a) **Identification of test laboratory:**

Test laboratory	MGS Laboratories Ltd Unit 20 Hoeford Point Barwell Lane Gosport Hampshire PO13 0AU

b) **Identification of the Customer:**

Customer Name	Advanced Engineering Ltd
Customer Address	Guardian House Stroudley Rd Basingstoke Hampshire RG24 8NL

c) **Identification of the sample:**

Name of product	CondenCide
Batch number (and expiry date if available)	N/A
Manufacturer (or supplier)	Advanced Engineering Ltd
Date of delivery	01 Sep 16
Storage conditions	Room temperature and darkness
Product diluent recommended by the manufacturer for use	Not stated
Active substance(s) and their concentration(s) (optional)	Didecyl dimethyl ammonium chloride - Pre-diluted: 6.25g/100g (0.173mol/L).
Appearance of the product	Clear pale blue liquid

d) **Test method and its validation:**

MGS procedure reference	WIN-1000.050-07
Method	Dilution neutralisation
Neutraliser	Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Details of validation of the neutraliser	Neutraliser validation performed according to 5.5.2 of EN 1276:2009.

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

e) **Experimental conditions:**	
Period of analysis	05 Sep 16 to 12 Sep 16
Product diluent used during the test	Standard Hardness Water
Product test concentrations	1:6 Dilution (1-part product:6 parts water)
Appearance of product dilutions	Clear pale blue liquid
Contact time	5 minutes ± 10s
Test temperature range	20°C ± 2°C
Interfering substance	3g/l Bovine albumin
Stability of the mixture	Precipitate absent throughout test
Temperature of incubation	36°C ± 2°C
Identification of the bacterial strains used	*Pseudomonas aeruginosa* ATCC 15442 *Escherichia coli* NCTC 10418 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541
f) **Results:**	
Test results	1) Controls and validation 2) Evaluation of bactericidal activity
g) **Conclusion:**	Based on EN 1276 (2009), the product CondenCide, when tested 1 part product: 6 parts water, possesses bactericidal activity in 5 minutes at 20°C under dirty conditions for the referenced strains of *E. coli*, *E. hirae*, *S. aureus* and *P. aeruginosa*.
h) **Deviations:**	None
i) Special remarks:	All controls and validations were within basic limits No precipitate was formed during the test
J) Comments:	This report replaces TRB-2016-124-01

Re-issued by: Linda James

Name: Linda James

Position: Laboratory Manager

Date: 22 SEP 16

Approved by:

Name: Kim Morwood

Position: Technical Director

Date: 22 SEP 16

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

The MGS procedure WIN-1000.050 is a laboratory method based on the EN 1276 (2009) standard; the minor deviations from the standard, which do not affect the overall results, are detailed below:

- EN 1276 states an allowed tolerance of 36°C ±1°C or 37°C±1°C, MGS laboratories equipment is validated to ±2°C therefore MGS procedures state ±2°C. The tests are self validating so any stress caused to the organism will be reflected in the validations.
- Organisms are prepared by swabbing plates and adding to 9ml diluent to form a suspension, rather than adding loopfuls of organism to 10ml diluent with beads, shaking for 3 minutes, aspirating and adding to a new container. Swabbing forms a smooth suspension removing the need to shake with beads.
- The laboratory is regulated at 20°C; therefore for testing at 20°C a water bath is not used.
- Plates are incubated for the full time rather than performing an interim read; in addition the incubation period may be extended to a maximum of 4 due to business hours
- All tests performed include validation of neutralisation, but the neutraliser is not always pre-proved.
- Any part of the method may be altered to meet customer requirements; MGS does not insist on testing the standard conditions or three concentrations of product with replicates of the limiting organism

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate [ ]
Number of plates: 1 / ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 23.5°C
Test organism: *P. aeruginosa* ATCC 15442
Incubation temperature: 36°C ± 2°C
Interfering substances: 3g/l Bovine albumin
Date of Test: 05 Sep 16
Person responsible: Laura Taylor Signature:
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation and Controls

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutraliser Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	104	χ =100	Vc1	90	χ =87	Vc1	96	χ =94	Vc1	129	χ =127
Vc2	96		Vc2	84		Vc2	92		Vc2	125	
30 ≤ χ of Nv_0 ≤ 160? Yes [X] No [ ]			χ of A is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of B is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of C is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]		

Test suspension and test

Test suspension (N and N_0):	**N**	**Vc1**	**Vc2**	χ = 42 x 10^7; lgN= 8.63
	10^{-6}	>330	>330	N_0 = N/10; lgN_0 = 7.63
	10^{-7}	45	39	7.17 ≤ lg N_0 ≤ 7.70? Yes [X] No [ ]

Conc of the product	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	<14	<14	<140	<2.15	>5.48	5 minutes

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate [ ]
Number of plates: 1 / ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 23.5°C
Test organism: *E. coli* NCTC 10418
Incubation temperature: 36°C ± 2°C
Interfering substances: 3g/l Bovine albumin
Date of Test: 05 Sep 16
Person responsible: Laura Taylor Signature: pp E. Wo
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation and Controls

