Confidential Project Report **P2503E**

for

Thomas Goldschmidt

by

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P2503E

COMPARISON OF **THE** EFFICACY OF TEGOL 2000 WITH TEGO 51

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INTRODUCTION

Thomas Goldschmidt Ltd has developed a new disinfectant for use in the food industry and requested that the Research Association should assess the efficacy of the new product (Tegol 2000) and compare it with that of Tego 51.

Two series of tests were required. The first was to assess the effect of Tegol 2000 and Tego 51 on cultures of Campylobacter jejuni, Listeria monocytogenes and Yersinia enterocolitica, using the protocol described in the European Suspension Test, modified where necessary to ensure cultural conditions appropriate to the different test organisms (see Graham & Blood, 1987). A disinfectant passes the test if it achieves, at the lowest use-dilution recommended by the manufacturer, a microbiocidal effect of at least 5 logarithms reduction for each of the test organisms on two of three test occasions. Two soil levels are used.

The second series of tests was undertaken with an earlier Research Association modification of the European Suspension Test using six cultures (Bacillus cereus NCTC 2599; Pseudomonas aeruginosa NCIB 10421; Saccharomyces cerevisiae NCYC 87; Salmonella typhimurium NCTC 74; Staphylococcus aureus NCIB 9518, Streptococcus faecalis NCTC 8213) and a cocktail of the above cultures (excluding B. cereus). The disinfectant passes the test if it effects a 5 logarithmic reducion for all the test organisms except for spores of B. cereus, where a 1 logarithmic reduction must be achieved. The test must be passed on each of the two test occasions and a third decisive test is not permitted. The disinfectant is tested at the manufacturer's recommended use-concentration in the presence of one level of soil only.

MATERIALS AND METHODS

Details of methodologies used for C. jejuni, L. monocytogenes and Y. enterocolitica are described in the previous report (Graham & Blood, 1987). Methods for the other organisms are appended (Appendix I).

On some occasions It was necessary to take additional precautions to ensure that the test suspensions of organisms contained sufficiently high numbers to enable estimation of survivors after a 5 $\log_{10}$ kill had been effected. This was achieved by centrifugation of 10 x 10-ml broth cultures at 2000g for 10 min and resuspension of the deposits in a total of 10 ml sterile 0.852 m/V saline.

Except where indicated, all work was done at the manufacturer's recommended concentration of disinfectant, namely 0.52 m/V for Tegol 2000 and 1% m/V for Tego 51. Tests for the efficacy of the inactivation procedures with Tegol 2000 were undertaken with all nine cultures and with the five culture cocktails using methods previously described (Graham & Blood, 1987).

RESULTS

1. Inactivation Studies

Results shown in Table I indicated that complete inactivation of Tegol 2000 was achieved for all organisms.

2. Effects of Tego 51 and Tegol 2000 on C. jejuni, L. monocytogenes and Y. enterocolitica.

A greater than 5 $\log_{10}$ kill was obtained on all three cultures at the low (0.03% m/V) soil level for Tego 51 and Tegol 2000, which appeared to give comparable disinfection. Results are shown in Table II (C. jejuni Table III (L. monocytogenes) and Table IV (Y. enterocolitica).

At the high (12 m/V) soil level a 5 $\log_{10}$ kill was rarely obtained for any of the three organisms, on either test occasion (see Tables II, III and Iv). The extent of kill for L. monocytogenes ranged between 3.0 and 4.5 $\log_{10}$ reduction in number depending on occasion and disinfectant. Increasing the concentration of Tegol 2000 improved its efficacy against L. monocytogenes but a consistent 5 $\log_{10}$ kill was not achieved by

concentrations of 0.6% m/V or 0.7% m/V, the highest concentration tested. Results are shown in Table V.

3. Modified European Suspension Test on six Organisms and *Cocktail'

The results of these tests on Tego 51 and Tegol 2000 are summarised in Table VI and detailed results are given in Appendix II, (Tables AI and AII).

