SCIENTIFIC EVIDENCE OF POTOK AIR DECONTAMINATION TECHNOLOGY

potok®

CONTESTS

FOREWORD FROM POTOK

Mission of our company is to protect everyone from all types of bacteria, viruses and other harmful air-borne microorganisms and make breathing safe inside every premise all over the world

You are holding collection of reviews, research reports of Potok air decontamination solutions, that were conducted by a various internationally recognized leading research institutions, state medical facilities, food enterprises, schools and other social and commercial companies.

Potok Inter scientific and production company was founded in 1994 by Russian scientists, who had invented the Potok air decontamination technology, authors of scientific works and methodical instructions and holders of numerous patents.

The technology inactivates 99,99% of any types of airborne microorganisms (SARS-COV-2 and all other viruses, bacteria and mold).

Potok Inter ensures microbiological purity and reduces the level of microbial contamination of the air in orbital stations. In 1995 the Potok equipment solved the problem of colonies of mold fungi in the Mir orbital space station. In 2001 the Potok equipment was modified according to the cosmonauts' requests, delivered to the International Space Station (ISS), and installed in the Russian segment. In 2009 the Potok equipment was delivered to the US segment as ordered by NASA: in one week of operation, the unit reduced the level of molds in the air to zero (tenfold contamination excess was previously recorded).

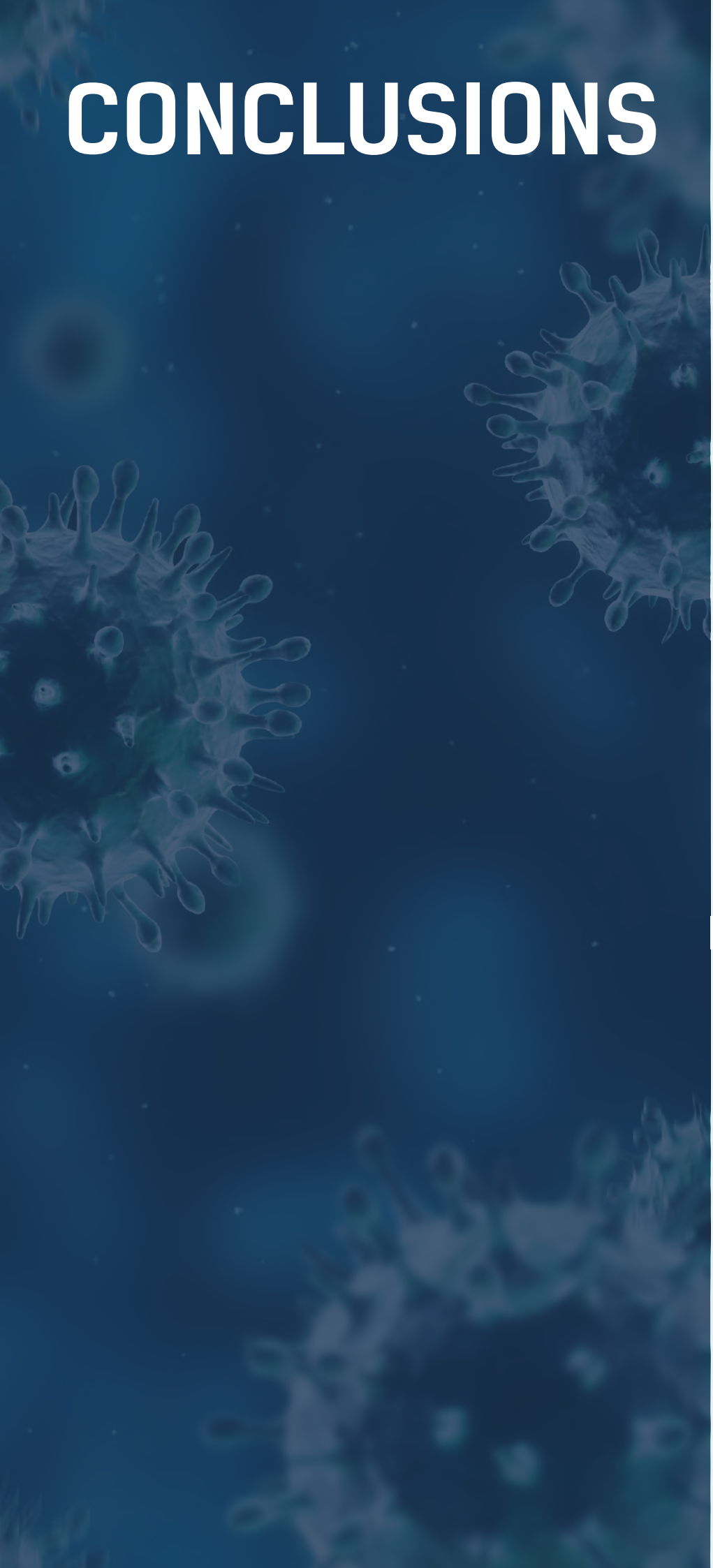
In addition to space technologies, Potok Inter has been dealing with problems on Earth for more than 25 years - conducting research and developing and manufacturing equipment for all spheres, where air purity is a matter of life and death.

The leadership position imposes on us special obligations for information transparency and openness of the company. We are confident that constant and effective cooperation with the media is the key to the successful development of our business. That is why we encourage you to familiarize yourselves with our main publications in scientific journals and mainstream media.

The tests are conducted by using the same Potok technology with a different types of air decontamination units. More detailed information about research methods is explained in each report.

We are hoping this publication offers you the required information about Potok technology, that has no analogues in the world.

CONCLUSIONS



HARVARD SCHOOL OF PUBLIC HEALTH

Department of Environmental Health
Environmental Science and Engineering Program

4 February 2003

Progress Report II, Phase I, Potok 150-M-01 Test Program

This is a report of progress to date on a program of microbiological tests performed on an Potok 150-M-01 stand-alone unit. The tests use microbiological aerosols produced by nebulizing selected organisms from an aqueous suspension containing synthetic saliva. After air drying, they are introduced into the entry of the unit under test. Representative samples of the airborne microorganisms are taken simultaneously up- and downstream of the unit under test with identical 6-stage Anderson Biological Cascade Impactors. Analysis of viable microorganisms collected by the impactors gives total numbers in the air up- and downstream of the unit, particle size of the microorganisms, and efficiency of the unit for removing microorganisms from the air passing through it.

Tests were conducted using the unit's low and high electrical settings and at the unit's rated air flow of 77 cubic feet per minute (CFM) and at 100 CFM..

Detailed test results are shown in the five pages of attached tables with statistical analyses that indicate the confidence intervals around the averages of the multiple replicate tests. They are well within acceptable limits for microbiological aerosol studies. The results for the five microorganisms tested up to this date are summarized as follows:

(1) B. subtilis spores, a commonly used surrogate for anthrax spores. When the unit was operated at its characteristic airflow rate (77 CFM) and at the high voltage setting it destroyed 99.5% of the spores. At the same airflow rate and at low electrical setting the kill rate was 94.4%. When operated at 100 CFM and the high electrical setting, spore killing efficiency was 98.8%. These comparative results are in the expected direction, greater lethal effect at higher voltage and at lower airflow rate, i.e., longer treatment time inside the unit.

(2) Serratia marcescens, a vegetative organism commonly found in nature and frequently used in microbiological aerosol studies. At high electrical setting and characteristic airflow rate, reduction in viable bacteria was 99.4%. At the same airflow rate but at the low electrical setting, bacteria reduction was 97.3%. At the higher airflow rate (100 CFM), removal efficiency at the high electrical setting was 99.0% and at the low electrical setting, 92,4%. The relative efficiencies were in an expected mode.

665 Huntington Avenue, Boston, Massachusetts 02115 Program Office (617) 432-1170 Fax (617) 432-3349

(3) Staphylococcus aureus, an organism commonly found in medical settings and currently under suspicion as a pathogen. At characteristic airflow rate and high electrical setting bacterial removal efficiency was 99.8%. At the same airflow rate but at the low electrical setting the removal efficiency was 97.6%. At an airflow rate of 100 CFM, removal efficiency was 99.4% at the high electrical setting and 97.0% at the low electrical setting.

(4) Pseudomonas aeruginosa, a vegetative bacterium sometimes found in medical settings and under suspicion as a pathogen. Unit efficiency for this microorganism at characteristic airflow rate and high electrical setting was 99.4%; at low electrical setting it was 98.0%. At the higher airflow rate, efficiency at high electrical setting was 99.5%. Although the kill percentage was slightly higher at the higher airflow rate in this case, the differences are well within the confidence intervals of each and within the variable nature of microbiological aerosol measurements.

(5) Aspergillus niger spores, a widely dispersed fungus in nature and frequently found in mold infestations in buildings, is a cause of respiratory illnesses. Unit efficiency for these spores was indistinguishable from total destruction when operated at low electrical setting with characteristic and elevated airflow rates. Tests were conducted with spores aerosolized from aqueous suspensions and with dry spores dispersed into the air stream. Efficiency was lower with dry spores. The reason for the difference is not known. Some additional testing may be called for here.

Results at the characteristic airflow rate (77 CFM) and high electrical setting are excellent and could probably be made better still by increasing electrical voltage inside the instrument and increasing retention time by making the treatment chamber larger.

We are nearly ready to test the performance of the unit with a vaccine, namely Vaccinia, the usual surrogate for smallpox.

Best wishes,

Melvin W. First

Melvin W. First, Sc. D.
Professor of Environmental Health
Engineering, Emeritus

Enclosure

UNIVERSIDAD DE GRANADA
FACULTAD DE FARMACIA
Departamento de Microbiología

ESTUDIO DEL TEST REALIZADO A "AIR-CLEANER UNIT POTOK 150"

Departamento de Microbiología.
Facultad de Farmacia.
Universidad de Granada.

Granada, Octubre 1995

UNIVERSIDAD DE GRANADA
FACULTAD DE FARMACIA
Departamento de Microbiología

ESTUDIO DEL TEST REALIZADO A "AIR-CLEANER UNIT POTOK 150"
(Departamento de Microbiología. Facultad de Farmacia. Universidad de Granada.)

Equipo utilizado:

- Unidad Potok 150
- Medio Nutritivo Tripticasa-Soja-Agar (T.S.A. Difco)
- Placas Petri esteriles de 90mm
- Bomba de vacio portatil Millipore
- Atomizador (diametro aproximado de particula en el aerosol de 2 micrones)
- Solución salina fisiológica

El ensayo se realizó en el ambiente de una habitación cerrada de aproximadamente 40 m^3 y con una media de dos personas trabajando en ella durante todo el tiempo de duración del ensayo.

Como microorganismo test se utilizó una cepa de *Micrococcus luteus* (ATCC 13513)

Metodología:

Previo al ensayo se realizó un estudio sobre el número de microorganismos presentes en la habitación en la que se realizaría el test. Para ello se distribuyeron placas con medio de cultivo TSA en distintas zonas de la habitación, y se mantuvieron abiertas los mismos tiempos que se emplearian en el test de la unidad Potok 150. A este estudio se le denominó ensayo control.

Posteriormente el estudio se realizó en dos etapas:

-Etapa I denominada SP (sin utilizar la unidad POTOK)

-Etapa II denominada CP (con el empleo de la unidad POTOK)

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FACULTAD DE FARMACIA
Departamento de Microbiología

En ambas etapas se utilizó una suspensión del microorganismo *M. luteus* en solución salina fisiológica con aproximadamente 10^8 células/ml. Dicha suspensión (15 ml) fué dispersada por la habitación por medio del atomizador durante 1 minuto y sin conectar la unidad POTOK. En el caso de la etapa II a partir de este momento fué conectada la unidad POTOK.

Tanto en la etapa I como en la II, posteriormente y a lo largo de 5 horas, con intervalos de 30 min y 60 minutos, se colocaron 4 placas Petri abiertas con medio nutritivo TSA en distintas zonas de la habitación a cada tiempo del ensayo.

Una vez realizado el ensayo las placas se incubaron a 37°C durante 24 horas.

Resultados:

Los resultados obtenidos se muestran en las tablas 1, 2 y 3.

Tabla 1 . Resultados obtenidos en el ensayo control (n° de microorganismos presentes de forma regular en la habitación de ensayo)

	Número de colonias en placa			
	Zona 1	Zona 2	Zona 3	Zona 4
	9	10	11	13

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FACULTAD DE FARMACIA
Departamento de Microbiología

Tabla 2. Resultados obtenidos en la etapa I (sin utilización de la unidad POTOK).

Solución de microorganismos de partida 1,94 x 10^8 células/ml.

Tiempo en minutos	Número de colonias en placa			
	Zona 1	Zona 2	Zona 3	Zona 4
1	2128	2180	2240	2228
30	1076	1008	997	1044
90	133	110	109	134
150	38	37	46	35
210	33	35	44	33
270	12	11	18	13

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FACULTAD DE FARMACIA
Departamento de Microbiología

Tabla 3. Resultados obtenidos en la etapa II (con utilización de la unidad POTOK).

Solución de microorganismos de partida 2,25 x 10^8 células/ml.

Tiempo en minutos	Número de colonias en placa			
	Zona 1	Zona 2	Zona 3	Zona 4
1	2680	2840	2768	2640
30	288	296	352	332
90	10	12	14	15
150	5	8	5	9
210	1	3	4	4
270	0	1	1	0

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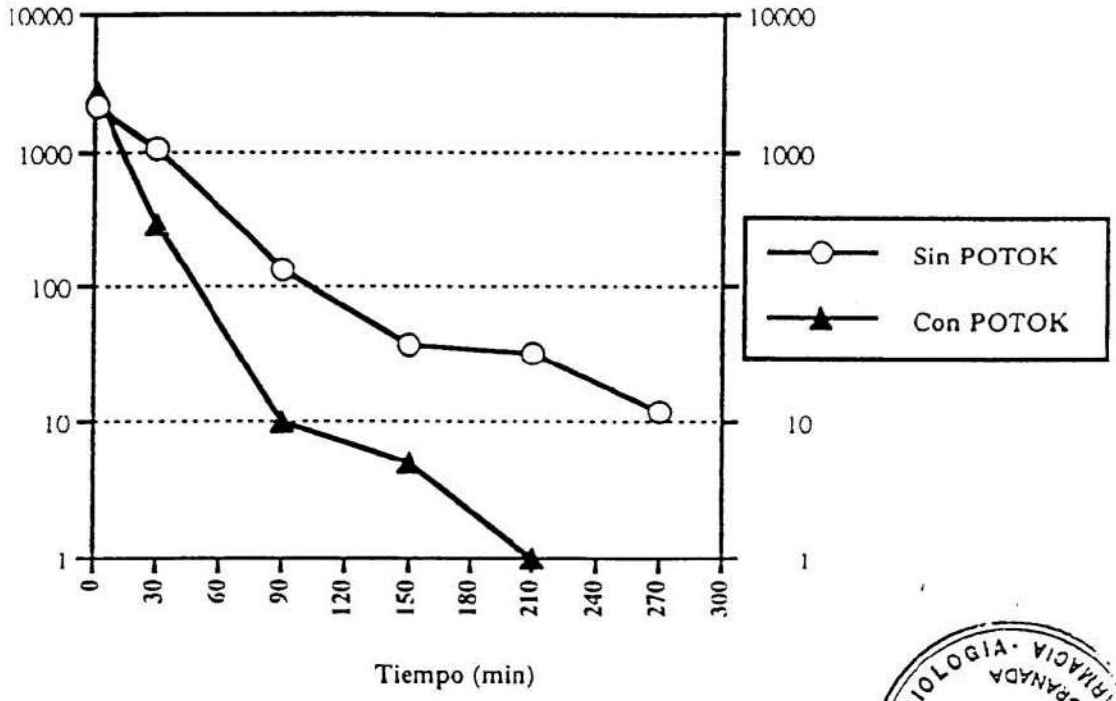
Departamento de Microbiología

Fig.1 Número de colonias



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FACULTAD DE FARMACIA
Departamento de Microbiología

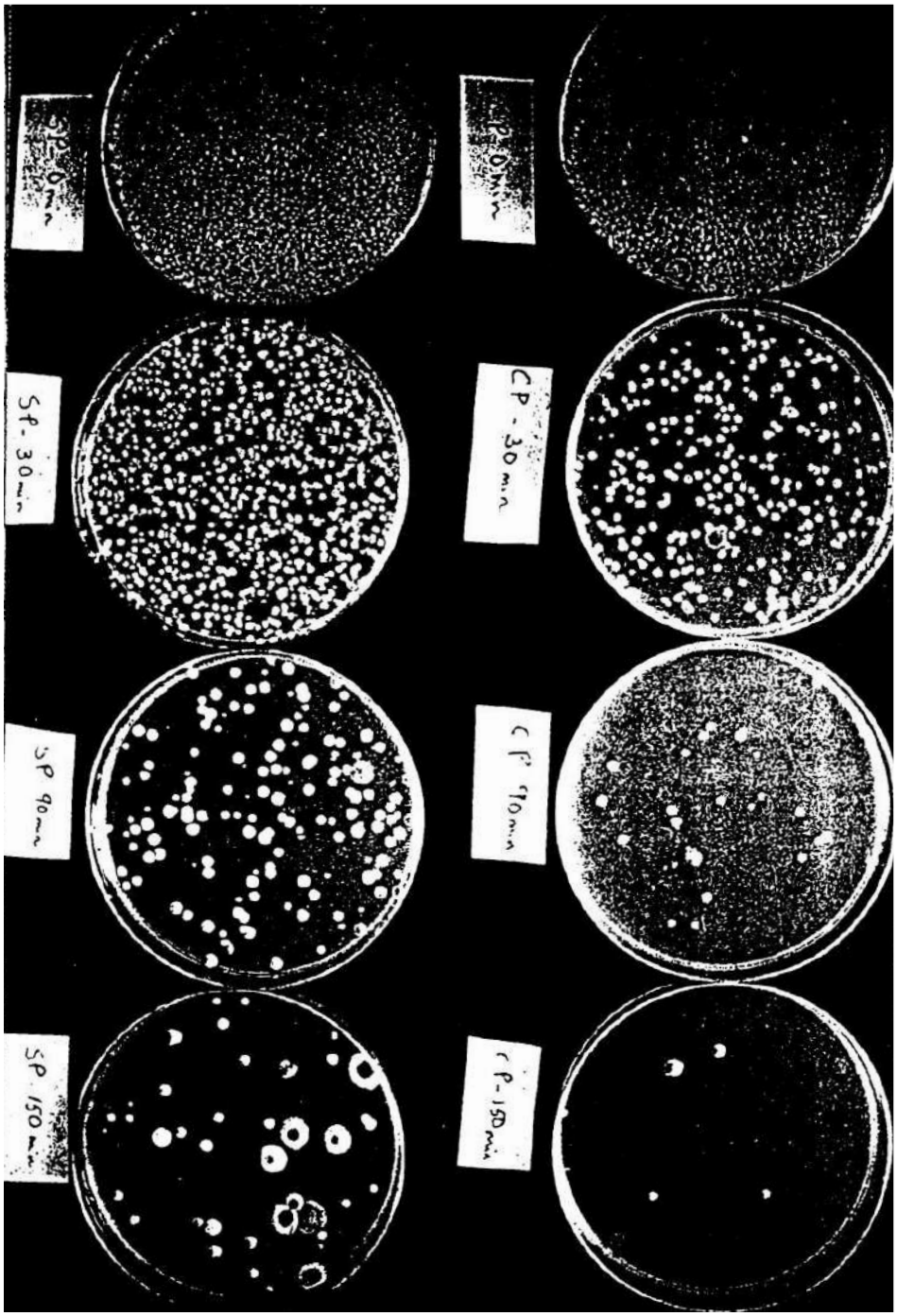
Conclusión

En relación a los resultados obtenidos en nuestro ensayo podemos deducir que el empleo de la unidad POTOK 150 origina una redución de la microbiota presente en el aire de aproximadamente 1/200 en 90 minutos, mientras que la reducción normal sin utilización de la unidad se encuentra sobre 1/20 en el mismo tiempo (Figura 1). Este resultado implica que es posible emplear la unidad POTOK 150 para crear ambientes limpios de microorganismos en un tiempo no demasiado largo y mantener el ambiente en dichas condiciones mientras que la unidad POTOK 150 se encuentra en funcionamiento.

DPTO. DE MICROBIOLOGIA - UNIVERSIDAD DE GRANADA - FACULTAD DE FARMACIA

Fdo.:Alberto Ramos Cormenzana
Catedrático de Microbiología

Fdo.: Mercedes Monteoliva Sanchez
Prof. Titular de Microbiología


SP-0 min
SP- 30 min
SP 90 min
SP 150 min
CP-0 min
CP - 30 min
CP 90 min
CP-150 min

ercem

Référence : GS 103024 01
Etb / Bac : 34881/2
Type C.R.A : N

Président Directeur Général : Philippe GUY

Intervention réalisée par : B. LIGNON
Unité Opérationnelle Rhône Auvergne
14 Rue Gorge du Loup Bât. C Bureau 123
69004 LYON
Tél : 78 47 00 10 Fax : 78 47 00 20

AD
Messieurs Cappeaux et Carbonel
17 Rue de BOULOGNE
[illegible]

Grenoble, le 24 Août 1995

COMPTE-RENDU D'ANALYSE MICROBIOLOGIQUE

Échantillons : APPAREIL DE TYPE POTOK ISOM
Identification du lot : GS 103024 01

Objet : Vérification de la production d'air stérile

RESULTATS

Objectif de l'étude :

Tester l'appareil vis-à-vis d'une souche de Bacillus Subtilis afin de vérifier qu'il produit bien de l'air stérile en sortie d'appareil.

Protocole

Une souche de Bacillus Subtilis est pulvérisée à l'entrée de l'appareil à raison de 5.10 4 micro-organismes par pulvérisation.
Après fonctionnement de l'appareil, un dispositif de réception de micro-organismes (boîte de pétri + milieux de culture) est installé à la sortie de l'appareil. Ce dispositif se trouvant dans un manchon de plastique parfaitement étanche, 5 passages de micro-organismes sont réalisés, les instructions d'usage étant fournies par le client.

Résultats

On note l'absence de culture sur les cinq passages.

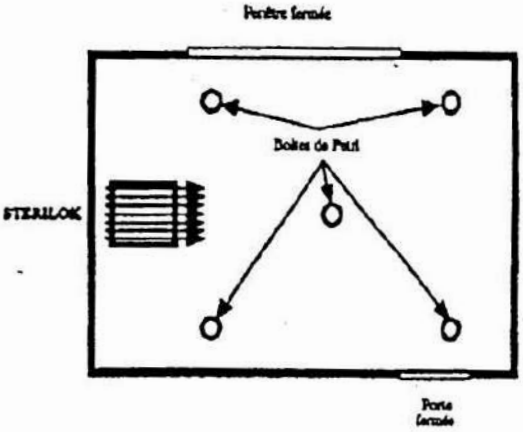
B. LIGNON

TESTS du Laboratoire ERCEM

LYON, le 27 juin 1995

Le laboratoire ERCEM de Lyon a effectué le 27 juin 1995, des mesures pour contrôler l'efficacité de l'appareil STERILOK, pour stériliser une pièce de 36,8 m3.

Le STERILOK était placé dans la pièce suivant le schéma ci-contre :



Modalités du test

Cinq boîtes de prélèvement de 23,7 cm2 contenant un milieu de culture PCA sont mises à 1 m de hauteur dans une pièce fermée de 36,8 m3. L'appareil STERILOK est placé dans cette pièce suivant schéma, à 1 m du sol.

Le test a été répété 4 fois :

- 1 heure après la mise en fonctionnement
- puis 2,3,4 heures après

Les germes recherchés sont des germes aérobies Mésophiles (ensemble des bactéries se développant en milieu présentant de l'oxygène et à température moyenne optimum de croissance : de + 20°C à + 40°C)

Les milieux ont ensuite été incubés 48 heures à 30°C.

Nota: la température de la pièce était de 27,8°C.

RESULTATS

REFERENCES	HEURES	RESULTAT G.A.M.	CONCLUSION
GH 9792801	H+1	Stérile	Excellent
GH 9792802	H+1	Stérile	Excellent
GH 9792803	H+1	Stérile	Excellent
GH 9792804	H+1	Stérile	Excellent
GH 9792805	H+1	Stérile	Excellent
GH 9792806	H+2	Stérile	Excellent
GH 9792807	H+2	Stérile	Excellent
GH 9792808	H+2	Stérile	Excellent
GH 9792809	H+2	Stérile	Excellent
GH 9792810	H+2	Stérile	Excellent
GH 9792811	H+3	Stérile	Excellent
GH 9792812	H+3	Stérile	Excellent
GH 9792813	H+3	Stérile	Excellent
GH 9792814	H+3	Stérile	Excellent
GH 9792815	H+3	Stérile	Excellent
GH 9792816	H+4	Stérile	Excellent
GH 9792817	H+4	Stérile	Excellent
GH 9792818	H+4	Stérile	Excellent
GH 9792819	H+4	Stérile	Excellent
GH 9792820	H+4	Stérile	Excellent

c/c Mᵉ CAPIAUX

Référence : GH 97928
Type C.R.A : N

Intervention réalisée par : J. DELPECH
Unité Opérationnelle 3
14 rue gorge de loup - Bat C - Bur 123
69008 LYON
Tel : 78.47.66.18 Fax : 78.47.66.23

RESULTATS DES ANALYSES BACTERIOLOGIQUES D'ATMOSPHERE DU 27 JUIN 1995

Appareil testé : POTOK

1 - MODALITÉ DU TEST :

Cinq boites de prélèvements de 23,7 cm2 contenant un milieu PCA sont mises à 1 mètre de hauteur dans une pièce fermée.

Les boites sont disposées de la manière suivante :

1 boite au centre de la pièce et 1 boite dans chaque angle.

Le test a été répété 4 fois :

1 heure après la mise en état de fonctionnement de l'appareil POTOK puis 2 heures, 3 heures et 4 heures.

L'apparcil POTOK est situé à 1 mètre du sol au milieu des 2 boites placées dans des angles.

2 - RESULTATS

REFERENCES	HEURES	RESULAT GAM	CONCLUSION
GH 9792801	H+1	Absence	Satisfaisant
GH 9792802	H+1	Absence	Satisfaisant
GH 9792803	H+1	Absence	Satisfaisant
GH 9792804	H+1	Absence	Satisfaisant
GH 9792805	H+1	Absence	Satisfaisant
GH 9792806	H+2	Absence	Satisfaisant
GH 9792807	H+2	Absence	Satisfaisant
GH 9792808	H+2	Absence	Satisfaisant
GH 9792809	H+2	Absence	Satisfaisant
GH 97928010	H+2	Absence	Satisfaisant
GH 97928011	H+3	Absence	Satisfaisant
GH 97928012	H+3	Absence	Satisfaisant
GH 97928013	H+3	Absence	Satisfaisant
GH 97928014	H+3	Absence	Satisfaisant
GH 97928015	H+3	Absence	Satisfaisant
GH 97928016	H+4	Absence	Satisfaisant
GH 97928017	H+4	Absence	Satisfaisant
GH 97928018	H+4	Absence	Satisfaisant
GH 97928019	H+4	Absence	Satisfaisant
GH 97928020	H+4	Absence	Satisfaisant

Les germes recherchés sont :

Les Germes Aérobies Mésophiles
(ensemble des bactéries se développant en milieu présentant de l'oxygène et à température moyenne optimum de croissance : +20 à +40°C).

