TESTS PROTOCOL

Date and number of protocol	12.01.2021, protocol № 1
Place:	IKO Science laboratory, Laki 32, 12915, Tallinn, Estonia
The content of the work:	Microbiological analysis of air
Measurement parameters:	Measurement of the number of biological aerosols with the BD500 biodetector
The methodology used:	Dosing of protein aerosols and measurement with the BD500 biodetector
Devices used:	1. Biodetector BD500, S/N 500000408; 2. Gas flow meter TSI model 5300-1, S/N 53002005004, next calibration 31.01.2021; 3. Compressor-evaporator Omron NE-C28P, S/N 20200403737UF; 4. IKA Vortex Genius 3 S000 laboratory vortex shaker, S/N 100057169 5. Eppendorf Research Plus 1-channel automatic pipette, 100-1000 µL.
Samples used:	1. Bovine serum albumin (Albumine bovine serum, manufacturer Acros Organics, batch A0404646, code 268130100, molecular weight 66kDa, hydrodynamic diameter 6 nanometers); Weighing protocol – Annex 1. 2. α-Lactalbumin (α-Lactalbumin from bovine milk, manufacturer SIGMA Aldridge, batch SLC83030, code 1002007215, molecular weight 14.2kDa, dimension 2.3x2.6x4.0 nanometers); 3. Distilled water, manufacturer B.Braun, batch 201068001, best before 02.2023; 4. Synthetic scientific air (Synthetische Luft Scientific), specification – Annex 2.
Dates of tests:	04.12.2020 – 15.12.2020.

Signatures:

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This document consists of a Tests Protocol with a summary of results and conclusions on eight (8) pages and Annexes on two (2) pages in one (1) signed copy.

BD500 description.

The biodetector BD500 is intended for measurement of concentration of biological aerosols in rooms with a limited concentration; the basic model has a sensitivity limit of eight particles per cubic meter, the version for surgical rooms - four particles per cubic meter.

For testing, biodetector BD500 with S/N 500000408 had been used (photo 1).

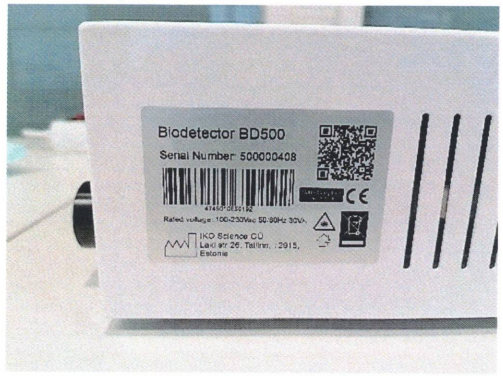


Photo 1.

Principle of BD500 operation.

The principle of operation is based on the registration of the fluorescence of the amino acid tryptophan as a marker of presence of proteins in aerosols, the excitation radiation wavelength is 280nm, the fluorescence is recorded by a photon counter in a 40nm band with an average wavelength of 357nm.

Algorithm of BD500 operation.

The device is adjusted for 30 seconds without pumping air through the detection zone. Thereafter the fans are turned on, in 5 minutes 25±2% liters of air pass through the detection zone. Thereafter the calibration-measurement cycles (30 sec+300 sec) are repeated automatically.

Test preparation

Biodetector BD500 had been turned on 90 minutes prior to testing start. After turning on the device, the process of temperature stabilization begins, maximum sensitivity and reliability of measurement results is guaranteed after 90minutes of device operation.

After stabilization is complete, the biodetector is connected to the test unit using a tube (black with a white stripe, photo 2)

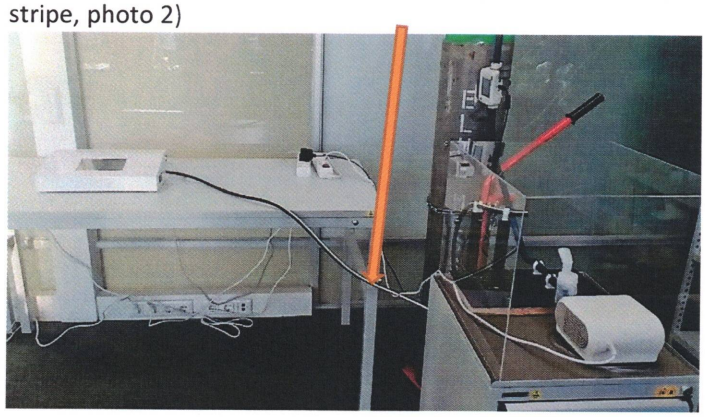


Photo 2.

