



LIETUVOS  
NACIONALINIS  
AKREDITACIJOS  
BIURAS

Nr. LA.01.138

**MICROBIOLOGICAL TESTING DEPARTMENT  
OF NATIONAL PUBLIC HEALTH SURVEILLANCE LABORATORY**

Zolyno street 36, LT-10210 Vilnius, Lithuania, Tel. +370 5 234 40 03, fax +370 5 210 54 05 E-mail [priimamasis.zolyno@nvspl.lt](mailto:priimamasis.zolyno@nvspl.lt)

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**MICROBIOLOGICAL TEST REPORT No.** MA 2769 (MA 7503)/2020

25 May 2020

Customer, address: JSC "Liventra", Kaunas, Užnėrio str. 52B

[E]

Agreement (mark X) [ X ] there is no [ ] is date 20 \_\_\_\_\_ No \_\_\_\_\_

Phone: 867407243 E-mail: a.voitechovicius@gmail.com

Pickup Act-Order No: V 4097

Date and time of delivering the samples and sample temperature (if required)

2020-05-19, 10.30 h

Information supplied by the customer:

Samples supplied (title of the sample, method of packing, amount of sample supplied (kg,l), producer, method, by which test sample is produced, batch size, production date, date of realization, time, other information supplied by the customer):

15% ethereal amber oil in walnut oil, 100 g, JSC "Liventra"

Place of the selecting Kaunas, Užnėrio str. 52B

the samples,

Sampling report No: \_\_\_\_\_ (object name and address)

Samples selected by: Andžejus Voitechovičius

(institution, division, position, name)

Date and time of sample selecting, sample

temperature, identification No. of the document:

2020-05-19, 8.00 h, No doc. of sampling: MA 2769

Samples delivered by: Andžejus Voitechovičius

(institution, name)

Testing started on: 2020-05-19

Test results:

Sample name 15% ethereal amber oil in walnut oil

Sample registry No.	Testing performed by the method	Searched microorganism	Test results	± U
<u>MA 7503</u>	<u>LST EN ISO 21149:2017</u>	<u>Enumeration of aerobic mesophytic bacteria</u>	<u>KSV /g/ml</u>	<u>&lt;10</u>
	<u>LST EN ISO 22717:2016</u>	<u>Detection of Pseudomonas aeruginosa</u>	<u>1 g/ ml</u>	<u>absent</u>
	<u>LST EN ISO 18416:2016</u>	<u>Detection of Candida albicans</u>	<u>1 g/ ml</u>	<u>absent</u>
	<u>LST EN ISO 22718:2016</u>	<u>Detection of Staphylococcus aureus</u>	<u>1 g/ ml</u>	<u>absent</u>
	<u>LST EN ISO 16212:2017 (N)</u>	<u>Enumeration of yeasts and moulds</u>	<u>KSV /g/ml</u>	<u>&lt;10</u>

Supplementary data, remarks: not indicated

Date of performing tests: 2020-05-25

Test performed by Microbiology specialist Irina Iljina

(position, name and surname)

Approve: Medical microbiologist Jordana Saleikiene

(position, name and surname, signature)

Explanations:	<ol style="list-style-type: none"><li>1. U – the presented expanded uncertainty is calculated by multiplying the standart uncertainty by the coverage factor k=2, which when the normal distribution applies provides a level of confidence of 95 %.</li><li>2. Test results related only to the particular samples tested.</li><li>3. N -not accredited method.</li><li>4. Test report or parts thereof (annexes) can't be reproduced without the consent of the head of division and/or subdivision.</li><li>5. Handing over of the test report [E]-by e-mail</li></ol>
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MICROBIOLOGICAL TEST REPORT No.