A greater than 5 $\log_{10}$ kill was achieved by both disinfectants on the two test occasions for Staph. aureus and for Strep. faecalis.

Culture suspensions of Sac. cerevisiae contained marginally insufficient numbers of cells for a 5 $\log_{10}$ reduction count to be demonstrated; however, Tego 51 effected a greater than 4.6 $\log_{10}$ reduction in all six replicates whilst Tego 2000 achieved a similar kill in five of six replicates.

Sporicidal effects against spores of B. cereus were adequate for both disinfectants on one only of the two occasions tested.

Tegol 2000 at 0.5% m/V was markedly superior to Tego 51 at 1.0% m/V in its bactericidal effect against Ps. aeruginosa but overall did not pass the requirements of the test, i.e. it achieved a 5 log,, kill on only two replicates on two of the three test occasions.

The activity of Tegol 2000 was again superior to that of Tego 51 against Sal. typhimurium, and achieved a greater than 5 $\log_{10}$ reduction in two of the three test occasions.

Overall, it is evident that the performance of Tegol 2000, at 0.5% m/V is better than that of Tego 51, as can be seen by the total score of >5 $\log_{10}$ kills shown in Table VI.

TABLE I

Performance of inactivation liquid

Organism	Soil level (% bovine albumin)	Log_{10} mean colony count (cfu/ml) After inactivation of Tegol 2000	WSH
Campylobacter jejuni	0.03	7.85	7.86
	1	7.86	7.66
Yersinia enterocolitica	0.03	7.89	7.89
	1	7.85	7.83
Listeria monocytogenes	0.03	7.62	7.59
	1	7.60	7.63
Pseudomonas aeruginosa	0.03	7.11	6.85
Salmonella typhimurium	0.03	7.68	7.84
Staphylococcus aureus	0.03	6.46	6.81
Streptococcus faecalis	0.03	7.32	7.30
Saccharomyces cerevisiae	0.03	5.93	5.97
Mixed culture (a)	0.03	7.0	6.99
Bacillus cereus spores	0.03	3.53	3.57

(a) not containing spores
WSH = water of standard hardness

TABLE II

Extent of disinfection against C. jejuni

Occasion	Soil level, bovine albumin (% m/V)	Test treatment (5 min @ 20°C)	mean colony count (cfu/ml)	Log_{10} mean colony count	DE 20/5
1	0.03	0.5% (m/V) Tegol 2000	<10	<1	6.3
		WSH	1.97×10^6	6.3	
		1% (m/V) Tego 51	1.0×10^1	1.0	5.3
		WSH	1.89×10^6	6.3	
2	0.03	0.5% (m/V) Tegol 2000	<10	<1	6.4
		WSH	2.8×10^6	6.4	
		1% (m/V) Tego 51	<10	<1	6.3
		WSH	2.16×10^6	6.3	
		0.52 (m/V) Tegol 2000	2.2×10^1	1.3	4.7
		WSH	9.68×10^5	6.0	
		1% (m/V) Tego 51	1.0×10^2	2.0	4.2
		WSH	1.5×10^6	6.2	
		0.52 (m/V) Tegol 2000	<10	<1	6.3
		WSH	1.8×10^6	6.3	
		1% (m/V) Tego 51	<10	<1	6.3
		WSH	1.94×10^n	6.3	

cfu/ml = colony forming units per ml
WSH = Water of Standard Hardness
DE 20/5 = Extent of disinfection after 5 min contact at 20° + 1°C

