330 000	>5,52	<2	1
	10_{-1}	>330	>330	>330	>330				
	10_{-2}	>330	>330	>330	>330				

Legend:

Vc = number per ml (one or more plates), k = average of Vc1 and Vc2 (1st and 2nd duplicate determinations);
Na = number of surviving cells per ml in test suspension at the end of the exposure period;
N = test suspension; No = N / 100 = number of cells per ml in test mixtures in 0 exposure period;
Nvo = Nv / 10 = number of cells per ml of suspension for validation in 0 exposure period;
Nvb = number of cells per ml of validation suspension for control B (neutralizing agent);
x_{wm} = average weight x; R = reduction ($\lg R = \lg N_0 - \lg Na$).

Calculations: At an exposure period of 5 minutes, the reduction refers to *Staphylococcus aureus CCM 4516* in logarithmic sequences:

For 97%: lg R = lg No - lg Na = 7.24 - 2.15 = > 5.37
For 50%: lg R = lg No - lg Na = 7.24 - 4.47 = > 2.37
For 0.5%: lg R = lg No - lg Na = 7.24 - 5.52 = <2

Attachment to Protocol no. 4: 6/DP/20

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	***Enterococcus hirae* CCM 4533**
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	16/03/2020

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Preparation of the basic suspension

Dilution of basic suspension	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Number of colonies on plate 1	>330	>330	>330	>330	>330	>330	>330	237	27
Number of colonies on plate 2	>330	>330	>330	>330	>330	>330	>330	223	21

Validation and control:

Validated suspension Nvo (Nv)				Experimental condition control (A)				Neutralizing agent control (B)				Validation method (C) product concentration: undiluted			
Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2	Number per plate		Vc1	Vc2
36	40	36	40	34	24	34	24	80	67	80	67	40	35	40	35
Arithmetic mean Vc1+Vc2: $\bar{x}$= 38				Arithmetic mean Vc1+Vc2 $\bar{x}$=29				Arithmetic mean Vc1+ Vc2 $\bar{x}$=73.5				Arithmetic mean Vc1+ Vc2 $\bar{x}$=37.5			
Is 30 ≤ $\bar{x}$of Nvo ≤ 160 ? yes - no				Is $\bar{x}$od A ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of B ≥ 0.5 x $\bar{x}$of Nvo ? yes - no				Is $\bar{x}$of C ≥ 0.5 x $\bar{x}$ of Nvo ? yes - no			
								41	26	41	26				
								Arithmetic mean Vc1+ Vc2 $\bar{x}$=33.5 Is 30 ≤ $\bar{x}$z NVB /1000 ≤ 160 ? yes - no							

Test

Product concentration (%)	Dilution	Number per plate		Vc1	Vc2	Na = mean $\bar{x}$ or weight average xwm $\bar{x}$ 10	lg Na = lg ($\bar{x}$ or $\bar{xwm}$) x 10	lg R = lg N0 - lgNa lg No = 7.43	Period of exposure (min)
97	10_0	15	14	15	14	<140	<2,15	>5,37	1
	10_{-1}	5	4	<14	<14				
	10_{-2}	3	0	<14	<14				
50	10_0	71	69	71	69	<140	<2,15	>5,37	1
	10_{-1}	43	66	43	66				
	10_{-2}	4	0	<14	<14				
0,5	10_0	>330	>330	>330	>330	>330 000	>5,52	<2	1
	10_{-1}	>330	>330	>330	>330				
	10_{-2}	>330	>330	>330	>330				

Legend:

Vc = number per ml (one or more plates), k = average of Vc1 and Vc2 (1st and 2nd duplicate determinations);
Na = number of surviving cells per ml in test suspension at the end of the exposure period;
N = test suspension; No = N / 100 = number of cells per ml in test mixtures in 0 exposure period;
Nvo = Nv / 10 = number of cells per ml of suspension for validation in 0 exposure period;
Nvb = number of cells per ml of validation suspension for control B (neutralizing agent);
x_{wm} = average weight x; R = reduction ($\lg R = \lg N_0 - \lg Na$).

