IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY PROTOCOL:	**Determination of the Antimicrobial Efficacy of a Treated Article against eMRSA 15, *Pseudomonas aeruginosa* and *Candida albicans* using a Simulated Splash Test**
CLIENT:	**Managed by Micramed Ltd on Behalf of:** **NitroPep** **Delves Lane Industrial Estate** **Consett** **DH8 9HU** **UK**
STUDY NO:	**IMSL2017/10/019.3B**
DATED:	**06th March 2018**

Study: Determination of the Antimicrobial Efficacy of a Treated Article against eMRSA 15, *Pseudomonas aeruginosa* and *Candida albicans* using a Simulated Splash Test

Client: Managed by Micramed Ltd on Behalf of:
NitroPep
Delves Lane Industrial Estate
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DH8 9HU
UK

Study No: IMSL2017/10/019.3B

The above study was conducted in the laboratories of Industrial Microbiological Services Ltd at Pale Lane Hartley Wintney, Hants, RG27 8DH, UK. This report represents a true and accurate account of the results obtained.

Start Date: 30th January 2018

Analysis Period: 31st January 2018 - 12th February 2018

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1. Introduction / Objective

The objective of this study is to assess the antimicrobial efficacy of a stainless steel surface which has been treated to covalently bind an antimicrobial agent (Chlorhexidine).

Freshly prepared stainless steel surfaces covalently bound with Chlorhexidine prepared in solvent solution, as well as a variant of the covalently bound Chlorhexidine prepared in solvent solution which has been surface wiped with a disinfectant to simulate real life exposure to cleaning agents or solvent-only treated stainless steel surfaces (i.e. treatment without the presence of Chlorhexidine) were analysed for their antimicrobial activity against eMRSA 15, *Pseudomonas aeruginosa* and *Candida albicans* in the presence of a soiling agent compared to standard stainless steel.

2. Test Materials

2.1 Test Product

Replicate (72) virgin Chlorhexidine stainless steel coupons, (72) Disinfectant wiped Chlorhexidine stainless steel coupons, (72) virgin solvent-only treated stainless steel coupons and (72) untreated (control) stainless steel coupons (10 mm x 10 mm) were supplied by the client. All test materials were stored at 20 $\pm$ 2°C in the dark prior to use. The samples were tested as received i.e. no surface sterilisation of the material was performed prior to analysis.

2.2 Test Neutralisers

Dey-Engley neutralising broth was used as the neutraliser based on the validation result obtained in Report IMSL2017/10/019.3A (Ref 4 and Appendix C).

2.3 Test Microorganisms

Three microbial species were employed for the study (see Table 1). Both bacteria were held as primary storage stocks on Trypcase Soya Agar (TSA) slopes at 2 - 8°C for the duration of the study.

The yeast was held as primary storage stocks on Malt Extract Agar (MEA) slopes at 2 - 8°C for the duration of the study.

Microorganisms were stored based on the principles described in EN 12353 (Ref 1).

Table 1: Test Microorganisms

Test Species	Strain
eMRSA 15	NCTC 13142
Pseudomonas aeruginosa	ATCC 15442
Candida albicans	ATCC 10231

2.3.1 Preparation of Test Inoculum

A fresh inoculum was prepared for the study and was used within 2 hours of preparation.

Individual suspensions of the microorganisms detailed in Table 1 were prepared in sterile, tryptone sodium chloride solution (see Appendix A) in the presence of a soiling agent (see section 2.4) from 24 hour culture plates for both bacterial strains and from a 48 hour culture plate for the yeast strain.

The cell count in each individual bacterial suspension was determined using a counting chamber (Thoma 1/400 mm^2 x 0.02 mm) and adjusted (using the suspending medium described in section 2.4) such that they contained between 1.0 x 10^7 to 5.0 x 10^7 cells ml^{-1}.

The cell count in each individual yeast suspension was determined using a counting chamber (Haemocytometer $^1/_{400}$ mm^2 x 0.1 mm) and adjusted using the suspending medium described in section 2.4) such that it contained between 1.0 x 10^7 to 5.0 x 10^7 cells ml^{-1}.

The number of colony forming units in each individual suspension was determined using dilution plate count (see Section 3.2.1). The cell counts as well as the colony forming units (CFU) ml^{-1} are shown in Appendix C.

2.4 Soiling Conditions

Both simulated clean and simulated dirty conditions were employed for this study (Ref 2 and 3).

The inocula described in section 2.3.1 was prepared in either a solution of tryptone sodium chloride containing 0.3 g L^{-1} Bovine Serum Albumin (BSA) (simulated clean conditions) or a solution of tryptone sodium chloride containing 3.0 g L^{-1} BSA and 3.0 ml L^{-1} sheep erythrocytes.(simulated dirty conditions)

3. Test Method

Antibacterial activity of the test samples was determined using a simulated splash test against eMRSA 15, *Pseudomonas aeruginosa* and *Candida albicans* (Appendix B).

Test panels were inoculated with either eMRSA 15, *Pseudomonas aeruginosa* or *Candida albicans* prepared in either a simulated clean or dirty soiling diluent (see section 2.4) to give an in-test concentration of *ca* 10^5 CFU surface and then incubated for up to 15 minutes at 20 $\pm$ 2°C.

The survival of these microorganisms on the surfaces was measured by determining the total viable count (as colony forming units) remaining on the surface of the samples.

Antimicrobial efficacy was determined by calculating the log values of the recovery on the treated samples and comparing it to the log values of the recovery from the untreated samples.

3.1 Inoculation and Recovery

Replicate aliquots (9 x 1 µl) of a log phase cell suspension of either eMRSA 15, *Pseudomonas aeruginosa* or *Candida albicans* prepared in tryptone sodium chloride inluding a soiling agent (see section 2.4) were inoculated on to 12 replicate surfaces (20 x 20 mm) of each treatment type contained in square petri dishes (100mm x 100mm) and allowed to stand at 20 $\pm$ 2°C for up to 15 minutes. Relative humidity was measured for the duration of each experiment (Signatrol - SL54TH-A) and found to be 53% $\pm$ 5%.

After a contact interval of 5 and 15 minutes, individual sub-samples (6) of each treatment/soiling type were placed into a sterile container filled with 10 ml of sterile Dey-Engley neutralising broth (see section 2.2) containing 5 x 4 mm sterile glass beads.

The containers were then vigorously agitated for 1 minute on a vortex mixer and then after a neutralising period of a further 4 minutes at 20°C, the viable cells in each suspension was enumerated by spiral dilution and by pour plate (See section 3.2) onto appropriate media (TSA for bacteria and MEA for yeast).

The test plates were then incubated at 35 ± 2°C (TSA) and 28 ± 2°C (MEA) for 48 hours and then the viable colonies counted.

The populations of surviving organisms per surface was calculated and the antimicrobial efficacy was determined by calculating the log values of the recovery on the treated samples and comparing it to the log values of the recovery from the untreated samples at each time interval.

3.2 Detection of Surviving Microorganisms

The number of colony forming units was determined by both dilution plate count (section 3.2.1) and pour plates (section 3.2.2).

3.2.1 Dilution Plate Count

The number of colony forming units in each solution and dilution was determined by spiral dilution (Spiral Systems Inc Model DU) onto appropriate agar plates (TSA for bacterial strains and MEA for yeast strains).

The plates were then incubated for at 35 ± 2°C for the bacterial strains (TSA) or at 28 ± 2°C for the yeast strain (MEA) for 48 hours and then enumerated.

The theoretical limit of detection of this method is 20 CFU sample.

3.2.2 Pour Plate Count

An aliquot (1.0 ml) from each neutralised solution was pipetted in to individual sterile petri-dishes (90 mm) to which molten (*ca* 48°C) of appropriate agar was added and mixed.

Once set, the plates were then incubated at 35 ± 2°C for the bacterial strains (TSA) or at 28 ± 2°C for the yeast strain (MEA) for 48 hours and then enumerated.

The theoretical limit of detection of this method is 10 CFU sample.

4. Results

The results are shown in Tables 2 - 4 and Figures 1, 4 and 7 below (raw data in Appendix C). The statistical analysis of the data is shown in Figures 2, 3, 5, 6, 8 and 9 as Confidence Intervals.

