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CERTIFICATE

Inactivation of pathogens by cold disinfection

in the cryostat CryoStar NX70

Study Director

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Date

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1. Summary

The cold disinfection procedure in the CryoStar NX70 involves fogging of a chemical disinfectant throughout the working space of the cryostat at temperatures down to -20 °C.

The efficacy of microbial inactivation was determined in analogy to the standard methods of the DGHM (German Society for Hygiene and Microbiology) [1] and the European standard EN 13697:2002-01 [2] for quantitative non-porous surface testing for the evaluation of chemical disinfectants with adaptations to the test item. The bacterium *Staphylococcus aureus* ATCC 6538 was used as a surrogate test organism. The instrument temperature during the tests was set to -20 °C.

“Sanosil® S010 spezial” (SANOSIL Service GmbH, Farchant, Germany) containing approximately 5 % hydrogen peroxide and 50 mg/L silver nitrate or 5 % hydrogen peroxide prepared from a stock solution were used as disinfectants.

By employing a 20 min fogging period followed by an additional 30 min contact time, a 99.996 % (4.5 $\log_{10}$ units) inactivation of *Staphylococcus aureus* was demonstrated. This corresponds to the reduction rate achieved in previous experiments with the cryostat HM550 using the same test strain. Accordingly, results on disinfection efficacy obtained with various pathogens in the HM550 may be transferred to the CryoStar NX70. The prolonged fogging period in the CryoStar NX70 (20 min) compensates for the different dimensions of the cryostat chamber compared to the HM550.

Thus a 20 min fogging period in the CryoStar NX70 should inactivate vegetative bacteria and the pathogenic yeast *Candida* albicans by 99.5 to 99.999 %. The tests included *Mycobacterium* sp., demonstrating mycobactericidal and tuberculocidal activity. Similarly, infectious titers of test viruses are expected to be reduced as in the cryostat HM550 (94 and 99.5 %).

Deposition of silver from the Sanosil® disinfectant onto the working space surfaces has an additional long-term bacteriostatic effect [3].

The test results also show that Sanosil® S010 spezial may be substituted by a 5 % hydrogen peroxide solution without compromising immediate disinfection efficacy.

The cold disinfection procedure in the CryoStar NX70 thus offers a safe and convenient way to significantly reduce microbial contamination in the cryostat working space and, therefore, infection risk.

However, the cold disinfection system cannot replace the safety precautions, regular cleaning and disinfection of the cryostat chamber according to the manufacturer´s instructions.

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Amtzell, 12 August 2011 Ingo Maier, PhD

2. Experimental

Detailed descriptions of the experimental conditions are given in a separate test report [7].

2.1 Test instruments

Microm CryoStar NX70, serial numbers Beta 04, Beta 06 and TP5

2.2 Test strains

The following test strain was used in the present study:

Staphylococcus aureus ATCC 6538

The test strain was originally received from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) and kept in the laboratory as a frozen stock culture.

2.3 Disinfectant

(a) Sanosil® S010 spezial, containing approx. 5 % hydrogen peroxide and 50 mg/L silver ion complex according to the supplier, Sanosil Service GmbH, Farchant, Germany

(b) 5 % hydrogen peroxide freshly prepared from a 35 % stock solution Carl Roth GmbH & Co. KG, Karlsruhe, Germany)

2.4 Test method

Suspensions of test organisms were prepared in dilution buffer containing 0.03 % bovine serum albumin as an additional organic load. 50 µl aliquots of the suspensions were dried onto stainless steel coupons (20 mm diameter) and placed in different positions in the cryostat working space. After cooling to -20 °C, the coupons were treated by fogging the cryostat chamber with disinfectant during a 20 min time period. After an additional contact period of 30 min at -20 °C, the coupons were transferred into tubes containing neutralizing solution and glass beads. The surviving test organisms were rinsed off the coupons on a shaker and cell numbers were determined by plate counting.

Tests were carried out with at least three replicates each. Reduction factors were determined in comparison to control experiments without treatment with disinfectant. Control experiments on the efficacy of neutralization and the toxicity of the neutralizing solution were carried out in previous investigations [4, 5].