Les milieux ont ensuite été incubés 48 heures à 30°C.

Remarque : La température de la pièce était de +27,8°C.
Ce résultat ne peut en aucun cas être interprété comme un comptage particulaire.

J. DELPECH

7220-1060-8752-3070

TEST REPORT

1. NO : CT18-092506

Reissuance(R1)
Date : 2018.12.10

2. Client
   - ○ Name : Loofen Co.,Ltd.
   - ○ Address : 916-ho, C-dong, 40, Imi-ro, Uiwang-si, Gyeonggi-do, Republic of Korea
3. Date of Test : 2018.08.24 ~ 2018.10.24
4. Use of Report : Quality control
5. Test Sample : Air-decontamination appliance(Potok 150-M-01)
6. Test Method
   (1) Client's requirement method

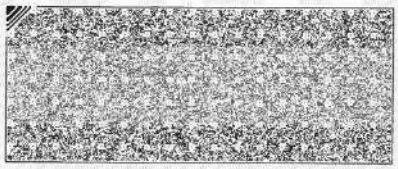
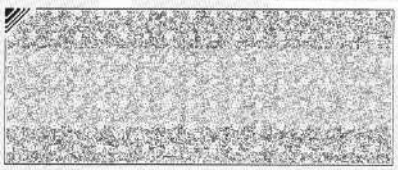
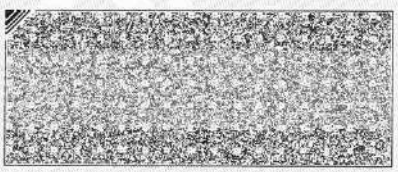
Affirmation	Tested By Name : Kye Seung Chang JKS	Technical Manager Name : Sang Bok Bae Sangbok

Our report apply only to the standards or procedures identified and to the sample(s) tested unless otherwise specified. The test results are not indicative of representative of the qualities of the qualities of the lot from which the sample was taken or of apparently identical or similar products. The authenticity of this test report can be checked on KCL website(www.kcl.re.kr).

2018.10.24

Korea Conformity Laboratories President Yoon, Kap Seok Yoon, Kapseok

Address : #805, I' VALLEY Gunpo,149,Gongdan-ro,Gunpo-si,Gyeonggi-do,15845,Korea 82-31-389-9100
Result Inquiry : The Center of Green Complex Technologies 82-31-389-9184

TEST REPORT

No : CT18-092506

7. Test Results

Test Items		Test method	Test Results			Testing Environment
			Before operating Conc.(CFU/㎥)	After operating Conc.(CFU/㎥)	Reduction rate of bacteria(%)	
Reduction test for Airborne microbes (*Escherichia coli*)	Air-decontamination appliance (Potok 150-M-01)	Client's requirement method	1.1×10^4	< 10	99.9	(23.2 ± 0.2) ℃ (50.3 ± 2.0) %R.H.

※ CFU : Colony Forming Unit

※ Test strain : *Escherichia coli* ATCC 25922

※ Chamber size : 8 ㎥

※ Measurement equipment : MAS-100 NT (MERCK, Flow rate : 100 L/min)

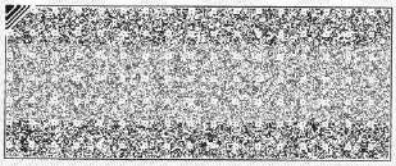
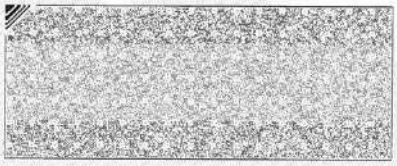
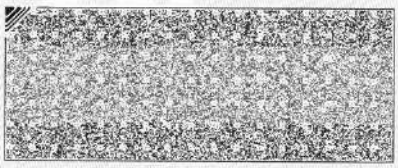
※ Sample : Air-decontamination appliance(Potok 150-M-01)

※ Operating time : 3 hours

※ Result concentration : Feller Conversion Table application

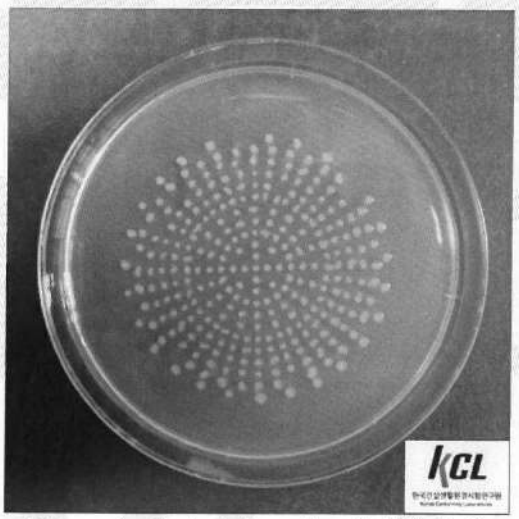
※ Client's requirement method : After injecting a constant concentration of target bacteria inside the test chamber and operating the sample for 3 hours, measure the reduction rate of bacteria.

※ Chamber environment and sampling method : KS I 2008:2013 Mod.

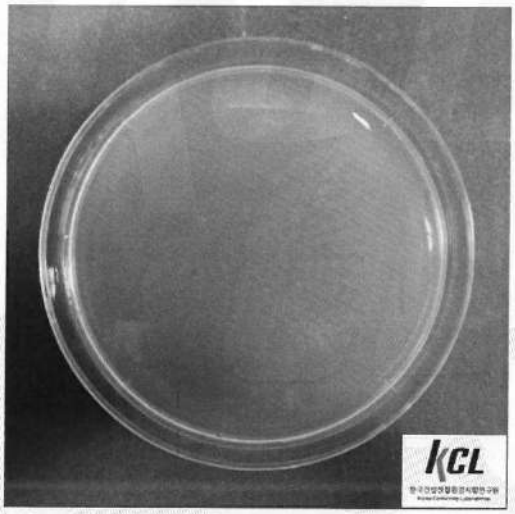




TEST REPORT

No : CT18-092506



<Picture 1. *Escherichia coli* - BLANK (0 h)>



<Picture 2. *Escherichia coli* - Air-decontamination appliance (Potok 150-M-01) (3 h)>

TEST REPORT

No : CT18-092506

<Picture 3. Sample[Air-decontamination appliance(Potok 150-M-01)]>

----- End of Report -----

National Public Health Institute
1097 Budapest, Albert Flórián út 2-6.

For G and G Instruments
Ltd. 1182 Budapest,
Hímesháza u. 12.

Reg. no.:KÖZ-7298-3/2017
Referent:
Dr. Szigeti Tamás, Dr. Magyar Donát
Subject: Testing effectiveness of air decontamination equipment

EXPERTISE

on testing effectiveness of „POTOK" air decontamination equipment, distributed by
G and G Instruments Ltd.

National Public Health Institute
2017.

National Public Health Institute
1097 Budapest, Albert Flórián út 2-6.

Content:

1. INTRODUCTION

The Air Hygiene and Aerobiology Department of the National Public Health Institute (further Institute received a request from G and G Instruments Ltd. (1182 Budapest, Hímesháza u. 12., further Principal) for testing „POTOK" air decontamination equipment distributed by the Principal on the efficiency of the operation of the equipment.
The Principal considered the investigation to be necessary because it would like to justify the effectiveness of the equipment on the basis of the Institute's investigations and expert opinion.

2. HISTORY

The Principal has provided the Institute with its promotional material, which states that the "POTOK" air decontamination device inactivates the airborne microorganisms (bacteria, viruses, molds) with 99% efficiency and removes the inactivated biological contaminants and fine aerosol particles from the air.
The Principal indicated that the use of the appliance is primarily intended to improve the air quality of medical offices and hospital premises.

3. OBJECTIVE/PURPOSE

The purpose of the air quality test shall be the determination of the concentration of aerosol particles with an aerodynamic diameter of less than 1, 2, 5 and 10 µm diameter (ranges $PM_{1,0;}$ $PM_{2,5}$, PM_{10}), volatile organic compounds, aldehydes, biological agents) in the airspace of a room of relevance for prior to use and after use of the equipment.

4. SAMPLING/TESTING PLAN

The staff of the Institute's Department of Air Hygiene and Aerobiology have designed a sampling / measurement plan for the efficiency of air purification equipment, which is used equally for all air purification equipment.

The sampling/testing plan is the following:

Determination of the mass concentration of aerosol particles with an aerodynamic diameter of less than 1, 2,5 és 10 µm with Grimm 1.108 aerosol spectrometer on a single point continuously during the test period (1 minute time resolution).

Active sampling of volatile organic compounds on a Tenax TA thermal desorption sampling tube at a sampling point two hours before activation of the air purification system two hours after switch-on, one-hour sampling time (sample volume of 4.8 liters) and analysis of samples by thermal desorption / capillary gas chromatography (according to ISO 16017-1: 2001).

Active sampling of aldehydes (sodium iodide and 2,4-dinitrophenylhydrazine coated silica-gel sampling tube) at a sampling point two hours before the air cleaner was switched on and two hours after the device was switched on, with a sampling time of one hour (volume of sample: 60 L) and sample analysis by liquid chromatography (according to ISO 16000-3: 2011 standard).

In the case of molds and bacteria, sampling was carried out with an Andresen-type (MAS 100) air sampler at the given test day 4 occasions:

a) Approx. two hours before the air decontamination device was switched on;
(b) The room volume of air has been exchanged once;
(c) After two times exchanged the air volume of the room;
d) After three times exchanged the air volume of the room.

During the air sampling operation, 100-100 L of air is sucked by the sampler, and the air intake is collided by the inserted medium, which adsorbs the bacteria/fungal spores from the air. To determine allergenic molds, chloramphenicol containing 2% malt extract agar was used, incubated at 25 ° C for 5 days. To detect all colony-forming bacteria, we used blood agar at 37 ° C for 3 days incubated. The results are given in colony-forming units (CFU / m^3).
During the evaluation, the total bacterial counts were determined (CFU). The number of colony-forming units was adjusted according to the Feller table assigned to the device.
For molds, each colony-forming unit was typed on a genus level, and a total number of colonies were given per sample. Here we also made the Feller correction

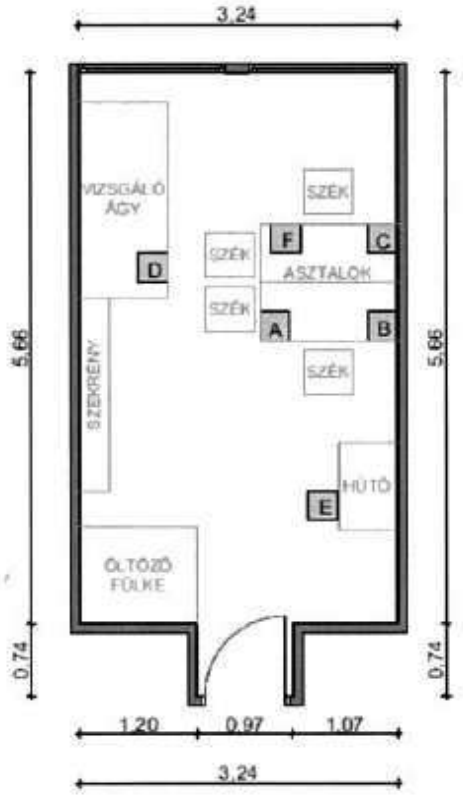
Measurement of temperature and relative humidity (IAQ-CALC indoor Air Quality Meters 7545; TSI Inc.) continuously on a test point during the test period (with 1 minute time resolution).

Further specifications, recommendations for sampling, measurements and evaluation, we considered:

- 1995. LIII. Act on the Protection of the Environment;
- 306/2010. (XII.23) Government Decree on Air Protection;
- 4/201 1. (1.14.) VM Regulation on limit values for airborne loads and emission limit values for stationary sources of air pollutants;
- MSZ 21460-1: 1988 Definitions of air purity protection. Definitions of general terms (MSZ – Hungarian Standard);
- MSZ ISO 4225: 1995 Air quality. General considerations. Concept Definitions;
- WHO: Guidelines for indoor air quality: selected pollutants, 2010.

Sampling / on-site measurements were performed in a medical office (Figures 1 and 2) at the National Public Health Institute on 3 November 2017. Nominal data of the equipment (130 m3/h air flow) and volume of room air space (57.18 m3) based on the air purifier unit for about 26 minutes once full air volume of the test room.

1. Fig. Lay - out of POTOK air decontamination equipment testing.



A: sampling bacteria and fungi
B: sampling of aldehydes
C: sampling of volatile organic compounds
D: POTOK air purification equipment
E: temperature, relative humidity measurement
F: mass concentration of aerosol particles

2. Fig. Sampling location

5. TESTING RESULTS AND CONCLUSIONS

The results of the studies are shown in Figures 1-4. and in Figure 3.

Table 1: Time variation of the concentration of aerosol particles with an aerodynamic diameter of less than 1, 2.5 and 10 µm.

	Prior to switch on	1 hour after switching	2 hours after switching on	3 hours switching on
PM_{10} [µg / m^3]	8,4	2,1	1,9	2,1
$PM_{2,5}$ [µg / m^3]	4,2	1,7	1,5	1,4
$PM_{1,0}$ [µg / m^3]	3,1	1,5	1,2	1,1

The concentration of aerosol particles in the room was low even before the equipment was switched on but the mass concentration of the aerosol particles continued to decrease during the intended operation (Table 1).

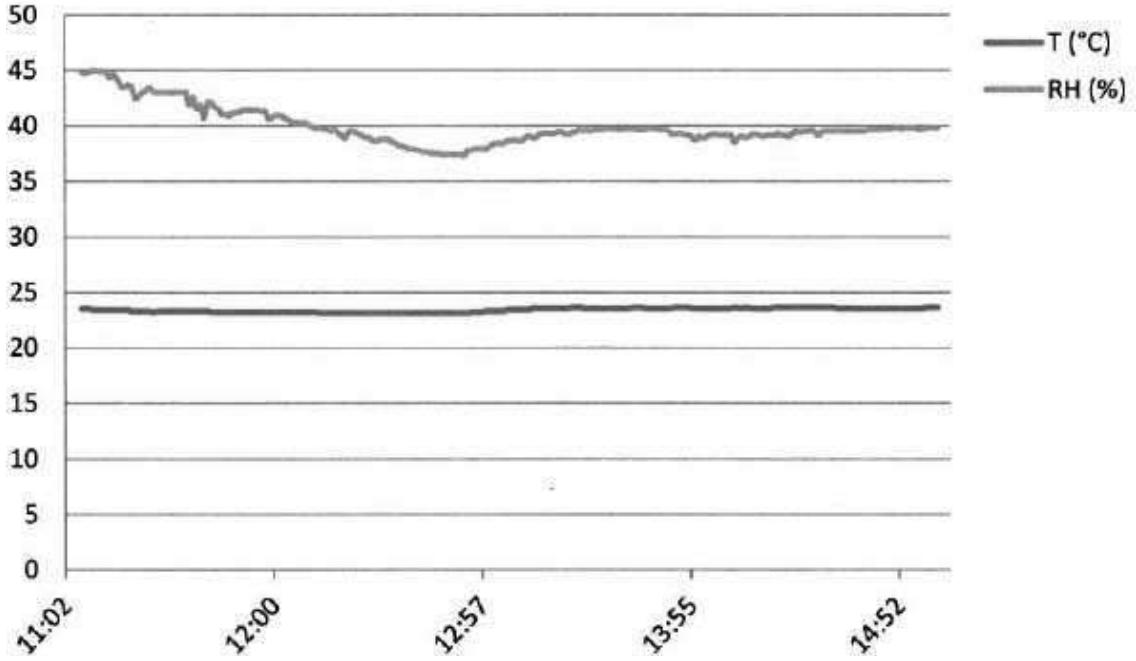
Table 2: Time-varying concentrations of selected volatile organic compounds and aldehydes.

Organic compounds	Before switching	After switching
Formaldehyde	17,0	16,9
Acetaldehyde	34,1	30,7
Benzaldehyde	1,7	<0,75
Hexaldehyde	3,2	3,2
Benzene	1,1	< 0,1
Toluene	3,4	2,8
Ethilbenzene	< 0,1	< 0,1
Xylene	1,2	< 0,3
Alpha-pinene	1,4	1,5
s-limonene	3,0	2,6
Naphtalene	<2,0	<2,0

The equipment did not reduce the concentration of volatile organic compounds and aldehydes during their intended use (Table 2).

During normal operation of the equipment, the temperature and relative humidity did not change significantly (Figure 3).

3. Fig.Changes in temperature and relative humidity during the test.



3. Table: Changes of total number of bacteria during the testing

Time of measurement	Marks of measurement	POTOK air decont. device test (PL=PAD)	Total number of Bacteria, CFU / m^3
2017.11.03_10:01	5LEG1B	Control PAD before testing	470
2017.11.03_13:02	5LEG2B	POTOK changed the air 1x in the room	150
2017.11.03_14:01	5LEG3B	POTOK changed the air 2x in the room	40
2017.11.03_15:01	5LEG4B	POTOK changed the air 3x in the room	80

All bacterial counts in the air samples significantly decreased by the use of POTOK air decontamination equipment. After three times the air reversal a 83% reduction of bacteria counts in atmospheric concentration was measured.

4.Table: Changes of total number of Molds during the testing

Time of measurement	Marks of measurement	POTOK Air decont. device tests (PL=PAD)	Total number of Molds, CFU / m^3
2017.11.03_10.06	5LEG1B	Control, before switching on POTOK	85
2017.11.03_13:05	5LEG2B	POTOK changed the air 1x in the room	75
2017.11.03_14:04	5LEG3B	POTOK changed the air 2x in the room	45
2017.11.03_15:05	5LEG4B	POTOK changed the air 3x in the room	25

A more detailed evaluation of the mold growth test is provided in the Annex.

In the air samples, the total number of molds in molds significantly decreased the use of POTOK air decontamination equipment. After three times the air revolutions, 70% of the atmospheric decreases in molds were measured.

5._SUMMARY

Based on the results of the tests carried out by the National Institute of Public Health, the "POTOK" air decontamination equipment, marketed by G and G Instruments, effectively reduces the concentration of small aerosol particles and the total number of bacteria and molds in the indoor air during normal use. The atmospheric concentration of volatile organic compounds and aldehydes will not be affected.

Based on the results of the examinations, the National Public Health Institute does not raise objections to the intended use of the device and recommends its use and shall award a certificate with serial number 2017/4.

Budapest, 2017. december 18.

……... ..…
Dr. Szigeti Tamás
témafelelős

.....
Dr. Pándics Tamás
igazgató

Appendix

Time of measurement	Marks of samples	POTOK Air Decontamination Device tests (PL = PAD)	Molds taxon	Total number of Molds [CFU /m^3]
2017.11.03. 10:06	5LEG1G			
			Cladosporium sp.	30
			Non spore forming	60
			Total	90
2017.11.03. 10:11	5LEG1Gí			
	Control, prior to switching on POTOK		*Aspergillus niger*	10
			Penicillium sp.	20
			Cladosporium sp.	20
			Scopulariopsis sp.	10
			Non spore forming	20
			Total	80
2017.11.03. 13:05	5LÉG2G			
	POTOK changed the air 1x in the room		*Cladosporium* sp.	30
			Penicillium sp.	10
			Yeast spp.	10
			Non spore forming spp.	20
			Total	70
2017.11.03. 13:09	5LEG2Gí			
	POTOK changed the air 1x in the room		*Cladosporium* sp.	50
			Penicillium sp.	20
			Non spore forming spp.	10
			Total	80
2017.11.03. 14:04	5LEG3G			
	POTOK changed the air 2x in the room		*Cladosporium* sp.	30
			Penicillium sp.	20
			Total	50
2017.11.03. 14:08	5LEG3Gi			
	POTOK changed the air 2x in the room		*Cladosporium* sp.	20
			Non spore forming spp.	20
			Total	40

National Public Health Institute
1097 Budapest, Albert Flórián út 2-6

Tine of measur	Marks of samle	POTOK Air Decontamination Device tests (PL	Molds taxon	Total number of Molds [CFU /m^3]
2017.11.03. 15:05	5LEG4G			
	POTOK changed the air 3x in the room		*Cladosporium* sp.	30
			Penícillium sp.	10
			Total	40
2017.11.03. 15:07	5LEG4Gi			
	POTOK changed the air 3x in the room		not sporulating spp.	10
			Total	10

Ostbayerische Technische Hochschule
Amberg-Weiden

Experiments for the evaluation of effectiveness for the Potok system

1. Introduction

Potok Inter Engineering Company is a renowned expert on air decontamination with a unique technology. It inactivates all types of airborne microorganisms (bacteria, viruses, fungi, mold, etc.) with approx. 99.999% efficiency within 1 second. The POTOK inactivation technology is based on a physical influence method with constant electric fields as well as with changing polarity and additionally fine filtration of inactivated biomass and aerosol particles. The Potok Inter Engineering Company designs and produces air decontamination devices of various types, but all based on inactivation technology:

• standalone units
• duct-in units
• laminar flow devices

All devices are patented in Russia, Ukraine, Japan, the US and some European countries.

The purpose of this work was to evaluate the decontamination potential of the Potok system both in an experimental setting in the research OR of the Ostbayerische Technische Hochschule Amberg-Weiden with standalone Air Decontamination Units (Potok 150-M-01) and in a clinical setting in an operating theater in a Moscow hospital where the laminar flow device is based on the Potok system.

2. Material and Methods

Two units of the system "Potok 150-M-01 standalone Air Decontamination Unit (ADU)" were available for the evaluation of the effectiveness at the Ostbayerische Technische Hochschule (Fig. 1).

Fig. 1: Potok 150-M-01 standalone Air Decontamination Unit (ADU)

The units were positioned in an experimental OR-setting according to the Swedish standard SIS-TS 39: 2015. The microbiological examination of the room air was done with the active air sampler Impaktor FH6 from Markus Klotz GmbH. Three parallel samplings were performed at predefined measuring points. These are located directly on the operating table (1.2m above the ground and ≤0.5m from the operating site), on the instrument table and in the periphery of the room near an exhaust opening. Sampling was done by impaction method, in which an air volume of 1000 liters was collected per 10 minutes via a columnar opening onto a blood agar plate. The culture media were then incubated for 3 days at 35 °C +/- 1 °C. After incubation the plates were photographed and colonies were counted manually and documented as colony forming units per cubic meter of air (cfu/m³).

Already in the pilot studies to this work the impact of the surgical clothing on the germ load in the operating room was investigated. On the basis of these results, Swedish surgical gowns were chosen as standard for further experiments. This so-called "Clean Air Suit" from Mölnlycke Healthcare is a disposable product and consists of polypropylene. (Fig. 2)

Fig. 2: Example of Swedish surgical clothing

The measurements were performed during a one-hour surgical simulation (6 measurements a 10 minutes). The simulated surgery was performed by 7 people. In order to simulate as much as possible a reality-oriented process, 4 persons represent the surgical team directly at the operating table, one person acts as an

anesthesiologist and 2 other persons move through the room during the surgical simulation. (Fig. 3)

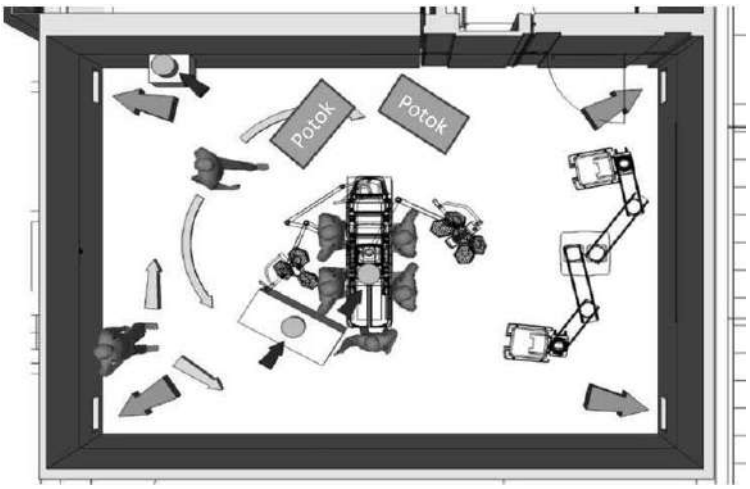


Fig. 3: Movement profile of the OR simulation, measurement points of the active air sampling and location of the Potok devices

In contrast to the standard systems the research OR at the Ostbayerische Technische Hochschule is equipped with a new temperature controlled airflow ventilation system (Opragon) that was developed by the Swedish company Avidicare AB. Opragon supplies the operative zone with slightly cooled, HEPA-filtered air (class H14) from an external air treatment unit equipped with a heating/cooling battery. The supply air is discharged through hemispherical air showers. To minimize the impact of the ventilation system it was set to at rest mode for the experiments (air exchange rate: 500m³/h). The OR in Weiden has a ground area of 41,78m² and a height of 2,90m. The Volume of the room amounts to 121,16m³.
In order to examine the influence of the Potok units on the bacterial burden of the room air and therefore the decontaminating capacity these experiments were initial performed without the Potok devices to obtain the background contamination of the research OR. Then the Potok units were switched on and after 24 hours a second identically measurement was done. It must be pointed out, that the standalone units are designed to provide local sterile zones at the OR table and the instrument board.

In order to compare the Potok technologies to other established ventilation systems the measurements were repeated in a clinical situation. Therefore the activity and

effectiveness of the Potok system was tested in a real-life setting in an operating theater in a hospital in Moscow. (Fig. 4)

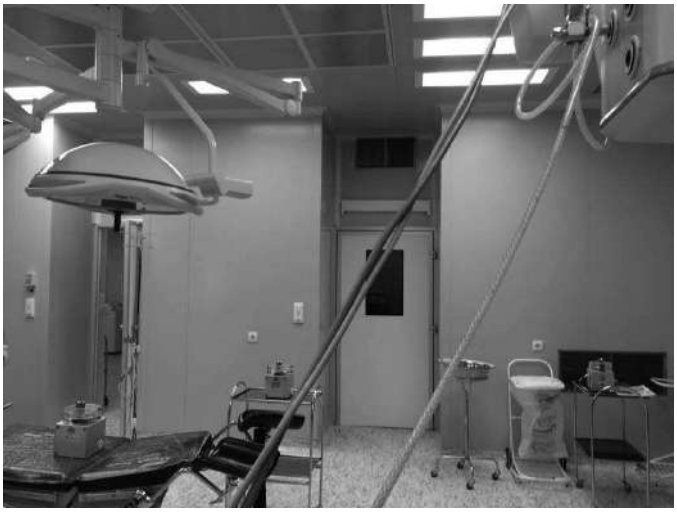


Fig. 4: Russian operating theater with installed Potok system

The OR was chosen after its technical details. (Fig. 5) Therefore it represents an average operating theater and ventilation system ideal for comparison with other technical solutions like temperature controlled airflow ventilation system [TAF], low turbulent uni-directional airflow [TAV] or turbulent mixed ventilation [TML].