Table 2: Activity Against eMRSA 15 (Geometric Mean of 6 Replicates as Colony Forming Units Sample^{-1})

Test Surface	Soiling	CFU Sample^{-1} ‡			Log_{10} CFU Sample^{-1}			Log Reduction From Initial	
		0	5 Mins	15 Mins	0	5 Mins	15 Mins	5 Mins	15 Mins
SS Blank	Clean	3.9×10^5	1.5×10^5	1.8×10^5	5.6	5.2	5.2	0.4	0.3
SS Blank	Dirty	4.4×10^5	1.8×10^5	2.0×10^5	5.6	5.2	5.3	0.4	0.3
SS Solvent	Clean	3.9×10^5	2.1×10^5	1.7×10^5	5.6	5.3	5.2	0.3	0.4
SS Solvent	Dirty	4.4×10^5	2.0×10^5	1.9×10^5	5.6	5.3	5.3	0.3	0.4
SS CHDG	Clean	3.9×10^5	< 10	$\leq$ 10	5.6	$\leq$ 1.0	$\leq$ 1.0	$\geq$ 4.6	$\geq$ 4.6
SS CHDG	Dirty	4.4×10^5	3.7×10^2	$\leq$ 10	5.6	2.6	$\leq$ 1.0	3.1	$\geq$ 4.6
SS CHDG + Disinfectant	Clean	3.9×10^5	4.7×10^1	1.1×10^1	5.6	1.7	1.1	3.9	4.5
SS CHDG + Disinfectant	Dirty	4.4×10^5	1.7×10^2	$\leq$ 10	5.6	2.2	$\leq$ 1.0	3.4	$\geq$ 4.6