Test unit, general view (photo 3).

Pressure reducer, set at a flow rate of 5 liters per minute

Gas flow meter TSI model 5300-1

Synthetic air cylinder 50l, 200 bar

The tube connecting the flow meter with the inlet air filter of the compressor-evaporator

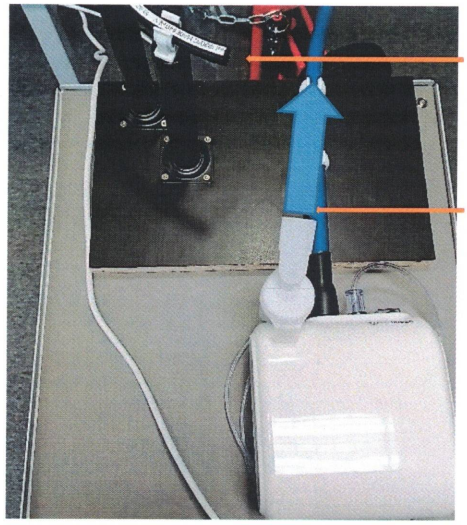
BD500 connection tube positioner

Compressor-evaporator- reservoir for liquids

Compressor-evaporator Omron NE-C28P

Photo 3.

Test unit, detailed view (photo 4).



The inlet of the tube-connection with BD500 is located approximately 12 cm from the edge of the nozzle of the compressor-evaporator and approximately 4-8 cm from the axis of the aerosol flow, which ensures that only small drops are absorbed into the tube, water from small drops will evaporate during the flight in 2- x meter tube and the BD500 will receive only dry bioaerosols.

Direction of movement of the aerosol flow.

Photo 4.

Test unit in the cover (photo 5).

A cover to prevent the air currents in the room from influencing the direction of aerosol flow from the compressor-evaporator.

Photo 5.

Test procedure.

Verification Test and Measurement Tests 1-2, 04.12.2020
Laboratory environment conditions: air temperature 23 ° C, relative humidity 38%.

Verification test: 2 ml of distilled water is poured into the reservoir of the compressor-evaporator. After the start of a 5-minute measurement cycle, the compressor-evaporator is switched on for 30 seconds. At the end of the 5-minute measurement cycle, the result is read. For the reliability of the result, the verification test is repeated 3 times.

Verification test	Result
1	0
2	0
3	0

For measuring tests, 0.6mg of bovine serum albumin (Albumine bovine serum, manufacturer Acros Organics, batch A0404646, code 268130100, molecular weight 66kDa, hydrodynamic diameter 6 nanometers), dissolved in 5ml of distilled water, is injected into the reservoir of the compressor-evaporator. This amount of solution is sufficient for two tests.
Thus, for the tests we used:
Test 1-2 - vial number 1
Test 3-4 - vial number 13
Test 5-6 - vial number 14
Vial weighing protocol is attached.

Measurement tests 1-2: After the start of a 5-minute measurement cycle, the compressor-evaporator is switched on for a fixed evaporation time (Table Test 1, column 2 "Injection time"). The amount of evaporated aerosol depends on the duration of the evaporation time. At the end of the 5-minute measurement cycle, the result is read, which is indicated in the Test 1 table, column 3 "Quantity, in 10^3", showing the number of detected particles in 1 m^3 of air (i.e., based on aerosol evaporation for 5 seconds, 240 particles had been detected in 1 m^3 of air).

Conversion to 1 m^3 is made according to the formula

$$N_{m^3}=n*k,$$

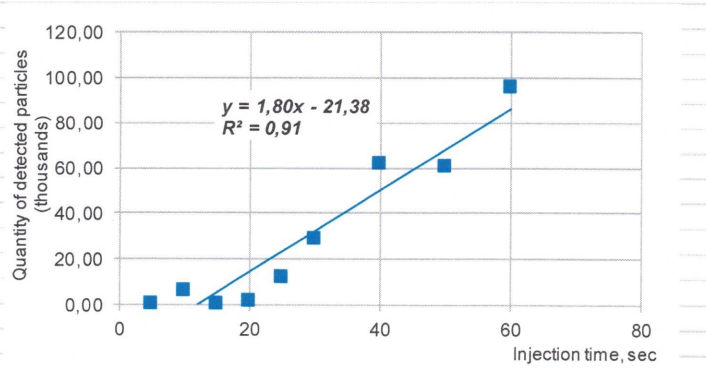
where:

