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Leica Instruments GmbH
Postfach 11 20
Heidelberger Str. 17-19

69222 Nußloch

Hygienist's expertise on the aptitude of the preparation

"Cryofect"

- in the following referred to as test substance - regarding its efficiency as a surface disinfectant for use in cryostats at -20 °C.

According to your order, the test substance has been tested on its disinfecting efficiency using a combination of standard test germs which can be considered model germs for certain groups of infective agents in man.

The test was made according to the guidelines for the examination of chemical disinfectants of the Deutsche Gesellschaft für Hygiene und Mikrobiologie, dated 1981 and 7/12/91.

Specification of the test substance according to the manufacturer (Henkel Hygiene GmbH):

Basic active ingredients:	ethanol and chlorhexidinedigluconate
Colour:	blue
Consistency:	liquid
Solubility:	good
Smell:	alcoholic
pH-value of concentrated and ready-for-use solution:	conc. 8.71

Lot number: solution no. 9

The following standardized test germs have been used:

Staphylococcus aureus	ATCC 6538
E. coli	ATCC 11229
Proteus mirabilis	ATCC 14153
Pseudomonas aeruginosa	ATCC 15442
Candida albicans	ATCC 10231
E. faecium	ATCC 6057
S. typhimurium	
S. enteritidis	
V. cholerae	
K. pneumoniae	
M. terrae	ATCC 15755
A. fumigatus	
Cryptococcus	

As culture media a solution of pancreatic digest of casein / papaic digest of soya bean and an agar of pancreatic digest of casein / papaic digest of soya bean have been taken. For the trials with fungi we additionally used Sabouraud glucose agar and for tests with *M. terrae* Löwenstein-Jensen culture medium was taken.

I. Finding out the optimal inactivation substance

Investigation of the optimal inactivation substance was made according to the guidelines of the DGHM. All three combinations of neutralizer substances showed a neutralizing effect, which was most significant when using the neutralizer combination III (3% Tween 80, 0.3% lecithine, 0.1% histidine, 0.5% Na-thiosulphate). For all further examinations we used this combination of inactivating substances in all test culture media and rinse liquids.

II. Determination of recovery rates of test germs at room temperature (22°C +/-1.5°C) and at low temperature (-20°C +/- 1°C)

The tests were made in 50% glycerin. For use at low temperature the 50% glycerin was cooled down to -20°C, then the germ suspension was applied and remained in place during the corresponding contact times of 5, 10, or 15 min. Parallel investigations were made at room temperature. After incubation time, 0.5 ml glycerin-germ mixture was applied to Casobouillon containing the neutralizer combination and then test strains were counted.

Table 1 shows the results for *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

EVALUATION:

There were no significant differences in the recovery rates of test germs achieved at room temperature or at -20°C.

III. Rating the efficiency by means of a quantitative suspension test

The tests were made with:

S. aureus
E. coli
E. faecium
K. pneumoniae
P. aeruginosa
C. albicans
S. enteritidis
S. typhimurium
A. fumigatus
Cryptococcus
V. cholerae
M. terrae

In addition to the original concentration (x) of the test substance, a 10% higher concentration as well as 10% and 20% lower concentrations without protein load and with a protein load of 0.2% albumine were tested, using incubation times of 5, 10, and 15 min.

The results have been captured in tables 2 - 7.

EVALUATION:

When using the original concentration of the test substance reduction rates of $\geq \log 5.0$ were achieved with and without protein load in testing:

Staphylococcus aureus at an incubation time of 5 min (table 2).

E. coli at an incubation time of 5 min (table 2)

E. faecium at an incubation time of 10 min (table 3)

K. pneumoniae at an incubation time of 5 min (table 3)

Ps. aeruginosa at an incubation time of 5 min (table 4)

Vibrio cholerae at an incubation time of 5 min (table 7)

M. terrae at an incubation time of 15 min (table 7)

and a reduction rate of $\geq \log 4.0$ in testing

Candida albicans at an incubation time of 5 min (table 4)

Cryptococcus at an incubation time of 5 min (table 6)

and fungicidal efficiency in testing

Aspergillus fumigatus at an incubation time of 15 min (table 6).