‡ The theoretical limit of detection is 10 CFU Sample^{-1}

Figure 1: Results as Log_{10} CFU cm^{-2} - eMRSA 15

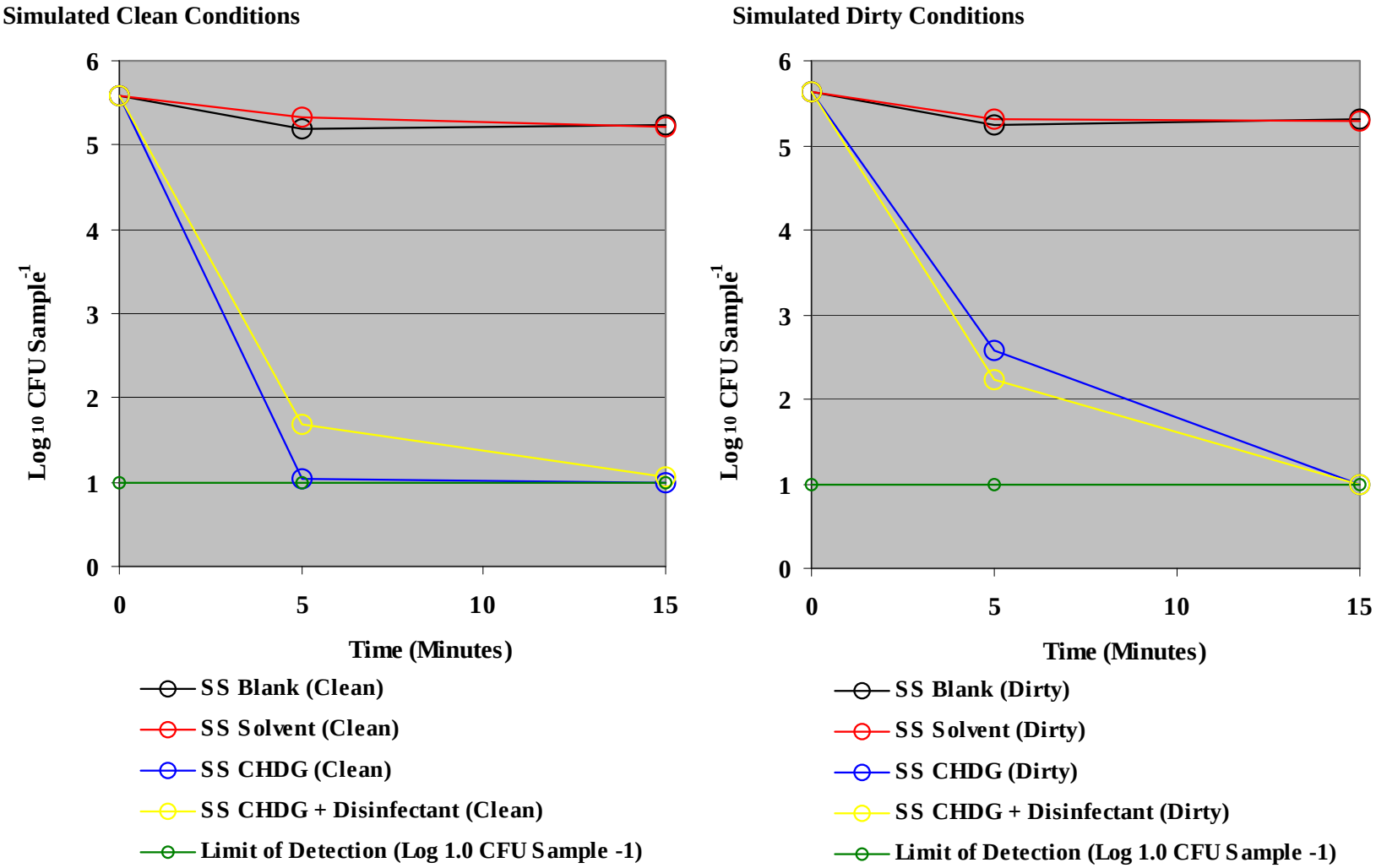

Figure 2: Confidence Intervals of the Data After 5 Minutes - eMRSA 15

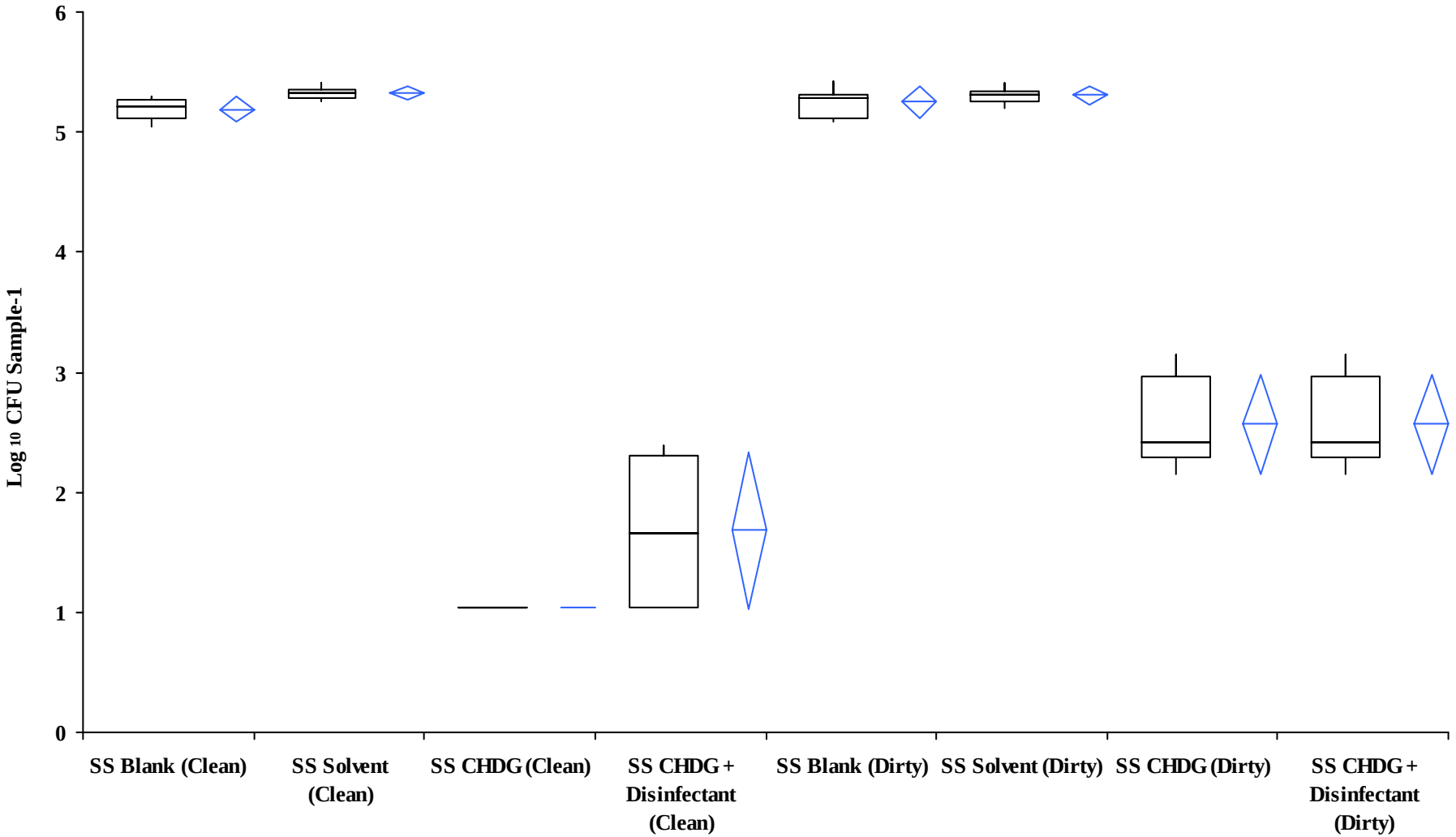

Figure 3: Confidence Intervals of the Data After 15 Minutes - eMRSA 15

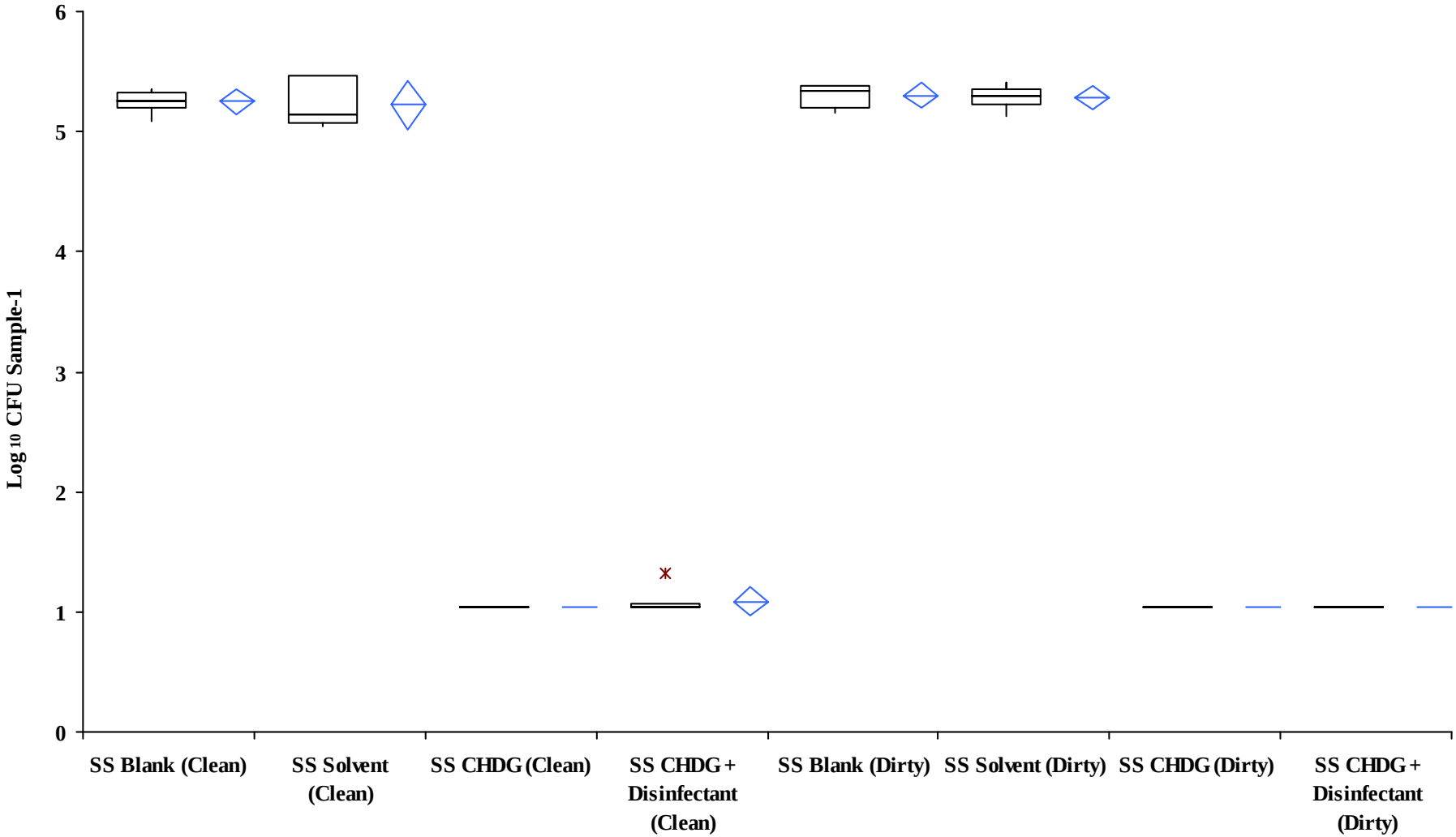

Table 3: Activity Against *Pseudomonas aeruginosa* (Geometric Mean of 6 Replicates as Colony Forming Units Sample^{-1})

Test Surface	Soiling	CFU Sample^{-1} ‡			Log_{10} CFU Sample^{-1}			Log Reduction From Initial	
		0	5 Mins	15 Mins	0	5 Mins	15 Mins	5 Mins	15 Mins
SS Blank	Clean	2.1×10^5	1.7×10^5	1.5×10^5	5.3	5.2	5.2	0.1	0.1
SS Blank	Dirty	2.2×10^5	2.9×10^5	1.8×10^5	5.3	5.5	5.3	-	0.1
SS Solvent	Clean	2.1×10^5	1.4×10^5	1.5×10^5	5.3	5.1	5.2	0.2	0.2
SS Solvent	Dirty	2.2×10^5	1.9×10^5	1.4×10^5	5.3	5.3	5.1	0.1	0.2
SS CHDG	Clean	2.1×10^5	8.7×10^1	≤ 10	5.3	1.6	≤ 1.0	3.4	≥ 4.3
SS CHDG	Dirty	2.2×10^5	2.1×10^1	≤ 10	5.3	0.7	≤ 1.0	4.0	≥ 4.3
SS CHDG + Disinfectant	Clean	2.1×10^5	1.7×10^2	≤ 10	5.3	2.2	≤ 1.0	3.1	≥ 4.3
SS CHDG + Disinfectant	Dirty	2.2×10^5	8.9×10^1	≤ 10	5.3	1.6	≤ 1.0	3.4	≥ 4.3

‡ The theoretical limit of detection is 10 CFU Sample^{-1}

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Figure 4: **Results as Log_{10} CFU cm^{-2} - *Pseudomonas aeruginosa***

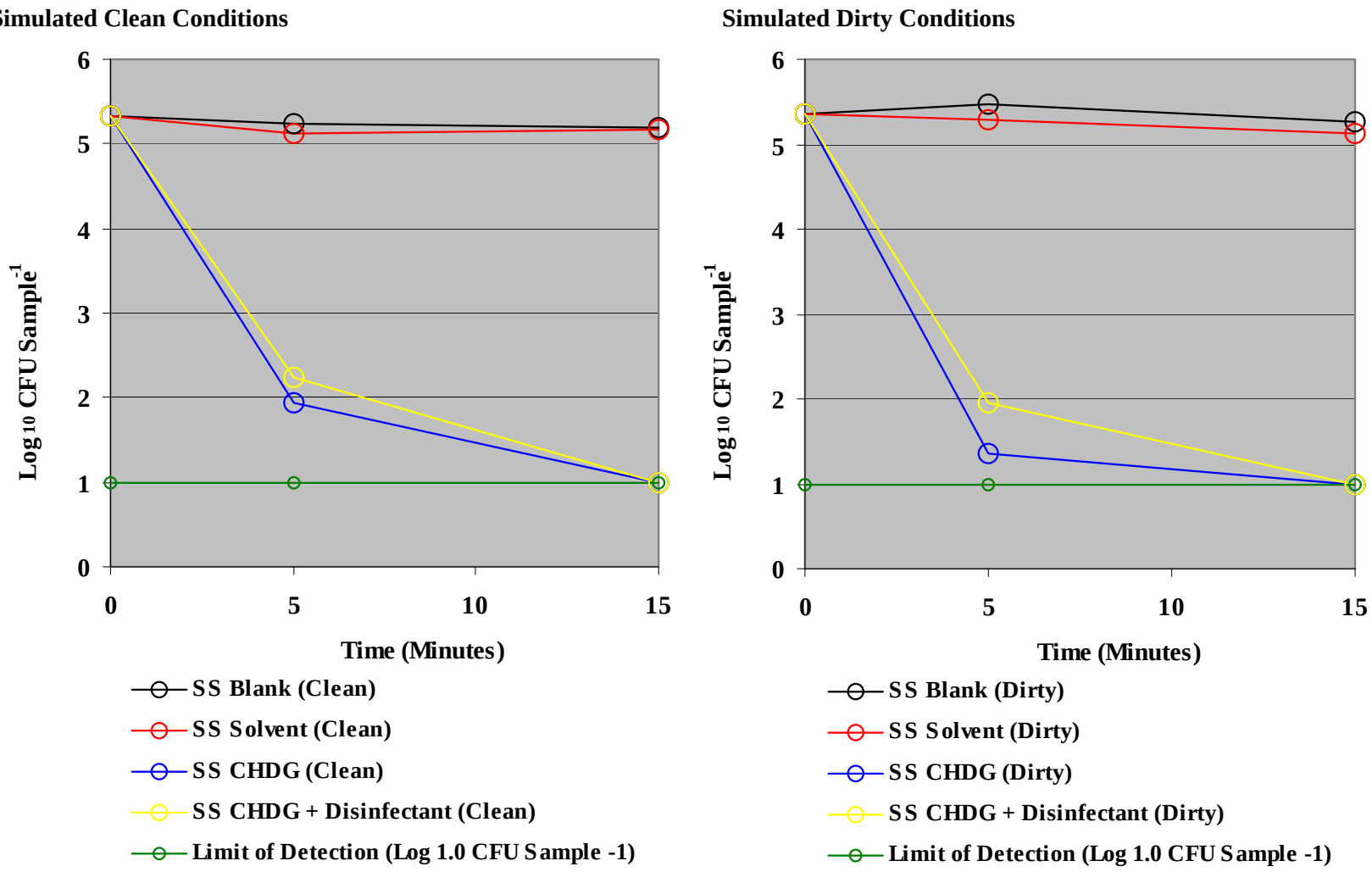


Figure 5: Confidence Intervals of the Data After 5 Minutes - *Pseudomonas aeruginosa*