MA 2751 (MA 7470)/2020

23 May 2020

Customer, address: **JSC "Lifestra", Kaunas, Užnėrio str. 52B** [E]

Agreement (mark X)  there is no  is date 20 \_\_\_\_\_ No

Phone: **867407243** E-mail: **a.voitechovicius@gmail.com** Pickup Act-Order No: **V 4073**

Date and time of delivering the samples and sample temperature (if required) **2020-05-19, 10.30 h**

Information supplied by the customer:

Samples supplied (title of the sample, method of packing, amount of sample supplied (kg,l), producer, method, by which test sample is produced, batch size, production date, date of realization, time, other information supplied by the customer):

**100% ethereal amber oil, 80 g, JSC "Lifestra"**

Place of the selecting **Kaunas, Užnėrio str. 52B**

the samples,

Sampling report No: \_\_\_\_\_ (object name and address)

Samples selected by: **Andžejus Voitechovičius**

(institution, division, position, name)

Date and time of sample selecting, sample temperature, identification No. of the document: **2020-05-19, 8.00 h, No doc. of sampling: MA 2751**

Samples delivered by: **Andžejus Voitechovičius**

(institution, name)

Testing started on: **2020-05-19**

Test results:

Sample name **100% ethereal amber oil**

Sample registry No.	Testing performed by the method	Searched microorganism	Test results	± U
<b>MA 7470</b>	LST EN ISO 21149:2017	Enumeration of aerobic mesophytic bacteria	KSV /g/ml	<10
	LST EN ISO 22717:2016	Detection of Pseudomonas aeruginosa	1 g/ ml	Absent
	LST EN ISO 18416:2016	Detection of Candida albicans	1 g/ ml	Absent
	LST EN ISO 22718:2016	Detection of Staphylococcus aureus	1 g/ ml	Absent
	LST EN ISO 16212:2017 (N)	Enumeration of yeasts and moulds	KSV /g/ml	<10

Supplementary data, remarks: **not indicated**

Date of performing tests: **2020-05-23**

Test performed by **Microbiology specialist Irina Iljina**

(position, name and surname)

Approve: **Medical microbiologist Jordana Saleikiene**

(position, name and surname, signature)

Explanations:	1. U – the presented expanded uncertainty is calculated by multiplying the standard uncertainty by the coverage factor k=2, which when the normal distribution applies provides a level of confidence of 95 %. 2. Test results related only to the particular samples tested. 3. N -not accredited method. 4. Test report or parts thereof (annexes) can't be reproduced without the consent of the head of division and/or subdivision. 5. Handing over of the test report [E]-by e-mail
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**MICROBIOLOGICAL TEST REPORT No. MA 2751 (MA 7471)/2020**  
**29 June 2020**

Customer, address: **JSC "Livestra", Kaunas, Užnero str. 52B**

[E]

Agreement (mark X) [ X ] there is no [ ] is date 20 \_\_\_\_\_ No \_\_\_\_\_

Phone: **867407243** E-mail: **a.voitechovicius@gmail.com** Pickup Act-Order No: **V 4073**

Date and time of delivering the samples and sample temperature (if required) **2020-05-19, 10.30 h**

Information supplied by the customer:

Samples supplied **Cosmetic and personal hygiene product, 1 samples**

Place of the selecting the samples, **Kaunas, Užnero str. 52B** (object name and address)

Sampling report No: \_\_\_\_\_

Samples selected by: **Andžejus Voitechovičius**

Date and time of sample selecting, sample temperature, identification No. of the document: (institution, division, position, name)

**2020-05-19, 8.00 h, No doc. of sampling: MA 2751**

Samples delivered by: **Andžejus Voitechovičius** (institution, name)

Testing started on: **2020-05-27**

Test results:

Sample registry No.	Title and identification of the sample	Testing performed by the method	Searched microorganism	Test results	±U
1	2	3	4	5	6
<b>MA 7471</b>	<i>15% ethereal amber oil in walnut oil</i>	LST EN ISO 11930:2019 (N)	Evaluation of neutralisation with <i>Candida albicans</i> Log	Neutralisation effective	
		LST EN ISO 11930:2019 (N)	Evaluation of neutralisation with <i>Aspergillus brasiliensis</i> Log	Neutralisation effective	
		LST EN ISO 11930:2019 (N)	Evaluation of neutralisation with <i>Pseudomonas aeruginosa</i> Log	Neutralisation effective	
		LST EN ISO 11930:2019 (N)	Evaluation of neutralisation with <i>Staphylococcus aureus</i> Log	Neutralisation effective	
		LST EN ISO 11930:2019 (N)	Evaluation of neutralisation with <i>Escherichia coli</i> Log	Neutralisation effective	
		LST EN ISO 11930:2019 (N)	Log Reduction of <i>Candida albicans</i> ATCC 10231 7 d.	R=5,4	
		LST EN ISO 11930:2019 (N)	Log Reduction of <i>Candida albicans</i> ATCC 10231 7 d.	R=5,4	
		LST EN ISO 11930:2019 (N)	Log Reduction of <i>Candida albicans</i> ATCC 10231 7 d.	R=5,4	
		LST EN ISO 11930:2019 (N)	Log Reduction of <i>Aspergillus brasiliensis</i> ATCC 16404 14 d.	R=4,6	
		LST EN ISO 11930:2019 (N)	Log Reduction of <i>Aspergillus brasiliensis</i>	R (Log)	R=4,6