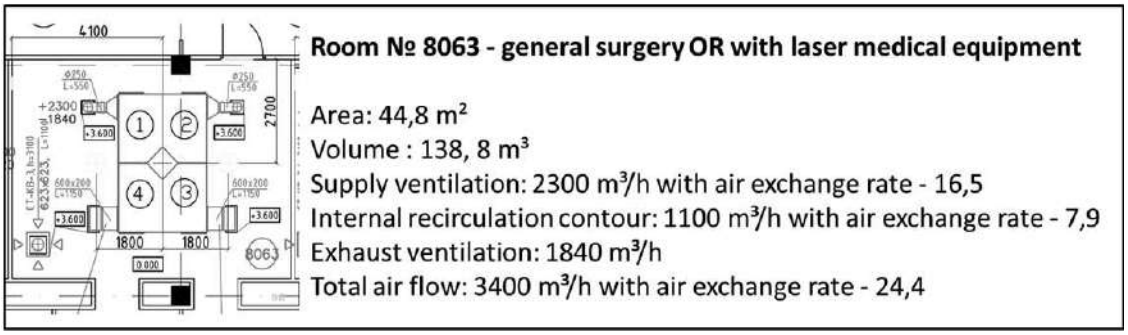
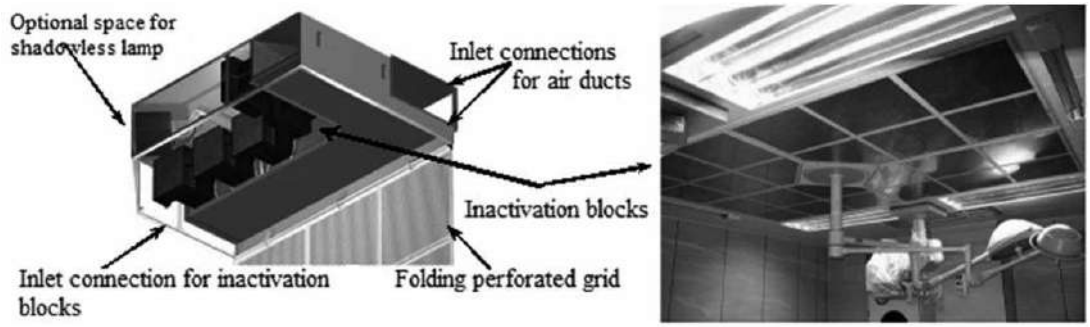


Fig. 5: Technical details of the Moscow operating theater

The OR is equipped with a TAV system based on POTOK technology. The unit consists of 4 inactivation blocks (to be installed as a part of hermetic false ceiling) with optional space for shadow less lamp. (Fig. 6)



Power Supply	220 V / 50 Hz
Power consumption of 4 blocks assembled	Max. 40 W
Capacity of 4 blocks assembled	2160 m^3/h - 3600 m^3/h
Inactivation efficiency rate	99.999%
One pass inactivation time	1 sec
Filtration class	H11-H14 (max.)
Aerodynamic resistance at nominal capacity	Max. 110 Pa
Inactivation efficiency control	Automatic
Dimensions of 4 blocks assembled	3600x2400x350 mm
Dimensions of inlet connections	600x200 mm
Warranty	5 years
Service life	Min. 10 years
Expendables	None

Fig. 6: Composition of the inactivation blocks and technical specifications

Again this was done according to the Swedish standard SIS-TS 39: 2015. The measurements were done for two hours during the surgical simulation (12 measurements a 10 minutes). The first measurements were done with the ventilation system of the OR switched off. Because of the technical situation of the OR the supply ventilation could not be turned off completely and led to an air circulation of 2300 m³/h. After that the complete system with the Potok devices was started again and after 24 hours the experiment was repeated adequately.

3. Results

Our experiments showed an impact of the Potok 150-M-01 standalone Air Decontamination Unit (ADU) on the bacterial contamination of the room air. For the

initial measurements in the research OR at the Ostbayerische Technische Hochschule in Weiden this could be shown by a decrease of the bacterial burden at all three different measurement points. It is to mention that the bacterial burden at the OR table and the instrument board are under the threshold level for the Swedish standard of ≤5 cfu/m³ (Fig. 7)

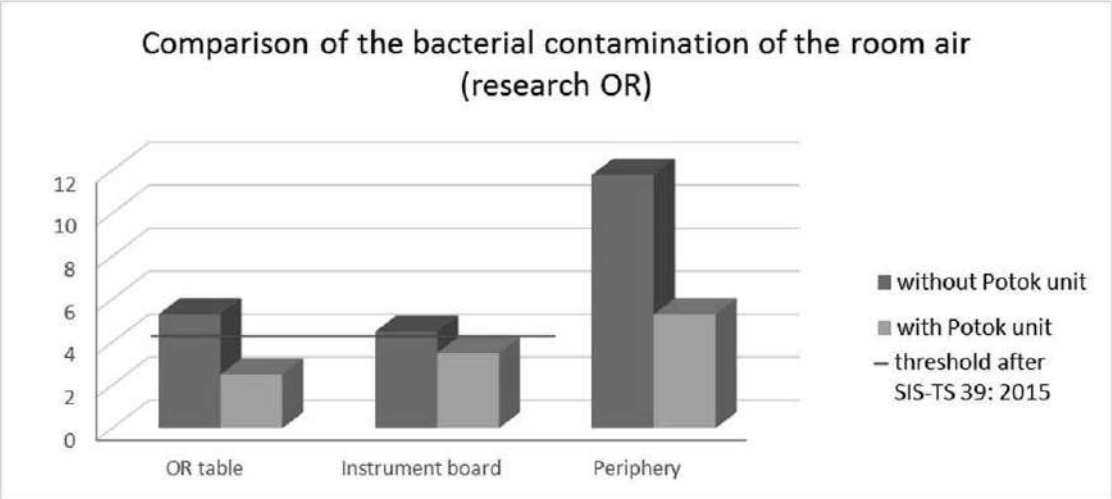


Fig. 7: Comparison of the bacterial contamination of the room air in the research OR

For the measurement directly on the OR table the activity of the units led to a decrease of the bacterial air contamination from 5 cfu/m³ to 3 cfu/m³ in average. For the Instrument table and the Periphery of the room this reduction was from 5 cfu/m³ and 12 cfu/m³ down to 4 cfu/m³ and 5 cfu/m³ respectively. (Tab. 1)

	without Potok unit		with Potok unit	
OR table		5		3
Instrument board		5		4
Periphery		12		5

Tab. 1: Average cfu/m³ for the 3 different measurement locations

Also the subsequently done measurements in the Moscow hospital verified this decontaminating effectivity of the Potok system. Because of the more realistic setting of the experiment the results of these measurements seem to be more conclusive. In this case the initial bacterial background of the operating theater was higher than in the research OR in Germany. This bacterial burden could be

effectively decreased by the use if the installed Potok based TAV ventilation system. (Fig. 8)

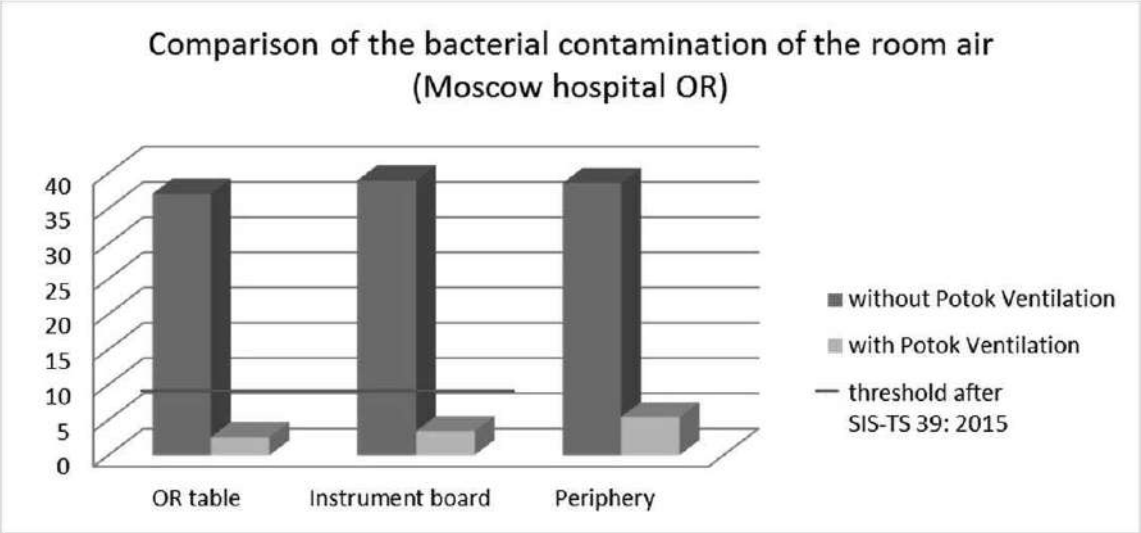


Fig. 8: Comparison of the bacterial contamination of the room air in the OR of the Moscow hospital

For the three different measurement locations our results showed a decrease of more than 87%. The initial bacterial burden of 37 cfu/m³ on the OR table and 39 cfu/m³ on the instrument board and the periphery of the room had been reduced to ≤5 cfu/m³ in average for every measurement point. (Tab. 2)

	without Potok unit		with Potok unit	
OR table		37		3
Instrument board		39		3
Periphery		39		5

Tab. 2: Average cfu/m³ for the 3 different measurement locations

4. Discussion

Our results showed a significant effect of the Potok system on the bacterial burden of the room air. In the experimental setup at the Research OR of the Ostbayerische Technische Hochschule Weiden this effect was rather slight due to the minimal initial microbiological contamination of the room and because of the use of only mobile units. However a decrease could be observed for every measurement point.
This decontaminating effect of the Potok technology was confirmed by the results of the measurements in the real-life setting of the operating theater in a Russian

hospital. Here the Potok ventilation system showed a significant and strong decrease of the airborne bacteria. In average the microbiological burden could be reduced down to ≤5 cfu/m³ for each measurement point. Compared with the results of measurements of already established ventilation systems (e.g. temperature controlled airflow ventilation system [TAF], low turbulent uni-directional airflow [TAV] or turbulent mixed ventilation [TML]) with the same experimental setup the Potok system proofed to be capable of achieving similar effectiveness as TAV and TAF system. The investigated operating theaters (TAF/TML/TAV) were certificated after DIN 1946 4:2008-12. They are classified as Ia and Ib and have an overall air exchange rate of 4120 m³/h (TML) and 9200 m³/h (TAV). The OR at the Hochschule Weiden (TAF) has an air exchange rate of 7700 m³/h. All Operating theaters are comparable regarding room size and furniture. (Fig. 9)

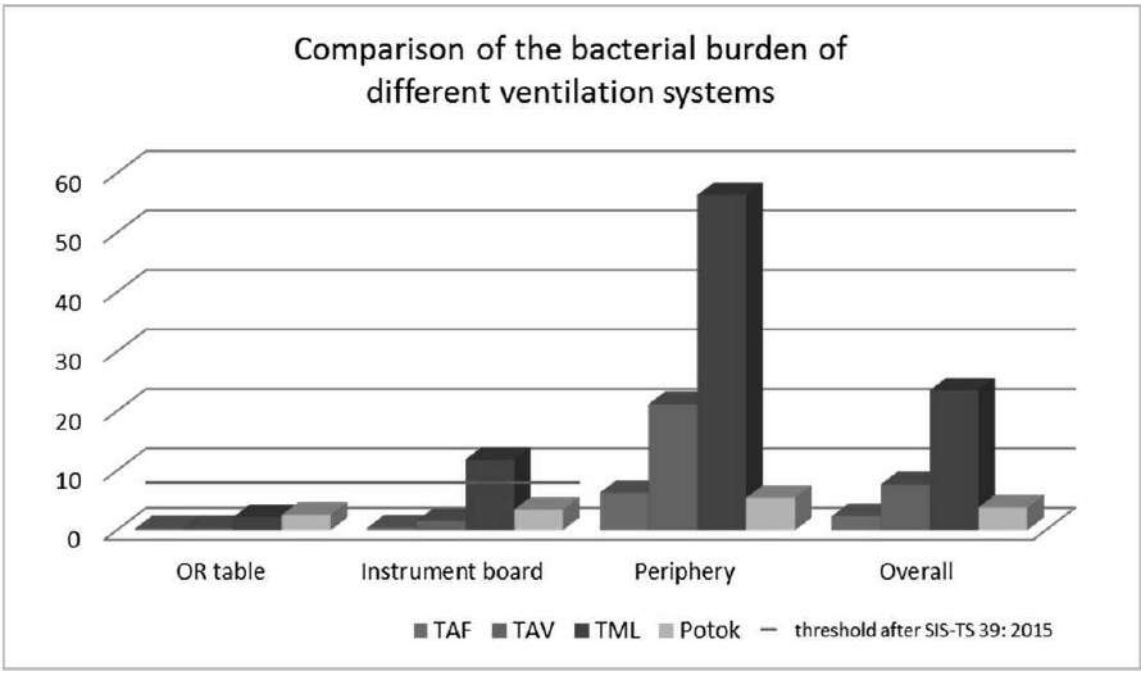


Fig. 9: Comparison of the bacterial burden in cfu/m³ for current types of ventilation systems

Additionally it is to point out that the bacterial burden on the OR table is reduced to ≤5 cfu/m³ in our measurements with the Potok decontamination system. This means that the Potok system is able to fulfill the specification for ventilation systems in the OR according to the Swedish SIS-TS 39: 2015.

With its high rate of decontamination and the following low germ contamination in the room the solution could also find use in the field of clean room technology. According to the EU GMP-Guide the threshold for a class B clean room is 10 cfu/m³. In our experiments this standard could be fulfilled with the Potok technology.

Another advantage of the Potok system is that there is no need for HEPA-filters. This means a reduced maintenance effort for the operator because there is no regular filter change.

A notable point is the energy saving aspect of the system. In our tested OR the total air exchange rate of the room was 3400m³/h with the Potok system. A comparable OR with a TAV technology has approximately an air exchange rate of 9200m³/h. And even the more energy efficient TAF system at the Ostbayerische Technische Hochschule has an total air exchange rate of 7700m³/h running with the 22 air shower set-up (air volume per shower = approx. 350 m³/h).

Based on our findings with the Potok system it should be discussed whether the technology has to be considered as a viable alternative to other currently used ventilation systems and whether it represents another potential solution for infection control of airborne microbiological burden of operating theaters.

Expert's report on the indiscriminate effect of the air decontamination unit "Potok 150-M-01" on all types of airborne microorganisms

Impact assessment of the air decontamination unit "Potok 150-M-01" on various types of airborne microorganisms was based on an analysis of the following materials:

1. The air decontamination method and unit for its implementation [Text]: Pat. 2541004 Russian Federation: IPC A 61 L 9/22, B03C 3/01 / Nagolkin A.V., Volodina E.V.; Applicants and patent holders Nagolkin A.V., Volodina E.V. - N 2013152726/03; declared 11/17/13; publ. 02/10/15, Bull. N 4. - 15 p.: Ill.

2. Device for inactivation and fine filtering of viruses and airborne microorganisms [Text]: Pat. 2344882 ROS. Federation: IPC B03C 3/14 / Volo effectiveness din A. M.; Applicant and patent holder A. Volodin M. - N 2007118919/12; declared 05/21/07; publ. 01/27/09, Bull. N 3. - 16 p.: Ill.

3. The study on the effectiveness of the air decontamination unit "Potok 150-M-01" on the inactivation of microorganisms and the impact on the structure of microbial cells [Text]: report on research / Research Center "BioResources and Ecology", Institute of Biochemistry and Physiology of Microorganisms named after G.K. Scriabin RAS (IBPM RAS); hands. V.A. Dmitrieva, A.M. Boronin, Doctor of Biological Sciences T.V. Kulakovskaya - Pushchino, 01.30.2012. - 16 p.

4. Investigations of the Potok air decontamination unit on inactivation of non-specific microflora and mycobacterium tuberculosis [Text]: report on research (conclusion): 89-309 / Central Research Institute of Tuberculosis RAMS; hands. V.V. Erokhin - Moscow, 03/26/2001. - 1 p.

5. Microbiological studies of the air decontamination unit "Potok 150-M-01" [Text]: report on the research study / RAMS of the GUNII of epidemiology and microbiology named

after honorary academician N. F. Gamalei; I. S. Tartakovsky; Performer: V.V. Petrosov - Moscow, 2002 .-- 28 p.

6. The effectiveness of the air decontamination unit "Potok 150-M-01" on the air decontamination which is used for removing avian influenza [Text]: report on research (conclusion) / Bioton LLC; hands. A.N. Sergeev - Novosibirsk, September 15, 2005. - 2 p.

7. The effectiveness of the air decontamination unit "Potok 150-M-01" which is used for removing the vaccinia virus [Text]: report on research (conclusion) / Bioton LLC; A.N. Sergeev - Novosibirsk, 10/05/2005. - 2 p.

8. Effectiveness evaluation of air decontamination with the “Potok 150-M-01” units in the premises of the Research Institute of Pediatric Oncology and Hematology of the Federal State Budget Scientific Center named after N. N. Blokhin "Ministry of Health of the Russian Federation [Text]: report on research / FSBI" RONTs . N. N. Blokhin "; A.V. Popa, G.L. Mentkevich - Moscow, 05.16.2016. - 2 p.

Examination of the documents submitted lets us make a conclusion, that the air decontamination unit "Potok 150-M-01" inactivates all types of airborne microorganisms (destroys them), as the technology is used to decontaminate air by exposing microbial cells or virus receptors to constant electric fields of a given orientation and tension. The value of the electric field is designed to destroy any microorganisms and viruses, regardless of the type.

Microorganisms are repeatedly exposed to constant electric fields that rapidly vary in intensity, gradient and ions of opposite signs, which completely destroy the microbial cells and cellular receptors of viruses.

Depending on the type of microorganism irreversible degradation of cellular structures is expressed in the following:

• in yeast - in the complete destruction of membrane organelles, plasmalemma and cytoplasm;

• in gram-negative bacteria - in the appearance of multiple local zones of rupture in the cytoplasm and the outer membrane;

• in gram-positive bacteria - in the appearance of single, but extensive zones of cytoplasm ruptures and rejection of cell wall fragments;

(The study on the effectiveness of the air decontamination unit "Potok 150-M-01" on destruction of microorganisms and impact on the microbial cells structure p. 15).

The influence of a constant electric field on viruses leads to the fact, that the positive mass particles of nucleic acid molecules (for example, that are part of the virion, full-fledged virus consisted of a nucleic acid and a capsid and located outside a living cell) go to the negative electrode, and negatively charged to the positive.

As a result of multiple recharges, intermolecular bonds are broken, thereby violating the protein's tertiary and secondary structure. This leads to the destruction of not only the membranes (cell membranes), but also the irreversible degradation of the protein structures of non-enveloped microorganisms, regardless of their type and resistance to chemical disinfectants.

Conclusion

The air decontamination technology used in air decontamination unit "Potok 150-M-01" is non-selective. The impact of this unit on microorganisms does not depend on their structure and degree of resistance to disinfectants.

In view of the above, it may be considered appropriate, to recommend the appliance of the air decontamination unit "Potok 150-M-01" for inactivation (destruction) of all types of airborne microorganisms, including:

- bacteria, including sanitary representative microorganisms of the intestines (Escherichia coli, Enterococcus spp., Proteus mirabilis, Pseudomonas aeruginosa, etc.) and the upper respiratory tract (Staphylococcus spp., Streptococcus spp., Etc.), including those resistant to antibiotic strains;
- mold fungi and yeast, including Aspergillus niger, Mucor ramosissimus, Saccharomyces cerevisiae, etc.;
- viruses, including Influenzavirus, Grippus avium, Coronaviridae, etc.

Acting Director of Research institute of influenza (Russia)

D. A. Lioznov

RUSSIAN ACADEMY OF MEDICAL SCIENCES

STATE INSTITUTION
N.F. GAMALEYA RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY
of the Order of the Red Banner of Labor

APPROVED BY
I.S. Tartakovsky */Signature/*
Head of Legionellosis Laboratory,
Doctor of Biological Sciences, professor
April 17, 2002

/Seal:
Moscow
State Institution No. 30030
N.F. Gamaleya Research Institute of Epidemiology and Microbiology
Russian Academy of MEDICAL Sciences/

REPORT
on the results of microbiological studies of Potok 150-M-01 air disinfection unit

Moscow, 2002

SUMMARY

1. Potok 150-M-01 unit, after working for 60–90 minutes in a room with a volume of up to 100 m^3, both in the absence of people and with people working, reduced the contamination of the air to 0 CFU/m^3 with the initial concentration of up to 10^9 CFU/m^3.
2. Efficiency of air disinfection unit (ADU) indoors does not change in continuous operation at both low and high concentrations of microorganisms (up to 10^9 CFU/m^3).
3. Potok 150-M-01 ADU performs single-pass complete inactivation of microorganisms.
4. Potok 150-M-01 ADU performs complete inactivation of microorganisms after a single pass of the air through its active element without a precipitation section.
5. Potok 150-M-01 ADU can be effectively used for air sterilization in locations, as well as for creation of "clean" workspaces when working with various biological objects.

CONCLUSIONS

Results of the studies carried out in 1992-2000 can serve as a basis for drawing the following conclusions on the Potok technology used in Potok 150, Potok 150M and Potok 150-M-01 air disinfection units.

1. The technology ensures inactivation of airborne microorganisms with an effectiveness of up to 100% (above 99%).

2. No accumulation of living microorganism occurs inside the active element of the device.

3. Microorganism inactivation (sterilization) process is localized within the inactivation zone of the functional element.

4. A single Potok 150-M-01 device is capable of disinfecting rooms as large as 100 m^3, thus decreasing the airborne bacterial contamination level from 10^{11} CFU/m^3 down to 0 CFU/m^3 within 90 minutes.

Head of Immunoprophylaxis Team
of Legionellosis Laboratory,
senior research fellow,
Candidate of Medical Sciences *[Signature]* V.V. Petrosov

FINAL REPORT

on the effectiveness of Potok 150-M-01 unit in air disinfection and inactivation of the avian influenza virus.

Novosibirsk September 15, 2005

Effectiveness of Potok 150-M-01 unit was estimated based on the results of tests that were carried out using a purpose-made aerosol stand. There were three series of experiments using an aerosol containing avian influenza virus [strain A/Chicken/Suzdalka/Nov-11/2005 isolated by the specialists of Federal State Unitary Enterprise State Research Center of Virology and Biotechnology VECTOR 27.07.05 during epizootic outbreak of avian influenza among chickens in Novosibirsk region] with the initial activity of 8.0–7.0 lg TCD_{50}/ml by chick-embryo culture, with mass-average concentration of 0.23 g/m^3 and parameters of fraction-dispersion composition (FDC) of aerosol (MMAD ≅ 1.5 μm, $\sigma_g \cong 2.4$).

The organization has a license of the Ministry of Healthcare of the Russian Federation for the right to work with microorganisms of pathogenicity groups 1–4 (registration number of Russian Federation Oversight Committee for Sanitation and Epidemiology 117-2D dated June 11th, 2003) and individual sanitary-epidemiological permission of the Chief Sanitary Doctor of the subject and Koltsovo for the right to work with microorganisms of pathogenicity group 4, including aerosol works (registration number B-1-2002 dated October 24th, 2002).

A virus-containing suspension was used as a dispersible liquid. 10% (by volume) of glycerol and uranine with a final concentration of 10^{-4} g/ml were added to the suspension.

Biological activity was determined by the cytopathogenic effect (CPE) of the virus on MDCK sensitive cell cultures.

Uncoloured Hank's solution, with 2% by volume of bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin, was used as an absorbing fluid.

We used impinger samplers MTs-2 filled with 10 ml of absorbing fluid. Uptime of the samplers was 5 minutes, with an air flow through the samplers of 10±0.5 l/min. We began sampling in 1 minute after the puffer started to work, to conduct research at a constant aerosol concentration. Samplers were attached to the tubes and vacuum line wiring using rubber and silicone tubes.

The results of these tests are presented in the table below.

Table: Effectiveness of single-pass filtration and inactivation of the avian influenza virus aerosol with Potok 150-M-01 unit

Air disinfection unit operation mode	Air flow, m^3/h	Aerosol by weight filtration effectiveness ($E_{m,G} \pm I_{0.95,\ Em,G}$), %	Avian influenza virus inactivation effectiveness ($E_{h,G} \pm I_{0.95,\ Eh,G}$), %
"0" (off)	135	50.75±3.28	52.74±0.07
"II"	135	98.33±0.54	99.63±0.04

Summary

The following conclusions can be drawn from the results obtained from the work on phase 3:

- the modes of dispersion and sampling of aerosol containing the avian influenza virus were practiced;
- techniques of fluorescent and virological analysis of samples were practiced;
- parameters of FDC of aerosol were found to be almost identical before and after ADU operation (MMAD ≅ 1.5 μm, $\sigma_g \cong 2.4$);

- the values of parameters of the effectiveness of filtration and inactivation of highly concentrated aerosol of avian influenza virus in some experiments and three series corresponding to the two ADU operation modes, as well as their 95% confidence intervals, were determined;
- ADU operation mode “0” causes practically no inactivation of viral aerosol;
- ADU operation mode “II” with volume flow of 135 m^3/h provides in a single pass:

– aerosol filtration effectiveness by weight of up to 98.33%;

– avian influenza virus inactivation effectiveness of up to 99.63%;

– no significant differences in the structure of the avian influenza virus particles of aerosol, before and after ADU operation modes “0” and “II” with a flow of 135 m^3/h were found.

Conclusions

Potok-150-M-0,1 ADU in the operation mode “II” with an air flow of 135 m^3/h in a single pass of highly concentrated aerosol containing avian influenza virus, with an average MMAD of particles 1.5 μm provides:

- aerosol filtration effectiveness by weight of up to 98.33%;
- avian influenza virus inactivation effectiveness of up to 99.63%.

The test results suggest that Potok-150-M-0,1 ADU of recirculation type provides high aerosol filtration effectiveness and effectiveness of inactivation of avian influenza virus.

Head of the agreement,
Doctor of Medical Sciences, professor *[Signature]* A.N. Sergeyev

[Seal:
Bioton
Limited Liability Company
Russian Federation
Novosibirsk]

FINAL REPORT

on the effectiveness of Potok 150-M-01 unit in air disinfection and inactivation of the vaccinia virus.

Novosibirsk September 01, 2005

Effectiveness of Potok 150-M-01 unit was estimated based on the results of tests that were carried out using a purpose-made aerosol stand. There were four series of experiments using an aerosol containing vaccinia virus with the initial activity of 10^4–10^5 PFU/m^3, with mass-average concentration of 0.08-0.12 g/m^3 and parameters of fraction-dispersion composition (FDC) of aerosol (MMAD ≅ 1.5 μm, $\sigma_g \cong 2.4$).

The organization has a license of the Ministry of Healthcare of the Russian Federation for the right to work with microorganisms of pathogenicity groups 1–4 (registration number of Russian Federation Oversight Committee for Sanitation and Epidemiology 117-2D dated June 11, 2003) and individual sanitary-epidemiological permission of the Chief Sanitary Doctor of the subject and Koltsovo for the right to work with microorganisms of pathogenicity group 4, including aerosol works (registration number B-1-2002 dated October 24, 2002).

A tissue-culture virus-containing suspension made by roll-bottle method of the vaccinia virus cultivation (stain L-IVP) in continuous 4647 cell culture was used as a dispersible liquid. The virus activity in the sprayable suspension was 6.0-7.0 lg PFU/m3. 10% (by volume) of glycerol and uranine with a final concentration of 10^{-4} g/ml were added to the suspension.