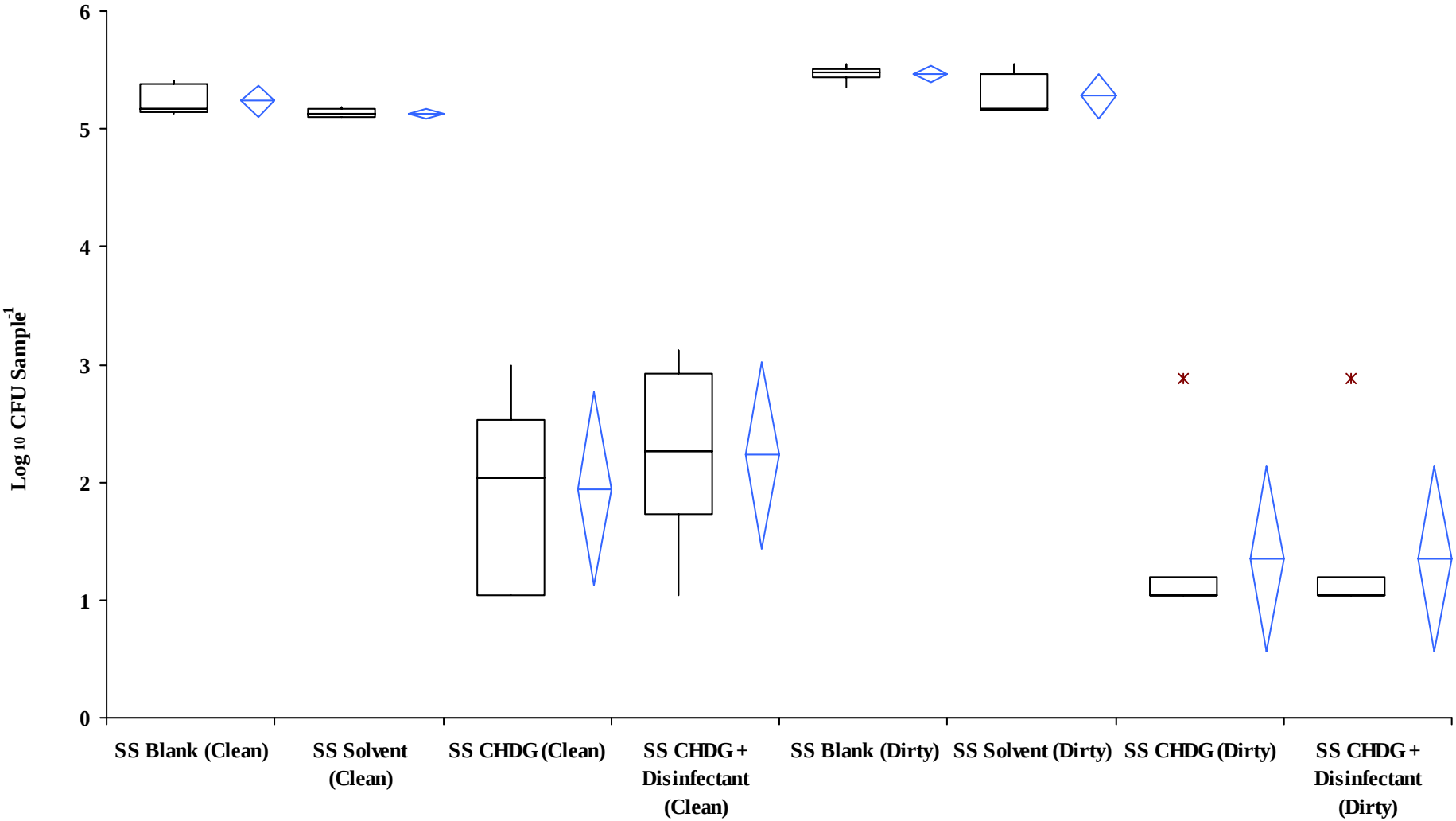


Figure 6: Confidence Intervals of the Data After 15 Minutes - *Pseudomonas aeruginosa*

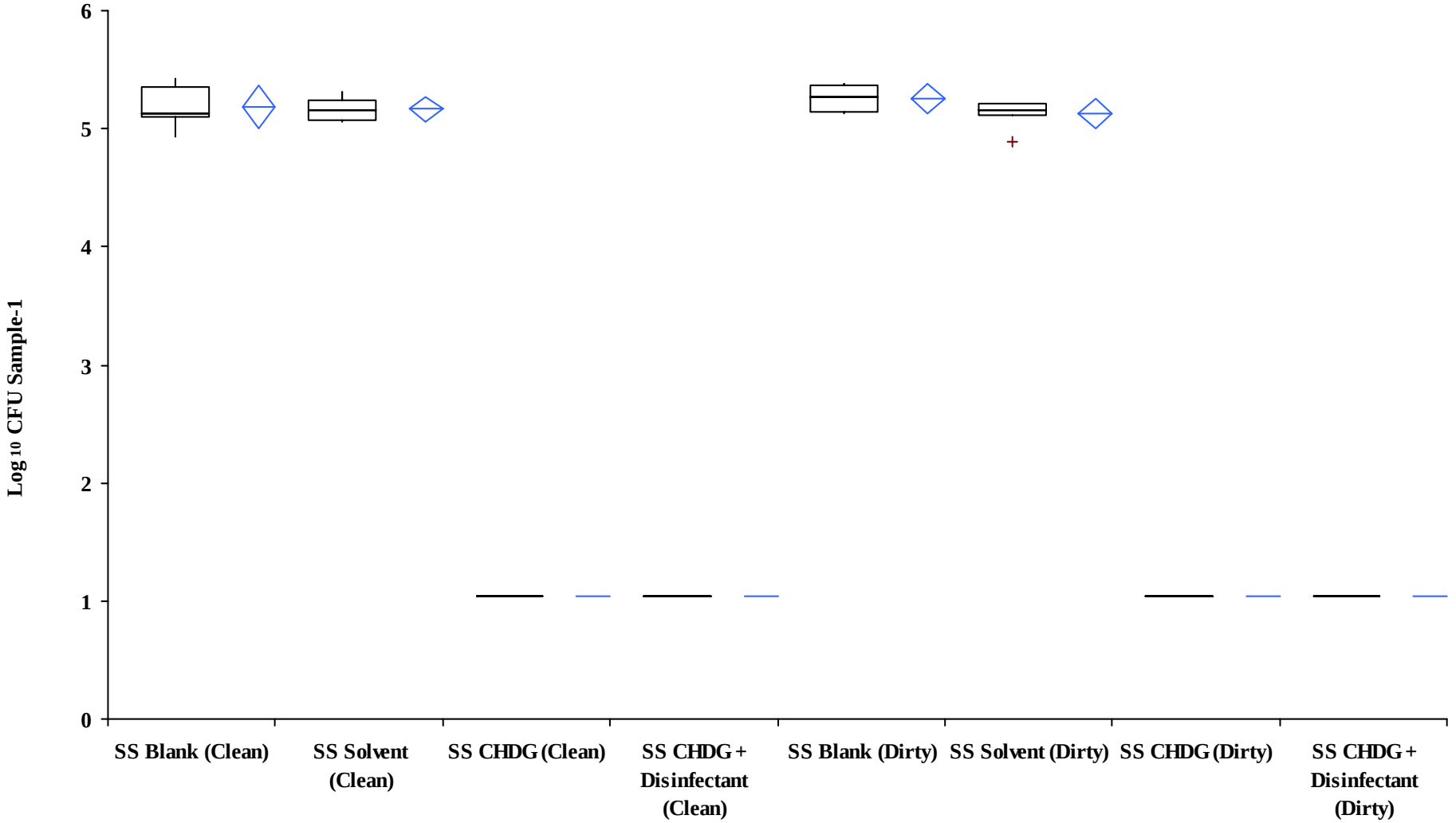


Table 4: Activity Against *Candida albicans* (Geometric Mean of 6 Replicates as Colony Forming Units Sample^{-1})