TABLE III

Extent of disinfection against L. monocytogenes

Occasion	Soil level, bovine albumin (% m/V)	Test treatment (5 min @ 20°C)	mean colony count (cfu/ml)	Log_{10} mean colony count	DE 20/5
1	0.03	0.52 (m/V)			
		Tegol 2000	<10	<1	6.3
		WSH	4.27×10^6	7.6	
		1% (m/V)			
		Tego 51	<10	<1	7.4
		WSH	2.55×10^7	7.4	
2	0.03	0.52 (m/V)			
		Tegol 2000	<10	<1	7.3
		WSH	1.82×10^7	7.3	
		1% (m/V)			
		Tego 51	<10	<1	7.1
		WSH	1.35×10^7	7.1	
	1	0.52 (m/V)			
		Tegol 2000	1.3×10^4	4.1	3.0
		WSH	1.4×10^7	7.1	
		1% (m/V)			
		Tego 51	7.2×10^2	2.9	4.1
		WSH	1.0×10^7	7.0	
2	1	0.5% (m/V)			
		Tegol 2000	2.86×10^3	3.5	3.8
		WSH	2.19×10^7	7.3	
		1% (m/V)			
		Tego 51	5.7×10^2	2.7	4.5
		WSH	1.79×10^7	7.2	

cfu/ml = colony forming units per ml
WSH = Water of Standard Hardness
DE 20/5 = Extent of disinfection after 5 min contact at 20" + 1°C

TABLE IV

Extent of disinfection against Y. enterocolitica

Occasion	Soil level, bovine albumin (% m/V)	Test treatment (5 min @ 20°C)	mean colony count (cfu/ml)	Log_{10} mean colony count	DE 20/5
1	0.03	0.52 (m/V)			
		Tegol 2000	<10	<1	7.6
		WSH	4.46 x 10^7	7.6	
		1% (m/V)			
		Tego 51	<10	<1	7.6
		WSH	4.0 x 10^7	7.6	
2	0.03	0.52 (m/V)			
		Tegol 2000	<10	<1	7.1
		WSH	1.29 x 10'	7.1	
		1% (m/V)			
		Tego 51	<10	<1	7.5
		WSH	3.26 x 10^7	7.5	
	1	0.52 (m/V)			
		Tegol 2000	2.9 x 10"	2.5	5.1
		WSH	3.66 x 10^7	7.6	
		1% (m/V)			
		Tego 51	9.36 x 10^2	2.0	4.5
		WSH	3.2 x 10^7	7.5	
2	1	0.52 (m/V)			
		Tegol 2000	9.62 x 10^2	3.0	4.5
		WSH	3.56 x 10^7	7.5	
		1% (m/V)			
		Tego 51	2.68 x 10^3	3.4	4.2
		WSH	3.74 x 10^7	7.6	

cfu/ml = colony forming units per ml
WSH = Water of Standard Hardness
DE 20/5 = Extent of disinfection after 5 min contact at 20" + 1°C

TABLE V

Efficacy of different concentrations of Tegol 2000 and Tego 51 **(1%** m/V) **against L. monocytogenes at 1% (m/V) soil level**

Occasion	Test treatment (5 min **@ 20°C)**	**Mean** colony count **(cfu/ml)**	**Log_{10}** mean colony count	DE **20/5**
1	0.5% (m/V) Tegol 2000	2.27 x 10^4	4.4	3.1
	WSH	3.44 x 10^7	7.5	
	0.6% **(m/V)** Tegol 2000	2.15 x 10^3	3.3	4.2
	WSH	3.18 x 10'	7.5	
	0.7% (m/V) Tegol 2000	6.6 x 10'	1.8	5.9
	WSH	5.5 x 10^7	7.7	
	1% (m/V) Tego 51	4.1 x 10'	1.6	5.9
	WSH	2.9 x 10'	7.5	
2	0.52 (m/V) Tegol 2000	2.95 x 10^3	3.5	4.2
	WSH	4.86 x 10^7	7.7	
	0.6% (m/V) Tegol 2000	2.0 x 10'	1.3	6.3
	WSH	4.4 x 10'	7.6	
	0.72 (m/V) Tegol **2000**	5.2 x 10^2	2.7	4.9
	WSH	4.16 x 10^7	7.6	
	1% (m/V) **Tego** 51	1.2 x 10'	3.1	4.4
	WSH	3.29 x 10'	7.5	

cfu/ml = colony forming units per ml
WSH = Water of Standard Hardness
DE **20/5** = Extent of disinfection after 5 min contact at **20° + 1°C**