IV. Determination of the practical disinfecting efficacy by means of a surface disinfection test

As a test carrier steel plates of 2 cm x 2 cm size, fixed on styrofoam plates with countersunk magnets were used.

Contamination was made by distributing 10 microliters of germ suspension on the steel plates. Then, the plates were stored for approx. 20 min at -20°C (in the cryostat). After that, a layer of 0.2 ml of the corresponding concentrations of the test substance were applied on the steel plates (in the cryostat) and distributed by means of a diascope. Once the corresponding reagent times were over, the contaminated steel plates were removed from the magnet and immersed in culture media containing neutralizer combination. For the recovery of the colony forming units van Klingerens method was used (see guidelines of the DGHM, dated 7/12/91).

The results are shown in tables 8 - 12.

EVALUATION:

As shown in the tables the test substance concentration of the original product achieved the required reduction rates of $\geq \log 5.0$ when testing:

Staph. aureus at an incubation time of 5 min (table 8).

E. coli at an incubation time of 10 min (table 8)

Ps. aeruginosa at an incubation time of 5 min (table 9)

Proteus mirabilis at an incubation time of 10 min (table 9)

E. faecium at an incubation time of 10 min (table 10)

Candida albicans at an incubation time of 10 min (table 10)

Klebsiella pneumoniae at an incubation time of 10 min (table 11)

S. typhimurium at an incubation time of 5 min (table 11)

M. terrae at an incubation time of 10 min (table 12)

Aspergillus fumigatus at an incubation time of 10 min (table 12).

CONCLUSION:

The test results proved that

"CRYOFECT"

in its composition as concentrated original test substance can be recommended as a surface disinfectant agent in cryostats (-20°C) at an incubation time of 15 min for the purpose of infection prophylaxis against human pathogenic bacteria and fungi.



(Prof. Dr. med. H.-G. Sonntag)

Table 1: Recovery rates of test germs achieved at room temperature ($22^{\circ}\text{C} \pm 1,5^{\circ}\text{C}$) in comparison to freezing temperature ($-20^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

germ species	temperature	incubation time 5 min KBE/ml	incubation time 10 min KBE/ml	incubation time 15 min KBE/ml
Staph. aureus	room temperature	$7,3 \times 10^5$	$7,2 \times 10^5$	$1,1 \times 10^6$
	-20°C	$7,6 \times 10^5$	$7,5 \times 10^5$	$1,7 \times 10^6$
E. coli	room temperature	$3,9 \times 10^6$	$3,7 \times 10^6$	$2,3 \times 10^6$
	-20°C	$1,7 \times 10^6$	$2,5 \times 10^6$	$2,6 \times 10^6$
Ps. aeruginosa	room temperature	$5,6 \times 10^6$	$1,0 \times 10^7$	$8,8 \times 10^6$
	-20°C	$2,8 \times 10^6$	$8,7 \times 10^6$	$5,8 \times 10^6$
Cand. albicans	room temperature	$7,6 \times 10^4$	$2,2 \times 10^5$	$1,4 \times 10^5$
	-20°C	$1,6 \times 10^5$	$2,0 \times 10^5$	$1,8 \times 10^5$

Table 2: Rating of efficiency by means of a quantitative suspension test of „Cryofect“

