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DECONTAMINATION OF A DIVING SUIT

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ABSTRACT

When working in chemical or biological environments, contamination is an extremely dangerous issue for the rescue services of the fire department, police and the army.
Modern protective overalls worn by fire fighters or dry "Viking" diving suits made from neoprene or nylon covered with polyurethane, have been proven to ensure sufficient protection. However, once the contaminated area is left, there is a need to perform decontamination of the external and internal surfaces of the protective overalls; in order to ensure the clothing continues to offer a high level of comfort and to retain the durability of said protective clothing, it is of course also necessary to perform a drying procedure.
Moreover, there is a risk of a transfer of pathogenic micro-organisms between persons utilising the same protective clothes, particularly in the case of expensive specialist suits. Micro-organisms which may potentially spread through clothing include intestinal bacteria, such as: Salmonella, Shigella, Campylobacter, E. coli (including E. coli O157), C. difficile, viruses inducing infections of the upper respiratory tract and alimentary tract (noraviruses, rotaviruses, adeno and astroviruses). The risk of infection also involves the presence of the flu viruses, herpesviruses and pathogens transferred through skin, such as S. aureus (including MRSA), yeast-like fungi (Candida albicans), fungal strains inducing Tinea pedis and Tinea corporis [1]. Pathogenic micro-organisms can easily transfer from fabric surface onto the body of a person wearing protective clothing.
From the numerous available techniques of decontamination of surfaces, equipment and protective clothing we propose to use for this purpose gaseous hydrogen peroxide (H_2O_2), a very effective biocidal agent. In field conditions, typical for the activities of rescue crews of the fire department, police and army we assume utilisation of a portable decontamination chamber enabling performance of a complete decontamination process.
The process lasting approximately 3 hours encompasses 3 phases:

- Drying phase;
- Decontamination with gaseous hydrogen peroxide;
- Catalytic combustion phase of hydrogen peroxide residues to a level safe for the environment.

The integrated humidity and H_2O_2 level sensors ensure automatic control of the entire process and the unique distribution system of gaseous H_2O_2 secures full accessibility of the biocidal agent to the external surface of protective clothing as well as its interior. Moreover, the container allows for the conduction of the complete decontamination of the rescue equipment, night vision devices, binoculars, field telephones, radio stations, etc. Upon decontamination cycle completion, we obtain a completely dried suit which can be safely used by another crew member.
Key words: diving, diving suit, decontamination.

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INTRODUCTION

Working in chemical or biological environment contamination is extremely dangerous for the rescue services of the fire department, police and the army. Modern protective overalls worn by fire fighters or dry "Viking" diving suits made from neoprene or nylon covered with polyurethane, have been proven to ensure sufficient protection. However, once the contaminated area is left, there is a need to perform decontamination of the external and internal surface of protective overalls; in order to ensure the clothing continues to offer a high level of comfort and to retain the durability of said protective clothing, it is of course also necessary to perform a drying procedure.

Additionally, there is a risk of a transfer of pathogenic micro-organisms between people using the same clothing, particularly in the case of expensive specialist overalls. Micro-organisms which may potentially spread through clothing include intestinal bacteria, such as: Salmonella, Shigella, Campylobacter, E. coli (including E. coli O157), C. difficile, viruses inducing infections of the upper respiratory tract and alimentary tract (noraviruses, rotaviruses, adeno and astroviruses). The risk of infection is moreover connected with the flu viruses, herpesviruses and pathogens transferred through skin, such as S. aureus (including MRSA), yeast-like fungi (Candida albicans), fungal strains inducing Tinea pedis and Tinea corporis [1].

It is estimated that a human can daily emit into the environment approximately one million dead skin cells which may contain fungi and bacteria, including *S. aureus* [2].

The survival rate of micro-organisms on various surfaces depends on fabric type, humidity and temperature as well as initial pathogen concentration. Neeley and Maley have studied the survival rate of 22 Gram-positive bacteria species on such materials as: 100% cotton, 60% cotton+40% polyester, 100% polyester. All micro-organisms survived at least 1 day, whilst some even up to 90 days on selected materials. Generally, the survival rate of bacteria, viruses and fungi on hydrophobic smooth surfaces is at least 2-4 times higher as compared with such materials as pure cotton (smooth, terry fabrics) or cotton/polyester mixtures [3,4,5].

Pathogenic micro-organisms can easily transfer from a fabric surface onto the body of a person wearing protective clothing. In the work by Sattar et al. it has been indicated that one of the most crucial factors determining the speed of transfer of a pathogen from fabric surface onto a person's hands consists in fabric humidity. Drying of a surface may reduce the transfer of a micro-organism up to 10 times in relation to the transfer from a humid surface onto a humid skin [6].

From the numerous available techniques of decontamination of surfaces, equipment and protective clothing we decided to use for this purpose gaseous hydrogen peroxide (H_2O_2), a very effective biocidal agent applied, for instance, in the eradication of such dangerous pathogens as Mycobacterium tuberculosis or Clostridium difficile [7,8].