«N_{m^3}» - calculated number of particles in 1 m^3 (Quantity, in thousands)

«n» - number of particles registered in a 5-minute cycle in 25 liters of air

«k» = $\frac{1000l}{25l} = 40$

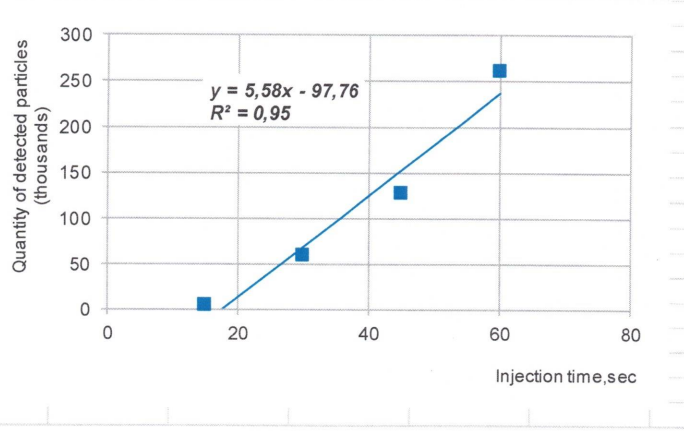
TEST 1	Testing time:	10.30-11.15
№	Injection time, sec	Quantity, in 10^3
1	5	0,24
2	10	5,76
3	15	0,32
4	20	1,40
5	25	11,92
6	30	28,36
7	40	61,40
8	50	60,60
9	60	95,72



Due to the fact that test 1 was carried out with a short duration of nebulization immediately after protein dissolution, and the homogenization of the solution has not yet been completed, the graph shows a significant scatter in the results of the first 4 measurements.

Therefore, test 2 was carried out on the same solution with increased duration of spray discretization from five to 15 seconds. The correctness of the choice is clearly shown by the results of test 2.

TEST 2	Testing time:	11.30-11.55
№	Injection time, sec	Quantity, in 10^3
1	15	3
2	30	57,8
3	45	125,92
4	60	259,32

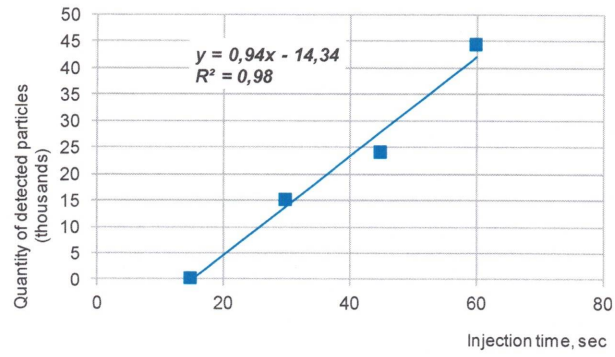


Measurement tests 3-4, 09.12.2020

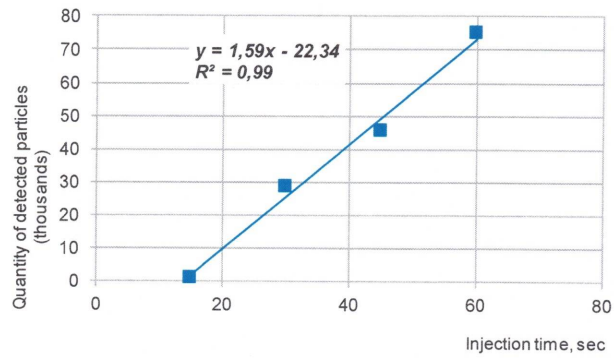
Laboratory environment conditions: air temperature 21.5 ° C, relative humidity 42%.

In tests 3-4, the inlet of the BD500 connection tube was located approximately 12 cm from the tip of the compressor-evaporator nozzle and approximately **8 cm** from the axis of the aerosol stream.