Test Surface	Soiling	CFU Sample^{-1} ‡			Log_{10} CFU Sample^{-1}			Log Reduction From Initial	
		0	5 Mins	15 Mins	0	5 Mins	15 Mins	5 Mins	15 Mins
SS Blank	Clean	2.5×10^5	2.5×10^5	1.6×10^5	5.4	5.4	5.2	-	0.2
SS Blank	Dirty	2.4×10^5	2.1×10^5	2.3×10^5	5.4	5.3	5.4	0.1	-
SS Solvent	Clean	2.5×10^5	1.8×10^5	1.9×10^5	5.4	5.3	5.3	0.1	0.1
SS Solvent	Dirty	2.4×10^5	2.3×10^5	2.0×10^5	5.4	5.4	5.3	-	0.1
SS CHDG	Clean	2.5×10^5	8.2×10^1	2.1×10^1	5.4	1.9	1.3	3.5	4.1
SS CHDG	Dirty	2.4×10^5	1.2×10^3	4.3×10^1	5.4	3.1	1.6	2.3	3.7
SS CHDG + Disinfectant	Clean	2.5×10^5	2.5×10^2	4.9×10^1	5.4	2.4	1.7	3.0	3.7
SS CHDG + Disinfectant	Dirty	2.4×10^5	3.9×10^3	5.0×10^1	5.4	3.6	1.7	1.8	3.7

