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Per E-Mail

Expert Statement
Conformity of Diosol Generator® and Diosol® Liquid to European Requirements of Disfection

Dear Sirs,

The Diosol-System nebulizes hydrogen peroxide stabilized and activated by silver ions.

The use of hydrogen peroxide fog for room disinfection until now has not found an analogue in European Standards. Only in France is established a standard for fogging disinfectants (NF T 72-281).

The experiments to evaluate the fitness of this process for use in hospitals and other medical institutions including homes for the elderly started in the early 80ties of the last century. At this time no EN-Standards were available for disinfectant processes, each country (and sometimes each laboratory) used own techniques to evaluate the reduction of germs and viruses. In Germany the methods recommended of the German Society of Microbiology and Hygiene (Deutsche Gesellschaft für Hygiene und Mikrobiologie, DGHM) and later of the Association for practical Hygiene (Verbund für angewandte Hygiene, VAH). These methods remained unchanged up to the early first decade of this millennium. In the last years a harmonization of the standards of the European countries took place including some changes in test procedures.
As a consequence, the development of disinfectants was divided in some steps as indicated following:

Phase	Step	Testinhalt
-	-	Toxicity tests, classification of hazardous substances if needed
1	-	Estimation of antimicrobial action on Staphylococcus aureus ATCC 5638 and Pseudomonas aeruginosa ATCC 15422 (Standard EN 1040), Candida albicans ATCC 10231 and Aspergillus niger (brasiliensis) ATCC 16404 (Standard EN 1275), Bacillus subtilis ATCC 6633 and Bacillus cereus ATCC 12826 Standard EN 14347)
2	1	Suspension Test (Microorganisms are “swimming” in disinfectant, “clean” and “dirty” conditions
	2	Practical test using standardized test surfaces, Institutions of foodstuff processing, industrie and official institutions
-	-	Four fields test for wipes

In 2013 the German Institute of Healthcare and Infection Prevention (Robert-Koch-Institut, Berlin) has added the method of fogging hydrogen peroxide in the list of recommended disinfection procedures in case of orders of healthcare officers. Therefore, it was accepted, that this method will be usefully even

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in cases of more dangerous, contagious germs and viruses. Here a validation is mandatory.

In the case of biocidal products scope in medical units, for proving the efficacy the following statements are wished:
- TP1 - tests of effectiveness for hygienic hand disinfection by rubbing / washing (according to EN 1499 / EN1500) surgical hand disinfection, virucidal (according to EN 14476)
This is here contraindicated, because it is a surface disinfectant

- TP2 - tests of effectiveness bactericidal, fungicidal, virucidal (according to EN 14476) mycobactericidal (according to EN 14348) action. This is the main task of the following expert statement.

Unfortunately, it was published late about this time, that the influence of hydrogen peroxide on microorganisms and viruses depends on factors like room temperature and air humidity. So in older expert statements basing on measurements of the effect of the method on different pathogens this two factors were not protocolled regular. However, that does not mean, that no accordance to EN-Standards are given and therefore results were not interpretable. This is due to the fact, that most of the experiments were taken out in Middle Europa with a defined range of usual temperatures and air humidity. In addition, as described below at 7., Diop has presented an advice to adjust the dose of substance to fog.

In the following statement different test protocols are presented and the grades of accordance to EN-Standards is elucidated. From more than 100 expert statements only examples are chosen showing data nearest the requirements of the European Standards.

1. Preliminary Risk Assessment
Before starting further evaluations, a toxicity test should be performed. Just presenting the results according to the German Chemical Security Act (GefStoffV) and work security list of maximal contrations of substances inhaled by personnel (MAK-Liste) concentrations of Diosol up to 6 % are not harmful. Higher concentrations used depending of target microorganism (up to 19 %) needs a careful handling using personal protection equipment. Furthermore, permanent room air concentration should not exceed 1 ppm, so a reduction of fog residues mixing with fresh air after end of proposed exposition time is mandatory.