TEST 3	Testing time:	9.30-10.15
№	Injection time, sec	Quantity, in 10^3
1	15	0,24
2	30	15,08
3	45	24,04
4	60	44,24



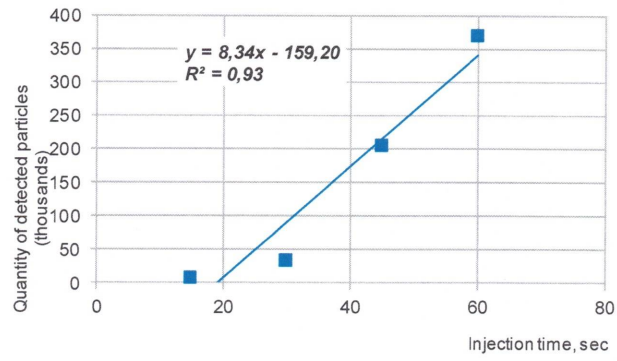
TEST 4	Testing time:	10.38-11.25
№	Injection time, sec	Quantity, in 10^3
1	15	0,92
2	30	28,48
3	45	45,4
4	60	75



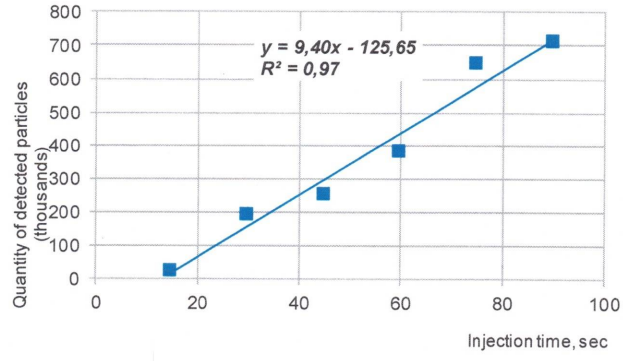
Measurement tests 5-6, 10.12.2020 r.
Laboratory environment conditions: air temperature 22°C, relative humidity 39%.

In tests 5-6, the inlet of the BD500 connection tube was located approximately 12 cm from the tip of the compressor-evaporator nozzle and approximately **4 cm** from the axis of the aerosol stream.

TEST 5	Testing time:	12.15-13.03
№	Injection time, sec	Quantity, in 10^3
1	15	8,44
2	30	33,44
3	45	203,72
4	60	368,72



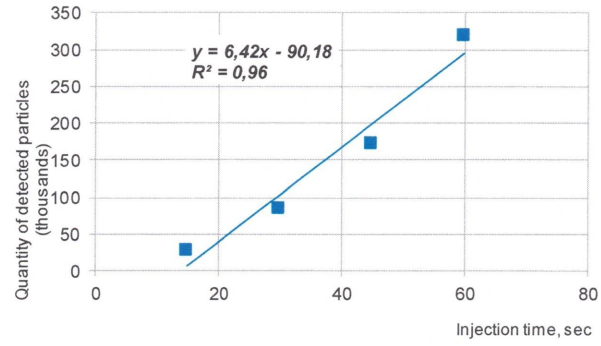
TEST 6	Testing time:	13.22-14.12
№	Injection time, sec	Quantity, in 10^3
1	15	23,24
2	30	190,72
3	45	253,16
4	60	382,6
5	75	646,64
6	90	710,84



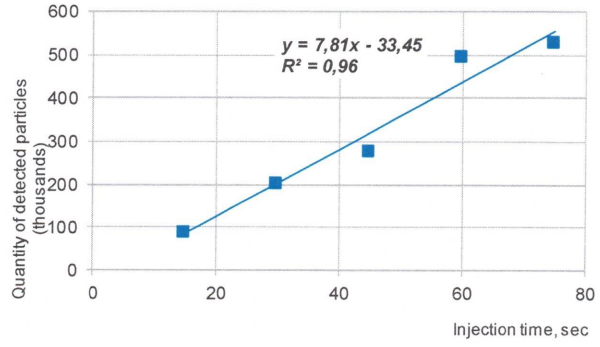
Tests with α-lactalbumin, 15.12.2020
Laboratory environment conditions: air temperature 21.5 ° C, relative humidity 41%.

For measuring tests with α-lactalbumin , 0.1mg of α-lactalbumin (α-Lactalbumin from bovine milk, manufacturer SIGMA Aldridge, batch SLC83030, code 1002007215, molecular weight 14.2kDa, dimension 2.3x2.6x4.0 nanometers [1]), dissolved in 5ml of distilled water, is injected into the reservoir of the compressor-evaporator. This amount of solution is sufficient for two tests.