TABLE VI

Summary of results for the modified European Suspension Test

Culture	Disinfectant: Tego 51 (1% m/V)	Disinfectant: Tegol 2000 (0.5% m/V)	Total no. replicates	No. replicates giving DE 20/5 in indicated range: >5	>4-5	>3-4	>2-3	>1-2	1 or less
Ps. aeruginosa	/		9[a]	0	0	8	1	0	0
		/	9[a]	4	1	4	0	0	0
Sal. typhimurium	/		9[a]	4	2	3	0	0	0
		/	9[a]	6	0	3	0	0	0
Staph. aureus	/		6[b]	6					
		/	6[b]	6					
Strep. faecalis	/		6[b]	6					
		/	6[b]	6					
Sac. cerevisiae	/		6[b]		6[c]				
		/	6[b]		5[c]	1			
B. cereus spores	/		6[b]	0	0	0	0	3	3
		/	6[b]	0	0	0	0	3	3
Cocktail	/		6[b]	0	0	6	0	0	0
		/	6[b]	0	3	0	3	0	0
Total	/		48	16	8	17	1	3	3
		/	48	25	6	11	0	3	3

[a] = 3 replicates on each of 3 occasions
[b] = 3 replicates on each of 2 occasions
[c] = insufficient cells in culture to enable a 5 log kill to be estimated. Results were >4.69 - >4.81

DISCUSSION

Although it appears that the performance of Tegol 2000 at 0.5% m/V is superior to that of Tego 51 at 1% m/V, the results are less conclusive than is desirable. This is because the performance of Tego 51 was less satisfactory than previous results obtained with this disinfectant had indicated.

At the present time, there is little published information on the effects of disinfectants on Campylobacter jejuni, Listeria monocytogenes or Yersinia enterocolitica. Our previous work (Graham & Blood, 1987) on the effect of Tego 51 on these organisms led us to expect that 1% m/V Tego 51 would effect at least a 5 $\log_{10}$ kill with C. jejuni, although the margin of success had been less pronounced. The present results with L. monocytogenes were perhaps less unexpected since previously it had been necessary to carry out the test on a third occasion because of failure on one occasion at the high soil level.

As far as we know, the European Suspension Test has not been applied elsewhere to these organisms, so there is no other information available on the robustness or even the application of this test for the above organism. Ideally it should be subjected to more extensive trials to establish repeatability and reproducibility within and between occasions and between operators. Van Klingeren, Leussink & Van Wijngaarden (1977) have discussed statistical aspects of the Dutch Standard Suspension Test for the evaluation of disinfectant, the precursor of the European Suspension Test (see also Abbiss & Jarvis, 1980; Blood, Abbiss & Jarvis, 1981).

The modification of the European Suspension Test used in the present work for the other micro-organisms differs from the original method in several ways - choice of organism, level of organism in the test suspension, use of one soil level (0.03% m/V) only, and in the requirement to pass the test, i.e. to achieve a 5 $\log_{10}$ kill or greater on each of two occasions. Also, there is no latitude allowed, i.e. there is no third, decisive test. This modification was devised at the

Research Association in collaboration with some disinfectant manufacturers at the request of a large supermarket chain.

The objective was to assess disinfectant for use in high risk areas as alternatives to Tego 51. The method was never subject to collaborative trials to determine the variations discussed above; it must be assumed that they differ little from those of the parent test, which are known to be considerable, as with other microbiological tests for disinfectant activity.

Certainly, results of intermittent tests done at the Research Association over a period of several years had led us to expect a better performance from Tego 51.

The present work has highlighted some deficiencies in the method appended, since it gives no indication that, in order to achieve the initial high level of organisms in the test suspension, it is necessary to concentrate the cultures used.