2. Phase 1

<u>2.1 Demonstration of bactericidal effect in general</u>
The further proceed is based on standards collected in CEN/TC 216. In Phase 1 EN 1040 (Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics. Test method and requirements - Phase 1). This first test does not represent the fogging effect, it just demonstrates the effect in general. So within a defined time (so called exposure time) the substance has to kill inside swimming bacteria 100,000:1, meaning a reduction factor of 5. In this Phase two bacteria strains are engaged: Staphylococcus aureus ATCC 5638 representing gram positive germs and Pseudomonas aeruginosa ATCC 15422 representing gram negative germs. At least two different expert statements of independent laboratories are required.
This very first evaluation showed that the required reduction was shown under the following conditions: concentration of 6 % and exposition time of 30 min or 2 % and exposition time of 60 min.

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The expert statements showing these effects are as follows:

1. IKI, Siemensstr. 18, 35394 Gießen, dirty conditions (0,3 % Bovine Serum Albumine, BSA), 2004

2. Bionovis Hygieneinstitut OHG, Kerkrader Str. 7, 35394 Gießen, clean conditions (without BSA), 2006
3. Dr. habil. P. Trenner, Hüttenweg 18, 16230 Chorin, 2004, clean conditions (0,0% and 0,03 % BSA), concentration 12 %, exposure time 60 min).

2.2. Demonstration of fungicidal effect in general

The fungicidal effect was preliminary tested according to EN 1275 (Quantitative suspension test for the evaluation of basic fungicidal or basic yeasticidal activity of chemical disinfectants and antiseptics. Test method and requirements - phase 1) employing the test isolates Candida albicans ATCC 10231 as representant of yeasts and Aspergillus niger (brasiliensis) ATCC 16404 representing molds. The following results were obtained in cited expert statements:

1. Dr. habil. P. Trenner, Hüttenweg 18, 16230 Chorin, clean conditions (0,0% and 0,03 % BSA), concentration 5 %, exposure time 60 min, Reduction factor 4 – 10,000:1), 2004.

2. IKI, Siemensstr. 18, 35394 Gießen, dirty conditions (0,3 % BSA), concentration 6 %, exposure time 60 min, Reduction factor 7 – 10,000,000:1), 2004

2.3. Demonstration of sporicidal effect in general

While spores of fungi react on chemical disinfectants nearly like native bacteria, bacterial spores are extreme resistant against most disinfectants including thermodisinfection. Because of the rising count of cases of Clostridium difficile associated diarrhoe a sporicidal effect is quite desirable. The according European standard for Phase 1 is EN 14347 (Basic sporicidal activity. Test method and requirements - phase 1, step 1). Here the two test strains Bacillus subtilis ATCC 6633 and Bacillus cereus ATCC 12826 are engaged.
The following results were obtained:
Concentration: 25,8%, Exposure time: 5 bis 6 min at a pH: around 7 and temperature: 24°C
Clean conditions. The application methods were spraying, rinsing, and diving.
However, one has to note that the RKI ordered the use of spores of Geobacillus stearothermophilus to validate the fogging process. This spores are routinely used in sterilization checks.

2.4. Demonstration of mycobactericidal effect in general

No Phase 1 Test is established.

3. Phase 2

3.1 Further demonstrations of bactericidal effect

In Phase 2 EN 1276 (Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas- Test method and requirements - phase 2, step 1). Here an extended test panel of microorganisms is employed, including in addition to the isolates used in phase 1 Escherichia coli and Enterococcus hirae. A reduction factor of 5 is required at clean conditions (0,03 % BSA) and dirty conditions (0,3 % BSA) as well. The concentration

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found bactericidal in both conditions are12 %, exposure time required are 60 min, this was proved in different expert statements.

3.2. Further demonstration of fungicidal effect

The fungicidal effect was further tested according to EN 1650 (Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Phase 2, step 1) Here a reduction factor of 4 should be reached under clean and dirty conditions. Here the following results were obtained:
Clean Conditions: Conzentration 5 %, Exposition time 60 min

Dirty Conditions: Conzentration 6 %, Exposure time 60 min (Reduction factor 7 was found!)

1. Dr. habil. P. Trenner, Hüttenweg 18, 16230 Chorin, clean conditions (0,0% and 0,03 % BSA), concentration 5 %, exposure time 60 min, Reduction factor 4 – 10,000:1), 2004.