Uncoloured Hank's solution, with 2% by volume of bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin, was used as an absorbing fluid.

We used impinger samplers MTs-2 filled with 10 ml of absorbing fluid. Uptime of the samplers was 5 minutes, with an air flow through the samplers of 10±0.5 l/min. We began sampling in 1 minute after the puffer started to work, to conduct research at a constant aerosol concentration. Samplers were attached to the tubes and vacuum line wiring using rubber and silicone tubes.

The virus-containing samples were titrated in 4647 cells culture by method of plague-forming cells [Leparc-Goffart I., Poirier B., Garin D. at al. Standardization of a neutralizing anti-vaccinia antibodies titration method: an essential step for titration of vaccinia immunoglobulins and smallpox vaccines evaluation // J. of Clinical Virology.-2005-Vol.32 P.47-52], the only difference being that the infected cells incubated within 3 days and 0.001% solution of gentian violet made on the basis of fixative solution.

The results of these tests are presented in the table below.

Table: Effectiveness of single-pass filtration and inactivation of the vaccinia virus aerosol with Potok 150-M-01 unit

Air disinfection unit operation mode	Air flow, m^3/h	Aerosol by weight filtration effectiveness ($E_{m,G} \pm I_{0.95,\ Em,G}$), %	Vaccinia virus inactivation effectiveness ($E_{h,G} \pm I_{0.95,\ Eh,G}$), %
"0" (off)	150	38.8±12.5	40.3±9.6
"I"	150	85.6±12.0	95.9±1.1
"II"	150	86.6±5.6	97.5±0.9
"II"	100	98.9±0.9	99.6±0.1

Summary

The following conclusions can be drawn from the results of the experiment:

- the modes of dispersion and sampling of aerosol containing the vaccinia virus were practiced;
- techniques of fluorescent and virological analysis of samples were practiced;
- parameters of FDC of aerosol were found to be practically identical before and after ADU operation (MMAD ≅ 1.5 μm, $\sigma_g \cong 2.4$);
- the values of parameters of the effectiveness of filtration and inactivation of highly concentrated aerosol of avian influenza virus in some experiments and four series corresponding to the two ADU operation modes at air flow of 150 and 100 $m^{3/}$h, as well as their 95% confidence intervals, were determined;
- ADU operation mode "0" causes practically no inactivation of viral aerosol;
- ADU operation mode "I" with volume flow of 150 m^3/h provides:

– aerosol filtration effectiveness by weight of (85.6±12.0)%;

– vaccinia virus inactivation effectiveness of (95.9±1.1)%;

– ADU operation mode "II" with volume flow of 150 m^3/h provides:

– aerosol filtration effectiveness by weight of (86.6±5.6)%;

– vaccinia virus inactivation effectiveness of (97.5±0.9)%;

– ADU operation mode "II" with volume flow of 100 m^3/h provides:

– aerosol filtration effectiveness by weight of (98.9±0.9)%;

– vaccinia virus inactivation effectiveness of (99.6±0.1)%;

Conclusions

Potok-150-M-0,1 ADU in a single pass of highly concentrated aerosol containing vaccinia virus, with an average MMAD of particles 1.5 μm provides high aerosol filtration effectiveness by weight and vaccinia virus inactivation effectiveness in the air.

The values of aerosol filtration effectiveness by weight and vaccinia virus inactivation effectiveness in Potok-150-M-0,1 ADU operation modes "I" and "II" with volume flow of 150 m^3/h were practically the same (did not differ significantly) and were 86.2% and 96.7% respectively.

Decreasing of volume flow from 150 m^3/h to 100 m^3/h in the Potok-150-M-0,1 ADU operation mode "II" led to increasing of aerosol filtration effectiveness by weight from 86.6% to 98.9% and vaccinia virus inactivation effectiveness from 97.5% to 99.6%, which is caused by increasing of the time during which the aerosol was in the electrical fields of ADU by factor of 1.5.

The test results suggest that Potok-150-M-0,1 ADU of recirculation type provides high aerosol filtration effectiveness and effectiveness of inactivation of avian influenza virus.

Head of the agreement,
Doctor of Medical Sciences, professor *[Signature]* A.N. Sergeyev

No. 89-309
26.03.01

Russian Academy of Medical Sciences
CENTRAL TUBERCULOSIS
RESEARCH INSTITUTE
Russian Federation 107564, Moscow
Yauzskaya alleya, 2, Tel. +7095/268 49 60,
268 1441 Fax +7095/963 80 44

Attn: A.V. Nagolkin,
Director General of
Research and Production Company
POTOK INTER LLC

FINAL REPORT

In 1993 and 1997, the studies of nonspecific microflora and Mycobacterium tuberculosis inactivation by means of Potok 150 M air disinfection unit were carried out in the Microbiology Department of CTRI of the Russian Academy of Medical Sciences. The studies were carried out in two stages.

The first stage, 1993: determination of the suitability of the unit for use in laboratory premises for air disinfection — inactivation of nonspecific microflora. After recording baseline air indices that showed the presence of Staphylococcus epidermidis, *Klebsiella* bacteria species, as well as non-fermenting gram-negative bacteria *Pseudomonas species*, Potok 150 M unit was turned on. As a result, under conditions of the unit in operation, within a given time the indices of air bacterization were reduced to zero. Thus, Potok 150 M unit provides complete air sterilization and offers effective protection of employee's workplace through continuous 4-hour operation.

The second stage, 1997: determination of the effectiveness of the unit on the specially designed stand under extreme conditions — the concentration of microbial cells as high as $5x10^8$ in 1 ml of suspension of *Mycobacterium bovis* BCG standard strain. Filtration element of Potok 150 M unit showed high efficiency of air disinfection — inactivation of *Mycobacterium bovis* BCG standard strain of 99.8% in demanding conditions of the tests.

M. bovis mycobacterium species are typical members of the *Mycobacterium* genus and are capable, at high microbial burden, of causing infectious diseases in humans and animals. In view of the above, the results of the tests carried out using *Mycobacterium bovis* BCG as a test strain can be considered reasonably applicable to all pathogenic members of the *Mycobacterium* genus, including so-called wild strains secreted from TB patients.

CONCLUSIONS

Potok 150 M air disinfection unit (ADU) can be used in the presence of people as a device for air disinfection in TB hospitals in laboratories, operating rooms, dressing rooms, wards, vivaria and other departments where pathogenic material is handled. Due to the high incidence of tuberculosis in the system of Federal Penitentiary Service, it is advisable to use the unit in reception centers, special isolation wards, prisons and similar institutions that have inadequate sanitary and hygienic properties.

Potok 150 M TEST REPORT on 4 pages is enclosed.

Director of CTRI RAMS,
Doctor of Medical Sciences, professor *[Signature]* V.V. Erokhin

[Seal:
Moscow
Central Tuberculosis Research Institute
Russian Academy of Medical Sciences]

APPROVED BY
I.B. Ushakov */Signature/*
Director of the State Research Centre of the Russian Federation
Institute of Medical and Biological Problems
of the Russian Academy of Sciences,
corresponding member of the Russian Academy of Sciences
05.11.2012

/Seal:
Russian Academy of Sciences
Federal State Budgetary Research Institution
State Research Centre of the Russian Federation
Institute of Medical and Biological Problems of the Russian Academy of Sciences
(SRC RF IMBP of the RAS)/

FINAL REPORT
on the results of performance study of Potok 150MK
and Potok 150-M-01 air disinfection units

Performance of Potok 150MK and Potok 150-M-01 air disinfection units (ADU) in cleaning the air from fine aerosol, allergens and trace components, and inactivation of microorganisms was evaluated based on the results of studies conducted by the Institute of Medical and Biological Problems of the Russian Academy of Sciences on board of Mir orbital space station and the International Space Station, in experiments on the long-term isolation SFINCSS'99 and MARS 500.

The following conclusions can be drawn from the results of the studies:

1. Potok units provide highly effective filtration of solid and liquid aerosols with the efficiency equivalent to that of highly effective filters of classes up to H14.
2. Physical mode of Potok ADU operation is based on the complex technology of air disinfection, which enables to carry out non-selective inactivation (destruction) with an efficiency of up to 100% of the various kinds of microorganisms: gram-positive bacteria, including spore-forming forms, gram-negative bacteria, microscopic mold fungi, including microorganisms resistant to antimicrobial agents and UV light.
3. Results of long-term microbiological studies carried out on board of Mir orbital space station and the International Space Station have shown that Potok 150MK air disinfection units represent an effective tool providing air biological cleanliness and safety. The units provide inactivation of microorganisms forming the microflora of human environment in manned spacecraft and allow maintaining standard sanitary and microbiological condition of the air.
4. Available results of performance studies of Potok air disinfection units and practical experience of use of ADU in manned spacecraft enable to draw a conclusion about the high efficiency of ADU in air disinfection and ensuring air microbiological safety in confined spaces and facilities for various purposes.
5. Potok 150-M-01 ADU can be recommended for use in healthcare institutions, microbiological laboratories, transport, public places; it is advisable to use these units in air ventilation, conditioning and recirculation systems in buildings and facilities for

various purposes.

Currently, at the International Space Station two Potok 150 MK units work satisfactorily in two modules; this allows maintaining optimum sanitary and microbiological state of air and ensuring protection of crew members from the negative influence of microbial factor.

Head of the Laboratory
"Microbiology of human environment and antimicrobial protection",
Doctor of Biological Sciences *[Signature]* N.D. Novikova
05.11.2012

Перевод с русского языка на английский язык
Translation from Russian into English

APPROVED BY
CEO of Potok Inter Engineering Company, LLC
/Signature/ A.V. Nagolkin

/Seal: Moscow, Limited Liability Company,
State Registration No. 707816, Potok Inter Engineering Company/

Report
on the results of estimation of the efficiency of air disinfection with Potok 150-M-01 units in premises of Pediatric Oncology and Hematology Research Institute of Federal State Scientific Institution of the RAMS N.N. Blokhin Russian Cancer Research Center (RCRC)

Moscow April 5, 2016

Objective:

Determination of the efficiency of air disinfection with Potok 150-M-01 air disinfection unit (ADU) in a joint stay ward and in the ward air lock chamber of the hematology unit of Pediatric Oncology and Hematology Research Institute of Federal State Scientific Institution of the RAMS N.N. Blokhin Russian Cancer Research Center (RCRC)

Procedure:

In the course of works, the total microbial count (TMC) and the mold count in the air of premises was determined before Potok 150-M-01 ADU activation and several days after the activation of the unit. The works were performed in accordance with MUK (Methodical instructions) 4.2.2942-11 Methods of sanitary-bacteriological studies of the environment, air and sterility control in medical organizations. The air sampling was implemented with PU-1B aspirator (Ximko, Russia). The efficiency of air disinfection with Potok 150-M-01 units was estimated by comparison of the data on the air microbial content before and after the unit activation.

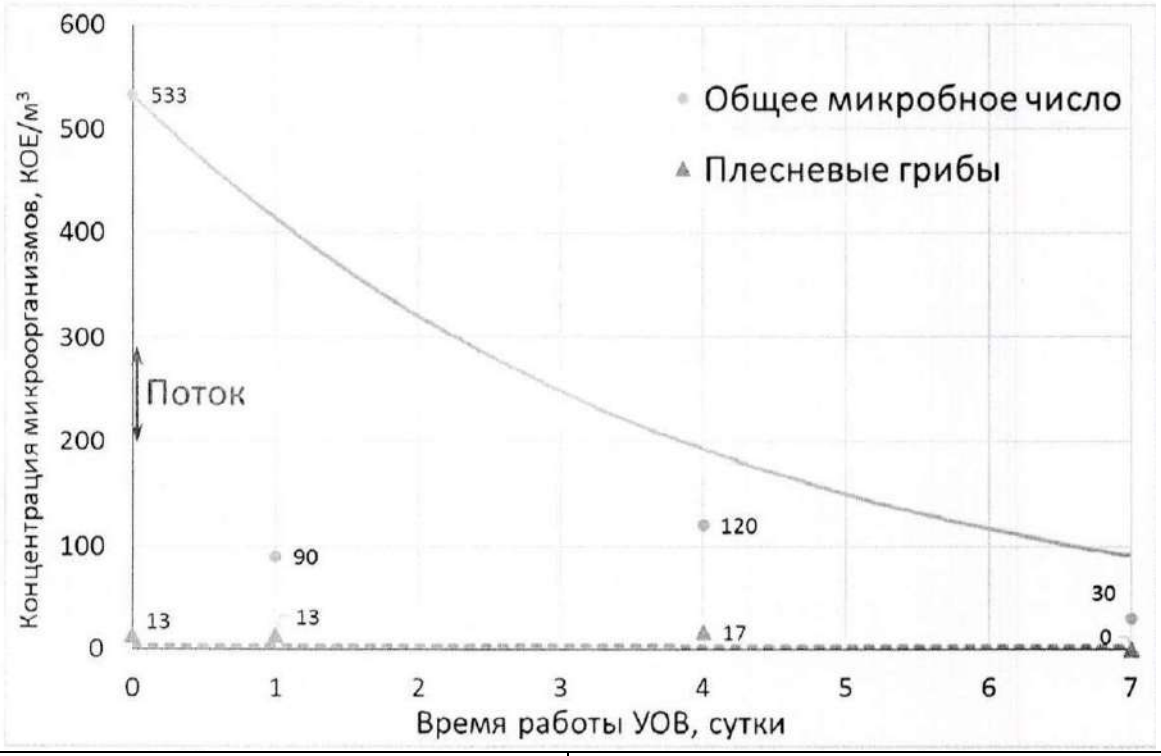
STUDY RESULTS.

Ward air lock chamber.

The air lock chamber is a transition area between the contaminated area (unit corridor) and clean area (joint stay ward); therefore, the air quality in the air lock chamber affects the air quality in the ward. Consequently, the concentration of microorganisms in the air of the air lock chamber was estimated. For measuring the background level of microorganisms (before the ADU activation) 6 air samples were collected and analyzed, in particular, 3 aerosol samples were precipitated on plates with meat infusion agar to determine the total microbial count, and the remaining 3 aerosol samples - on plates with Sabouraud agar to determine the yeast and mold count. Prior to the experiments the TMC average background concentration in the air of the air lock chamber was 533 CFU/m^3, the yeast and mold concentration was 13 CFU/m^3 (Fig. 1).

The measurements taken in 24 hours after the activation of Potok 150-M-01 air disinfection unit demonstrated that the TMC in the air of the air lock chamber decreased by a factor of 6 (from 533 to 90 CFU/m^3). 7 days after the unit operation the TMC decreased to 30 CFU/m^3, 22 days after it decreased to 3 CFU/m^3. The total TMC decrease in the air of the air lock chamber within 22 days of the unit operation was by a factor of 178 (from 533 to 3 CFU/m^3).

The mold concentration in the air of the air lock chamber within the first 4 days after the ADU activation scarcely changed, remaining at the level of 13-17 CFU/m^3. 7 days after the unit operation the mold concentration in the air of the air lock chamber dropped to zero (molds were found in none of the 3 air samples). On day 22 the mold concentration in the air of the air lock chamber was 3 CFU/m^3, which was probably caused by the mold ingress from the adjacent premises (corridor).



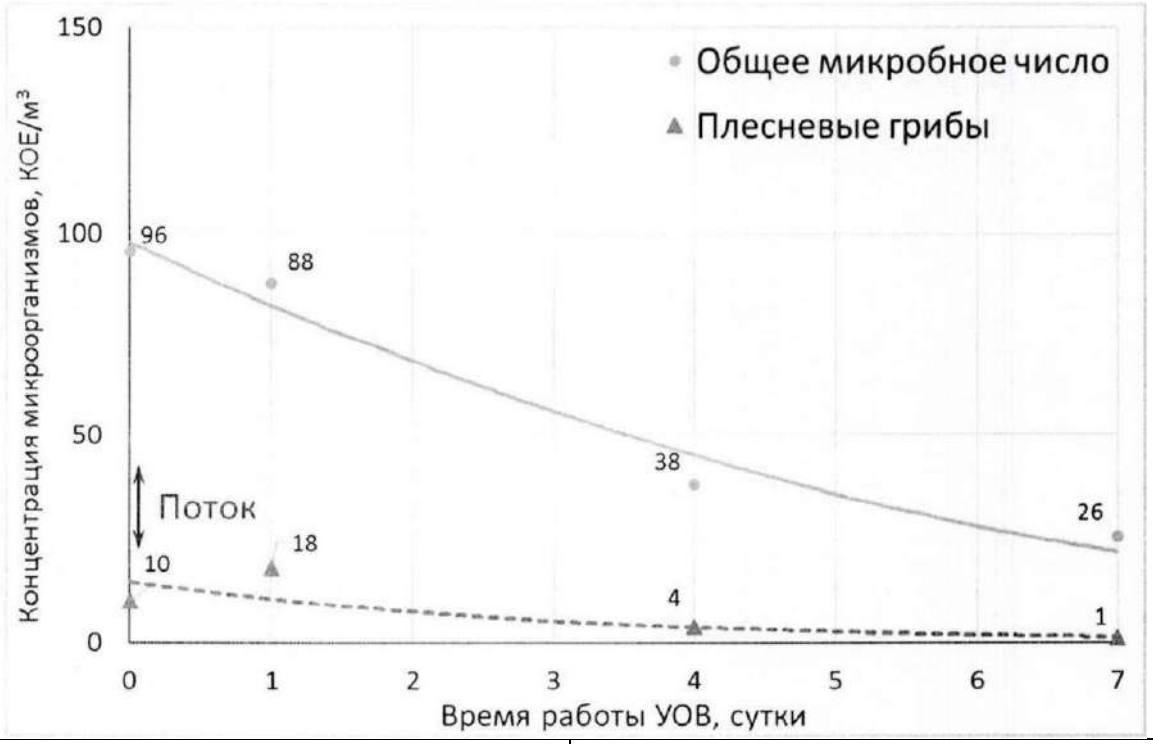
Концентрация микроорганизмов, КОЕ/м^3	Concentration of microorganisms, CFU/m^3
Общее микробное число	Total microbial count
Плесневые грибы	Molds
Поток	Flow
Время работы УОВ, сутки	ADU operation time, days

Figure 1. The concentration of microorganisms in the air of the air lock chamber before and after Potok 150-M-01 ADU activation.

Joint stay ward

For measuring the background level of microorganisms in the ward (before the ADU activation) 18 air samples were collected and analyzed, in particular, 9 samples were precipitated on plates with meat infusion agar to determine the total microbial count, and the remaining 9 samples – on plates with Sabouraud agar to determine the yeast and mold count. Prior to the experiments the TMC average background concentration in the air of the ward was 96 CFU/m^3, the yeast and mold concentration was 10 CFU/m^3.

The preliminary identification of mold species made it possible to find a significant diversity of molds (approximately 6 species), among which predominantly there were aspergilli: *Aspergillus terreus, Aspergillus sydowii, Aspergillus fumigatus, Aspergillus versicolor.*



Концентрация микроорганизмов, КОЕ/м^3	Concentration of microorganisms, CFU/m^3
Общее микробное число	Total microbial count
Плесневые грибы	Molds
Поток	Flow
Время работы УОВ, сутки	ADU operation time, days

Figure 2. The concentration of microorganisms in the air of the joint stay ward before and after Potok 150-M-01 unit activation.

The measurements taken in 24 hours after the activation of Potok 150-M-01 ADU demonstrated that the TMC in the air of the ward decreased from 96 to 88 CFU/m3. Subsequently, there was the continued TMC decrease, and 7 days after the unit operation it decreased by a factor of 4 (from 96 to 26 CFU/m^3).

The mold concentration in the air of the ward within 24 hours of operation increased from 10 to 18 CFU/m^3. It was probably related to the fact that in the course of the ADU operation the indoor air exchange increases, and the mold spores entered the total indoor airspace from stagnant areas. 4 days after the unit operation there was the mold concentration decrease in the air of the ward to 4 CFU/m^3, 7 days thereafter – to 1 CFU/m^3, 22 days thereafter – to 0 CFU/m^3.

It must be noted that the use of Potok 150-M-01 ADU resulted in the decrease in the mold culture species diversity in the air of the ward – before the ADU activation there were about 6 mold species identified (predominantly, representatives of *Aspergillus spp.),* and 4 days after the ADU operation there was only 1 mold species found, which was probably directly related to the ADU operation.

CONCLUSIONS

1) The studies conducted demonstrated that the use of Potok 150-M-01 air disinfection units (ADU) made it possible to reduce the total microbial count in the air of the air lock chamber by a factor of 178 (from 533 to 3 CFU/m^3), and the mold concentration by a factor of 4 (from 13 to 3 CFU/m^3).
2) The use of Potok 150-M-01 ADU made it possible to reduce the total microbial count in the air of the joint stay ward by a factor of 4 (from 96 to 26 CFU/m^3), and the mold concentration – from 10 CFU/m^3 to zero.
3) The use of Potok 150-M-01 ADU resulted in the decrease in the number of mold species in the air of the joint stay ward from 6 to 1.

Chief of the laboratory
of Potok Inter Engineering Company *[Signature]* E.N. Kobzev

Перевод с русского языка на английский язык

/Logo of Wimm-Bill-Dann company/

Separate Subdivision of WBD JSC
352720, Krasnodar Territory, st Medvedovskaya ul. Krasnaya d8
Tel.: **+7 (86130) 908-00; Fax: +7(86130) 908-05**
www.wbd.ru

From 2003 to the present day our production facility has been using the automatic filling machines of fermented milk products Pastpack-4L and Pastpack-2L of Tauras-Phoenix Company with the built-in air disinfection units of Potok Inter Engineering Company (NPF "Potok Inter" LLC).

No microbiological problems were detected for the period of 13 years of operation of air disinfection units "Potok-150-M-01" in the automatic filling machines.

The company has proved to be a reliable partner; its staff provides full mutual understanding with the wishes of the customer, even if they are beyond their capabilities.

The units of Potok Inter Company have substantially improved the efficiency of the automatic filling machines: they ensure product protection from microbiological contamination and increase product shelf life. The use of this solution has allowed improving the quality of the final products.

Head of Service Department	/*signed*/	Petlyuk S.V.
PCS Lead Engineer	/*signed*/	Shabelnik R.N.

/Logo of PepsiCo corporation/

Final report

On the effectiveness of Potok 150-M-01 air disinfection unit (ADU)
in microorganisms inactivation and impact on the structure of microbial cells

Pushchino January 30, 2012

The effectiveness of Potok 150-M-01 ADU in microorganisms inactivation was evaluated based on the results of microbiological studies carried out in the laboratories of BioResources and Ecology Research Center and G.K. Skryabin Research Institute for Biochemistry and Microbial Physiology, Russian Academy of Sciences *(License of the Federal Service for Consumer Rights Protection 77.99.18001 L 000154.11.04 for activities involving the use of infectious agents. Operations with microorganisms of pathogenicity group 4)*.

The studies on the effectiveness of *Pseudomonas fluorescens*, *Micrococcus luteus* and *Saccharomyces cerevisiae* inactivation and effect on their structure showed the following:

1. Potok 150-M-01 ADU provides effective (close to 100%, NLT 99.99% with due regard to experimental uncertainty) single-pass inactivation of highly concentrated lyophilized aerosol containing *Pseudomonas fluorescens*, *Micrococcus luteus* and *Saccharomyces cerevisiae*, within a contact time of about 0.5 seconds.

2. In Potok 150-M-01 ADU, cells are inactivated by means of exposure of their structure to constant electric field and ions with opposite charges resulting in strong destructive changes in their ultrastructural organization (electroporation) such as:

- In yeasts: complete destruction of membrane organelles, plasmalemma and cytoplasm;
- In gram-negative bacteria: multiple local plasma membrane and outer membrane perforations;
- In gram-positive bacteria: isolated but vast plasma membrane perforations and rejection of the cell wall fragments.

3. The nature of cell structure damage of different types of microorganisms that occurs as a result of Potok 150-M-01 ADU exposure significantly differs from that caused by other known impacts (UV bactericidal irradiation, ozone or other chemicals).

4. Based on the results of the studies conducted and available reports of specialized microbiology laboratories, we can recommend Potok 150-M-01 ADU for use in air disinfection systems that provide not only filtration but inactivation of microorganisms of pathogenicity groups 1–4.

The work was carried out at:

Laboratory of microorganisms cytology, G.K. Skryabin Research Institute for Biochemistry and Microbial Physiology, RAS

Laboratory of biochemical processes regulation, G.K. Skryabin Research Institute for Biochemistry and Microbial Physiology, RAS

Laboratory of environmental biotechnology, BioResources and Ecology Research Center

Director of BioResources and Ecology RC */Signature/* V.A. Dmitrieva

Director of G.K. Skryabin
Research Institute for Biochemistry and Microbial Physiology, RAS
Corresponding member of RAS,
Doctor of Biological Sciences */Signature/* A.M. Boronin

Chief of Laboratory of biochemical processes regulation
of G.K. Skryabin Research Institute for Biochemistry

and Microbial Physiology, RAS,
Doctor of Biological Sciences /Signature/ T.V. Kulakovskaya

Responsible officer
senior research fellow
of Laboratory of microorganisms cytology,
Candidate of Biological Sciences /Signature/ V.V. Dmitriev

/Seal:
RUSSIAN FEDERATION
MOSCOW REGION
PUSHCHINO
NONPROFIT PARTNERSHIP
BIORESOURCES AND ECOLOGY RESEARCH CENTER/

/Seal:
Russian Academy of Sciences
G.K. Skryabin Research Institute for Biochemistry and Microbial Physiology, RAS
OGRN 1025007171491/

Перевод с русского языка на английский язык
Translation from Russian into English

APPROVED

by Director of
the State Research Centre of the Russian Federation
the Institute of Biomedical Problems
of the Russian Academy of Sciences
Associate member of the Russian
Academy of Sciences
/*Signature*/ I. B. Ushakov
November 5, 2012
*/Seal: The Russian Academy of Sciences * The Federal state Government-financed Research Institution * The State Research Centre of the Russian Federation * The Institute of Biomedical Problems of the Russian Academy of Sciences * Primary State Registration Center (OGRN) 1027739333710 */*

REPORT
on the research results of the operating efficiency of the air decontamination systems
Potok 150MK and Potok 150-M-01

Operating efficiency of the air decontamination systems Potok 150-M-01 and Potok 150MK on cleansing of the air from bland aerosol, allergens and trace substances, and on inactivation of microorganisms was assessed according to the results of the researches carried out by the Institute of Biomedical Problems of the Russian Academy of Sciences aboard the Mir Orbital Space Station and the International Space Station in experiments on long-term isolation SFINCSS'99 and MARS 500.

According to the research results, we can make the following conclusions:

1. The Potok systems provide high-performance filtration of solid and liquid aerosol with the efficiency which meets the standards of the high-performance filters of classes up to H14.

2. The physical principle of operation of the Potok air decontamination devices is based on the complex technology of the air decontamination, that allows carrying out non-selective inactivation (elimination) of different kinds of microorganisms with the 100% efficiency: gram-positive bacterium including spore-formers, gram-negative bacterium, microscopic mold fungi, including microorganisms which are resistant to antimicrobial agents and ultraviolet light.

3. Long-term microbiological studies carried out aboard of the Mir Orbital Space Station and the International Space Station showed that the air decontamination devices Potok 150MK are the efficient means to provide microbiological cleanliness and safety of the air. They carry out inactivation of the microorganisms forming microflora of the manned spacecraft environment and allows supporting the controlled sanitary microbiological status of the air.