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APPENDIX I

RESEARCH ASSOCIATION MODIFICATION OF EUROPEAN SUSPENSION TEST ORGANISMS AS INDICATED IN TEXT OF REPORT

Materials and Methods

1. Apparatus

Incubator operating at 30 ± 2°C

Water bath operating at 20 ± 0.5°C

Vater bath operating at 46 ± 1°C

centrifuge, e.g. MSE Bench centrifuge

Centrifuge tubes, 10 ml capacity

Pipettes: of appropriate capacity,

for bacteriological purposes: BS700, Type 4 (blow-out)

for disinfectant preparation: BS700, Type 2, Class B

Volumetric flasks

Erlenmeyer flasks, 100 ml capacity, .

McCartney bottles (28 ml capacity) with screw caps

Universal bottle6 (28 ml capacity) with screw caps

Test tube6 with caps*

200 ml capacity glass bottles with screw cap6

90 mm internal diameter Petri dishes

Glassware should be washed so that all residues of disinfectants are completely removed.

*Broth and agar elope cultures may be carried out in bottles of suitable dimensions,

2. Media and Reagents

2.1 Agar media

The media for pour-plating should be dispensed into bottles in 200 ml volumes before sterilizing. The media for slope6 should be dispensed into test tubes (or Universal. bottles) in 10 ml volume6 before sterilizing.

2.1.1 For bacteria and *spores*

Tryptone - soya broth + agar

Tryptone soya broth (Oxoid CU 129)	30 g
Agar No. 3 (Oxoid L13)	15 g
distilled water	1 l.

Sterilize by autoclaving at 121°C for 15 min.

2.1.2 For yeasts

Halt extract-yeast extract-peptone-glucose-agar

Halt extract (Oxoid L39)	3 g
Yeast extract (Oxoid L21)	3 g
Peptone, mycological (Oxoid L40)	5 g
Glucose	10 g
Agar No.3 (Oxoid L13)	20 g
distilled water	1 l.

Adjust pH to 5.6 - 5.8 and sterilize by autoclaving at 121°C for 15 min.

2.2 Broth media

The media should be dispensed into test tubes (or McCartney bottles) in 10 ml volumes before sterilizing.

2.2.1 For bacteria and spores

Tryptone soya broth (Oxoid CU 129)

Prepare and sterilize as directed,

2.2.2 For yeasts

As 2.1.2 but without agar.

2.3 Diluent

Bovine albumin (Sigma A4503)	0-3 g
¼-Strength Ringer solution (2.4)	1 l.

Sterilize by filtration and dispense aseptically into sterile bottles,

2.4 &Strength Ringer solution

&Strength Ringer solution tablets (Oxoid BR52)

Prepare and sterilizc as directed, After sterilization, dispense aseptically into sterile McCartney bottles in 9ml volumes.

2.5 . Standard hard water (WHO, 1973)

342 ppm hardness (expressed a6 $CaCO_3$)

$CaCl_2$	0.304 g
$MgCl_2\ 6H_2O$	0.139 g
distilled water	1 1

Sterilize by autoclaving at 121°C for 15 min.

2.6 Bovine Albumin solution

Bovine albumin (Sigma A4503)	1.5 g
distilled water	100 ml.

Sterilize by filtration and dispense aseptically into bottles,

2-7 Neutralizing solution

Lecithin (BDH 29053)	3 g
Polysorbate 80 (Tween 80)	30 ml
L-histidine	1 g
0.25N phosphate buffer (2.8)	10 ml
distilled water	to1 1

Sterilize by autoclaving at 121°C for 15 min. Add 50 ml aseptic horse serum per litre and dispense aseptically into McCartneys in 9 ml volumes,

2.8 0.25N Phosphate buffer

KH_2PO_4	3-4 g
distilled water	50 ml

Adjust the pH to 7-2 ± 0.1 with 1N NaOH

distilled water	to 100 ml

Sterilize by autoclaving at 121°C for 15 min.