‡ The theoretical limit of detection is 10 CFU Sample^{-1}

Figure 7: Results as Log_{10} CFU cm^{-2} - *Candida albicans*

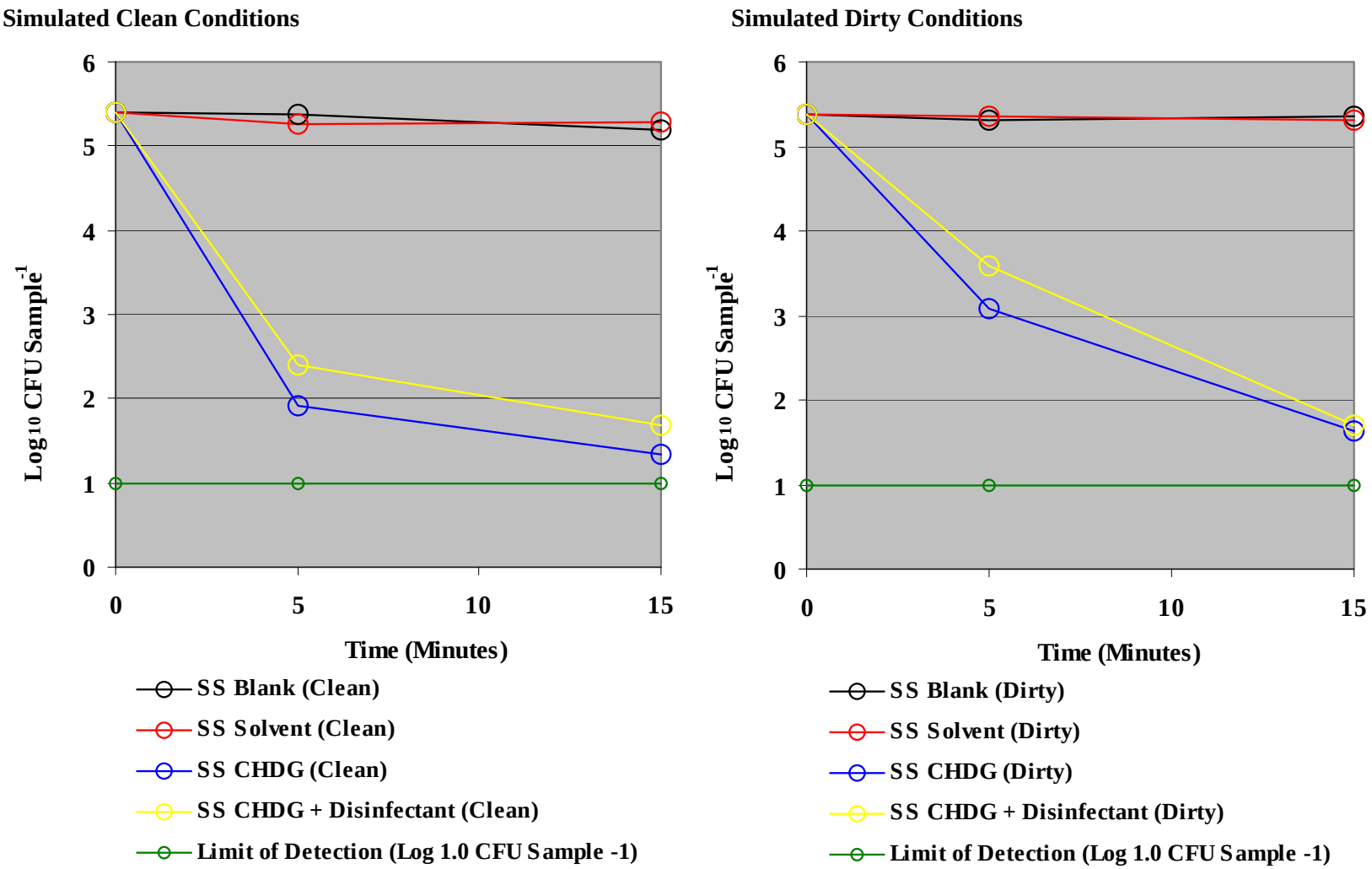


Figure 8: Confidence Intervals of the Data After 5 Minutes - *Candida albicans*

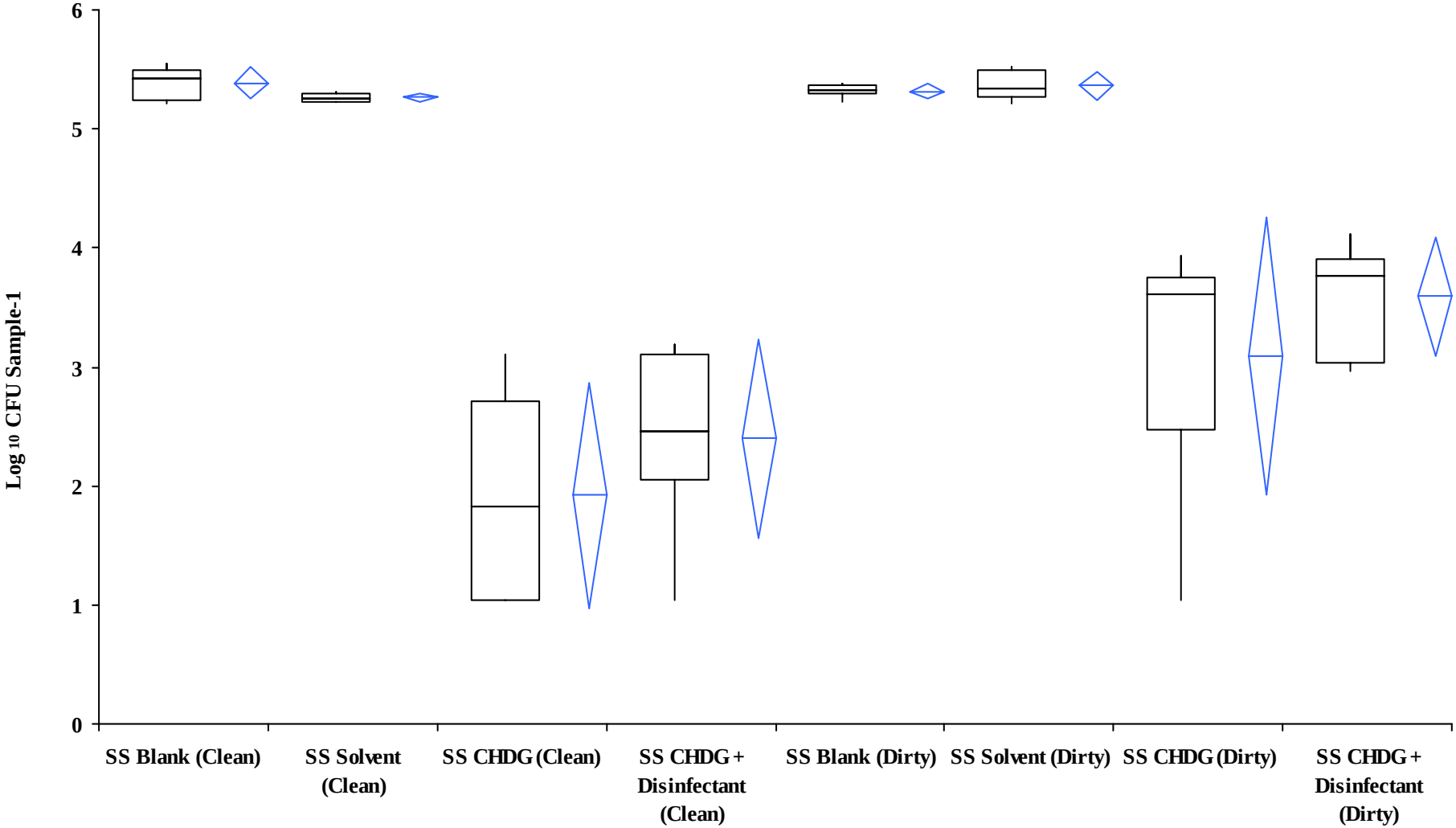
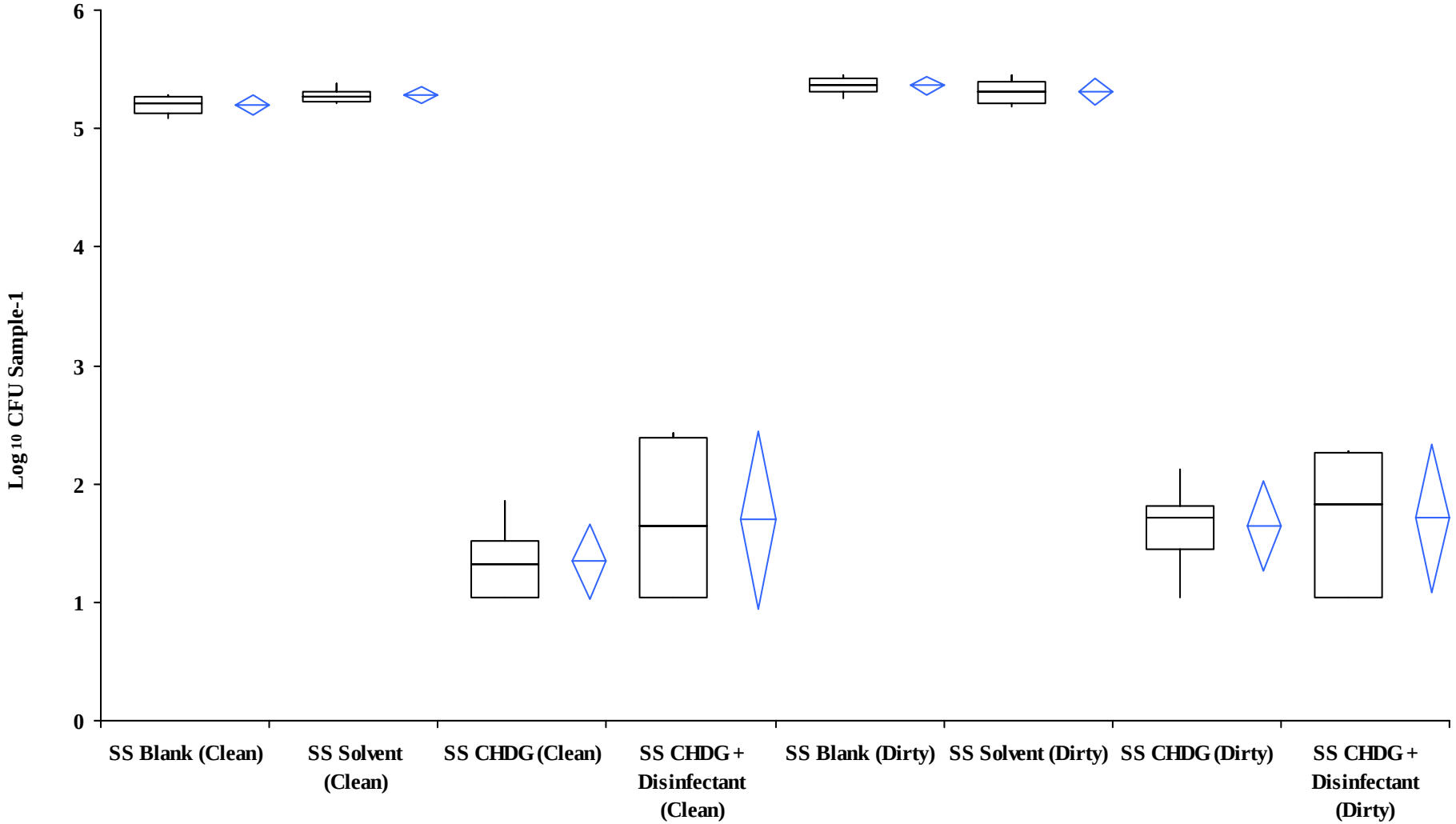


Figure 9: Confidence Intervals of the Data After 15 Minutes - *Candida albicans*



5. Discussion / Conclusion

In can be seen from the results in Table 2 above, that the populations of eMRSA 15 prepared in either a solution of tryptone sodium chloride containing 0.3 g L^{-1} Bovine Serum Albumin (BSA) (simulated clean conditions) or a solution of tryptone sodium chloride containing 3.0 g L^{-1} BSA and 3.0 ml L^{-1} sheep erythrocytes (simulated dirty conditions) showed a Log reduction of 0.4 after 5 minutes and a Log reduction of 0.3 after 15 minutes compared to the initial population.

Similarly, the populations of eMRSA 15 prepared under clean and dirty conditions held in contact with the samples of SS Solvent showed a Log reduction of 0.3 after 5 minutes and a Log reduction of 0.4 after 15 minutes compared to the initial population.

In contrast, the populations of eMRSA 15 prepared in simulated clean conditions held in contact with SS CHDG showed a Log reduction of $\geq$ 4.6 to below the limit of detection after 5 minutes and under dirty conditions a Log reduction of 3.1 after 5 minutes and a Log reduction of $\geq$ 4.6 to below the limit of detection after 15 minutes compared to the initial population.

The populations of eMRSA 15 prepared in simulated clean conditions held in contact with SS CHDG + Disinfectant showed a Log reduction of 3.9 after 5 minutes and a Log reduction of 4.5 after 15 minutes. Under dirty conditions eMRSA showed a Log reduction of 3.4 after 5 minutes and a Log reduction of $\geq$ 4.6 to below the limit of detection after 15 minutes compared to the initial population.

In can be seen from the results in Table 3 above, that the populations of *Pseudomonas aeruginosa* prepared in simulated clean conditions and simulated dirty conditions showed a Log reduction of 0.1 after 15 minutes compared to the initial population.

Similarly, the populations of *Pseudomonas aeruginosa* prepared under simulated clean and dirty conditions held in contact with the samples of SS Solvent showed a Log reduction of 0.2 after 15 minutes compared to the initial population.

In contrast, the populations of *Pseudomonas aeruginosa* prepared in simulated clean conditions held in contact with SS CHDG showed a Log reduction of 3.4 after 5 minutes and a Log reduction of $\geq$ 4.3 after 15 minutes to below the limit of detection compared to the initial population. Under simulated dirty conditions, the population of *Pseudomonas aeruginosa* showed a Log reduction of 4.0 after 5 minutes and a Log reduction of $\geq$ 4.3 to below the limit of detection after 15 minutes compared to the initial population.

Similarly, the populations of *Pseudomonas aeruginosa* prepared in simulated clean conditions held in contact with SS CHDG + Disinfectant showed a Log reduction of 3.1 after 5 minutes and a Log reduction of $\geq$ 4.3 to below the limit of detection after 15 minutes. Under dirty conditions, the populations of *Pseudomonas aeruginosa* showed a Log reduction of 3.4 after 5 minutes and a Log reduction of $\geq$ 4.3 to below the limit of detection after 15 minutes compared to the initial population**.**

In can be seen from the results in Table 4 above, that the populations of *Candida albicans* prepared in simulated clean conditions showed a Log reduction of 0.2 after 15 minutes and under simulated dirty conditions, the populations of *Candida albicans* remained constant after 15 minutes compared to the initial population.

Similarly, the populations of *Candida albicans* prepared under simulated clean and dirty conditions held in contact with the samples of SS Solvent both showed a Log reduction of 0.1 after 15 minutes compared to the initial population.

In contrast, the populations of *Candida albicans* prepared in simulated clean conditions held in

contact with SS CHDG showed a Log reduction of 3.5 after 5 minutes and a Log reduction of 4.1 after 15 minutes compared to the initial population. Under simulated dirty conditions, the population of *Candida albicans* showed a Log reduction of 2.3 after 5 minutes and a Log reduction of 3.7 after 15 minutes compared to the initial population.

Similarly, the populations of *Candida albicans* prepared in simulated clean conditions held in contact with SS CHDG + Disinfectant showed a Log reduction of 3.0 after 5 minutes and a Log reduction of 3.7 after 15 minutes. Under dirty conditions, the populations of *Candida albicans* showed a Log reduction of 1.8 after 5 minutes and a Log reduction of 3.7 after 15 minutes compared to the initial population.