2. IKI, Siemensstr. 18, 35394 Gießen, dirty conditions (0,3 % BSA), concentration 6 %, exposure time 60 min, Reduction factor 7 – 10,000,000:1), 2004

3.3. Further demonstration of sporicidal effect

The according European standard for Phase 2 is EN 13 704 (Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements -phase 2, step 1). Here a reduction factor of 3 (1,000:1) is accepted.
Results found were
Concentration: 8%, Exposure time: 5 bis 6 min at a pH: around 7 and temperature: 24°C
Clean conditions.
Concentration 2 % 30 min (Only Bacillus subtilis ATCC 6633, clean conditions)
Concentration 3 % 10 min (Only Bacillus anthracis, no defined strain, clean conditions)

3.4. Demonstration of mycobactericidal effect

These tests have to be performed according to EN 14 348 (Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants. Test methods and requirements - phase 2, step 1). Here the result was clean condition, suspension test: 6 % exposition of 60 min (Microbiological Laboratory Dr. Zdenka Kotarski, Zagreb, clean conditions (0,0 % BSA),1998). Under fogging conditions (clean surfaces with bacterial contamination an adequate reduction factor was found using 10 % solution and an exposition time of 90 min

4. Data of fogging procedures

4.1 Test according to NF T 72-281

This French standard is designed especially for fogging based disinfection technologies. A test panel of

some mikroorganisms is engaged as follows: Pseudomonas aeruginosa (CIP 103-467), Staphylococcus aureus (CIP 4.83), Enterococcus hirae (CIP 5855), Escherichia coli (CIP 54127)
Sporizide action: Bacillus subtilis (CIP 52 62)
Fungizidal action: Candida albicans (IP 4872), Aspergillus niger (IP 1431-83)
Levurocidic action: Candida albicans (IP4872).

This tests are always done in defined rooms, with defined points for the generator positions. Bacteria or spores are used as bioindicators. The suspensions are brought on metal or other standardized carriers. The simulation of clean or dirty conditions is performed by addition of BSA and sterile sheep erythrocytes in different concentrations. These carriers and further chemoindicators are placed around the generator in the room to demonstrate the operating range. Sometimes positions difficult to reach for the fog were chosen, for example nearly closed drawers.

At Göttingen University Medical Department this test was performed at 22 °C and an air humidity of initial 50 % and 70 % after application of fog (F. Schmelz, 2013)

Test organism	**Reduction factor clean conditions**	**Reduction factor dirty conditions**
Staphylococcus aureus	5,54	5,30
Enterococcus faecalis	5,44	5,32
Pseudomonas aeruginosa	5, 04	4,32
Escherichia coli	5,51	4,97
Candida albicans	5,15	5,05
Trichophyton rubrum	4,46	4,17
Aspergillus niger	5,17	4,90

As acceptable reduction factor 4 was given here, so the test was passed. The concentration used was 3 %, with no given exposition time. Clean conditions mean in this study 0,0% BSA and erythrocytes. Dirty conditions were defined using 3 % BSA and 0,3 % erythrocytes.

The Bionovis Institute found employing ca. 3 x 10E3 Escherichia coli and ca. 2 x 10E4 Staphylococcus aureus a total elimination at most of test points (Gießen 9/2013).

4.2 Modified Test by Schwarzkopf Institute in collaboration with Labor L+S AG, Bad Bocklet, Germany
The application of 1 % BSA and erythrocytes (three times of dirty conditions in the usual standards) on non-pourous surfaces showed a well visible contamination, no mechanical cleaning was done before fogging. The Diosol Generator was placed according to the instructions of the manufacturer. Diosol fog volumina were adjusted correlating to the room volume.
Fresh prepared test surfaces were placed even in places difficult to reach by fog. Test bacterium was Enterococus faecium ATCC 6057 in a concentration of 10^6 colony forming unit (cfu) per test surface. Within the fog area a germ reduction of RF 5 (100.000 cfu to 1 cfu) of test germ was required. The lowest RF found was RF 2 in surfaces in a far position outside the fog area. This is analogue to a well done cleaning process. Employed were 3 and 6 % product with 90 min exposure time.

5. Virucidal Activity of Diosol®-System
Until now there is one harmonized standard to prove virucidal activity of disinfectants, EN 14476. In

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Germany now two classes of activtity (in near future three) are established:

Limited virucidal activity means activity against enveloped viruses like Hepatitis B, C, HIV, Herpes Family and Influenza for example.

Virucidal activity means full activity against non-enveloped viruses like Adeno-Group, Noro, Rota, Hepatitis A, E and others.