3 Test Organisms

Bacillus cereus	NCTC 2599'; ATCC 14579
Pseudomonas aeruginosa	NCIB 10421; ATCC 15442
Saccharomyces cerevisiae	NCYC 87; ATCC 9763
Salmonella typhimurium	NCTC 74; ATCC 13311
Staphylococcus aureus	NCIB 9518; ATCC 6538
Streptococcus faecalis	NCTC 8213

. proposed neotype.

3.1 Storage of culture6

Maintain strains on two agarslopes (2.1) stored at 5 ± 2°C. Use one as the working stock and the other as the master stock. Subculture monthly from the master stock onto fresh slopes (2.1). Incubate these at 30 + 2°C for 24 h and then store at 5 ± 2°C.

3.2 Cultivation of test organisms

For all tests, use the 4th to the 14th subculture, The number of broth and agar culture6 needed to obtain cell (3.3.1) and spore suspensions (3.3.2) of sufficient volume should be determined-

3.2.1 All *strains* except B. cereus

Subculture from the working Stock (3.1) onto a fresh agar slope (2.1), incubate at 30 + 2°C for *24* + 1 h then subculture into 10 ml broth (2.2) and incubate at 30 ± 2°C for 24 ± 1 h.

3.2.2 B. cereus

Subculture from the working stock (3.1) onto a fresh agar slope (2.1), incubate at 30 + 2°C for 24 ± 1 h then subculture onto another agar slope (2.1) and incubate at 30 ± 2°C for 24 ± 1 h.

3.3 Preparation of suspensions

3.3.1 Vegetative cell suspension6 (all except B. cereus)

Centrifuge the 24 h broth *cultures* (3.2.1) at 2000 g *for* 20 ❑ in-
Decant the supernatant and wash the cells with diluent (2.3).
Centrifuge and resuspend in diluent (2.3) to obtain a concentration of ca. 10^{10} cells ml^{-1} determined with the aid of a nephelometer.

3.3.2 Spore suspension (B. cereus)

Collect the growth on the agar slopes (3.2.2) in diluent (2.3) and centrifuge at 2000 g for 20 min. Decant the supernatant and wash the cells with diluent (2.3). Centrifuge and resuspend in diluent (2.3) to obtain a concentration of ca. 5 x 10^6 cell6 ml", determined with the aid of a nepholometer. Heat suspensions, whilst shaking, in a water bath to 80 ± 2°C and maintain at that temperature for 60 + 5 6. Cool under running water.

The spore suspension (ca. 2 x 10^6 spores ml^{-1}) thus prepared may be stored for 1 month at 5 ± 2°C.

3.3.3 Hold& Time

The cell suspension6 (3.3.1) should be used within 15 min of the time of final preparation.

4 Assessment of Disinfectant6

Controls (4.2 and 4.3) must be carried out at the same time and with the same cell or spore suspension as the teat disinfectant.

4.1 Test disinfectant system

Dispense 24 ml of test disinfectant, diluted to the manufacturer6 recommended strength with standard hard water (2.5), into 3 sterile Erlenmeyer flasks of 100 ml capacity. Equilibriate to 20 ± 0.5°C.

Mix equal. volume6 of vegetative cell (3.3.1) or spore suspension (3.3.2) and albumin solution (2.6). For the test systems with mixed cell suspensions, mix equal volumes of each vegetative cell suspension then mix equal volumes of the combined suspension and the albumin solution. Equilibriate to 20 ± 0.5°C for 2 min ± 2 s. Add 1 ml to each of the 3 flasks containing disinfectant and incubate, with shaking, at 20 + 0.5°C for 5 min ± 5 s.

After this time, pipette 1 ml of the test suspension into 9 ml of neutralizing solution (2.7) at 20 ± 0.5°C, mix, leave for 5 min ± 5 s at 20 ± 0.5°C, then make decimal serial dilutions in &strength Ringer solution (2.4) and prepare five' 1 ml pour plates with medium (2.1) tempered at 46 + 1°C for each dilution. Incubate at 30 ± 2°C for 48 h and determine the viable count.

• ⁺ᵐˡ 5 replicate plate6 for plating 10^{-1} dilution, i.e. the neutralized material and use 3 replicate plate6 for all other dilutions.