X = original product

germ species	concentr. in %	experimental set-up	incubation time 5 min KZ/ml	log	RF	incubation time 10 min KZ/ml	log.	RF	incubation time 15 min KZ/ml	log.	RF
Staph. aureus 1,2 x 10 ⁹ KBE/ml	X + 10%		<20	1,30	5,08	<20	1,30	5,11	<20	1,30	5,04
		+0.2% Alb.	<20	1,30	5,18	<20	1,30	5,11	<20	1,30	5,08
	X		<20	1,30	5,08	<20	1,30	5,11	<20	1,30	5,04
		+0.2% Alb.	<20	1,30	5,18	<20	1,30	5,11	<20	1,30	5,08
	X - 10%		<20	1,30	5,08	<20	1,30	5,11	<20	1,30	5,04
		+0.2% Alb.	<20	1,30	5,18	<20	1,30	5,11	<20	1,30	5,08
	X - 20%		5,0 x 10 ⁴	4,70	1,68	1,4 x 10 ²	2,15	4,26	7,6 x 10 ²	2,88	3,46
		+0.2% Alb.	2,8 x 10 ²	2,45	4,03	60	1,78	4,63	60	1,78	4,60
	control		2,4 x 10 ⁶	6,38	-	2,6 x 10 ⁶	6,41	-	2,2 x 10 ⁶	6,34	-
		+0.2% Alb.	3,0 x 10 ⁶	6,48	-	2,6 x 10 ⁶	6,41	-	2,4 x 10 ⁶	6,38	-
E. coli 2,9 x 10 ⁹ KBE/ml	X + 10%		<20	1,30	5,10	<20	1,30	5,58	<20	1,30	5,46
		+0.2% Alb.	<20	1,30	5,26	<20	1,30	5,49	<20	1,30	5,52
	X		<20	1,30	5,10	<20	1,30	5,58	<20	1,30	5,46
		+0.2% Alb.	<20	1,30	5,26	<20	1,30	5,49	<20	1,30	5,52
	X - 10%		<20	1,30	5,10	<20	1,30	5,58	<20	1,30	5,46
		+0.2% Alb.	<20	1,30	5,26	<20	1,30	5,49	<20	1,30	5,52
	X - 20%		1,9 x 10 ⁵	5,28	1,12	1,1 x 10 ³	3,04	3,84	1,0 x 10 ³	3,00	3,76
		+0.2% Alb.	1,1 x 10 ⁵	5,04	1,52	4,1 x 10 ⁴	4,61	2,18	6,0 x 10 ³	3,78	3,04
	control		2,5 x 10 ⁶	6,40	-	7,5 x 10 ⁶	6,88	-	5,8 x 10 ⁶	6,76	-
		+0.2% Alb.	3,6 x 10 ⁶	6,56	-	6,2 x 10 ⁶	6,79	-	6,6 x 10 ⁶	6,82	-

Table 3: Rating of efficiency in the quantitative suspension test of "Cryofect"

X = original product

germ species	concentr. in %	experimental set-up	incubation time 5 min KZ/ml	log	RF	incubation time 10 min KZ/ml	log.	RF	incubation time 15 min KZ/ml	log.	RF
E. faecium	X + 10%		1,4 x 10 ²	2,15	4,72	<20	1,30	5,18	<20	1,30	5,36
		+0.2% Alb.	2,8 x 10 ²	2,45	4,06	<20	1,30	5,38	<20	1,30	5,46
	X		6,4 x 10 ²	2,81	4,06	<20	1,30	5,18	<20	1,30	5,36
		+0.2% Alb.	1,6 x 10 ²	2,20	4,31	<20	1,30	5,38	<20	1,30	5,46
	X - 10%		6,0 x 10 ³	3,78	3,09	1,2 x 10 ²	2,08	4,40	<20	1,30	5,36
		+0.2% Alb.	2,4 x 10 ³	3,38	3,13	1,0 x 10 ²	2,00	4,68	<20	1,30	5,46
	X - 20%		3,0 x 10 ⁴	4,48	2,39	6,2 x 10 ³	3,79	2,69	2,2 x 10 ²	2,34	4,32
		+0.2% Alb.	3,3 x 10 ⁴	4,52	1,99	2,8 x 10 ³	3,45	3,23	4,5 x 10 ²	2,65	4,11
	control		7,4 x 10 ⁶	6,87	-	3,0 x 10 ⁶	6,48	-	4,6 x 10 ⁶	6,66	-
		+0.2% Alb.	3,2 x 10 ⁶	6,51	-	4,8 x 10 ⁶	6,68	-	5,8 x 10 ⁶	6,76	-
Klebs.	X + 10%		<20	1,30	5,18	<20	1,30	5,04	<20	1,30	5,11
pneum.		+0.2% Alb.	<20	1,30	5,08	<20	1,30	5,11	<20	1,30	5,15
	X		<20	1,30	5,18	<20	1,30	5,04	<20	1,30	5,11
KBE/ml		+0.2% Alb.	<20	1,30	5,08	<20	1,30	5,11	<20	1,30	5,15
	X - 10%		<20	1,30	5,18	<20	1,30	5,04	<20	1,30	5,11
		+0.2% Alb.	<20	1,30	5,08	<20	1,30	5,11	<20	1,30	5,15
	X - 20%		1,4 x 10 ⁶	6,15	0,33	3,0 x 10 ⁵	5,48	0,86	3,5 x 10 ⁵	5,54	0,87
		+0.2% Alb.	1,0 x 10 ⁵	5,00	1,38	1,0 x 10 ⁵	5,00	1,41	2,5 x 10 ⁵	5,40	1,05
	control		3,0 x 10 ⁶	6,48	-	2,2 x 10 ⁶	6,34	-	2,6 x 10 ⁶	6,41	-
		+0.2% Alb.	2,4 x 10 ⁶	6,38	-	2,6 x 10 ⁶	6,41	-	2,8 x 10 ⁶	6,45	-