3. Results

The following table summarizes the test results on contaminated coupons placed on the cryobar of the CryoStar NX70. The fogging period was 20 min, the total contact time 50 min. Details are given in a separate test report [7].

Disinfectant	Mean cell number[1]		Reduction factors[1]	
	Control	Treatment	log_{10}	%
Sanosil® S010 spezial	6.18×10^7	2.669	4.5	99.996
5 % hydrogen peroxide	1.12×10^8	250	5.7	99.9998

[1] mean values from five to six replicates, respectively

Cold disinfection in the CryoStar NX70 with a fogging period of 20 min inactivated the standard test bacterium *Staphylococcus aureus* to the same extent than the disinfection routine with 15 min fogging in the HM550, both with Sanosil® S010 spezial and a total contact time of 50 min. Accordingly, results on disinfection efficacy obtained with various pathogens in the HM550 may be transferred to the CryoStar NX70. The prolonged fogging period in the CryoStar NX70 (20 min) compensates for the different dimensions of the cryostat chamber.

This applies to the following, experimentally determined reduction factors taken from separate test reports [4 - 7]. Test position was the cryobar in each case.

Microorganism	Reduction factors	
	log_{10}	%
Bacteria/Mycobacteria		
Staphylococcus aureus	4.5	99.996
Staphylococcus aureus	4.3	99.99
Pseudomonas aeruginosa	≥ 5.2	≥ 99.999
Enterococcus hirae	2.3	99.5
Escherichia coli	≥ 4.4	≥ 99.996
Mycobacterium terrae	4.1	99.993
Mycobacterium avium subsp. avium	4.9	99.999
Yeast		
Candida albicans	3.7	99.98
Viruses		
Bovine Diarrhoevirus (BVDV)	≥ 2.3	≥ 99.5
Bovine Parvovirus (BPV)	1.2	94

The test results also show that Sanosil® S010 spezial may be substituted by a 5 % hydrogen peroxide solution without compromising disinfection efficacy.

4. References

[1] Gebel J, Werner H-P, Kirsch-Altena A, Bansemir K. Standardmethoden der DGHM zur Prüfung chemischer Desinfektionsverfahren. 14 Flächendesinfektion - praxisnaher Versuch. 14.1 Überprüfung der bakteriziden und fungiziden Wirkung auf nicht porösen Oberflächen, mhp Verlag, Wiesbaden, Germany, 2001.

[2] DIN EN 13697:2002-01. Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements without mechanical action (phase 2/step 2).

[3] Monteiro DR, Gorup LF, Takayama AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB 2009. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int. J. Antimicrob. Agents 34(2), 103-110.

[4] Maier I 2010. Prüfbericht: Kaltdesinfektion im Kryostaten Microm HM550 - Prüfung der bakteriziden und fungiziden Wirkung Teil 1 [Test report: Cold disinfection in the cryostat Microm HM550 - Test for the evaluation of bactericidal and fungicidal efficacy Part 1] (20 Dec 2010).

[5] Maier I 2011. Kaltdesinfektion im Kryostaten Microm HM550. Prüfung der bakteriziden und fungiziden Wirkung Teile 2-3 [Test report: Cold disinfection in the cryostat Microm HM550 - Test for the evaluation of bactericidal and fungicidal efficacy Parts 2-3] (15 Feb 2011).

[6] Maier I 2011. Kaltdesinfektion im Kryostaten Microm HM550. Viruzide Wirkung gegen Bovine Diarrhoevirus (BVDV) / Bovine Parvovirus (BPV) [Test report: Cold disinfection in the cryostat Microm HM550 - Virucidal activity against Bovine Diarrhoevirus (BVDV) / Bovine Parvovirus (BPV)] (15 April 2011).

[7] Maier I 2011. Prüfbericht: Kaltdesinfektion im Kryostaten CryoStar NX70 – Prüfung der antimikrobiellen Wirkung [Test report: Cold disinfection in the cryostat CryoStar NX70 – Test for the evaluation of antimicrobial efficacy] (3 Aug 2011).