4.2 Control disinfectant system

Proceed as in 4.1 but using 24 ml volume6 of the control disinfectant (Tego 51) diluted to 1% (manufacturers recommended strength) with standard hard water (2.5).

4.3 Blank system

Proceed as in 4.1 but using 24 ml volumes of standard hard water (2.5).

5 Calculation of the Extent of Disinfection

The extent of disinfection after 5 min contact time at 20°C ($DE\ ^{20}_{5}$) is expressed by the formula:

$$DE\ ^{20}_{5} = \log_{10} N_B - \log_{10} N_D$$

where N_B = number of viable organisms ml of the system without disinfectant

and N_D = number of viable organisms ml^{-1} of the system with disinfectant-

APPENDIX II

DETAILED RESULTS *OF* THE MODIFIED EUROPEAN SUSPENSION TEST

Table **AI**

Extent of disinfection (DE **20/5)** achieved by Tego 51 (1% m/V)

Organism	Extent of disinfection (DE **20/5**) Occasions (3 replicates)			**Range**	**Mean**
	1	2	3(a)		
Pseudomonas aeruginosa	**<3.63**	3.59	3.24	2.99 - 3.67	3.33
	<3.63	3.66	2.99		
	<3.63	3.67	3.09		
Salmonella typhimurium	x3.38	**>6.21**	5.25	**<3.38 ->6.21**	4.79
	<3.38	**>6.21**	4.65		
	<3.38	**>6.21**	4.97		
Staphylococcus aureus	**>6.1**	**>5.73**		**>5.73 ->6.1**	5.92
	>6.1	**>5.73**			
	>6.1	**>5.73**			
Streptococcus **faecalis**	**>6.16**	**>6.35**		**>6.16 ->6.35**	6.25
	>6.16	**>6.35**			
	>6.16	**>6.35**			
Saccharomyces cerevisiae	**>4.69**	**>4.81**		**>4.69 ->4.81**	4.75
	>4.69	**>4.81**			
	>4.69	**>4.81**			
Mixed **culture**(b)	**<3.21**	**<3.15**		**<3.15 -<3.21**	3.18
	<3.21	**<3.15**			
	<3.21	**<3.15**			
Bacillus cereus spores	1.04	0.56		0.56 - 1.2	0.88
	1.2	0.9			
	1.1	0.88			

(a) carried out at later date using 'new' cultures of Ps. aeruginosa and S. typhimurium

(b) not containing spores

Table AII

Extent of disinfection (DE 20/5) achieved by Tegol 2000 (0.5% m/V)

Organism	Extent of disinfection (DE 20/5) Occasion6 (3 replicates) 1	2	3[a]	Range	Mean
Pseudomonas aeruginosa	<3.63	>6.25	5.39	<3.63 ->6.25	4.94
	<3.63	>6.25	3.41		
	<3.63	4.44	5.6		
Salmonella typhimurium	<3.38	>6.21	>7.57	<3.38 ->7.57	5.48
	<3.38	>6.21	>7.57		
	<3.38	>6.21	>7.57		
Staphylococcus aureus	>6.1	>5.73		>5.73 ->6.1	5.92
	>6.1	>5.73	-		
	>6.1	>5.73			
Streptococcus faecalis	>6.16	>6.35	-	>6.16 ->6.35	6.25
	>6.16	>6.35			
	>6.16	>6.35			
Saccharomyces cerevisiae	3.62	>4.81		3.62 ->4.81	4.22
	>4.69	>4.81			
	>4.69	>4.81			
Mixed culture[b]	<3.21	>6.15		<3.21 ->6.15	4.68
	<3.21	>6.15			
	<3.21	>6.15			
Bacillus cereus spores	1.16	0.76		0.65 - 1.16	0.9
	1.09	0.65			
	1.02	0.68			

[a] carried out at later date using new cultures of Ps. aeruginosa and S. typhimurium

[b] not containing spores