4. According to the data of the researches of the Potok air decontamination systems operating efficiency and practical experience of their use in the manned spacecraft we can make conclusion on their high efficiency on air decontamination and providing of microbiological cleanliness and safety of the air in the enclosed volumes and premises of different purposes.

5. The air decontamination systems Potok 150-M-01 can be recommended for use as standard equipment in medical units and microbiological laboratories, in transport, in public places,; it is expedient to use these devices in the ventilation, air conditioning and recycling systems for buildings and structures of different purposes.

At present, two devices Potok 150MK are properly functioning in two modules of the International Space Station that allows supporting optimal sanitary microbiological status of the air and provide protection of the crew members from negative influence of the microbial factor.

Head of the "Microbiology of the Living
Environment and Antibacterial Defense" Laboratory,
Doctor of Biological Sciences *November 5, 2012 /Signature/* N. D. Novikova

"All-Russian Scientific Research Institute of Poultry Processing Industry" - a branch of Federal State Budgetary Scientific Institution of the Federal Scientific Center "All-Russian Research and Technological Institute of Poultry Farming" of the Russian Academy of Sciences (VNIIPP)

UDC

State Registration No.

Reg. No.

APPROVED

Director of VNIIPP
Cand.Sc.(Eng.)
/*signed*/ I.V. Mokshantseva
"___" _____2016

REPORT

ON THE SCIENTIFIC RESEARCH WORK

Subject: "Testing of stand-alone air disinfection unit "Potok 150-M-01" manufactured by NPF "POTOK INTER" LLC
(final version)

Head of Project
Chief Researcher
Laboratory of Sanitary-Hygienic Assessment
of Raw Materials and Products
Dr.Sc.(Biol.) /*signed*/ S. S. Kozak

Rzhavki 2016

LIST OF CONTRIBUTORS

Head of Project Chief Researcher Dr.Sc.(Biol.)	/*signed*/ signature, date	Kozak S.S. (general management, report writing)
Responsible Person Researcher Dr.Sc.(Biol.)	/*signed*/ signature, date	Dogadova N.L. (experiments, report writing)

ABSTRACT

The report contains 16 pages, 3 tables, 14 sources used.

Keywords: air disinfection, air disinfection unit "Potok 150-M-01".

The object of the research is air disinfection unit "Potok 150-M-01" (hereinafter referred to as the Unit), carrying out highly effective disinfection of air flow by inactivating any microbial species (at least 99%), viruses and subsequent filtration of aerosols.

The goal of the work is to evaluate the effectiveness of unit "Potok 150 M-01" at a poultry processing facility.

Research objective: to evaluate the effectiveness of the microflora inactivation by air disinfection unit "Potok-150 M-01", to investigate the impact of microbiological air composition on the product quality of a poultry factory.

The methodology of the work consists of two stages. In the first stage of the research the screening of microflora at all stages of poultry processing was carried out. In the second stage we investigated the effectiveness of air disinfection by unit "Potok 150-M-01" in the poultry processing and its impact on the quality of the products.

The findings: testing of stand-alone air disinfection unit "Potok 150-M-01" at a poultry processing plant.

Application: poultry processing industry.

Contents

This research report includes the following definitions and abbreviations: CFU/g (cm3) - the number of colony forming units in 1 gram (cubic centimeter) of the product, QMAFAnM - quantity of mesophilic aerobic and facultative anaerobic microorganisms, CGB - Escherichia coli group bacteria.

INTRODUCTION

For Russia the food production problem is of paramount importance, as well as for other countries. The high efficiency of production of poultry meat, which is considerably cheaper than beef, pork or lamb, requires less consumption of feed, energy, labor costs and provides an economic advantage over other sectors of the animal husbandry.

Poultry meat is cheaper than other types of meat, and this is very important for consumers in view of their limited paying capacity. Therefore, many segments of the population prefer poultry meat compared to other, more expensive products containing animal protein. Furthermore, the civilized countries are now characterized by a tendency to reduce the content of unsaturated fatty acids and cholesterol in the diet [1].

At each stage of development of society the requirements to the quality of products change that are in direct proportion to the achievements of science, technical level of production and the level of socio-economic development. These factors are reflected in the development of methodological approaches to the analysis of product quality, the most important of which is the microbiological control [2].

However, one of the important issues of expansion of poultry meat production is the limited shelf life because the meat is a positive breeding ground for microorganisms.

The designed standard GOST R 52702-2006 "Chicken meat (carcasses of chickens, broiler-chickens and their parts). Specifications" provides a recommended shelf life of chilled chicken meat at the air temperature in the refrigerator compartment from 0°C to 2°C inclusive: carcasses - not more than 5 days, parts of carcasses - not more than 2 days from the date of production. Increasing the shelf life of carcasses and carcass parts of broiler chicken is an urgent task.

With an increase in shelf life the supply of poultry meat over long distances is extended, realization time is increased, which is important for trade and industrial processing, refrigerating power, freezing chambers, transport costs are reduced, etc.

The increase in shelf life is affected by bacterial content of poultry carcasses at the stage of primary processing of poultry. A high level of hygiene in poultry processing factories, regular and efficient cleaning of equipment, the use of machines, ensuring minimal cross-contamination - all these measures prevent the spread of pathogenic bacteria at the factory and help to reduce bacterial contamination of products. Also the reduction of the bacterial content of carcasses is contributed by the air purity in the areas of poultry processing factory. In this study we conducted air microflora screening at all stages of poultry meat processing and examined the effectiveness of air disinfection by the unit "Potok 150-M-01" in the processing of poultry and its impact on the quality of products.

MAIN SECTION

1. Selection of research direction

The work was performed in two stages. In the first stage of the research the air microflora screening was carried out at all stages of processing of poultry meat. At the second stage we examined the effectiveness of air disinfection by the unit "Potok 150-M-01" in the processing of poultry and its impact on the quality of products.

2. Materials, equipment and research methods

2.1 Air disinfection unit (ADU) "Potok 150-M-01"

In accordance with the operational documents the air disinfection unit "Potok 150-M-01" provides highly efficient air flow disinfection by inactivation of any microbial species (at least 99%), viruses and subsequent filtration of aerosols.

Airflow processing in the unit is carried out in two stages. In the inactivation area (the first stage) the repeated depolarization of the membranes of microorganism cells by constant electric fields of a predetermined orientation and tension is carried out, which leads to the disintegration of their structure. In the filtration zone (the second stage) the trapping of microbial debris and aerosol particles in the treated air stream is carried out.

2.2 Automatic air sampler of biological aerosols (aspirator) PU-1B (CJSC "Khimki")

The automatic air sampling device of biological aerosols (aspirator) PU-1B ver. 1 is intended for automatic sampling of biological air aerosols during air-sanitary monitoring of various areas. Aspirator provides sampling of aerosols on solid medium by impact deposition. The samples are analyzed in the laboratory using standard procedures, duly approved.

Operating conditions of the device:
ambient temperature - from plus 10 to plus 35°C;
relative humidity - 80% at 25°C;
atmospheric pressure - 630-800 mm Hg

Limits of basic relative error of aspirator in the collection of the given sample volume $\delta^{v}_{0} = \pm 10\%$.

Power consumption - not more than 4 W (when powered by an autonomous power source).

2.3 Air sampling method

Preparation of dishes

Prepare Petri dishes in accordance with the approved procedure (fill a standard Petri dish with 20-21 ml of nutrient medium).

Take off the upper part of the sampling device by turning the knob counterclockwise. Take off the protective cover. Moisturize the multi-nozzle mesh with ethanol on both sides and flame it in a spirit lamp flame. Install the dish with a nutrient medium in aspirator holders, remove the cover from it and screw on the upper body. The device is ready for operation.

Turn on the device, set the desired volume of the sample and turn on a device to begin sampling. The centrifugal fan sucks air from the atmosphere through the multi-nozzle mesh of impactor. Aerosol particles of a certain size, contained in the air sample, impact the solid medium filled in a standard Petri dish. Then air is discharged into the atmosphere through the annular slot of the housing. The volume of samples taken is controlled automatically by an electronic counting device mounted on the circuit board. When a certain turning number of a fan corresponding to a predetermined volume of a sample is reached, the fan is automatically switched off.

After sampling remove the Petri dish, close the lid and place in a constant-temperature cabinet for colony formation.

Sample analysis is performed by visual counting of microorganism colonies on the agar surface, the number of which corresponds to the number of particles containing living microorganisms in a selected volume of air. With the number of colonies not above 35 the most probable number of particles equals to the number of colonies. With the increase in the number of colonies in the sample taken the calculations must be made using a special table.

Determination of the concentration of microorganisms (per m^3)

The concentration of microorganisms in the test air is determined by the formula:

$$C = 1000 \times \frac{P}{Q},$$

where:

C - concentration of airborne particles, particles/m^3;

P - probable number of particles in the sample;

Q - collected sample volume, l.

2.4 Methods of bacteriological tests

2.4.1 Method for detection of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM)

Detection of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) is carried out in accordance with GOST R 50396.1-2010 "Poultry meat, edible offal and ready-to-cook poultry meat. Method for quantity determination of mesophilic aerobic and facultative-anaerobic microorganisms", GOST 10444.15-94 "Food products. Methods for determination quantity of mesophilic aerobes and facultative anaerobes", GOST 26670-91. "Food products. Methods for cultivation of microorganisms".

2.4.2 Detection of Listeria
Detection of L.Monocytogenes is carried out according to GOST 32031-2012 "Food products. Methods for detection of Listeria monocytogenes".

2.4.3 Detection of coliform bacteria (CGB)
Detection of coliform bacteria (coliform bacillus) is carried out according to GOST R 54374-2011 "Poultry meat, edible offal and ready to cook products. Methods for detection and quantity determination of coliform bacteria", GOST 31747-2012 "Food products. Methods for detection and quantity determination of coliforms".

2.4.4 Detection of Salmonella
Detection of Salmonella is carried out in accordance with GOST 31468-2012 "Poultry meat, edible offal and poultry meat ready-to-cook. Method for detection of Salmonellae".

2.4.5 Detection of Staphylococcus aureus
Detection of staphylococci is carried out according to GOST R 52815-2007 "Food products. Methods for detection and quantity determination of coagulase-positive staphylococcus and Staphylococcus aureus".

2.4.6 Detection of yeasts and mold fungi
Detection of yeast and mold fungi is carried out according to GOST 10444.12-2013 "Microbiology of food and animal feeding stuffs. Methods for the detection and colony count of yeasts and molds".

3. Findings and discussion

3.1 Screening microflora at all stages of poultry meat processing.
Studies of air in industrial premises of poultry processing factories were conducted at two poultry farms of Moscow Region. To do this the process flow diagram, the layout of production facilities was analyzed and a visual inspection of production facilities was carried out to identify potential sources of bioaerosols and evaluation of the scope of surveys.

Samples were taken at the height of the product, at a distance of 20-30 cm from the product by means of an automatic air sampler of biological aerosols (aspirator) PU-1B. After sampling the Petri dishes were placed in a thermostat and incubated at optimal temperature for the growth of this group of microorganisms.

The results are given in Table 1.

Table - 1 Air microbiological data of the packing room of the slaughter and processing complex

Air sampling site	Microbiological data		
	QMAFAnM, CFU/m^3	Mold, CFU/m^3	Yeast, CFU/m^3
Sorting line	$4*10^1$	$4.4*10^2$	not detected
Placing carcasses in the packing	$1.4*10^2$	$1.8*10^2$	not detected
Cutting carcasses	$1.0*10^2$	$3.2*10^2$	not detected
Filleting line	$2*10^1$	$2.4*10^2$	not detected
Transitional gateway	$4.4*10^2$	$1.12*10^3$	not detected
Input-output	$2.4*10^2$	$4.2*10^2$	not detected

Table 1 shows that the mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) and mold were detected in the air of the packing room in all air sampling points. Therefore the air of industrial premises can be a source of microbial contamination of poultry carcasses, which affects the quality of the products and reduces the shelf life of poultry meat.

We consider it expedient to use air disinfection units at this section of the slaughter and processing complex.

3.2 Investigation of the efficiency of air disinfection by unit "Potok-150 M-01" in the poultry processing

After placing the "Potok" units in the specific process area (vacuum packing line) control measurements of air contamination were performed. Sampling method was the same as described above. Once the "Potok" units were turned on an experimental measurement of air contamination after one hour of their operation was performed.

Evaluation of the efficiency of "Potok" unit was determined by comparing the values of bacterial contamination of the air before the start of the unit operation (background level) with the contamination level after one hour of unit operation.

The results are given in Table 3. The yeast in this study was not detected, so the number is not reflected in the table.

Table - 2 Microbiological air parameters at the vacuum packing line before processing (background) and after one hour of operation of "Potok-150 M-01" unit. The volume of packing room - 356 m^3.

Air sampling site	Microbiological data			
	QMAFAnM, CFU/m^3		Mold, CFU/m^3	
	Background	After one hour of unit operation	Background	After one hour of unit operation
Smoked chicken packing table	$1.2*10^2$	$6.0*10^1$	$3.4*10^3$	$1.3*10^3$
Sausage packing line	$1.8*10^2$	<10	$1.2*10^3$	$8.2*10^2$
Smoked chicken labeling	$1.4*10^2$	$8.0*10^1$	$1.8*10^3$	$4.2*10^2$
Sausage labeling	$4.0*10^1$	$2.0*10^1$	$1.4*10^3$	$2.4*10^2$
Finished products weighing	$1.2*10^2$	$6.0*10^1$	$9.0*10^2$	$3.6*10^2$

Table 2 shows that after one hour of unit "Potok-150 M-01" operation the number of MAFAnM and molds decreased by 2-10 times, which indicates the high unit efficiency.

Conclusion

It was found that the air contaminated by microorganisms can be a source of microbial contamination of poultry carcasses, which affects the quality of the products and reduces the shelf life of poultry meat.

In the second stage we conducted comparative tests of air pollution before the start of unit "Potok-150 M-01" operation and one hour after its work. The result showed that the number of microorganisms (fungi and MAFAnM) decreased by 2-10 times after one hour of unit operation. This demonstrates the high efficiency of unit "Potok 150-M-01."

Air disinfection unit "Potok-150 M-01" is recommended for permanent use in poultry and egg processing shops.

List of references

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2 Rabinovich G.Y., Sulman E.M. Sanitary-microbiological control of environmental objects and food products with the basics of General Microbiology: Textbook. 1st ed. Tver: TSTU, 2005. 220 p.

3 Instruction on the sanitary-bacteriological evaluation of poultry carcasses and poultry products in the poultry and poultry processing factories, Moscow 1990

4 GOST R 53597-2009 "Poultry meat, edible offal and ready-to-cook poultry meat. Sampling methods and preparing of samples for examinations";

5 GOST 31467-2012 "Poultry meat, edible offal, and ready-to-cook poultry meat. Sampling methods and preparing of samples for examinations";

6 GOST R 50396.0-2013 "Poultry meat, edible offal and semi-prepared products from poultry meat. Sampling methods and preparing for microbiological examinations";

7 GOST R 50396.1-2010 "Poultry meat, edible offal and ready-to-cook poultry meat. Method for quantity determination of mesophilic aerobic and facultative-anaerobic microorganisms";

8 GOST 10444.15-94 "Food products. Methods for determination quantity of mesophilic aerobes and facultative anaerobes";

9 GOST 26670-91. "Food products. Methods for cultivation of microorganisms";

10 GOST 32031-2012 "Food products. Methods for detection of Listeria monocytogenes";

11 GOST R 54374-2011. "Poultry meat, edible offal and ready to cook products. Methods for detection and quantity determination of coliform bacteria";

12 GOST 31747-2012 "Food products. Methods for detection and quantity determination of coliforms";

13 GOST 31468-2012 "Poultry meat, edible offal and poultry meat ready-to-cook. Method for detection of Salmonellae";

14 GOST 10444.12-2013 "Microbiology of food and animal feeding stuffs. Methods for the detection and colony count of yeasts and molds";

JOINT STOCK COMPANY "Kaluga Poultry"

s. Lva Tolstogo, Dzerzhinsky District, Kaluga region, Russia, 249842

Tel. (48434) 4-41-84, fax (48434) 7-14-75

PROTOCOL №49

Research of "Potok 150 M-01" standalone air decontamination units (ADU) effect

on the duration period of products

1. Name of Enterprise: Joint Stock Company "Kaluga Poultry"
2. Address: s. Lva Tolstogo, Dzerzhinsky District, Kaluga region, Russia, 249842
3. Name of sample: semi-finished products of poultry meat
4. Place of sampling: cutlets production department
5. Volume of the room: 231m^3
6. Staff quantity: 7
7. Scheduled operations: microbiological analysis of the products
8. Conditions: indoor temperature +12 °C, humidity 80%
9. Regulatory Documents: Technical Regulations of the Customs Union "TR TC 021/2011 "Concerning safety of food products""
10. Research results
11. Concentration QMAFAnM (CFU/g) in the sample without working ADUs

Storage Days	Poultry cutlets "Fileinyie"	Poultry rissoles, creamy
1	**$1,9*10^6$**	$7*10^5$
5	$9*10^5$	$6,3*10^5$
7	**$1,5*10^6$**	**$2,2*10^6$**
10	**$1,7*10^6$**	**$6,5*10^7$**
11	**$>1*10^6$**	**$9,1*10^7$**

Concentration QMAFAnM (CFU/g) in the sample with two working air decontamination units "POTOK"

Storage days	Poultry cutlets "Po-kyievski"	Poultry schnitzel chopped
1	$1,0*10^4$	$5,0*10^5$
5	$4,0*10^4$	$3,0*10^5$
7	$7,0*10^5$	$9,0*10^5$
10	$8,0*10^5$	**$1,4*10^6$**
11	**$>1,0*10^6$**	**$5,9*10^6$**

Quantity of mesophilic aerobic and facultative-anaerobic microorganisms, according to the Technical Regulations of the Customs Union "TR TC 021/2011 "Concerning safety of food products"", must be no more than $1*10^6$ CFU/g.

Name of the tester_____/Totskaia O.S./
Name of the PTL Head _____/ Titov D.V./

Conclusion:

1. The duration period of the products without “POTOK” air decontamination units equaled to 5 days.
2. While using of the “POTOK” air decontamination units in the cutlets production department, the duration period of the products was extended to 7 days including the reserve coefficient 1,5, corresponding to “STO 00508187-018-2016”. Thus, with the use of air decontamination units, the duration period of products was extended by 40%.

Head of quality management Nazarova I.
Joint Stock Company "Kaluga Poultry"

Head of food industry department Subbotin S.V.
LLC SMF “Potok Inter”

Protocol accepted:

_____	_____	_____
Post	signature	name

Note: this test protocol concerns the tested samples only. The conditions of tests conducted comply with the requirements of the Regulatory Documents and are registered in the corresponding log.

Перевод с русского языка на английский язык

/*The coat of arms of the Russian Federation*/
RUSSIA
Federal State Unitary Enterprise
"Experimental Cheese Factory"
(FSUE "ECF")
152613, Uglich, Yaroslavl Region, Rybinskoye shosse, 22-V
telephone / fax (48532) 5-39-42 – Sales Department, fax 5-39-43.
telephone 5-39-40, 5-39-45 E-mail: secretary@uglich-syrzavod.ru
Settlement account No. 40502810703000000010 with the Yaroslavl Branch of "BANK SGB", Yaroslavl
INN Code 7612002423, BIC 047999782, c/a 30101810100000000782

July 01, 2016

CONCLUSION

On the efficiency of ADU "Potok-150-M-01" to reduce the level of air contamination in the production areas of dairy industry.

The efficiency of air disinfection unit "Potok-150-M-01" was estimated based on the results of microbiological tests of the air of the working premises at **FSUE Experimental Cheese Factory**. Testing and sampling were carried out in the butter packing room and cheese-making shop.

To improve the accuracy of the evaluation result, in addition to the main sampling method (sedimentation) a supplementary method was used, an aspiration method, which was carried out by using an automatic air sampler of biological aerosols (aspirator PU-1B).

During the tests the concentration of microorganisms was determined: QMAFAnM, yeast and mold fungi in the air of working areas of process equipment in the production line before turning on the "Potok 150 M-01" unit and after its operation.

The results of the research carried out (test report as of June "28", 2016) have shown that air disinfection unit "Potok-150-M-01" in the butter packaging area reduced the QMAFAnM concentration in the air by 40 times, mold fungi - by 15 times. In the second room, i.e. in the cheese-making area, the QMAFAnM concentration reduced by 106 times, mold fungi - by 18 times, and the yeast concentration was reduced to 0 CFU/m^3.

The tests have shown the efficiency of air disinfection unit "Potok-150-M-01" to significantly reduce the level of air contamination in the production areas in a short period of time.

Air disinfection units "Potok-150-M-01" are recommended for continuous use in production areas of dairy industry to reduce the level of airborne contamination, which in turn has a positive effect on safety of food products and duration of their shelf life.

Director /*signed*/ A. E. Golubev

*/Seal: Federal State Unitary Enterprise * "Experimental Cheese Factory" * (FSUE ECF) * Primary State Registration Number (OGRN) 1027601304246 */*

TEST RESULTS
FOR THE BUTTER PACKING ROOM

Table 1. Sedimentation method

No.	Sampling point	QMAFAnM, CFU per dish	Yeast, CFU per dish	Mold fungi, CFU per dish
1	Butter packing area before air filtration	1	0	6
2	Butter packing area after air filtration	0	1	0

Table 2. Aspiration method

No.	Sampling point	Concentration of microorganisms, CFU/50 l			
		QMAFAnM	Average for QMAFAnM	Mold fungi	Average for mold fungi
1	Butter packing area before air filtration	40	79	104	85
		102		90	
		96		61	
2	Butter packing area after air filtration	3	2	4	6
		2		9	
		1		4	

TEST RESULTS
FOR THE CHEESE-MAKING SHOP

Table 1. Sedimentation method

No.	Sampling point	QMAFAnM, CFU per dish	Yeast, CFU per dish	Mold fungi, CFU per dish
1	Cheese-making area before air filtration	31	16	8
2	Cheese-making area after air filtration	0	0	0

Table 2. Aspiration method

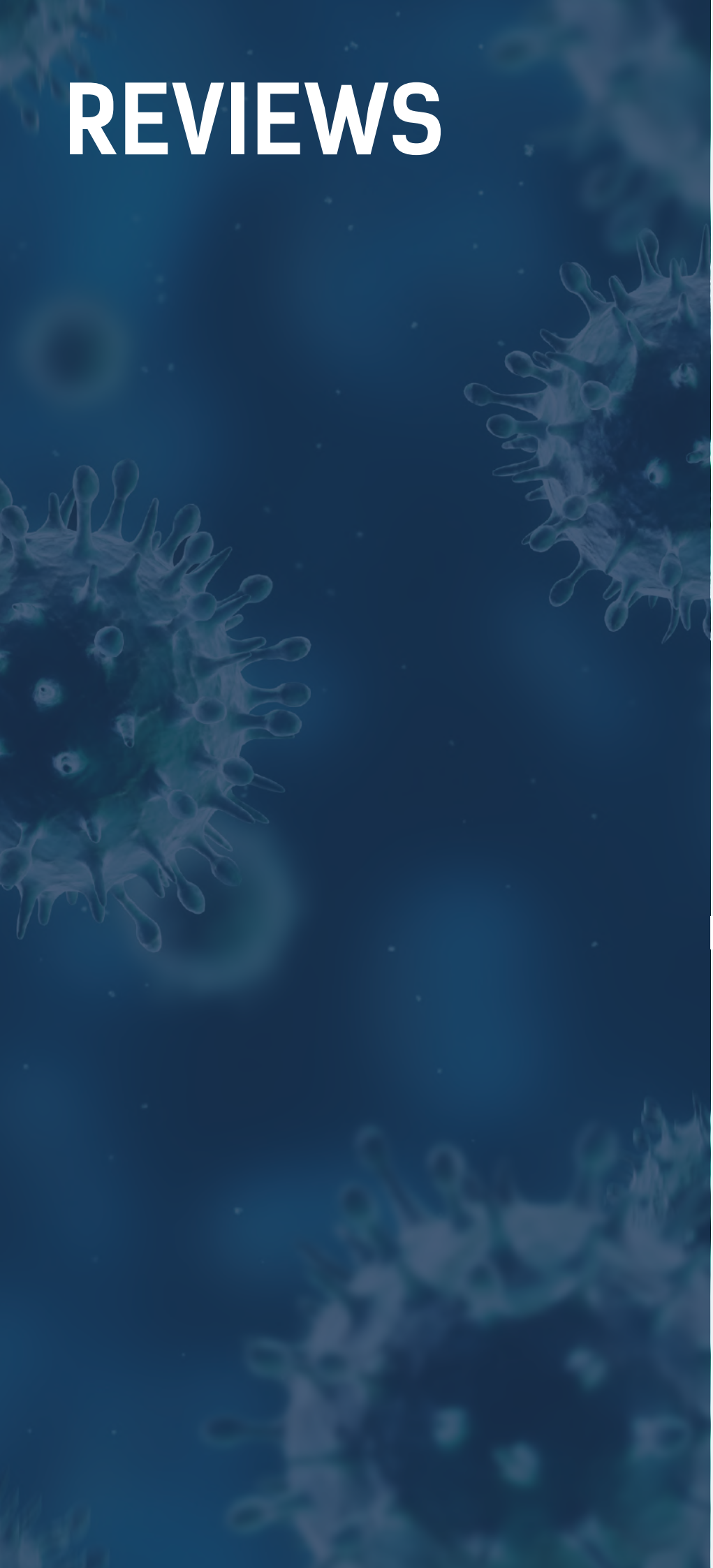
No.	Sampling point	Concentration of microorganisms, CFU/50 l			
		QMAFAnM	Average for QMAFAnM	Mold fungi	Average for mold fungi
1	Cheese-making area before air filtration	334	421	153	165
		488		172	
		442		169	
2	Cheese-making area after air filtration	6	4	23	9
		2		0	
		1		4	

Head of Production Laboratory /*signed*/ M. S. Kirillova

Chief Process Engineer /*signed*/ I. N. Bolshakov

*/Seal: Federal State Unitary Enterprise * "Experimental Cheese Factory" * (FSUE ECF) * Primary State Registration Number (OGRN) 1027601304246 */*

REVIEWS



ROCKET-SPACE CORPORATION

/Logo: S.P. Korolev ENERGIA/

141070
Korolev
Moscow region,
Ul. Lenina, 4-a
Telegraph GRANIT
Phone: (495) 513-86-55
Fax: (495) 513-88-70, 513-86-20, 513-80-20
E-mail: post@rsce.ru
http://www.energia.ru
___No.___
To No. ___ __

Review of the air decontamination unit (ADU) Potok150MK on board of the orbital station MIR and the International Space Station.

Air decontamination units (ADU) Potok 150MK developed by Research and Production Company Potok Inter LLC, Moscow, was used between 1998 and 2000 on board of the orbital station MIR, and since 2000 until now it has been used in the system for ensuring gas composition of the International Space Station.
During its use, ADU Potok 150MK has proven to be reliable high-performance equipment successfully meeting the challenges of ensuring the purity and safety of the air in the pressurized compartment of manned space stations. High efficiency of inactivation of any microbial species and filtration of high-dispersity aerosol carried out by ADU Potok 150MK quickly solved problems encountered in emergency situations related to excessive concentrations of microorganisms in the station air, emission of toxic "fire" aerosol, etc.
Given the positive experience, RSC Energia plans to keep using ADU Potok 150MK in the developed space programs.