Sample registry No.	Title and identification of the sample	Testing performed by the method	Searched microorganism	Test results	$\pm U$
1	2	3	4	5	6
			ATCC 16404 14 d.		
		LST EN ISO 11930:2019 (N)	Log Reduction of Pseudomonas aeruginosa ATCC 9027 7 d.	R (Log) R=6,8	
		LST EN ISO 11930:2019 (N)	Log Reduction of Pseudomonas aeruginosa ATCC 9027 7 d.	R (Log) R=6,8	
		LST EN ISO 11930:2019 (N)	Log Reduction of Pseudomonas aeruginosa ATCC 9027 7 d.	R (Log) R=6,8	
		LST EN ISO 11930:2019 (N)	Log Reduction of Staphylococcus aureus ATCC 6538 7 d.	R (Log) R=6,7	
		LST EN ISO 11930:2019 (N)	Log Reduction of Staphylococcus aureus ATCC 6538 7 d.	R (Log) R=6,7	
		LST EN ISO 11930:2019 (N)	Log Reduction of Escherichia coli ATCC 8739 7 d.	R (Log) R=7,0	
		LST EN ISO 11930:2019 (N)	Log Reduction of Escherichia coli ATCC 8739 7 d.	R (Log) R=7,0	
		LST EN ISO 11930:2019 (N)	Log Reduction of Escherichia coli ATCC 8739 7 d.	R (Log) R=7,0	

Supplementary data, remarks: **not indicated**

Date of performing tests: 2020-06-29

Test performed by Medical microbiologist Jordana Saleikiene

(position, name and surname)

Approve: Deputy Head of Microbiological testing department Vitalija Prasmutiene

(position, name and surname, signature)

Explanations:	1. U – the presented expanded uncertainty is calculated by multiplying the standart uncertainty by the coverage factor k=2, which when the normal distribution applies provides a level of confidence of 95 %. 2. Test results related only to the particular samples tested. 3. N -not accredited method. 4. Test report or parts thereof (annexes) can't be reproduced without the consent of the head of division and/or subdivision. 5. Handing over of the test report [E]-by e-mail
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**Cosmetic product: 15% ethereal amber oil in walnut oil**

**Normative reference:** LST EN ISO 11930:2019 Cosmetics - Microbiology - Evaluation of the antimicrobial protection of a cosmetic product

**Test strains:** *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404

**The counting method used:** pour plate.

**Culture media used:** for bacteria- tryptic soy agar (TSA), for *C. albicans*- Sabouraud dextrose agar medium (SDA) for *A. brasiliensis* - potato dextrose agar (PDA) .

**Diluents:** for bacterial suspension- 0,85 % NaCl; for preparation of *A. brasiliensis* spore suspension - polysorbate 80 solution

**Test temperature:** (22,5±2,5)°C.

**Incubation:** bacteria and *C. albicans* (32,5±2,5)°C for 48h to 72 h.; *A. brasiliensis* (22,5±2,5)°C for 3 to 5 days.

**Preparation of working culture:**

TSA for bacteria, SDA for *C.albicans* incubate at (32,5 ± 2,5) °C for 18 h to 24 h., PDA for *A. brasiliensis* (22,5±2,5)°C 1 week.

**Preparation of suspensions:** the number of cells in the suspension to  $1 \times 10^7$  cfu/ml to  $1 \times 10^8$  cfu/ml (bacteria) or  $1 \times 10^6$  cfu/ml to  $1 \times 10^7$  cfu/ml (*C. Albicans* and *A. brasiliensis*) using the diluent.

**Final concentration in the formulation:**  $1 \times 10^5$  cfu/ml and  $1 \times 10^6$  cfu/ml or g for bacteria, and between  $1 \times 10^4$  cfu/ml and  $1 \times 10^5$  cfu/ml or g for *C. albicans* and *A. brasiliensis*.