The data against the 3 test species employed in this study shows that the populations recovered from the SS Blank after 15 minutes on the surface under simulated clean and dirty conditions did not show a significant reduction in viability thus demonstrating that the test is valid.

Similarly, the bacterial and yeast populations applied to the surfaces of SS coated with solvent showed no significant reduction in viability after 15 minutes compared to the initial population, demonstrating that the coating procedure without chlorhexadine does not have an antimicrobial effect.

In contrast, bacteriologically significantly smaller bacterial and yeast populations were recovered from the stainless steel coupons treated with CHDG during the 5 and 15 minute contact intervals under simulated clean and dirty conditions compared to the populations recovered from the SS Blank samples.

It can also be seen that the bacterial and yeast populations recovered from the stainless steel coupons treated with CHDG and then surface wiped with a disinfectant were also bacteriologically significantly smaller than those recovered from the ss Blank samples. A Log reduction of $\geq$3.7 compared to the initial population was observed against all 3 test species during the 15 minute contact interval following wiping with disinfectant.

6. Raw Data

The raw data for this study will be held in files IMSL2017/10/019 in the Archive of IMSL at Pale Lane, Hartley Wintney, Hants, RG27 8DH, UK for 12 years from the date of this report.

7. References

1 EN 12353:2006 - Chemical disinfectants and antiseptics. Preservation of test organisms used for the determination of bactericidal (including *Legionella*), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity

2 ECHA – BPR Efficacy Working Group Document, Appendix 4, March 2017 - Overview of (EN) standards, test conditions, and pass criteria

3 EN 13727:2012 - Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants for instruments used in the medical area. Test method and requirements (Phase 2/Step 1)

4 Report IMSL2017/10/019.3B: Determination of the Antimicrobial Efficacy of a Treated Article against eMRSA 15, Pseudomonas aeruginosa and Candida albicans using a Simulated Splash Test

Appendix A: **Microbiological Media**

Neutraliser Recipe

Dey-Engley neutralising broth (D3435 Sigma-Aldrich)	Casein enzymatic hydrolysate	5 g
	Yeast extract	2.5 g
	Dextrose	10 g
	Sodium thiosulfate	6 g
	Sodium thioglycollate	1 g
	Sodium bisulfite	2.5 g
	Lecithin	7 g
	Polysorbate 80	5 g
	Bromocresol purple	0.02 g

Made up to 1 litre and autoclaved at 121°C for 15 minutes.

Standard Diluent

Tryptone Sodium Chloride	Tryptone	1 g
	Sodium Chloride	8.5 g

Made up to 1 litre and autoclaved at 121°C for 15 minutes.

Growth Medium

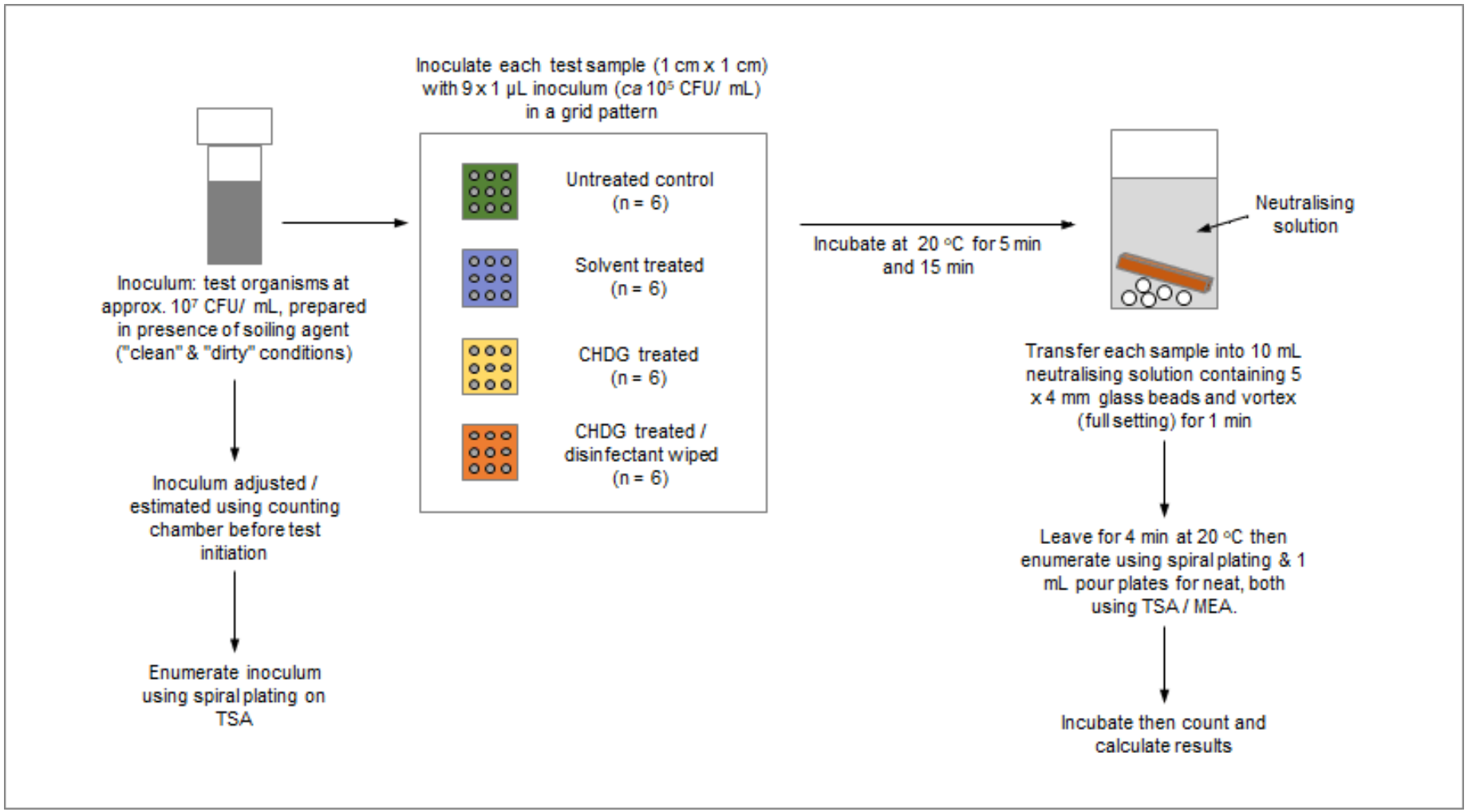
Trypcase Soya Agar	Trypcase	15g
	Soyase	5 g
	Sodium Chloride	5 g
	Agar	15g

Made up to 1 litre and autoclaved at 121°C for 15 minutes.

Malt Extract Agar	Malt Extract	30 g
	Mycological Peptone	5 g
	Agar	15g

Made up to 1 litre and autoclaved at 115°C for 10 minutes.

Appendix B: Method Overview (Micramed Ltd)



Appendix C: Raw Data

Table 5: Neutraliser Validation Data - eMRSA 15 (Ref 4)

Test Solution	CFU ml^{-1}	Recovery (%)
NV^{-1}	129	-
NTV (DE)	138.5	107.36
DNV Chlorhexidine (DE)	131	101.55
DNV Peptide (DE)	127	98.45

Key: NV - Neutraliser validation inoculum, NTV - Neutraliser toxicity validation, DNV - Dilution neutralisation validation. If NTV and DNV recovery is $\geq$ 50% of the expected the neutraliser is considered valid by the standard (Ref 3).

Table 6: Neutraliser Validation Data - *Pseudomonas aeruginosa* (Ref 4)

Test Solution	CFU ml^{-1}	Recovery (%)
NV^{-1}	103	-
NTV (DE)	139.5	135.44
DNV Chlorhexidine (DE)	127	123.3
DNV Peptide (DE)	128.5	124.76

Key: NV - Neutraliser validation inoculum, NTV - Neutraliser toxicity validation, DNV - Dilution neutralisation validation. If NTV and DNV recovery is $\geq$ 50% of the expected the neutraliser is considered valid by the standard (Ref 3).

Table 7: Neutraliser Validation Data - *Candida albicans* (Ref 4)

Test Solution	CFU ml^{-1}	Recovery (%)
NV^{-1}	134	-
NTV (DE)	134.5	100.37
DNV Chlorhexidine (DE)	134	100
DNV Peptide (DE)	112.5	83.96

Key: NV - Neutraliser validation inoculum, NTV - Neutraliser toxicity validation, DNV - Dilution neutralisation validation. If NTV and DNV recovery is $\geq$ 50% of the expected the neutraliser is considered valid by the standard (Ref 3).