Calculations: At an exposure period of 5 minutes, the reduction refers to *Staphylococcus aureus CCM 4516* in logarithmic sequences:

For 97%: lg R = lg No - lg Na = 7.24 - 2.15 = > 5.20
For 50%: lg R = lg No - lg Na = 7.24 - 4.47 = > 3.31
For 0.5%: lg R = lg No - lg Na = 7.24 - 5.52 = <1.84

Attachment to Protocol no. 5: 6/DP/20

Procedure according to SOP 3037 - ČSN EN 13727 + A2 - Quantitative suspension test for bactericidal activity in healthcare sector - phase 2 / level 1

Product name:	Dezanol
Manufacturer:	AQUASYSTEM s.r.o.
Storage conditions (temp, etc.):	5 °C-30 °C, dry, dark
Solvent:	hard water
Number of treated plates:	2 x 1 ml
Test method:	Neutralization-dilution
Neutralizing agent:	Polysorbate 80 30.0 g / l + sodium thiosulfate 15 g / l + lecithin 3 g / l
Tested product concentration:	100% (actually tested 97%), 50%, 0.5%
Exposure period:	5 min
Stability and appearance of the mixture during the procedure:	clear solution
Test temperature:	23 ± 2 °C
Interfering substances:	bovine albumin 0.3 g/l + sheep erythrocytes
Tested organism:	***Staphylococcus aureus* CCM 4516**
Incubation temperatures and time:	36 ± 1 °C, 48 h
Test date:	26/03/2020

Prepared by: Irena Willerthová

Controlled by: MUDr. Linda Stryjová

Signature:

Test suspension

Repetition	Dilution	Number on plate		Vc1	Vc2	N = x_{wm} =sum of values(Vc)C : 2,2 x10^7 No = N/100
1 (16. 3. 2020)	10-7	258	275	258	275	N = 2,71 x 10^9 lg N = 9,43
	10-8	36	28	36	28	No = 2,71 x 10^7 lg No = 7,43
2	10-7	208	209	208	209	N = 2,1 x 10^9 lg N = 9,32
	10-8	22	23	22	23	No = 2,1 x 10^7 lg No = 7,32
3	10-7	170	152	170	152	N =1,64 x 10^9 lg N = 9,21
	10-8	25	12	25	<14	No = 1,64 x 10^7 lg No = 7,21
4	10-7	190	198	190	198	N = 1,93 x 10^9 lg N = 9,28
	10-8	18	18	18	18	No = 1,93 x 10^7 lg No = 7,28
5	10-7	149	176	149	176	N = 1,60 x 10^9 lg N = 9,20
	10-8	14	12	14	<14	No = 1,60 x 10^7 lg No = 7,20
6	10-7	181	192	181	192	N = 1,85 x 10^9 lg N = 9,27
	10-8	16	18	16	18	No = 1,85 x 10^7 lg No = 7,27

Testing with limited test organisms:

Repetition (for 100 % concentration)	Dilution	Number on plate		Vc1	Vc2	Na = mean x^- or weight average xwm^-x 10	lg Na = lg (x^-ili xwm^-) x 10	lg R = lg N0 - lgNa	Period of exposure (min)
1 (16. 3. 2020)	100	0	0	<14	<14	<140	<2,15	>5,28	1
10-1	0	0	<14	<14					
10-2	0	0	<14	<14					
2	100	0	0	<14	<14	<140	<2,15	>5,17	1
10-1	0	0	<14	<14					
10-2	0	0	<14	<14					
3	100	0	0	<14	<14	<140	<2,15	>5,06	1
10-1	0	0	<14	<14					
10-2	0	0	<14	<14					
4	100	0	0	<14	<14	<140	<2,15	>5,13	1
10-1	0	0	<14	<14					
10-2	0	0	<14	<14					
5	100	0	0	<14	<14	<140	<2,15	>5,05	1
10-1	0	0	<14	<14					
10-2	0	0	<14	<14					
6	100	0	0	<14	<14	<140	<2,15	>5,12	1
10-1	0	0	<14	<14					
10-2	0	0	<14	<14					
Mean reduction:								**>5,14 lg**	
Standard deviation:								**± 0,045 lg**	

No part of this report may be reproduced in any form without the written approval of the testing laboratory. The test results refer only to the received test sample. Clinical Laboratory Center - Trial Laboratory no. 1554 ČIA accredited as per ČSN EN ISO / IEC 17025: 2018. A list of accredited methods is available at www.zuova.cz. The sample was tested in accordance with SOP no. 3037