RSC ENERGIA /*signature/* S.Yu. Romanov

September 30, 2010

*/Seal: S.P. Korolev Rocket-Space Corporation Energia Open Joint-Stock Company*Russian Federation*Korolev*Moscow Region*Primary State Registration Number 1025002032538, administrative office/*

Moscow Healthcare Department
N.I. Pirogov City Clinical Hospital No.1.
119049, Moscow, Leninsky pr.,dom 8
Tel. (495) 236-60-69, fax 236-65-28
16.03.12 No.449
To No.__ of __

Research and Production Company Potok Inter LLC
111250, Moscow, ul. Krasnokazarmennaya, d.12., str.9.
A.V. Nagolkin

In response to your inquiry No.23KO/12 dated 05.03.2012 we inform that since 2006, the 5th surgical building of N.I. Pirogov CCH NO.1, ADUs Potok 150-M-01 built in the ventilation system have been ensuring clean air in operating rooms and intensive care rooms. Throughout the period of their operation, the levels of air contamination in these areas are within the ranges stipulated by the requirements of sanitary and epidemiological rules (Sanitary Regulations and Standards 2.1.3.2630-10).
According to the hospital technicians, they are easy to use, require no consumables and there has not been a single case of equipment failure. The efficiency of the system is controlled by the remote control and monitoring consoles.
Thus, the experience in the use of air disinfection units Potok 150-M-01 at our hospital showed its sufficient effectiveness corresponding to the parameters stated by the manufacturer.

Chief Physician */signature/* A.V. Shabunin

Prepared by prof. V.V. Kuzin t. 9586003

Moscow Healthcare Department
Emergency Children's Surgery and Traumatology Research Institute
(ECST RI)
B.Polyanka ul., d.20, Moscow, 119180
Tel./fax 959-27-79, tel./fax 959-48-81
E-mail: leonid-roshal@lamport.ru
Russian Business and Organization Classification71635506, Primary State Registration Number 1037789054687, Taxpayer Identification Number 7706517001/ Tax Registration Reason Code 770601001

11.01.12 No.5
To No.__ of __

To General Director
Research and Production Company Potok Inter LLC
A.V. Nagolkin

Dear Aleksandr Vladimirovich,

In 2006, our Emergency Children's Surgery and Traumatology Research Institute had its ventilation system improved. It included air decontamination units (ADU) Potok 150-M-01 produced by the Moscow Research and Production Company Potok Inter. The units have been operating continuously for 5 years in the operating unit and an intensive care unit, and we can make some positive conclusions based on the operating results. Air sampling in the premises where air decontamination units (ADU) Potok 150-M-01 are used showed the absence of pathogenic organisms in the air. We believe that this factor significantly reduces the risk of infection and complications in the postoperative period. The quality of the air samples is fully compliant with existing sanitary and epidemiological rules and regulations.
Units are constantly monitored by automatic equipment. Information on the status of units is received by maintenance service professionals on consoles that are located in the adjacent room. Potok units are easy to use, consume little power and do not require consumables, which significantly reduces operating costs and other expenses. There are no complaints about their work.
According to our information, technology used in Potok units is innovative and has received positive reviews and recommendations from the Ministry of Health and Social Development of the Russian Federation. We are extremely grateful that such high-end equipment has been successfully operating in our hospital for many years.
We wish the Russian developer of Potok units continued success and congratulate on a high medical award - National Best Doctors of Russia Award "Prizvanie" in the nomination "For contribution to medical progress made by the representatives of fundamental science and non-medical professions".

Kind regards,

Director, Professor */signature/* L.M. Roshal

Moscow Healthcare Department
State Budgetary Institution Scientific and Practical Center for Children with Craniofacial Abnormalities and Congenital Nervous System Diseases

119620, Moscow Tel. 8 (499) 730 98 43
Ul. Aviatorov, 38, Fax 8 (499) 730 98 27
September 25, 2012
No.700

To General Director
Research and Production Company Potok Inter LLC
A.V. Nagolkin

Review
of the air decontamination system with units Potok 150-M-01

Since 2004 until now, Scientific and Practical Center for Children with Craniofacial Abnormalities and Congenital Nervous System Diseases of the Moscow Healthcare Department has been using air decontamination system based on units Potok 150-M-01. Potok systems are used in the supply ventilation of 9 rooms, including in operating room of cleanliness class A and CT and X-ray rooms of cleanliness class B. Autonomous recirculating units Potok 150-M-01 are used as additional equipment.
During the use of the air decontamination system Potok in our medical facility, we experienced its high efficiency in ensuring the sanitary and epidemiological purity of air and creating biologically safe conditions for patients and medical staff.
The results of sanitary and microbiological indicators tests regularly held by the Epidemiological Service of the Center confirm the effectiveness of the system. Bacteriological indicators of microbial contamination for the entire period of operation do not exceed the norms established by the Sanitary Regulations and Standards 2.1.3.2630-10 4
Operation Service notes stable system reliability. Management and control of its efficiency is carried out automatically. The absence of any consumables makes Potok unit cost-effective.
Based on the available operating experience, we can confidently assert that the air decontamination system Potok is technically elaborated state-of-the-art equipment helping to solve the problem of ensuring sanitary and epidemiological safety of air for healthcare facilities and creating the necessary conditions for effective treatment.

Deputy Director for Equipment of Scientific
and Practical Center for Children /*signature*/ A.Yu. Pundikov

*/Seal: State Budgetary Institution Scientific and Practical Center for Children with Craniofacial Abnormalities and Congenital Nervous System Diseases*Moscow Healthcare Department*State Budgetary Healthcare Institution of Moscow*Primary State Registration Number /illegible//*

Prepared by A.Yu. Pundikov 8(499)727-76-95

Translation from Russian into English

JOINT STOCK COMPANY "Kaluga Poultry Farm»

s. Leo Tolstoy, Dzerzhinsky District, Kaluga region, Russia, 249842 Tel. (48434) 4-41-84, fax (48434) 7-14-75

CONCLUSION

On the effectiveness of the ADU "Potok-150-M-01" to reduce air contamination level in the areas of poultry production

/Logotype of the JSC "Kaluga Poultry farm"/

Kaluga **«__» _____2016**

The efficiency of the air disinfection unit "Potok-150-M-01" was estimated based on the results of the air microbiological tests in the working premises at the JSC "Kaluga Poultry farm" (AO “Ptitsefabrika Kaluzhskaya”). Testing and sampling were carried out in the cutlet production and vacuum packaging departments.

To improve the accuracy of the evaluation result, in addition to the main sampling methods (sedimentation), the supplementary method was used - an aspiration method, which was applied by means of automatic sampling of biological air aerosols (aspirator PU-1B).

The aim of the tests was to determine the concentration of total bacterial count (TBC), yeasts and mold fungi in the air of process equipment working zones in the course of production process of operable ADU "Potok 150 M-01" and when off.

The results of these studies (Minutes No 1 as of May 25, 2016 and Minutes No 2 as of June 7, 2016) have shown that the air disinfection units "Potok-150-M-01" in the cutlet production department have made the TBC concentration 4.5-12 times less, yeasts and mold fungi - 11-66 times less. In vacuum packaging department TBC concentration was 2-7 times less, yeasts and mold fungi - 0-1.5 times less.

Tests carried out have shown the effective operation of the air disinfection unit "Potok-150-M-01" to significantly reduce the level of air contamination in the production areas in a short period of time.

Air disinfection units "Potok-150-M-01" are recommended for permanent use in areas of meat production in order to reduce the level of air contamination, which in turn has a positive effect on food safety and longer shelf life.

Head of Quality Department Of JSC "Kaluga Poultry farm" */Signature/* Nazarova I.A.

Head of production and process laboratory */Signature/* Titov D.V.

/Seal: Dzerzhinsky District, Kaluga region, Russia Joint Stock Company "Kaluga Poultry farm", for documents № 4 TIN 4004001997 RRC 400401001/

Signature

Translated from Russian into English
Перевод с русского языка на английский язык

APPROVED
by the Head of the Central Naval Clinical Hospital No. 32
Medical Service Colonel
***/Signature/* A. E. Tkachev**
December 14, 1999.

/Seal: Central Naval Clinical Hospital No. 32/

REVIEW
of the air sterilization systems Potok 150-M-01

The air decontamination units (ADU) Potok 150-M-01 have been used by the Vascular Neurology Ward of Central Naval Clinical Hospital No. 32 since October 1998.
When dealing with the restoration of disturbed brain function through the intratissual reconstruction (work nominated for the 1999 State Prize of Russia), we faced a serious problem of ensuring protection of the patients with damaged immune status from secondary infection. To solve this problem, UV lamps, which are the most common in our country, cannot be used due to severe restrictions on their operation in the presence of people. Use of the HEPA filtration air purification systems is also problematic because of high cost, difficulty in installation and high maintenance costs.
Use of ADU Potok 150-M-01 in the operating rooms, intensive care units and rooms solved this problem. The number of postoperative complications has decreased by 15%. We also used ADU to create and maintain sterile air in the laboratories where nerve cell cultures are grown. Check air studies have confirmed its high sterility. This allowed achieving the effect of recovery of brain and its functions.
Given the abovementioned, as well as ease of use and a high degree of reliability, we believe that ADU Potok 150-M-01 is an efficient means of preventing complications of vascular and neurological profile.

Head of Vascular Neurology Ward
Medical Service Colonel /*Signature*/ A. S. Bryukhovetskiy

Moscow Healthcare Department
City Clinical Hospital No.24
127015, Moscow, Pistsovaya ul., d.10
Tel.613-04-08, fax 613-04-34.
17.11.11 No.650
To No.__ of __

To General Director
Research and Production Company Potok Inter LLC
A.V. Nagolkin

REVIEW
of the air decontamination system with units Potok 150-M-01

Air decontamination systems with ADU Potok 150-M-01 has been used since September 2009 until now as a part of ventilation and air conditioning systems in 7 operating surgical wards of City Clinical Hospital No.24.
These systems provide the necessary air quality that meets the requirements of Sanitary Regulations and Standards 2.1.3.2630-10 "Sanitary requirements for organizations engaged in medical activities", as evidenced by regular testing of air swabs and samples in operating rooms, which are held by Federal State Healthcare Institution Federal Service for Supervision of Consumer Rights Protection and Human Well-Being. There are no complaints about their effectiveness and reliability.
Air decontamination systems with ADU Potok 150-M-01 is easy to operate, efficient and reliable in operation, provides continuous monitoring of the units. The absence of any consumables and low power consumption allows reducing operating costs significantly.
During the operation of these systems, we observed reduction in the cases of postoperative complications and sepsis.
According to the results of operation of air decontamination systems with ADU Potok 150-M-01,
I consider it possible to recommend their use in healthcare, since they increase the effectiveness of treatment, simplify maintenance and significantly reduce operating costs.

Chief Physician /*signature*/ V.B. Aleksandrov

*/Seal: City Clinical Hospital No.24 of Moscow Healthcare Department*State Budgetary Healthcare Institution of Moscow*Primary State Registration Number 1037739726771*Registered in the Seal Register No. 14809004006/*
Prepared by V.I. Koltsov 613-07-05

/*signature*/

/Wimm-Bill-Dann logo/

Open Joint-Stock Company "Wimm-Bill-Dann"
(OJSC "WBD")
127591, Moscow, Dmitrovskoye shosse, 108
Tel.: **+7 (495) 745-80-80**
Fax: **+7 (495) 483-00-47**
www.wbd.ru

Attn. A.V. Nagolkin,
Director General
of Potok Inter Engineering Company LLC

REPORT ON THE RESULTS OF "POTOK 150 M-01" AIR DECONTAMINATION DEVICE APPLICATION

In May 2014 OJSC "Wimm-Bill-Dann" finished the installation of "Potok 150 M-01" air decontamination device in Hassia diary products packing line of Lianozovsky Diary Plant OJSC, Moscow, and put it into operation.

Use of "Potok 150 M-01" air decontamination device enabled to protect the packaged products against microbiological contamination in the packaging area which helped to avoid defective products and increase the products shelf life.

Use of "Potok" air decontamination device enabled to considerably reduce the operating costs due to absence of consumables and low energy consumption which also made it possible to no longer use the expensive HEPA filters at the air intake to the filling tunnel of packing machine.

Following the operation results we recommend the use of "Potok 150 M-01" air decontamination device for food facilities as an effective solution providing biological air purity in packing, bottling and sorting departments.

We also recommend Potok Inter Engineering Company LLC as a reliable supplier of innovative air decontamination solutions.

Chief Engineer

MARCH 11, 2015

/Seal: Moscow, Open Joint Stock Company "Wimm-Bill-Dann", OJSC "WBD", Primary State Registration Number (OGRN) 1027739768924, Taxpayer Identification Number (INN) 7713085659/

/S.A. Korol/

/Signature/

/Pepsico logo/

THE MINISTRY OF CULTURE OF THE RUSSIAN FEDERATION
/*logo*/
The State Research Institute for Restoration
107014, Moscow, Gastello Street, 44. Tel. 214 48 11. S/A 200903 opened with Moscow Business Bank Branch. MFO 291100. Code 17

To Mr. A.V. Nagolkin,
Director,
POTOK Ltd.,
Research and Production Company

April 28, 1995 No. _____
Ref.No. _____

OPINION
on the possible use of the Potok-150M portable recirculation unit in cultural institution facilities

The Opinion is based on the results of examination of the materials presented under the study into air biological purification (by D.I. Ivanovsky Institute of Virology, the Institute of Epidemiology and Microbiology and others) and hygienic safety when the unit is used indoors (by A.N. Sysin Research Institute of Human Ecology and Environmental Hygiene).

The Potok-150M unit is designed to maintain continuous fine air cleaning from dust, spores and other contaminants. Having reviewed the unit documentation containing basic technical details and specifications, unit description, operating principle, operation procedure and safety precautions, we come to the conclusion that the unit is in line with its purpose and meets the requirements set to devices used to clean air environment indoors. We have to underline that the unit's specific design features pose no threat to humans during its operation.

As it follows from the test results, when the device is used in filtration mode the air is ozonized within the maximum contamination levels (MCL) for ozone at a distance of 0.5 m from the unit for rooms of at least 20 m^3 and shows no increase during 12-hour continuous operation.

Electric discharge to generate ozone in a tested device may result in nitrogen oxides. The monitoring has showed that the air concentrations of these components exceed no maximum contamination levels when the unit runs in continuous filtration mode for 12 hours in a room of at least 60 m^3.

The unit's optimum operating conditions are to be determined and corrected with due account for both (ozone and nitrogen oxide) indicators.

The unit performance results in almost 2-time decrease in the level of toxic substances (benzene, ethylene-benzene, xylene), and such compositions as butyl acetate disappear completely.

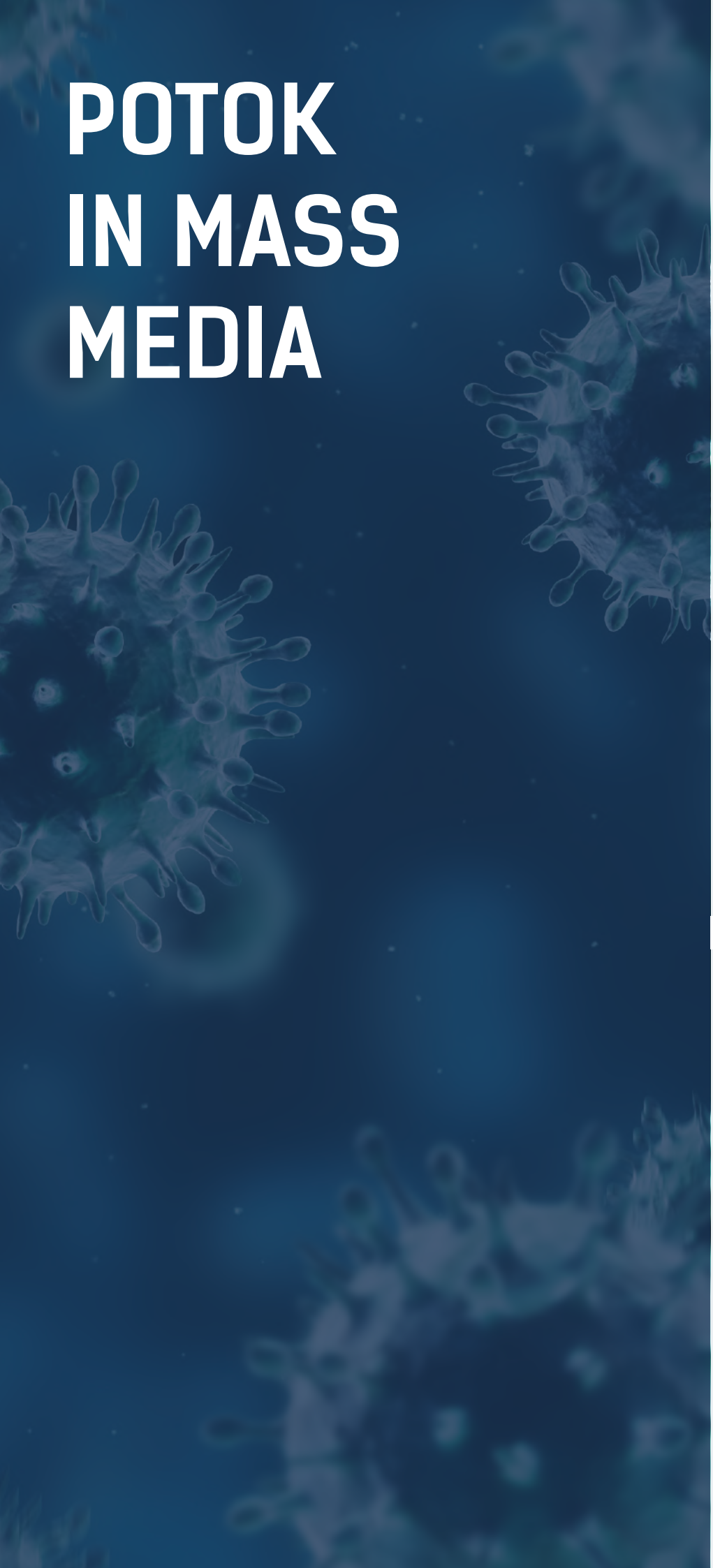
Hence, when the unit continuously operates in rooms of at least 60 m^3 for 12 hours the ozone and nitrogen oxide content is less than the maximum contamination levels, and the concentration of

toxic substances is decreased, which results in “clean” working zones under 100% biological purification with minimum expenses.

Based on the above said, it makes sense to conclude that the Potok-150M unit can be used to clean air in storage facilities for plastic arts, furniture and books in good condition which are generally stored in old buildings without air conditioning system where toxic substances generated by material destruction processes tend to accumulate in the air.

Head of the Center for Cultural Property Safety,
The State Research Institute for Restoration /*signed*/ L.I. Dushkina

POTOK IN MASS MEDIA



potok

Long-term spaceflight and microbiological safety issues

Natalia D. Novikova
Institute of Biomedical Problems of the Russian Academy of Sciences

Elena A. Deshevaya
Institute of Biomedical Problems of the Russian Academy of Sciences

Svetlana V. Poddubko
Institute of Biomedical Problems of the Russian Academy of Sciences

During the last few decades there have been significant developments in Russian astronautics, one of the most visible results of which has been the creation of long-term orbital space stations. The multi-year operation of these orbital stations have taught scientists and engineers many lessons as well as highlighting issues connected with crew safety and equipment reliability. Problems posed by microorganisms that are able to live and reproduce in space are one of the key challenges currently being addressed.

Microorganisms are record-breakers when it comes to the longest period an organism has survived in space. They not only live on orbital stations but they develop, adapt to the space environment and are able to reproduce. The longer spacecraft operate the more bacteria and microscopic fungi appear and, according to Russian scientists, there are currently over 250 types of microorganism living in space.

As is well known, microorganisms are a unique life form. They're characterised by unprecedented numbers and variations, ubiquity, extensive interaction with their environment and their ability to have a significant affect on the latter.

Microorganisms' activity and their role in the circle of life on Earth is well-known. Their inter-relationships with animals and humans can be either mutually advantageous (mutualism) or parasitical, and sometimes take on the most

► Fig 1: Mould growth on intercom apparatus, on the wire insulation.

negative form of all - infectious diseases, including the most dangerous ones on the planet.

They are incredibly resilient and malleable, and can be found in the most extreme environments. They can survive under conditions that prove lethal to other life forms - high and low temperatures, radiation doses that are deadly to humans, very little nutrition, etc.

The fact that microorganisms are capable of establishing residence in the material of an orbiting module is of critical importance

As such the ability of microorganisms to inhabit orbital space stations is practically limitless. The artificial environment that is created and maintained inside a spacecraft or space station presents optimal living conditions for humans, and is therefore even more comfortable for most known microorganisms, which are far less particular about their living conditions.

The source of microorganisms inside spacecraft can be either astronauts (including their epidermis and mucous membranes) or various cargo shipments - equipment, consumable materials, etc, that are regularly delivered by cargo and transport ships into orbit.

Obviously, this process cannot be completely avoided, as every time a person talks, coughs, exerts themselves physically, or even simply breathes, a significant number of microbes are emitted into the environment.

It is also impossible to fully sterilize all cargo and transport ships, including the cargo they deliver, even though much has been done to minimise transmission. Despite our best efforts, microorganisms continue to make it into space stations and spaceships.

So what are the possible consequences of this process as far as spaceflight safety is concerned? In reality, the situation is quite dire. First of all, in cases of a weakened immune system, some microorganisms that are usually benign may cause infections or allergies.

But there is another side to the problem - so-called microbial omnivory, which is the ability of such microorganisms to break down the most varied chemical compounds. Coming into contact with different materials, some microflora, most often bacterial and fungal combinations, adapt quickly to new environments and commence their vital life activities, which may include aggressive metabolic product emanation.

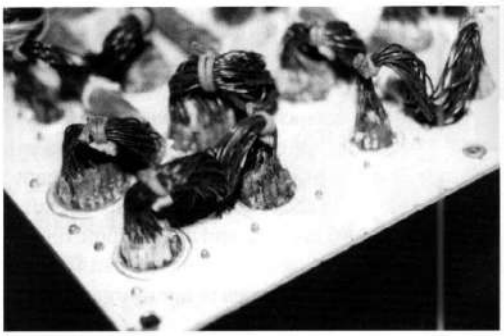
As a result, affected materials may change colour, and their mechanical resistance, pressurised sealing qualities, dielectric and other characteristics may become compromised. Most importantly, these processes could lead to the breakdown of equipment, which is unacceptable in working space stations.

The situation is exacerbated by the fact that in conditions lacking flow-through ventilation in enclosed spaces, air moisture may condense in certain spots that contain chemicals that microorganisms may feed on. A microorganism's development may also be spurred on by physical factors of spaceflight - periodic fluctuations in solar activity, radiation levels, or magnetic field gradients, etc.

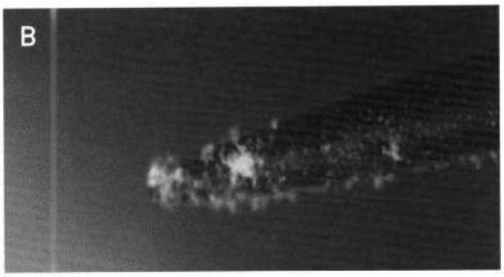
Russian orbital space station operational experience shows that processes like the growth of microorganisms can damage polymer-based construction materials. Metal bio-corrosion, the formation of biofilms and thrombus in the hydraulic water regeneration lines should be considered constant ecological risk factors.

Research on the issue of microbiological damage of construction materials began at the Medical and Biological Problems Institute of the Russian Academy of Sciences (MBPI RAS) back when Salyut 6 orbital station was in operation. The fifth main crew of the station discovered a white substance in some parts of the interior, on exercise equipment rods and some other parts of the living modules. Samples delivered back to Earth and studied were found to contain mould fungi - penicillium (black mould fungus) and aspergilli.

During the fifth main expedition to the Salyut 7 orbital station, a message was received from the cosmonauts about the discovery of visible mould in the interior, joints and cables of a work module.



► Fig 2: Mould growth on the intercom apparatus, on the wire insulation connectors.



◀ Fig 3: Mould growth on the fire suppression gauge - inside the casing (A) and needle bio-corrosion (B).

Again, samples were collected and brought to Earth for investigation. Visual examination showed that 25 to 50 per cent of sample surfaces were covered with the mould mycelia. When examined under the microscope, changes in the samples' structure were discovered, and some materials, particularly adhesive tape, had noticeable penetration defects.

The case of the navigational viewport on one of the Soyuz transport ships, that was used for six months at the Mir orbital station is of particular interest. During their mission, members of the third main crew observed an on-going reduction in the viewport's optical characteristics.

Research was conducted once the transport vehicle came back to Earth, and the following was discovered: the central window and most of the viewport's peripheral windows, which were made of heavy-duty quartz glass, as well as the enamel cover of the titanium rim, had evidence of mould mycelia, with one spot having a visible growing fungal colony.

Mycelia created an etching effect on the glass. Visually, it seemed that the source of the fungi was the paronite (rubber) seal that had been used to affix the glass to the titanium rim. A combination of microorganisms, including spore-forming bacteria and fungi were found in affected areas.

Another example of microbiological equipment damage is the breakdown of the control switchboard that was delivered back to Earth with the return of the 24th Mir mission. Actively growing mould was found under the metal cover of the mechanism, including on the conduit tubes, connectors, reinforced poly-urethane (Fig. 1). This process caused simultaneous corrosion of copper wires in areas where the insulation was damaged.

Other instances of microbiological equipment damage have also occurred during orbital station operations. Multiple malfunctions in the water regeneration system were noted - these were caused by gel-like thrombi that formed due to bacterial and fungal growths in the gaps between hydraulic lines, which were used to get condensation to the regeneration section of the mechanism.

A fire detector unit broke down at the International Space Station (ISS) because of bio-corrosion of the needle that received the electric charge (Fig. 3). Visible growth of mould was recorded by astronauts multiple times, especially on equipment located on the outer panels (Fig.4).

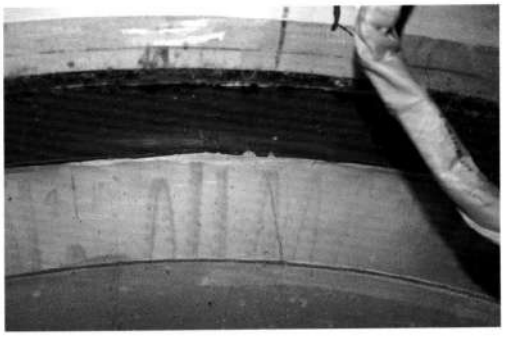
In some instances these processes were accompanies by damage to materials, including mould growth on aluminium, which led to its bio-corrosion and holes in the material (Fig. 5).

There are two main types of microorganism aggression in relation to construction materials: 'direct influence' (ie fermentative decay of materials as they're used as a source of nutrition) and 'indirect influence' - growth on contaminated materials, with abjection of organic acids and other by-products.