EXPERIMENTAL MODEL

a) Working conditions of an air and surface disinfection system

- Room: area 9 m^2, height 3.55 m, cubature 32 m^3.
- Distance between NOCOSPRAY device and control points: between 80 cm and 350 cm.
- Device emitting H_2O_2/water/air aerosol: Nocospray manufactured by Oxypharm.
- Aerosol emission speed: 80 m/s.
- Drop size: 5 microns.
- Decontamination liquid consumption: 1000 ml/hour; 16.6 ml/min.
- Decontaminating agent:
  - Nocolyse, stabilised solution 6 % H_2O_2, neutral odour;
  - Nocolyse, stabilised solution 6 % H_2O_2, mint odour;
  - Nocolyse One Shot, stabilised solution 12 % H_2O_2, neutral odour;

b) Experimental model

A diving suit was placed on a rack in the decontamination room (fig. 1) along with other suit components (fabric samples, metal and rubber parts) to perform material compatibility tests – resistance to a high and long-lasting H_2O_2 concentration in the air (fig. 2).

Fig. 1. A rack with the diving suit.

Fig. 2. Particular diving suit components subjected to decontamination.

Spatial concentration of gaseous H_2O_2 was monitored with the use of Nocotest strips.

The colour change from green to brown enables determination of the concentration of gaseous hydrogen peroxide in the decontamination room (green: 0.5-1.00 ppm H_2O_2, dark brown above 50 ppm).

Fig. 3. The placement of H_2O_2 concentration test strips.

a) Micro-organism tests

In order to determine the biocidal efficiency of gaseous H_2O_2 in relation to bacteria, fungi and yeasts, the tests were conducted with the use of micro-organisms specified in table 1. 10 points were assigned on the external surface of the diving suit (see arrows in fig.1), and an additional 8 points in the internal part for the placement of 100 µl of an overnight micro-organism culture solution to perform experimental decontamination. 30 minutes from sample inoculation on the right part of the suit (marked with letter K in fig. 1) the contamination level was tested with the use of the Orion imprint test. Moreover, the level of inactivation of the spores of the Geobacillus stearothermophilus bacteria species was determined by use of a vial biological indicator for hydrogen peroxide sterilisation testing, Bionova H_2O_2 (BT91) manufactured by Terragene.

Tab. 1.

The tested micro-organisms.

Bacteria	
Acinetobacter baumannii	ATCC 17978
Escherichia coli	ATCC 8739
Legionella pneumophila	ATCC 33152
Legionella longbeachae	ATCC 33462
Pseudomonas aeruginosa	ATCC 9027
Staphylococcus aureus,	ATCC 6538
Spore-forming bacteria	
Geobacillus stearothermophilus	ATCC 7953
Bacillus subtilis,	ATCC 6333
Fungi, yeasts	
Aspergillus brasiliensis	ATCC 16404
Candida albicans	ATCC 10231

Hygicult imprint tests are designed for a fast monitoring of microbiological hygiene and/or initial micro-organism identification (total count of bacteria, yeasts, mould and intestinal bacilli) on various surfaces. The test may be conducted on-site or with the use of a substrate as a convenient transport medium for samples.

The Hygicult TPC test is covered on both sides with Total Plate Count agar, which enables a fast growth of the majority of common micro-organisms. The test is dedicated for the detection of an elevated total count of micro-organisms. The second Hygicult TPC test is covered on both sides with Malt agar, which enables a fast growth of yeast and mould. Bacteria growth is inhibited. The test is dedicated for the detection of an elevated total count of fungi on surfaces.

Hygicult tests provide initial information on microbiological purity as well as the type of a micro-organism which causes contamination (depending on the selected Hygicult test). The samples were incubated at the temperature of 35-37^{0}C for 24 hours. The contamination level was determined according to the provided model in jtk/cm^2. Biological indicators Bionova H_2O_2 (BT91) enable monitoring of hydrogen peroxide decontamination

processes. Each indicator consists of a plastic vial with a glass ampoule with a culture medium placed inside.

Geobacillus stearothermophilus bacteria spores were provided at the bottom of the plastic vial in the quantity of 2.3 x 10^5 spores in a single test. Moreover, the test includes a decontamination process indicator provided on the label of each vial, which changes its colour during sterilisation from violet to green.

Before the incubation, the ampoule is to be crushed and the culture medium is to be spread over the bottom of the vial. The micro-organism growth is observable with a change in the culture medium colour from violet to yellow. The final result is obtained after the lapse of 24 hours of incubation at the temperature of 60^0C.

d) Decontamination procedure

The instruction attached to the Nocospray device provides for the use of 1 ml of 6% hydrogen peroxide solution (Nocolyse, stabilised solution 6 % H_2O_2, neutral odour) on an area of 1 m^3 of the room subject to decontamination.

With the cubature of 31 m^3 it was assumed that following approximately 2 minutes of emission, a 30-minute period of hydrogen peroxide activity should be ensured on all surfaces in the room, with particular attention paid to the diving suit. Hydrogen peroxide penetration of internal surfaces of the suit was secured with an air circulation system operated by a fan. This was followed by a 30-minute ventilation of the entire room through an active fume hood system. Entry into the room was permitted upon H_2O_2 concentration reduction in the air to the level below 1 ppm.