Table 8: Inoculum cell counts

Test Species	Cell Count (CFU $ml^{-1)}$	
	Clean	Dirty
eMRSA 15	4.4×10^7	4.9×10^7
Pseudomonas aeruginosa	2.3×10^7	2.5×10^7
Candida albicans	2.8×10^7	2.7×10^7

Table 9: Individual Values for the Recovery of Microorganisms from Stainless Steel Surfaces by Dilution Plate Count - eMRSA 15

Test Surface	Soiling	Contact Time (Mins)	CFU Sample^{-1} Replicate						Geometric Mean
			1	2	3	4	5	6	CFU Sample^{-1}
SS Blank	Clean	5	2.0×10^5	1.7×10^5	1.8×10^5	1.1×10^5	1.3×10^5	1.5×10^5	1.5×10^5
SS Blank	Dirty	5	1.9×10^5	2.0×10^5	2.7×10^5	1.3×10^5	1.2×10^5	1.9×10^5	1.8×10^5
SS Blank	Clean	15	1.8×10^5	2.1×10^5	2.3×10^5	1.6×10^5	1.2×10^5	1.8×10^5	1.8×10^5
SS Blank	Dirty	15	1.4×10^5	2.4×10^5	2.3×10^5	2.1×10^5	2.4×10^5	1.6×10^5	2.0×10^5
SS Solvent	Clean	5	1.9×10^5	2.1×10^5	2.3×10^5	1.8×10^5	2.1×10^5	2.5×10^5	2.1×10^5
SS Solvent	Dirty	5	2.6×10^5	2.1×10^5	1.8×10^5	2.2×10^5	2.1×10^5	1.6×10^5	2.0×10^5
SS Solvent	Clean	15	3.0×10^5	1.3×10^5	1.5×10^5	2.9×10^5	1.2×10^5	1.1×10^5	1.7×10^5
SS Solvent	Dirty	15	2.2×10^5	2.2×10^5	1.4×10^5	1.7×10^5	1.7×10^5	2.5×10^5	1.9×10^5
SS CHDG	Clean	5	< 10	< 10	< 10	< 10	< 10	< 10	< 10
SS CHDG	Dirty	5	1.4×10^3	8.8×10^2	1.4×10^2	2.4×10^2	2.9×10^2	2.0×10^2	3.7×10^2
SS CHDG	Clean	15	< 10	< 10	< 10	< 10	< 10	< 10	< 10
SS CHDG	Dirty	15	< 10	< 10	< 10	< 10	< 10	< 10	< 10
SS CHDG + Disinfectant	Clean	5	2.0×10^1	2.4×10^2	1.0×10^2	1.0×10^1	< 10	2.0×10^2	4.7×10^1
SS CHDG + Disinfectant	Dirty	5	5.9×10^2	1.5×10^2	8.0×10^1	9.0×10^1	1.2×10^2	3.4×10^2	1.7×10^2
SS CHDG + Disinfectant	Clean	15	< 10	< 10	< 10	< 10	2.0×10^1	< 10	1.1×10^1
SS CHDG + Disinfectant	Dirty	15	< 10	1.0×10^1	< 10	1.0×10^1	< 10	< 10	< 10

‡ The theoretical limit of detection is 10 CFU Sample^{-1}

Table 10: Individual Values for the Recovery of Microorganisms from Stainless Steel Surfaces by Dilution Plate Count - *Pseudomonas aeruginosa*

Test Surface	Soiling	Contact Time (Mins)	CFU Sample^{-1} Replicate						Geometric Mean
			1	2	3	4	5	6	CFU Sample^{-1}
SS Blank	Clean	5	1.4×10^5	2.4×10^5	2.6×10^5	1.5×10^5	1.4×10^5	1.5×10^5	1.7×10^5
SS Blank	Dirty	5	3.2×10^5	2.8×10^5	2.2×10^5	3.6×10^5	3.2×10^5	2.8×10^5	2.9×10^5
SS Blank	Clean	15	1.4×10^5	2.6×10^5	1.3×10^5	1.4×10^5	2.3×10^5	8.5×10^4	1.5×10^5
SS Blank	Dirty	15	1.3×10^5	2.4×10^5	2.3×10^5	2.3×10^5	1.4×10^5	1.5×10^5	1.8×10^5
SS Solvent	Clean	5	1.3×10^5	1.5×10^5	1.4×10^5	1.6×10^5	1.3×10^5	1.3×10^5	1.4×10^5
SS Solvent	Dirty	5	1.4×10^5	1.5×10^5	2.9×10^5	1.5×10^5	1.4×10^5	3.5×10^5	1.9×10^5
SS Solvent	Clean	15	1.1×10^5	1.7×10^5	1.2×10^5	2.0×10^5	1.3×10^5	1.6×10^5	1.5×10^5
SS Solvent	Dirty	15	1.4×10^5	1.4×10^5	1.6×10^5	1.5×10^5	1.6×10^5	7.9×10^4	1.4×10^5
SS CHDG	Clean	5	9.8×10^2	3.1×10^2	< 10	< 10	1.5×10^2	8.0×10^1	8.7×10^1
SS CHDG	Dirty	5	7.7×10^2	1.0×10^1	< 10	< 10	< 10	< 10	2.1×10^1
SS CHDG	Clean	15	< 10	< 10	< 10	< 10	< 10	< 10	< 10
SS CHDG	Dirty	15	< 10	< 10	< 10	< 10	< 10	< 10	< 10
SS CHDG + Disinfectant	Clean	5	7.9×10^2	1.3×10^3	2.4×10^2	1.0×10^1	1.4×10^2	6.0×10^1	1.7×10^2
SS CHDG + Disinfectant	Dirty	5	2.7×10^2	6.0×10^1	1.7×10^2	< 10	1.6×10^3	< 10	8.9×10^1
SS CHDG + Disinfectant	Clean	15	< 10	< 10	< 10	< 10	< 10	< 10	< 10
SS CHDG + Disinfectant	Dirty	15	< 10	< 10	< 10	< 10	< 10	< 10	< 10

‡ The theoretical limit of detection is 10 CFU Sample^{-1}

Table 11: Individual Values for the Recovery of Microorganisms from Stainless Steel Surfaces by Dilution Plate Count - *Candida albicans*

Test Surface	Soiling	Contact Time (Mins)	CFU Sample^{-1} Replicate						Geometric Mean
			1	2	3	4	5	6	CFU Sample^{-1}
SS Blank	Clean	5	2.5×10^5	3.5×10^5	1.6×10^5	1.8×10^5	3.1×10^5	2.7×10^5	2.5×10^5
SS Blank	Dirty	5	2.0×10^5	2.1×10^5	2.4×10^5	2.3×10^5	1.7×10^5	2.1×10^5	2.1×10^5
SS Blank	Clean	15	1.6×10^5	1.9×10^5	1.7×10^5	1.9×10^5	1.4×10^5	1.2×10^5	1.6×10^5
SS Blank	Dirty	15	2.8×10^5	2.6×10^5	2.6×10^5	1.8×10^5	2.1×10^5	2.1×10^5	2.3×10^5
SS Solvent	Clean	5	2.0×10^5	1.7×10^5	1.8×10^5	1.7×10^5	2.0×10^5	1.8×10^5	1.8×10^5
SS Solvent	Dirty	5	1.6×10^5	2.2×10^5	3.3×10^5	2.1×10^5	1.9×10^5	3.1×10^5	2.3×10^5
SS Solvent	Clean	15	1.7×10^5	1.6×10^5	2.0×10^5	1.7×10^5	2.0×10^5	2.4×10^5	1.9×10^5
SS Solvent	Dirty	15	1.6×10^5	2.5×10^5	2.8×10^5	2.2×10^5	1.6×10^5	1.9×10^5	2.0×10^5
SS CHDG	Clean	5	2.1×10^2	1.0×10^1	2.0×10^1	1.3×10^3	< 10	4.8×10^2	8.2×10^1
SS CHDG	Dirty	5	4.9×10^3	< 10	5.5×10^3	8.6×10^3	3.5×10^3	4.0×10^2	1.2×10^3
SS CHDG	Clean	15	< 10	3.0×10^1	2.0×10^1	< 10	2.0×10^1	7.0×10^1	2.1×10^1
SS CHDG	Dirty	15	3.0×10^1	< 10	6.0×10^1	5.0×10^1	5.0×10^1	1.3×10^2	4.3×10^1
SS CHDG + Disinfectant	Clean	5	1.4×10^2	1.6×10^2	< 10	5.1×10^2	1.6×10^3	1.3×10^3	2.5×10^2
SS CHDG + Disinfectant	Dirty	5	6.9×10^3	1.3×10^4	1.1×10^3	7.8×10^3	9.3×10^2	4.9×10^3	3.9×10^3
SS CHDG + Disinfectant	Clean	15	< 10	< 10	1.7×10^2	< 10	2.4×10^2	2.7×10^2	4.9×10^1
SS CHDG + Disinfectant	Dirty	15	1.4×10^2	1.9×10^2	< 10	1.8×10^2	3.0×10^1	< 10	5.0×10^1

‡ The theoretical limit of detection is 10 CFU Sample^{-1}