Demonstration of the neutralizer efficacy :

Mikroorganisms	Validation N <sub>vf</sub> log	Control N <sub>vn</sub> log	Inoculum control N <sub>v</sub> log
	Evaluation: N <sub>vf</sub> ≥ 0,5 N <sub>vn</sub>		Evaluation: N <sub>v</sub> and N <sub>vn</sub> has to be close
<i>Candida albicans</i>	2,1	2,1	2,2
<i>Aspergillus brasiliensis</i>	1,5	1,5	1,6
<i>Pseudomonas aeruginosa</i>	2,6	2,7	2,5
<i>Staphylococcus aureus</i>	2,5	2,7	2,5
<i>Escherichia coli</i>	2,5	2,6	2,6

The number of microorganisms at time t<sub>0</sub>:

Mikroorganisms	N ksv/ml	N <sub>0</sub> ksv/ml (N <sub>0</sub> =N/100)
<i>Candida albicans</i>	$2,4 \times 10^7$	$2,4 \times 10^5$
<i>Aspergillus brasiliensis</i>	$4,3 \times 10^6$	$4,3 \times 10^4$
<i>Pseudomonas aeruginosa</i>	$6,7 \times 10^8$	$6,7 \times 10^6$
<i>Staphylococcus aureus</i>	$5,0 \times 10^8$	$5,0 \times 10^6$
<i>Escherichia coli</i>	$9,9 \times 10^8$	$9,9 \times 10^6$

Results evaluation according to A criteria R = N<sub>0</sub> - N<sub>x</sub> (during T<sub>x</sub> time)

Mikroorganisms	N <sub>0</sub> log (N <sub>0</sub> =N/100)	Reduction R = N <sub>0</sub> - N <sub>x</sub> (T time) log			Reduction effective/ ineffective
		T 7 ≥ 1	T 14 ≥ 1 ir NI	T 28 ≥ 1 ir NI	
<i>Candida albicans</i>	5,4	$5,4 - 0 = 5,4$	$5,4 - 0 = 5,4$	$5,4 - 0 = 5,4$	Effective
			<b>T 14 ≥ 0</b>	<b>T 28 ≥ 1 ir NI</b>	
<i>Aspergillus brasiliensis</i>	4,6	Not performed	$4,6 - 0 = 4,6$	$4,6 - 0 = 4,6$	Effective
			<b>T 7 ≥ 3</b>	<b>T 14 ≥ 3 ir NI</b>	
<i>Pseudomonas aeruginosa</i>	6,8	$6,8 - 0 = 6,8$	$6,8 - 0 = 6,8$	$6,8 - 0 = 6,8$	Effective
<i>Staphylococcus aureus</i>	6,7	$6,8 - 0 = 6,7$	$6,8 - 0 = 6,7$	$6,8 - 0 = 6,7$	Effective
<i>Escherichia coli</i>	7,0	$7,0 - 0 = 7,0$	$7,0 - 0 = 7,0$	$7,0 - 0 = 7,0$	Effective

$N_{vf}$  – number of microorganisms present in the test mixture with the neutralizer and formulation  
 $N_{vn}$  – number of microorganisms present in the test mixture with the neutralizer in the absence of the formulation  
 $N$  – quantity of the initial numbers of microorganisms  
 $N$  - number of microorganisms present in the calibrated suspensions in colony-firming units per millilitre  $N = C/(V \times d)$ , where  
C - is the mean number of colonies counted in duplicate over the plates,  
V - is the volume of inoculum applied to each dish, in ml,  
d - is the dilution factor.  
R - reduction values expressed in log units  $R = \log N_0 - \log N_x$ , where  
 $N_0$  - number of microorganisms inoculated at time  $t_0$   
 $N_x$  - number of surviving microorganisms at each sampling time  $t_x$   
NI - no increase in the count from the previous contact time T7, T14, T28 days

**Conclusion:** The test of 15% ethereal amber oil in walnut oil confirmed the efficacy of the antimicrobial protection of a cosmetic product.

Start of the test: 2020-05-27

End of the test: 2020-06-29

Medicinos mikrobiologijos  
Jolanta Šaleikiene