During the decontamination with the use of a 12% hydrogen peroxide solution (Nocolyse One Shot, stabilised solution 12 % H_2O_2, neutral odour) the reduction of H_2O_2 concentration level to 1ppm lasted 60 minutes.

The maximum hydrogen peroxide emission time in the performed experiments was 12 minutes, which corresponded to the use of 200 ml of 6% or 12% H_2O solution respectively.

RESULTS

The decontamination of the tested room with Nocolyse 6 % H_2O_2, solution with a 2-minute emission time and a 30-minute total decontamination time guaranteed hydrogen peroxide accessibility to all measurement points in the room. Unfortunately, the distribution of hydrogen peroxide concentrations varied. Also, the level of reduction of the presence of micro-organisms on the tested surfaces indicated partial ineffectiveness of the decontamination process, especially with regard to spore-forming bacteria. The application of Nycolyse 6% H_2O_2 was efficient only with a 12-minute emission period and total decontamination time of 6 hours.

The change in the concentration of emitted hydrogen peroxide from 6% to 12 %, i.e. the application of a Nocolyse One Shot preparation, resulted in a radical shortening of the effective decontamination time and allowed the obtainment of a 100% biocidal efficacy.

The experiment encompassed 5 decontamination procedures with the use of Nocolyse 6% and various emission times and an effective decontamination time lasting between 30 minutes and 24 hours. The decontamination with the use of Nocolyse One Shot encompassed 6 exposures with the emission time between 2 to 20 minutes and effective decontamination time ranging between 120 to 360 minutes. Upon completion of each decontamination procedure, the room was ventilated in order to remove trace levels of hydrogen peroxide (to a safe level below 1 ppm).

In order to determine material compatibility, i.e. the resilience of construction elements of the diving suit to a high and long-lasting H_2O_2 concentration in the air, the exposure with the use of Nocolyse One Shot will be continued in the year 2017 with the frequency of 1-2 experiments per week over a period of 6 months.

Tab. 2.

The effects of hydrogen peroxide decontamination – tests for the presence of micro-organisms on the surface of a diving suit.

Micro-organism	Location distance from Nocospray, cm	H_2O_2 %	Emission min	Time min	Result From 1+ to 5+, from 10^2 to 10^5 jtk pathogen at the sample point 0 – no pathogen	Comments
All from table 1	Suit, 150 cm	0	0	0	5+	Control
BT91 Test	table, 60 cm	0	0	0	5+	Control
BT 91 Test	Upper surface of a suspended cabinet, 200 cm	0	0	0	5+	Control
BT 91 Test	Window, 300 cm	0	0	0	5+	Control
All from table 1	Suit, 150 cm	6	2	0	Bacteria 0 B.subtilis 3+ G.stearotherm. 5+ fungi, yeasts, 0	H_2O_2
BT 91 Test	table, 60 cm	6	2	0	5+	H_2O_2
BT 91 Test	Upper surface of a suspended cabinet, 200 cm	6	2	0	5+	H_2O_2
BT 91 Test	Window, 300 cm	6	2	0	5+	H_2O_2
BT 91 Test	Suit, 150 cm, feet	6	2	0	5+	
All from table 1	Suit, 150 cm	12	12	20	0	H_2O_2 One shot
BT 91	table, 60 cm	12	12	20	0	H_2O_2
BT 91	Upper surface of a suspended cabinet, 200 cm	12	12	20	0	H_2O_2
BT 91	Window, 300 cm	12	12	20	0	H_2O_2
BT 91	Suit, 150 cm, feet	12	12	20	0	H_2O_2

DISCUSSION

The conducted experiments showed a high biocidal efficiency of the hydrogen peroxide/water/air mixture emitted by the Nocospray device manufactured by Oxypharm. A particularly effective preparation was Nocolyse One Shot containing 12% stabilised hydrogen peroxide solution. The degree of microbiological contamination of the tested surfaces following the decontamination procedure was greatly below the acceptable level in a hospital environment [9]. Manual surface decontamination (using generally available detergents combined with a chloride bleach) did not guarantee comparable biocidal properties, especially on the external surface of a diving suit. The observations were consistent with the work by Cooper et al. [10].

The use of the BT91 test as a routine biological indicator evaluating the biocidal effectiveness of hydrogen peroxide using *Geobacillus stearothermophilus* spores has confirmed the principle that the elimination of the presence of spore-forming bacteria from a surface guarantees liquidation of other bacteria, viruses and mould [11].

The application of a 12 % hydrogen peroxide solution with the emission of 5 to 10 ml Nocolyse One Shot per one m^3 of the tested room has guaranteed full penetrability of the biocidal agent even into very narrow spaces (diameter 9 mm, depth 45 mm).

The circulation of the decontamination mixture forced through air circulation inside the suit enabled a complete decontamination of the interiors of boots and other difficult-to-access areas of protective clothing.

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