Table 4: Rating of efficiency in a quantitative suspension test of "Cryofect"

X = original product

germ species	concentr. in %	experimental set-up	incubation time 5 min KZ/ml	log	RF	incubation time 10 min KZ/ml	log.	RF	incubation time 15 min KZ/ml	log.	RF
Ps. aerug.	X + 10%		<20	1,30	5,26	<20	1,30	5,18	<20	1,30	5,23
		+0.2% Alb.	<20	1,30	5,11	<20	1,30	5,11	<20	1,30	5,08
1,4 x 10 ⁹	X		<20	1,30	5,26	<20	1,30	5,18	<20	1,30	5,23
		+0.2% Alb.	<20	1,30	5,11	<20	1,30	5,11	<20	1,30	5,08
KBE/ml	X - 10%		<20	1,30	5,26	<20	1,30	5,18	<20	1,30	5,23
		+0.2% Alb.	<20	1,30	5,11	<20	1,30	5,11	<20	1,30	5,08
X - 20%	X		3,9 x 10 ⁴	4,59	1,97	6,7 x 10 ³	3,83	2,65	1,0 x 10 ⁴	4,00	2,53
		+0.2% Alb.	9,7 x 10 ³	3,99	2,42	5,6 x 10 ³	3,75	2,66	3,8 x 10 ³	3,58	2,80
control			3,6 x 10 ⁶	6,56	-	3,0 x 10 ⁶	6,48	-	3,4 x 10 ⁶	6,53	-
		+0.2% Alb.	2,6 x 10 ⁶	6,41	-	2,6 x 10 ⁶	6,41	-	2,4 x 10 ⁶	6,38	-
Cand.	X + 10%		<20	1,30	4,48	<20	1,30	4,70	<20	1,30	4,53
albicans		+0.2% Alb.	<20	1,30	4,45	<20	1,30	4,48	<20	1,30	4,30
	X		<20	1,30	4,48	<20	1,30	4,70	<20	1,30	4,53
KBE/ml		+0.2% Alb.	<20	1,30	4,45	<20	1,30	4,48	<20	1,30	4,30
	X - 10%		<20	1,30	4,48	<20	1,30	4,70	<20	1,30	4,53
X - 20%	X		2,6 x 10 ⁴	4,41	1,37	1,3 x 10 ⁴	4,11	1,89	8,5 x 10 ³	3,93	1,90
		+0.2% Alb.	1,3 x 10 ⁴	4,11	1,64	7,8 x 10 ³	3,89	1,89	7,8 x 10 ³	3,89	1,61
control			6,0 x 10 ⁵	5,78	-	1,0 x 10 ⁶	6,00	-	6,8 x 10 ⁵	5,83	-
		+0.2% Alb.	5,6 x 10 ⁵	5,75	-	6,0 x 10 ⁵	5,78	-	4,0 x 10 ⁵	5,60	-