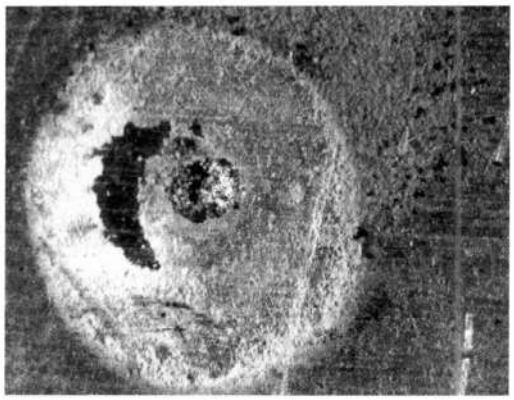
A good example of the latter is the damage to the heavy-duty quartz glass of an ISS viewing window. Of course the microorganisms did not use it for nutrition. They grew on the glass due to the lipid film, atmospheric moisture condensation and other contaminations, but at the same time, the secretions of their metabolic processes compromised the optical characteristics of the glass.

The fact that microorganisms are capable of establishing residence in the material of an orbiting module is of critical importance. Genetic research found the presence of this trait among a number of microorganisms taken from the Space Station's interior and equipment during spaceflight. It was proven that Penicillium

The longer spacecraft operate the more bacteria and microscopic fungi appear



◀ Fig 4: Fungal growth behind a service module panel.



► Fig 5: Microbiological damage on aluminium (appearance of holes).

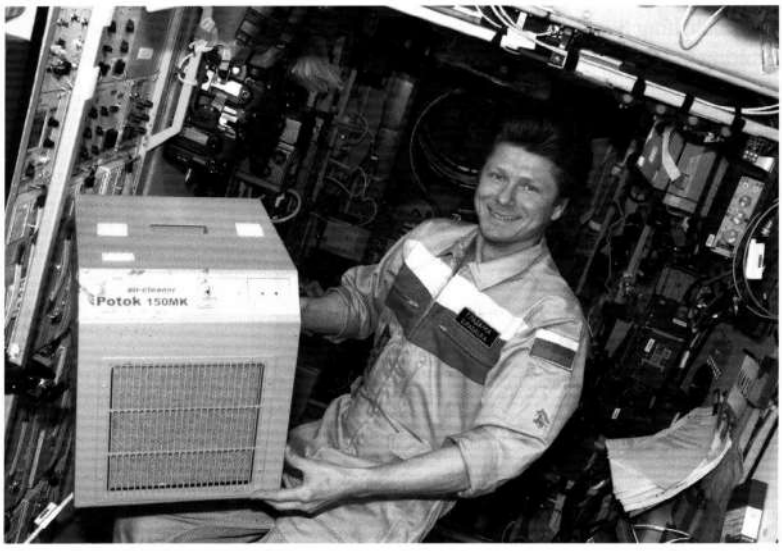
crysogenum fungi were constantly found in the interior of the Mir orbital station during the first 10 years of its operation, with certain fungal strains being the descendants of cultures found seven or eight years earlier.

Nevertheless, orbital space stations have been successfully operating for many years and the microbiological safety system developed by MBPI RAS plays an important part in this process. It includes, first and foremost, the constant screening and microbiological analysis of the air, the atmospheric moisture condensation, regenerated water, the surfaces of decorative and construction materials. In cases of even insignificant increase over the accepted microorganism norms, the crew is instructed to disinfect their environment. Cleaning and sanitation with vacuum cleaners and special antimicrobial wipes are conducted regularly onboard space stations, as is isolation and removal of waste with the use of cargo and transport ships.

The system cleans and disinfects the air by rendering microorganisms inactive with constant electric fields

The Potok 150 MK system has been used on the ISS since 2001 (and since 1995 on the Mir orbital station before it). The system cleans and disinfects the air by rendering microorganisms inactive with constant electric fields. This equipment is currently being used successfully on board the ISS in both the Russian Zvezda module and the American module - the functional cargo block. Using equipment with this technology in space medicine allowed astronauts to keep microorganisms in the air in a normal range for long-term spaceflights. Two Potok-150MK systems, which clean the air currents from aerosol particles and microorganisms, are turned on daily on the ISS (Fig. 6).

▼ Fig 6: Installing the Potok-150MK air cleaner on the ISS.



In order to prevent microbiological damage to construction materials and equipment, all materials are tested for their ability with withstand microorganisms prior to being used on space stations. Astronauts use special methodology to conduct periodic inspection of interior surfaces and station equipment, including outer panels. If suspicious areas are located, microflora samples are collected and the area is treated with a special chemical, Fungistat.

At the present time, MBPI RAS scientists are working on a number of applied solutions to increase the effectiveness of the current system of microbiological safety for piloted spaceflights. The most important of these are the development of methods that will ensure materials have antimicrobial properties created by surface modifications or antimicrobial film coating, and the creation of onboard instruments for early detection and diagnostics of microbiological damages. Solving these problems will create an advantageous ecological atmosphere on the International Space Station for a long time to come. ■

***Natalia D. Novikova**, PhD, Doctor of Biological Sciences, Academician of International Academy of Astronautics, Institute of Biomedical Problems of the Russian Academy of Sciences, Head of Laboratory of Environmental Microbiology and Antimicrobial Protection.*

***Elena A. Deshevaya**, PhD, Institute of Biomedical Problems of the Russian Academy of Sciences, Leading Researcher in the field of mycology. Laboratory of Environmental Microbiology and Antimicrobial Protection.*

***Svetlana V. Poddubko**, PhD, Institute of Biomedical Problems of the Russian Academy of Sciences, Leading Researcher Laboratory of Environmental Microbiology and Antimicrobial Protection.*

MAY - JULY 2017 Vol. 5 • No. 2

FOOD MANUFACTURING AFRICA

Journal for food and beverage manufacturers

Two *strong forces* collide

Custom processing all the rage

Food's fab **new flavours**

Harness **innovative technology** in CSD

A new era in *decontamination technology*

The All-Russian Research Institute of Poultry Processing Industry has published the results of a trail-blazing report. The object of this research was to investigate the impact of microbiological air composition on product quality. The effectiveness of a Potok decontamination unit at poultry processors was also evaluated.

Table 1: Microbiological air parameters at the vacuum packing line before processing (background) and after one hour of operation of unit.

Air sampling site	Microbiological data			
	QMAFAnM, CFU/m3		Mold, CFU/m3	
	Background	After one hour of unit operation	Background	After one hour of unit operation
Smoked chicken packing table	$1.2*10^2$	$6.0*10^1$	$3.4*10^3$	$1.3*10^3$
Sausage packing line	$1.8*10^2$	<10	$1.2*10^3$	$8.2*10^2$
Smoked chicken labeling	$1.4*10^2$	$8.0*10^1$	$1.8*10^3$	$4.2*10^2$
Sausage labeling	$4.0*10^1$	$2.0*10^1$	$1.4*10^3$	$2.4*10^2$
Finished products weighing	$1.2*10^2$	$6.0*10^1$	$9.0*10^2$	$3.6*10^2$

THE METHODOLOGY OF the work consisted of two stages. In the first, research on the screening of microflora at all stages of poultry processing was carried out. In the second, it investigated the effectiveness of air decontamination in poultry processing, and its impact on product quality.

Studies of air in industrial premises of poultry processing factories were conducted in the packing room of the slaughter and processing complex, and at the vacuum packing line of poultry meat. To facilitate this, the process flow diagram and layout of production facilities were analysed. A visual inspection of production facilities was also carried out to identify potential sources of bioaerosols.

Samples were taken at the height of the product, and at a distance of 20 to 30cm. An automatic air sampler of biological aerosols (aspirator) PU-1B was used. After sampling, petri dishes were placed in a thermostat and incubated at optimal temperature for the growth of this group of microorganisms.

*"It was found that **air contaminated by microorganisms** can be **a source of microbial contamination** of poultry carcasses"*

FINDINGS OF AIR SAMPLES

Mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) and mould were detected in the air of the packing room in all air sampling points. The air of industrial premises can be a source of microbial contamination of poultry carcasses. This affects the quality of products and reduces the shelf life of poultry meat.

INVESTIGATION OF THE EFFICIENCY OF AIR DECONTAMINATION

After placing the Potok units in the specific process area (vacuum packing line) control measurements of air contamination were performed. Once the units were turned on, an experimental measurement of air contamination after one hour of operation was performed.

Evaluation and efficiency of the unit was determined by comparing the values of bacterial contamination of the air before the start of the unit operation (background level) with the contamination level after one hour of unit operation (Table 1).

It was found that air contaminated by microorganisms can be a source of microbial contamination of poultry carcasses. This potentially affects the quality and shelf life of poultry meat. Comparative tests of air pollution before the start of the Potok-150 operation, and one hour after completion were conducted. Results indicate that the number of microorganisms (fungi and MAFAnM) decreased by two to 10 times after one hour of unit operation. This demonstrates the unit's high efficiency.

One of the important issues of expansion of poultry meat production is the limited shelf life, as meat is a positive breeding ground for microorganisms. With an increase in shelf life, the supply of poultry meat over long distances is extended, realisation time is increased and refrigeration, freezing chambers and transport costs reduced. •

Potok – www.potok-inter.ru

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Biodegradation of black oil by microflora of the Bay of Biscay and biopreparations

A.N. Shkidchenko [a], E.N. Kobzev [b,*], S.B. Petrikevich [a], V.A. Chugunov [b], V.P. Kholodenko [b], A.M. Boronin [a]

[a] *G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Moscow Region, 142292 Pushchino, Russia*
[b] *State Research Center of Applied Microbiology, Ministry of Health, Russian Federation, Moscow Region, 142279 Obolensk, Russia*

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Abstract

Recent research has shown that the bioremediation of oil-polluted soil by activation of microflora is not always advantageous when compared with the introduction of oil degrading microorganisms. Although microflora isolated from oil-polluted sites is adapted for growth under the above conditions, this does not imply a high rate of oil degradation. Indeed, it has been shown that the hydrocarbon-oxidative activity of isolated strains to black oil was 13.1–17.3% (incubation for 10 days at 24 °C), while the activity of a mixture of three aboriginal strains was 17.8%. At the same time, the hydrocarbon-oxidative activity of associations of the strains isolated from other regions was 24.0–30.0% (in 10 days).

Keywords: Biodegradation; Black oil; Aboriginal microflora; Introduction; Biopreparation

1. Introduction

In the spring of 2000, a black oil-carrying tanker was shipwrecked near the French shore in the Bay of Biscay. As a result, about 10,000 tonnes of black oil ran out into the ocean polluting the near-shore water area, cliffs and sand beaches of the Bay of Biscay.

Microorganisms play the main role in biodegradation of oil hydrocarbons, along with physicochemical processes. More than 70 genera of microorganisms capable of utilizing hydrocarbons as a sole carbon source are known at present [1–3].

There are two principal approaches to bioremediation of oil pollutions

- stimulation of the activity of aboriginal hydrocarbon-oxidative microflora by improvement of aeration, watering, introduction of biogenic elements and biologically active compounds [4,5];
- introduction of active strains of hydrocarbon-oxidative microorganisms and their associations into polluted objects as a biopreparation [6–8].

Introduction of oil degrading strains is most advisable under unfavourable conditions, e.g. in the northern regions with a short vegetation period or when oil gets into water, where the enrichment culture based on the natural microflora forms very slowly even under optimal conditions [3]. The introduction of biopreparation decreases the pollutant concentration and may be used at a pollution level of 20%.

At the same time, a number of scientists reject the introduction of biopreparations into a polluted environment, supposing it to disturb the natural ecological situation [9]. Soils with a low initial concentration of pollutant (up to 10 l/m^2 oil), in the opinion of some researchers, can be remediated only by stimulation of the activity of aboriginal hydrocarbon-oxidative microflora by loosening, watering, and introduction of the sources of biogenic elements and bioactivators [7–11].

In the opinion of some researchers, the aboriginal hydrocarbon-oxidative microflora of polluted sites has an indisputable advantage over the introduced microorganisms, because it is most adapted for growth under the prevailing conditions [9].

One of the methods of bioremediation is the use of biopreparations containing oil-degrading microorganisms isolated from oil-polluted soil samples and the growth of their

* Corresponding author. Tel.: +7-967-360001; fax: +7-967-360068.
E-mail address: e-kobzev@yandex.ru (E.N. Kobzev).

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biomass followed by introduction into a polluted object from where they had been isolated.

The goal of the present work was to study the hydrocarbon-oxidative activity of the aboriginal microflora of oil pollutions in the Bay of Biscay and to choose an optimal method of bioremediation of this pollution.

2. Materials and methods

The strains of aboriginal oil-degrading microorganisms have been isolated by a standard method of enrichment cultures from samples taken in the accident area a month after the tanker wreck.

The following strains from the collection of the sector "Biopreparations" of the Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (IBPM RAS): *Pseudomonas putida* Sh-1 and *Rhodococcus* sp. Sh-5 (isolated from oil-polluted soil samples of Tyumen), which constituted Biopreparation 1, and the strains from the collection of the Department of Ecological Biotechnology, State Research Center of Applied Microbiology (SRCAM): *Pseudomonas* sp. strain B, *Flavobacterium* sp. 415, and *Bacillus* sp. (isolated from water samples of the White Sea (the region of Arkhangelsk) polluted with oil products), which constituted Biopreparation 2, have also been used in experiments. All strains are able to actively assimilate diesel fuel, oil and black oil as the sole carbon and energy source.

Oil-degrading activity was determined by culturing the strains isolated in the course of research, as well as Biopreparations 1 and 2, in flasks with a fluid synthetic nutrient medium containing black oil as the sole carbon and energy source. The medium composition has been described in the previous paper [12]. Black oil in the amount of 1 wt.% was introduced into flasks after sterilization of the nutrient medium. Then the flasks were inoculated either with microorganisms sorbed on a carrier (later sorbed by a biopreparation) or with a suspension of microorganisms. Cultivation proceeded at 24 °C on a shaker at 180 rpm during 10 days.

The carrier used in some experiments for immobilization of the biopreparations was pearlite with granules of 3–7 mm in size, foamed in a thermal furnace. The mineral composition of a sorbent was as follows: Al_2O_3: 13–14%, MgO: 13–14%, $K_2O + Na_2O$: 2–6%, CaO: 1–3%, H_2O: 2–4%, SiO_2: 59–69%.

To obtain a sorbed biopreparation, the cultures of oil-degrading microorganisms constituting the biopreparation were inoculated by lawn on LB agar. After 24 h of growth at 24–26 °C, cells were washed off with a mineral medium of the above composition and cell suspensions were mixed in equal proportions. This suspension of microorganisms was mixed with pearlite in a ratio 1:1 (by weight) and the sorbent with immobilized biopreparation was dried at 25 °C for 24 h. The humidity of the sorbed biopreparation was 1%, and the specific concentration of viable cells was about 10^9–10^{10} cells/g sorbent, depending on the biopreparation properties.

For a laboratory soil experiment, 2.5 wt.% black oil as a solution in hexane was introduced into loamy sand and thoroughly homogenized. Hexane was evaporated in an exhaust-hood at room temperature for 3 days with periodical mixing of the soil. Weighed portions of the soil prepared by the above method (40 g) were placed in Petri dishes, where nitrogen, phosphorus and potassium (NPP) salts were added in the form of azophoska as a source of biogenic elements. Tap water in the same volume was added into control variants instead of the salts' solution.

Biopreparation 2 was used in the soil experiment. The biopreparation immobilized on the carrier was introduced and thoroughly mixed with the soil.

These prepared soil samples were incubated in a thermostat at 23–25 °C. Soil humidity during the whole experiment was maintained at 60% total moisture capacity.

Samples for microbiological and chemical analyses were taken immediately after the beginning of the experiment (initial point) and then on Days 15, 30 and 45. The total quantity of microorganisms in the soil was determined by inoculation from dilutions on a surface of enriched agar medium (LB agar) by standard methods [12].

The content and fractional composition of residual hydrocarbons was assayed by the methods described previously [13]. To determine the proportion of black oil in the soil, the entire contents of one dish were used as one repetition (six repetitions per experiment).

3. Results and discussion

The accidental black oil spill resulted in pollution of not only the near-shore water area but also the cliffs and sand beaches of the Bay of Biscay. Such a large-scale ecological disaster could not but affect the microbiological condition of the region of the accident. With regard to the fact that the quantity of aboriginal microorganisms in the sea water, sand soils and on cliffs is usually rather low and that the accident took place in spring (cold season), it may be assumed that the total titre of microorganisms in samples from the site of accident would also be low. However, contrary to these assumptions, the quantity of microorganisms in the samples under study was 10^8–10^9 cells/g sample. Research data are presented in Table 1.

Such a high quantity of microorganisms in the samples under study is probably explained not only by the activation of aboriginal microflora due to the hydrocarbon substrate added but also by the introduction of microorganisms along with black oil. It is interesting that the titre of microorganisms in samples from the cliffs is by an order higher than in other samples. This is probably due to the more favorable thermal and air regimes of the cliffs, which is a significant factor of microflora development, particularly in the cold season.

Table 1
Total titre of microorganisms in the samples under study

Sample number	Polluted object	Total titre of microorganisms
1	Water from the near-shore water area	4.6×10^8 cells/ml
2	Black oil film from the water surface	1.6×10^8 cells/g
3	Sand from the beach (point 1)	1.1×10^8 cells/g
4	Sand from the beach (point 2)	1.6×10^8 cells/g
5	Scrape from the cliffs (point 3)	1.7×10^9 cells/g
6	Scrape from the cliffs (point 4)	6.0×10^9 cells/g

It is also necessary to mention the high titre of microorganisms in water samples from the near-shore water area. The above data contradict the data of other researchers, since it has been reported that oil-oxidizing microorganisms are actually absent in the open sea. The key role in elimination of oil exposed to the Mediterranean Sea in 10 m^3 containers for 5 months was probably due to physicochemical factors, mainly photooxidation. According to other data, in the water taken from the open part of the Gulf of Mexico after the accident on a near-shore well, the enrichment culture formed slowly and signs of degradation of oil-product film appeared only after 2 weeks [14].

Subsequent isolation of microorganisms from the samples under study and their investigation showed that 95% of the aboriginal microflora in all six samples is represented mainly by three morphologically different types of microorganisms. These microorganisms, designated as F_1, F_2 and F_3, were isolated as pure cultures and characterized by a number of parameters, such as the ability to grow on individual hydrocarbons, oil and black oil at different temperatures and pH values.

Three predominant groups of microorganisms in the samples under study on the background of the high content of microflora may be evidence of suppression of biota species diversity in a black oil-polluted object. These groups of microorganisms are probably best adapted to these extreme living conditions.

As the above three predominant groups were found in all samples, it may be assumed that these microorganisms could be introduced into the corresponding sites together with black oil.

The above mentioned suppression of microflora in oil-polluted objects may be associated with black oil toxicity. Fractional composition of the hydrocarbon part of black oil from the sites of pollution was studied to verify this assumption (Table 2). Fractional composition of a Russian black oil M-100 is given for comparison.

Table 2 shows that compounds of the aromatic fraction were predominant in black oil from the Bay of Biscay, and their portion of the total composition was 15.9% higher than in the composition of M-100 black oil commonly used in research. Most likely, the high content of aromatic compounds determines the toxicity of "Biscay" black oil [15].

Table 2
Fractional composition of the hydrocarbon part of black oils

Fraction	Black oil from the Bay of Biscay (%)	M-100 black oil (%)
Paraffin–naphthenes	28.0	42.0
Aromatic compounds	45.9	30.0
Resins	18.5	20.0
Asphaltenes	7.6	8.0

It would be logical to assume that oil-degrading strains isolated from the samples polluted with "Biscay" black oil may have a higher affinity for this pollutant.

The study showed that strains of group "F" isolated in the course of screening are active oil degraders. Their hydrocarbon-oxidative activity was quantitatively assessed in a series of laboratory experiments in a fluid culture by studying the degree of degradation of black oil, from which they were isolated.

In the course of these experiments a number of difficulties were encountered.

Preliminary research showed that the degree of "Biscay" black oil degradation in a fluid medium during 10 days was 50–70%. At the same time, the extraction of black oil from the culture liquid by chloroform was shown to yield no more than half of the initial weighed portion of black oil.

In this connection, the effect of different solvents on extractability of black oil was studied. With this purpose, certain weighed portions of black oil were placed in flasks with a synthetic nutrient medium (the final black oil concentration 2.5%), a solvent was added, black oil was extracted by shaking the mixture for an hour on a shaker, an organic phase containing the solvent and black oil was separated and the solvent–lack oil mixture was evaporated at room temperature.

The ratio of the initial weight of black oil portion and the weight after evaporation of the solvent, multiplied by 100%, is a value of the degree of black oil extractability. The data on extractability are given in Table 3.

The data from the table indicate that hydrocarbon extraction by chloroform, which was used in most experiments, in the case of "Biscay" black oil allows the extraction of only 45% of black oil introduced initially. Subsequent studies have shown that such high losses of black oil at extraction were determined by the presence of admixtures in "Biscay" black oil. Thus, black oil was found to comprise soluble salts

Table 3
Degree of black oil extract

Solvent	Degree of black oil extractability by solvent (%)
Chloroform	45.0
Carbon tetrachloride	46.4
Petroleum ether	64.8
Hexane	80.5
Diesel fuel[a]	94.7

[a] The amount of diesel fuel was 32% of the initial weight of black oil.

3%, sand 2%, and water about 50% of the total weight of black oil. The admixtures were most likely present because black oil for this study was taken not from the tanker (which probably was technically difficult to realize) but from a polluted beach.

The data on black oil extraction by petroleum ether, hexane, and diesel fuel are interesting (Table 3). If the amount of admixtures (about 55%) are subtracted from the values given in Table 3, then a certain difference will remain (about 9% for petroleum ether, 35% for hexane, and 40% for diesel fuel). This difference cannot be attributed to the better extractability of black oil by the above solvents. On the contrary, in this case not only dissolution of black oil in solvent but also dissolution of solvent in black oil was observed, because all the above solvents (petroleum ether, hexane and diesel fuel) are hydrocarbons. Thus the solvent–black oil mixtures obtained, in all appearances, are more stable at room temperature than the chloroform–black oil mixture. Therefore the solvents, such as petroleum ether, hexane and diesel fuel are not evaporated completely at room temperature.

It should be mentioned that an increase in the molecular weight of solvents in the series of petroleum ether—hexane—diesel fuel results in the higher stability of solvent–black oil mixture and lower evaporability of the solvent at room temperature.

Thus, although the extraction of hydrocarbons by chloroform yielded only 45% of the initial black oil, chloroform was used in further investigation of the degree of black oil degradation. At the same time, a correction taking into account the admixtures in black oil was introduced in the calculation of the degree of black oil degradation. The study of hydrocarbon-oxidative activity of isolated "F" strains in relation to "Biscay" black oil showed the degree of destruction 13–17% (Table 4). In this experiment, the activity of a mixture of these three strains in equal portions has been studied in addition to the pure culture.

As the degree of degradation of "Biscay" black oil by aboriginal "F" strains and their mixture was not significantly high, experiments to study the hydrocarbon-oxidative activity of associations formed on the basis of strains from the collection of the sector "Biopreparations", IBPM RAS, and Department of Ecological Biotechnology, SRCAM, towards "Biscay" black oil have been conducted.

Table 4
The dynamics of the quantity of microorganisms and degree of destruction of "Biscay" black oil by the pure culture of "F" strains and by their mixture in a fluid medium

Strain	Titre of microorganisms (cells/ml of medium)		Biodegradation (%)
	Initial	Final	
F1	3.4×10^8	1.7×10^{12}	17.3
F2	3.8×10^8	5.4×10^{11}	16.4
F3	1.4×10^9	7.7×10^{11}	13.1
Mixture	8.4×10^9	1.5×10^{12}	17.8

Table 5
Biodegradation of "Biscay" black oil by biopreparation 1 in a fluid medium

Variant	Titre of microorganisms (cells/ml of medium)		Biodegradation (%)
	Initial	Final	
Fluid	4.0×10^8	2.7×10^8	28.5
Adsorbed	3.3×10^7	5.3×10^8	24.0

The activity of consumption of "Biscay" black oil by an association of two strains (Sh-1 and Sh-5, Biopreparation 1) was studied in one of the experiments. In this experiment, the biopreparation was used in two forms: fluid (cell suspension) and sorbed (immobilized on pearlite). The results of these experiment are given in Table 5.

The analysis of the data from Table 5 shows that the degree of black oil degradation by Biopreparation 1 was almost 10% higher than in the association based on aboriginal strains.

In the course of the following experiment, the hydrocarbon-oxidative activity of Biopreparation 2 towards "Biscay" black oil was studied. In this experiment, as in the previous one, the efficiency of two forms of the preparation—fluid and sorbed—was studied. It should be noted that both sorbed and fluid preparation was introduced into flasks, counting the initial concentration of oil destructors in the medium as 5×10^7 cells/ml. At the same time, the degree of black oil biodegradation during the experiment was 30% with the fluid form and 28% with the sorbed form.

As in the previous experiment, the activity of the fluid biopreparation was higher than that of the sorbed one. However, it is necessary to take into account that the sorbed form yields a high initial concentration of cells during soil inoculation (the titre of microorganisms immobilized on pearlite is 2×10^{10} cells/g of dry weight). It is also known that the immobilized form not only significantly reduces the concentration of oil and oil products but also improves hydro-aerial, physical and agrochemical properties of the soil [11]. Besides, the sorbed biopreparation is more convenient for transportation than its fluid forms, therefore in some cases it is more preferable to use the biopreparation immobilized on a carrier.

Comparative analysis of the above data showed that the activity of Biopreparation 2 was a little higher than Biopreparations 1 and 2 was used in further investigation.

The next stage of research was to study the hydrocarbon-oxidative activity of Biopreparation 2 in a model soil system. As the accidental black oil spill in the Bay of Biscay resulted in the sand shore pollution, non-sterile sandy loam was used in a laboratory soil experiment. The results of the soil experiment are given in Fig. 1.

As might be expected, the quantity of microorganisms in the variant with the biopreparation was appreciably higher than in other variants. The initial titre of microorganisms in the control was low, which was most likely determined by depletion of aboriginal microflora in the sandy loam used in the experiment.

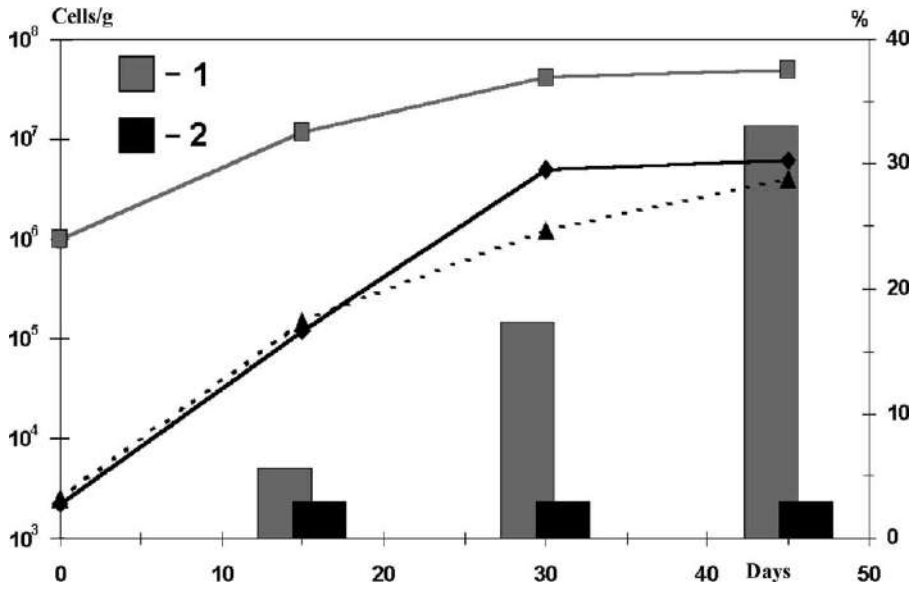

Fig. 1. Microorganisms kinetics and quantity of black oil in a laboratory soil experiment: (1) biopreparation + NPK; (2) NPK (the left axis of ordinates on the figure shows the quantity of microorganisms in 1 g of soil (graphs), the right axis of ordinates—biodegradation of black oil in percentage (hystograms), the axis of abscissas—the time after the first inoculation of microorganisms into soil in days; dotted line shows the quantity of microorganisms in the control).