TEST 1	Testing time	11.17-11.46
α-Lactalbumin	Molecular weight 14,2 kDa	
	dimension 2.3x2.6x4.0 nm	
№	Injection time, sec	Quantity, in 10^3
1	15	27,32
2	30	83,64
3	45	172,04
4	60	318,64



TEST 2	Testing time	11.58-12.39
α-Lactalbumin	Molecular weight 14,2 kDa	
	dimension 2.3x2.6x4.0 nm	
№	Injection time, sec	Quantity, in 10^3
1	15	89,04
2	30	202,08
3	45	275,92
4	60	494,16
5	75	528,72



CONCLUSION:

<u>As a result of the tests carried out, it was determined and / or confirmed:</u>

1. Optimal discretization of aerosol spraying duration - 15 seconds.
2. Correlation between aerosol spray volumes and measurements with the BD500 Biodetector is confirmed.
3. Since the tests used both a solution of bovine serum albumin (Albumine bovine serum, molecular weight 66kDa, hydrodynamic diameter 6 nanometers) and a solution of α-lactalbumin (α-Lactalbumin from bovine milk, molecular weight 14.2kDa, dimension 2.3x2.6x4.0 nanometers), the BD500's ability to detect bioaerosols with a diameter of 2-4 nanometers can be considered confirmed.

[1] https://books.google.ru/books?hl=en&lr=&id=0_ykbHl7yd0C&oi=fnd&pg=PR7&dq=alpha-Lactalbumin+dimensional&ots=1Nj4Bbf0PG&sig=X3WMxszcuuFOy_g_B0mraoAPwZk&redir_esc=y#v=onepage&q=alpha-Lactalbumin%20dimensional&f=false, p.11

PHARMAESTICA
manufacturing

WEIGHING PROTOCOL

Substance: Bovine serum albumin (Albumine bovine serum), manufacturer Acros Organics, batch A0404646, code 268130100

Used measuring instruments - analytical weight KERN ABJ 120-4NM, serial number WB14AK0218.
Date of previous calibration - 06/03/2020
Date of subsequent calibration - 06.2021

Weigh-in was carried out on 13.11.2020, 10.20

Weighing number and weight in grams:

1 - 0.0006 g
2 - 0.0023 g
3 - 0.0031 g
4 - 0.0014 g
5 - 0.0010 g
6 - 0.0014 g
7 - 0.0008 g
8 - 0.0011 g
9 - 0.0011 g
10 - 0.0009 g
11 - 0.0011 g
12 - 0.0006 g
13 - 0.0006 g
14 - 0.0006 g
15 - 0.0005 g
16 - 0.0012 g
17 - 0.0005 g
18 - 0.0002 g
19 - 0.0001 g
20 - 0.0004 g
21 - 0.0032 g

EESTI VABARIIK PharmaEstica Manufacturing OÜ reg. 10264929 TALLINN

O.Golubev
R&D Senior Specialist

Synthetische Luft N_2 / O_2

Bezeichnung / Kennzeichnung

Bezeichnung nach ADR

UN 1002 DRUCKLUFT, (LUFT VERDICHTET), 2.2, (E)

Behälterkennzeichnung

Schulterfarbe: leuchtend grün

Wesentliche Eigenschaften

verdichtetes Gas, farblos, geruchlos

Gefahrensymbole

Weitere Informationen entnehmen Sie bitte dem Sicherheitsdatenblatt O2-N2-024

Ventil / Armaturen

Ventilanschluss

DIN 477 Nr. 9: G 3/4"

Empfohlene Armaturen

Spectrolab FM 51, FM 52exact
Spectrocem FE 51 / FE 52exact

Spezifikation / Lieferformen

		Synthetische Luft 5.0 KW-frei	Synthetische Luft Scientific	
Zusammensetzung				
O_2	=	20,5	20,5	Vol.-%
N_2	=	79,5	79,5	Vol.-%
Nebenbestandteile				
CO_2	≤	0,5	0,1	ppmv
CO	≤	-	0,1	ppmv
KW (als CH_4)	≤	0,1	0,05	ppmv
NO_x	≤	0,1	0,01	ppmv
SO_2	≤	-	0,005	ppmv
H_2S	≤	-	0,005	ppmv
H_2O	≤	5	2	ppmv
Behälter/Inhalt				
F 10 200 bar		2,0	2,0	m^3
F 50 200 bar		9,8	9,8	m^3
F 50*12 200 bar		117,1	-	m^3

Hinweise

Herstelltoleranz +/- 0,5% abs.

In der Gaschromatographie Nullgas und Oxidationsgas für Flammen-Ionisations-Detektoren (FID)

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