Future class “Limited virucidal activity plus” means limited virucidal activity with additional effect of Noro- and Rotaviruses.

In the year 1988 a sort of Minimal Inactivating Concentration was performed employing the following viruses:
Poliovirus typ 1 (strain Mahony), Papova-Virus SV 40, Adenovirus 6 (Prototype strain) and Vaccinia virus. Here a minimal inactivating dose in suspension test (clean conditions) of 0,1 % was found for all viruses in an exposition time of 24 hours. So this data are not usable for nowadays standards. However, it was interesting, that in this experiments the use of dirty conditions (0,2 % BSA; 10 % fetal calf plasma) did not change the effect significantly (National Institute of Hygiene, 1966 Budapest, Gyali ut 2-6, Hungary, 1988).
Additional an expert statement from 1999 was performed according to the French Standard EF T 72-180. Test panel of viruses included Polio-Virus 1 Strain Sabin (Polioviruses LSc-2ab) humane Adenovirus Type 5 and Vaccinia-Virus. A 3% concentration inactivated $>10^4$ test viruses within 15 Minutes using clean conditions (Faculte´des Sciences Pharmaceutiques, Laboratoire de Bacteriologie, Virologie & Microbiologie Industrielle, Toulouse, 10/1999) A concentration of 12 % and an exposure time of 30 min were recommended.
Effect on covered viruses was shown using BVDV Virus (surrogate virus for Hepatitis C). Here a concentration of 3 % and an exposure time reached inactivation after 1 minute (1). Hepatitis B was tested employing Wild Duck-Hepatitis B Virus. An inactivation was obtained within 60 minutes with a concentration of 6 % (2).
Again dirty conditions showed nearly no difference in effect of inactivation in both expert statements. The soil in statement 2 was higher than required in EN 14476 (1,2). Using HIV- Viruses similar good results could be found (1). Employing concentrations of 12 % and 19 %, 90 min exposure time with a room temperature of 24 °C and air humidity of about 63 % a total inactivation of test viruses were obtained (3).

1: Victorian Infectious Disease Reference Laboratory, Fairfiled, Australien (10/2001)

2: Eurovir Hygiene-Institut, Luckenwalde (03/2005)

3. Micro-Lab GmbH, Bremen (07/2013)

6. Validation

To prove the effectiviness of Diosol®-System under local individual conditions, a validation is required. The Robert-Koch-Institute requires the following data for validation:

- Room description (air volume in cubic meters)
- Air humidity and room temperature (range of tolerances, adjustment of Generator dosage)

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- Point of generator position
- Close windows and top climate systems
- Volume, concentration and correlating exposure time
- Additional equipment (ventilators) if required
- Indicator systems for validation (bio indicators with Geobacillus stearothermophilus) and process check (data logger and/or chemical indicators)
- Personal Security Equipment (depends on employed concentration)

Based on the results of the validation a standard operating procedure is should be written for further uses of the system. This validation should be done for every room type (means no individual validation is required in case of rooms identical in construction) in any point of use.

7. Conclusion

More than 100 expert statements are available for the Diosol® liquid (Sanosil® in former times). In this expert statement a correlation to the actual European Standards was performed. The fogging system is well established and has been proved to reach germs, fungi and viruses even under dirty conditions. Different concentrations were available from manufacturer (3 – 19 %) and could be used depending on microorganisms to be fight against. The toxicity is stated less than that of formaldehyde used in former times to fog contaminated rooms. Unfortunately, not always air humidity and room temperature was given in the protocols of the tests done for the expert statements. However, due to the fact that no climate system could work while fogging and the expert statements were produced in Europe, a temperature from 20-25°C and air humidity from 30-65 % could be assumed as main test conditions. While fogging the air humidity rises about 15 – 20 percent points. Dated 16-08-30 Diop presented a list of different air humidities and adjusting factors for programming the fogging device.

So it could be shown in general, that fogging concentrations of 6 % and an exposure time of 60 min is bactericidal (mycobactericidal 10 % and 90 min) and fungicidal. Virucidal action will be archieved employing 6 % or 12 % using an exposure time of 60 min or 30 min respectively. Spores of Clostridium difficile should be inactivated using 19% in combination with an exposure time of 90 minutes.

Aura an der Saale, 16-9-22

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