The titre of microflora was shown to increase in all variants during the experiments, indicating the assimilation of black oil hydrocarbons by microorganisms. By the end of the experiment, on day 45 of incubation, there was a certain slowdown in the growth of the quantity of bacteria in all variants. The highest rate of black oil destruction was noted in the variant with the biopreparation, where the level of black oil biodegradation in 45 days of incubation was 33.1% (Fig. 1). The method of stimulation of aboriginal microflora was inefficient in this case and the hydrocarbon-oxidative activity of microorganisms in the variant without the biopreparation did not exceed 3%. Besides, the quantity of microorganisms in this variant actually did not differ from the quantity of the control.

4. Conclusions

The method of activation of aboriginal hydrocarbon-oxidizing microflora is far from always being advantageous as compared with the introduction of oil degrading microorganisms. Although the aboriginal microflora isolated from oil-polluted sites is the most adapted for growth in the above conditions, this does not imply that it has the highest rate of oil destruction.

Therefore, the choice of the optimal method of bioremediation of oil-polluted objects needs, first, a complex analysis of remediation conditions and, second, comparison of the efficiency of different methods of bioremediation, such as

- stimulation of aboriginal microflora;
- isolation of active aboriginal strains-destructors followed by production of their biomass and introduction into the polluted object where they had been isolated from;
- introduction of a biopreparation of oil degrading microorganisms.

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A microbial survey of the International Space Station (ISS)

Jenna M. Lang[1], David A. Coil[1], Russell Y. Neches[1], Wendy E. Brown[2,11], Darlene Cavalier[2,3,4], Mark Severance[2,4], Jarrad T. Hampton-Marcell[5,6], Jack A. Gilbert[7,8] and Jonathan A. Eisen[1,9,10]

[1] Genome Center, University of California, Davis, CA, United States of America
[2] Science Cheerleader, United States of America
[3] The Consortium for Science, Policy & Outcomes, Arizona State University, Tempe, AZ, United States of America
[4] Scistarter.org, United States of America
[5] Biosciences Division, Argonne National Laboratory, Lemont, IL, United States of America
[6] Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, United States of America
[7] Argonne National Laboratory, University of Chicago, Lemont, IL, United States of America
[8] Institute for Genomics and Systems Biology, Argonne National Laboratory, Lemont, IL, United States of America
[9] Evolution and Ecology, University of California Davis, CA, United States of America
[10] Medical Microbiology and Immunology, University of California, Davis, CA, United States of America
[11] Biomedical Engineering, University of California, Davis, CA, United States of America

ABSTRACT

Background. Modern advances in sequencing technology have enabled the census of microbial members of many natural ecosystems. Recently, attention is increasingly being paid to the microbial residents of human-made, built ecosystems, both private (homes) and public (subways, office buildings, and hospitals). Here, we report results of the characterization of the microbial ecology of a singular built environment, the International Space Station (ISS). This ISS sampling involved the collection and microbial analysis (via 16S rDNA PCR) of 15 surfaces sampled by swabs onboard the ISS. This sampling was a component of Project MERCCURI (Microbial Ecology Research Combining Citizen and University Researchers on ISS). Learning more about the microbial inhabitants of the ''buildings'' in which we travel through space will take on increasing importance, as plans for human exploration continue, with the possibility of colonization of other planets and moons.

Results. Sterile swabs were used to sample 15 surfaces onboard the ISS. The sites sampled were designed to be analogous to samples collected for (1) the Wildlife of Our Homes project and (2) a study of cell phones and shoes that were concurrently being collected for another component of Project MERCCURI. Sequencing of the 16S rDNA genes amplified from DNA extracted from each swab was used to produce a census of the microbes present on each surface sampled. We compared the microbes found on the ISS swabs to those from both homes on Earth and data from the Human Microbiome Project.

Conclusions. While significantly different from homes on Earth and the Human Microbiome Project samples analyzed here, the microbial community composition on the ISS was more similar to home surfaces than to the human microbiome samples. The ISS surfaces are species-rich with 1,036–4,294 operational taxonomic units (OTUs

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Corresponding author
Jonathan A. Eisen,
jaeisen@ucdavis.edu

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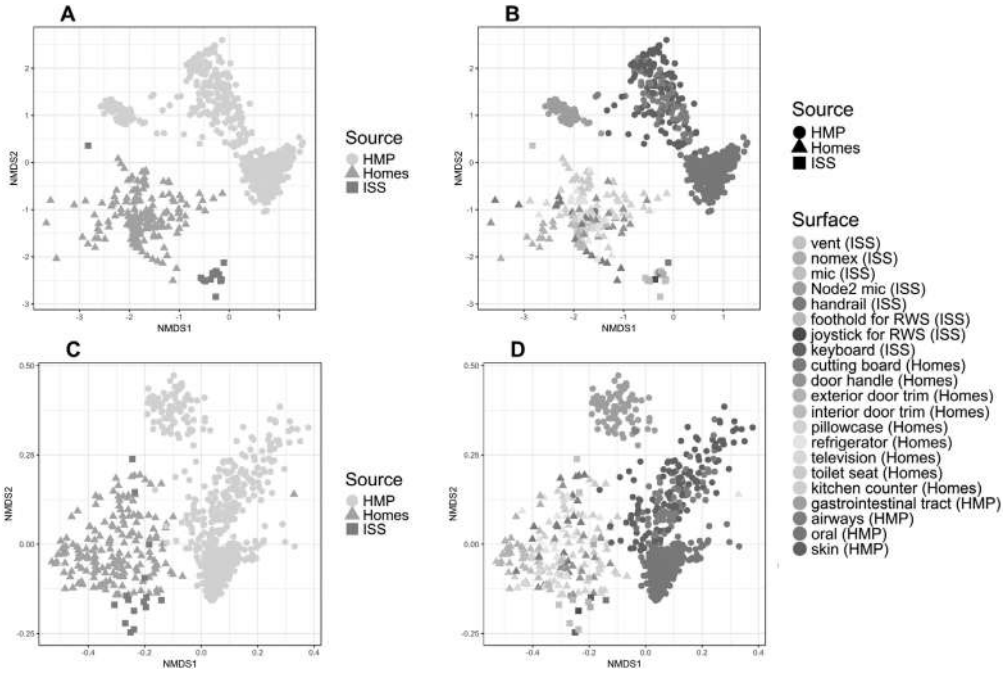

Figure 5 NMDS plots showing clustering of ISS, Earth homes, and Human Microbiome Project body sites. Non-metric multidimensional scaling (NMDS) ordination plots, based on Bray–Curtis (A and C) or Unweighted Unifrac (B and D) distances between samples obtained from the International Space Station, from homes on Earth, and from 13 body site from the Human Microbiome Project. The plots in (A) and (B) show identical data, as do the plots in (C) and (D). The points in (A) vs. (B) and (C) vs. (D) are colored differently as an aid for visualization. In these plots, points that are closer together have more similar microbial communities. The microbial communities associated with the ISS, homes on Earth, and the HMP samples were significantly different from each other (adonis, $R^2 = 0.08$, $P < 0.001$). Note: the crew and lab vent samples that are distinct from the other ISS samples in Fig. 2 are more similar to the human gastrointestinal tract samples from the HMP. This graph was produced using the Phyloseq package (*McMurdie & Susan, 2013*) in R (*R Core Team, 2014*).

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on Earth, and the HMP samples were significantly different from each other (adonis, $R^2 = 0.08$, $P < 0.001$) (Also see Fig. 5). We note that as with any meta-analysis, this difference could be also be partly due to differences in sample collection/preparation. However, the ISS communities are significantly more similar to the Earth home samples than the HMP samples (Student's t-test, $p < 0.00001$). This combined analysis also indicates that the starboard crew vent sample, which appears quite distinct from the rest of the ISS samples in Fig. 2A, is more similar to the human gastrointestinal HMP samples, which is corroborated by the dominance of animal gut-related OTUs found in that sample (see Fig. 3, and Table 2).

Finally, because the ISS is designed only to house six crew members, for a stay of six months each, only 220 individuals have visited the ISS since the year 2000. We hypothesized that there might be a relatively low microbial diversity on the ISS, either due to having a few total number of species, or due to the dominance of a very few species. In Fig. 6, we note that Shannon diversity (which takes into account both the number of species present, and

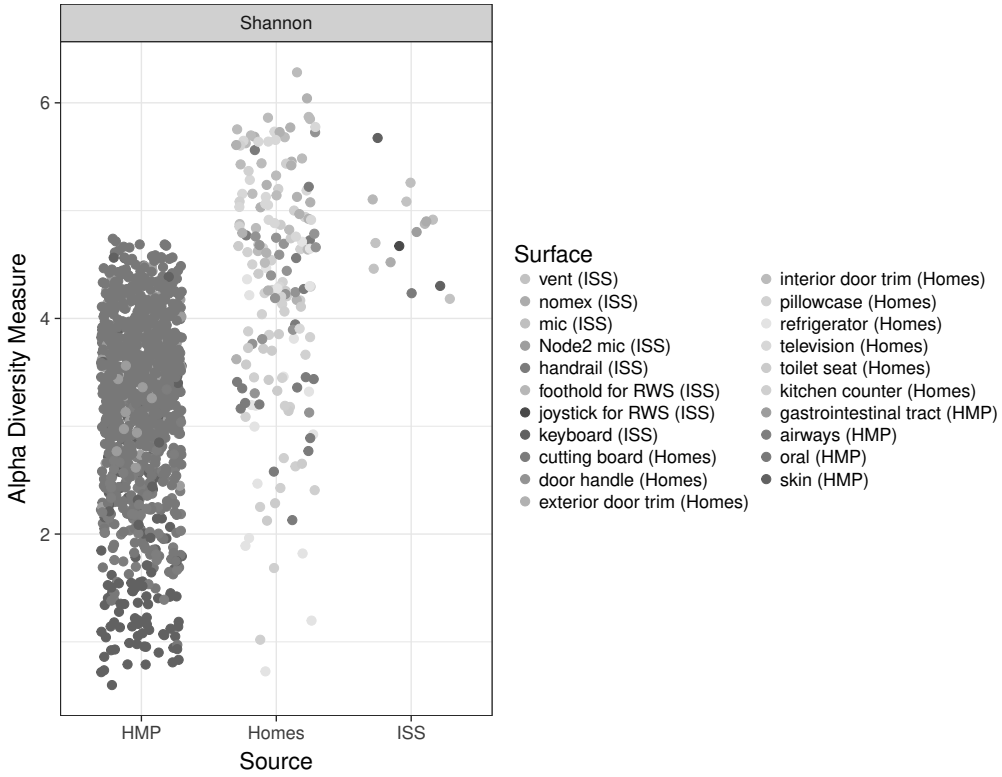

Figure 6 Comparison of Shannon diversity among the ISS, Earth homes, and HMP body sites. Shannon diversity, a measure of how many species there are as well as how evenly the counts of individuals are distributed across species is plotted for every sample. There is wide variation among the HMP samples, with the oral (blue) and gastrointestinal (green) samples typically having more diversity than the skin (pink) or airway (coral) samples. Surfaces on the International Space Station have relatively high Shannon diversity, on par with that of the most diverse HMP samples, and the average home sample. This graph was produced using the Phyloseq package (*McMurdie & Susan, 2013*) in R (*R Core Team, 2014*).

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how evenly our sequences are distributed throughout those species) is actually relatively high on the ISS.

Comparison to rooms with mechanical ventilation or open windows

Kembel et al. (2012), showed that rooms in a health-care facility that were primarily ventilated via an open window had greater phylogenetic diversity and lower proportion of OTUs closely related to known human pathogens than rooms that were mechanically ventilated. The only window on the ISS is never opened, and the doors are opened only briefly, every few months. Therefore, we hypothesized that for the samples from the ISS, the phylogenetic diversity would be lower and the proportion of OTUs closely related to known human pathogens would be higher than that seen for mechanically ventilated rooms. To test this hypothesis, we obtained the list of known human pathogens compiled by *Kembel et al. (2012)*, and followed their procedure to identify the proportion of OTUs in the ISS samples that were closely related to them (see Methods for details). Surprisingly,

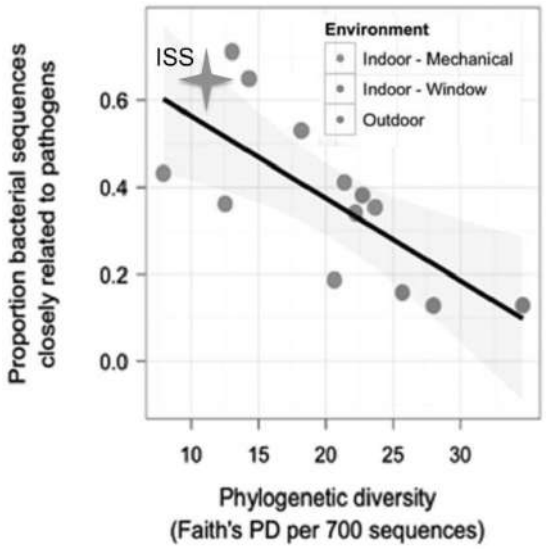

Figure 7 Proportion of OTUs found in the ISS samples that were closely related (97% sequence similarity) to human pathogens versus the phylogenetic diversity of those samples. This figure was modified from Fig. 4A of (*Kembel et al., 2012*). The pink star represents the ISS samples. The plot shows the proportion of OTUs that were closely related (97% sequence similarity) to human pathogens versus the phylogenetic diversity of those samples.

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but reassuringly, we found that the ISS samples are similar in both phylogenetic diversity and the proportion of OTUs closely related to known human pathogens as compared to the mechanically ventilated rooms in the health-care facility (Fig. 7). As with the studies above, some observed variance may be due to differences in sample collection/preparation.

CONCLUSION

This is the first time that the ISS has been analyzed in the broader context of the ''microbiology of the built environment'', and is the most in-depth comparison of the microbial communities found on the ISS to those found either in buildings or in the human microbiome. Perhaps surprisingly, given the extreme rarity of exchange with any external microbes, we found the ISS to be species-rich, and more similar to the surfaces of human homes on Earth than it is to human bodies. We found that the ISS is home to at least 12,554 distinct microbial species, including Archaea in very low abundance, and that the proportion of species that are closely related to known human pathogens is on par with similar built environments on Earth. Given the low number of samples in this study, no viability assessment, as well as the lack of sample preparation control we view these results as simply a starting place for more detailed future studies.

As outlined in the 2010 US National Space Policy and in the bipartisan NASA Authorization Act of 2010, NASA is targeting the 2030s for a manned spaceflight to Mars, with one ultimate goal of having people live and work on the Martian surface (see http://www.nasa.gov/exploration and http://www.nasa.gov/mars). We know that the

microbial communities found in our terrestrial built environments play an important role in human health. Therefore it is crucial to characterize and understand the microbial population of the only environment in which people are currently living and working in space. This study is one small step in that direction.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Jonathan A. Eisen is an Academic Editor for PeerJ.

Author Contributions

- Jenna M. Lang conceived and designed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- David A. Coil, Russell Y. Neches and Wendy E. Brown conceived and designed the experiments, reviewed drafts of the paper.
- Darlene Cavalier, Mark Severance and Jonathan A. Eisen conceived and designed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Jarrad T. Hampton-Marcell performed the experiments.
- Jack A. Gilbert performed the experiments, contributed reagents/materials/analysis tools.

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Atmospheric-pressure, nonthermal plasma sterilization of microorganisms in liquids and on surfaces*

Yuri Akishev[1,‡], Michail Grushin[1], Vladimir Karalnik[1], Nickolay Trushkin[1], Vasiliy Kholodenko[2], Vladimir Chugunov[2], Eugeniy Kobzev[2], Nadezhda Zhirkova[2], Irina Irkhina[2], and Georgiy Kireev[2]

[1]*State Research Center of RF TRINITI, Troitsk, Moscow region, 142190, Russia;*
[2]*State Research Center of RF for Applied Microbiology and Biotechnology, Obolensk, 142279, Russia*

Abstract: Gas discharge plasma inactivation of microorganisms at low (close to ambient) temperature is a promising area of investigation that is attracting widespread interest. This paper describes atmospheric-pressure, nonthermal plasma (NTP) methods for cold sterilization of liquids and thermal sensitive surfaces. These methods are based on the use of direct current (DC) gas discharge plasma sources fed with steady-state high voltage. Parameters characterizing the plasma sources used (plasma-forming gas, gas flow rate, electric power consumed, etc.) are given. The results for plasma sterilization of different microorganisms (vegetative cells, spores, fungi, biofilms) are presented. An empirical mathematical approach is developed for describing NTP inactivation of microorganisms. This approach takes into account not only the destruction of different components of the cells, but their reparation as well.

Keywords: plasma inactivation; nonthermal plasma; cold sterilization; direct current gas discharge plasma sources; nonthermal plasma inactivation of microorganisms.

INTRODUCTION

Development of effective and energy-efficient approaches for the destruction of biologically dangerous contaminants (pathogens and toxic chemicals) at low (close to ambient) temperature in gases, liquids, and on the surface of bodies is a challenge for modern science. In addition to sterilization, a complimentary application is a protection of different industrial materials, equipment, and electronic devices against biocorrosion and biodegradability. Indeed, corrosion of metals speeds up manifold when induced by thin films of microorganisms deposited on their surface. Usually, microorganisms forming such biofilms are very resistant to traditional sterilization methods.

Sterilization of objects consists of destruction or removal of the microorganisms, including vegetative cells, spores, viruses, etc. Traditional sterilization and disinfection methods use heating in dry and humid environments, filtration, radiation, and strong chemicals (biocides). These methods are

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‡Corresponding author

labor- and time-consuming and expensive as well (e.g., sterilization of biofilms with strong biocides takes more than 24 h). Besides, using biocides does not provide environmental safety.

Gaseous nonthermal plasma (NTP) has unique characteristics because it contains numerous biochemically active agents like UV photons, OH radicals, O atoms, electronically and vibration excited molecules, etc. A distinguishing property of NTP is that all the foregoing agents mentioned can be generated in gas or liquid without heating, close to ambient temperatures. By now, a lot of information devoted to the inactivation of microorganisms by various plasmas created at low and atmospheric gas pressure has been published (see, e.g., refs. [1–7] and the abundant literature cited therein). Many attempts have also been made to determine specific mechanisms responsible for plasma inactivation of microorganisms [2–5]. Nevertheless, from a scientific point of view this problem is still open for discussion.

This paper presents new results on cold sterilization at atmospheric pressure. One of the reasons why we have given particular attention to the development of plasma methods that work at atmospheric pressure is that this approach allows us to inactivate microorganisms not only on surfaces but in liquids as well. Another advantage of NTP inactivation at atmospheric pressure is the avoidance of expensive vacuum equipment required for plasma processing at low pressure. The paper also offers an empirical mathematical approach to describing the plasma inactivation process. The approach developed here takes into account not only the destruction of different elements of the cells but their reparation as well.

EXPERIMENTAL PLASMA DEVICES USED

We used two types of atmospheric-pressure plasma generators. One of them was used for sterilization (disinfection) of liquid media, another for processing of surfaces. Effective generation of biochemically active species (O, OH, etc.) inside a liquid is of great interest for many scientific and practical applications (water purification, biomedical applications, etc). In many cases, short-pulsed discharges forming streamers in the bulk of liquid or in gas above the liquid surface are used to do that [8–13]. Another promising approach allowing us to generate a lot of radicals in liquid is an electric discharge in the water filled with chaotically moving gas bubbles [14–17]. In such cases, active species are produced by cold plasma generated inside small bubbles (but not in a liquid itself), and after that they are transported due to diffusion from bubbles into the liquid.

Our device used to generate intensive NTP in bubbling water is shown in Fig. 1. The length of the glass tube in this figure is 50 cm, and the inner diameter of the tube is 3.6 cm. Cold plasma enables both the UV radiation and active species like O and OH radicals to be generated within the gas bubbles, which effectively destroys biologically harmful contaminants (not only pathogens, but toxic chemicals as well) in the treated water without attendant heating.

Three views in Fig. 2 show plasma jets in pure nitrogen, N_2 + 2 % O_2 mixture and air at atmospheric pressure used for sterilization of surfaces. The length of plasma jet is equal to 10–5 cm (a), 2–3 cm (b), and 1–2 cm (c). All plasma devices described above are fed by direct current (DC) high-voltage power suppliers.

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Name:
Nobuyasu Yamaguchi

Affiliation:
Associate Professor, Graduate School of Pharmaceutical Sciences, Osaka University

Address:
1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

Brief Career:
1993- Assistant Professor, Faculty of Pharmaceutical Sciences, Osaka University
2006- Associate Professor, Graduate School of Pharmaceutical Sciences, Osaka University

Selected Publications:
- "Global dispersion of bacterial cells on Asian dust," Scientific Reports, Vol.2, pp. 525-510, 2012.
- "Microbial monitoring of crewed habitats in space – current status and future perspectives," Microbes and Environments, Vol.29, pp. 250-260, 2014.
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Academic Societies & Scientific Organizations:
- American Society for Microbiology (ASM)
- Pharmaceutical Society of Japan (PSJ)
- Japanese Society for Bacteriology (JSB)

Name:
Masao Nasu

Affiliation:
Professor, Graduate School of Pharmaceutical Sciences, Osaka University

Address:
1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

Brief Career:
1989- Assistant Professor, Faculty of Pharmaceutical Sciences, Osaka University
1991- Associate Professor, Faculty of Pharmaceutical Sciences, Osaka University
1995- Professor, Faculty of Pharmaceutical Sciences, Osaka University

Selected Publications:
- "Environmental disease: environmental alteration and infectious disease," Ecological Research, Vol.26, pp. 893-896, 2011.
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Academic Societies & Scientific Organizations:
- American Society for Microbiology (ASM)
- Pharmaceutical Society of Japan (PSJ)
- Japanese Society for Bacteriology (JSB)

REFERENCE LIST

The Scientific & Manufacturing Firm «Potok Inter» (LLC), an established and reputable manufacturer of Potok Products (Air Decontamination Appliances), hereby indicates where the Potok Products are used to reduce the concentration of mold fungi and yeasts, bacteria and viruses in the air.

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Infectious diseases clinical hospitals No. 1 and No. 2
(Aseptic wards in coronavirus red zones, Russia)

City clinical hospital
(Resuscitation Halls, wards, Belarus)

City clinical hospital named after S. P. Botkin
(24 ORs, 1 Resuscitation Hall, Russia)

City clinical hospital №1 named after N. I. Pirogov
(3 Resuscitation Halls, 8 ORs, Russia)

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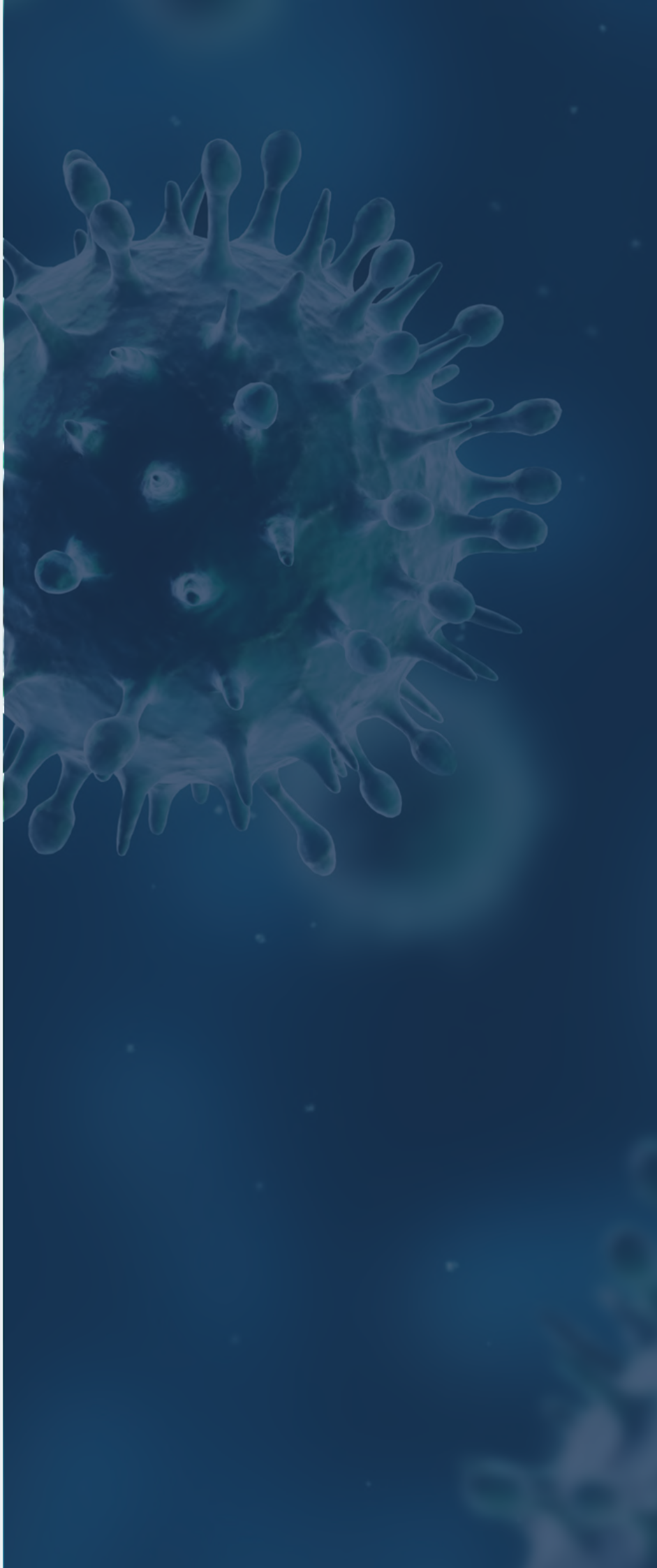
(chicken meat processing area)

Bogorodsky meat processing plant LLC
(sausages slicing and packaging area, Russia)

East Balt Bakery LLC
(refrigeration chamber for the semi-finished baked products, Russia)

Savushkin Product JSC
(one of the largest dairy manufacturers in the East European region, Potok units are used at cheese aging area, Belarus)



SCIENTIFIC–MANUFACTURING COMPANY POTOK INTER (LLC)
115162, MOSCOW,
RUSSIAN FEDERATION
18 KHAVSKAYA STR., BLD.2
TEL: +7(495) 025-20-20
WWW.POTOK.COM