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutraliser Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	87	χ =82	Vc1	67	χ =61	Vc1	89	χ =80	Vc1	57	χ =54
Vc2	76		Vc2	54		Vc2	70		Vc2	51	
30 ≤ χ of Nv_0 ≤ 160? Yes [X] No [ ]			χ of A is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of B is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of C is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]		

Test suspension and test

Test suspension (N and N_0):	**N**	**Vc1**	**Vc2**	χ wm = 285 x 10^6; lgN= 8.45
	10^{-6}	284	281	N_0 = N/10; lgN_0 = 7.45
	10^{-7}	32	29	7.17 ≤ lg N_0 ≤ 7.70? Yes [X] No [ ]

Conc of the product	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	<14	<14	<140	<2.15	>5.30	5 minutes

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate [ ]
Number of plates: 1 / ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 24.1°C
Test organism: *S. aureus* ATCC 6538
Incubation temperature: 36°C ± 2°C
Interfering substances: 3g/l Bovine albumin
Date of Test: 08 Sep 16
Person responsible: Edward Webber Signature: E. Webber
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation and Controls

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutraliser Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	44	χ =5	Vc1	72	χ =67	Vc1	62	χ =72	Vc1	58	χ =59
Vc2	55		Vc2	62		Vc2	82		Vc2	59	
30 ≤ χ of Nv_0 ≤ 160? Yes [X] No [ ]			χ of A is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of B is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of C is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]		

Test suspension and test

Test suspension (N and N_0):	**N**	**Vc1**	**Vc2**	χ wm = 220 x 10^6; lgN= 8.34
	10^{-6}	201	239	N_0 = N/10; lgN_0 = 7.34
	10^{-7}	21	22	7.17 ≤ lg N_0 ≤ 7.70? Yes [X] No [ ]

Conc of the product	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	<14	<14	<140	<2.15	>5.19	5 minutes

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate [ ]
Number of plates: 1 / ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 24.1°C
Test organism: *E. hirae* ATCC 10541
Incubation temperature: 36°C ± 2°C
Interfering substances: 3g/l Bovine albumin
Date of Test: 08 Sep 16
Person responsible: Edward Webber Signature: E. Webber
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation and Controls

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutraliser Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	71	χ =69	Vc1	67	χ =65	Vc1	74	χ =71	Vc1	45	χ =57
Vc2	67		Vc2	62		Vc2	67		Vc2	68	
30 ≤ χ of Nv_0 ≤ 160? Yes [X] No [ ]			χ of A is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of B is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of C is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]		

Test suspension and test

Test suspension (N and N_0):	**N**	**Vc1**	**Vc2**	χ wm = 245 x 10^6; lgN= 8.39
	10^{-6}	261	223	N_0 = N/10; lgN_0 = 7.39
	10^{-7}	30	25	7.17 ≤ lg N_0 ≤ 7.70? Yes [X] No [ ]

Conc of the product	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	<14	<14	<140	<2.15	>5.24	5 minutes

Explanations:

Vc = count per plate (one plate or more)
χ = average of Vc1 and Vc2 (1. + 2. duplicate)
χ wm = weighed mean of χ
R = reduction ($lgR = lgN_0 - lgNa$)
Na = number of survivors in the test mixture
N = number of cells in the test suspension
N_0 =N/10
Nv = number of cells in the validation suspension
Nv_0 = Nv/10

All test results have an associated uncertainty of measurement; for this test the expanded uncertainty is based on the estimated uncertainty multiplied by a coverage factor K=2 providing a level of confidence of approximately 95%. The uncertainty evaluation has been assessed in accordance with MGS laboratories' UKAS Accreditation and is available on request.

a) **Identification of test laboratory:**

Test laboratory	MGS Laboratories Ltd Unit 20 Hoeford Point Barwell Lane Gosport Hampshire PO13 0AU

b) **Identification of the Customer:**

Customer Name	Advanced Engineering Ltd
Customer Address	Guardian House Stroudley Rd Basingstoke Hampshire RG24 8NL

c) **Identification of the sample:**

Name of product	CondenCide
Batch number (and expiry date if available)	N/A
Manufacturer (or supplier)	Advanced Engineering Ltd
Date of delivery	01 Sep 16
Storage conditions	Room temperature and darkness
Product diluent recommended by the manufacturer for use	Not stated
Active substance(s) and their concentration(s) (optional)	Didecyl dimethyl ammonium chloride - Pre-diluted: 6.25g/100g (0.173mol/L).
Appearance of the product	Clear pale blue liquid

d) **Test method and its validation:**

MGS procedure reference	WIN-1000.051-05
Method	Dilution neutralisation
Neutraliser	Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Details of validation of the neutraliser	Neutraliser validation performed according to 5.5.2 of EN 1650:2008 + A1: 2013.

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

e) **Experimental conditions:**

Period of analysis	09 Sep 16 to 13 Sep 16
Product diluent used during the test	Standard Hardness Water
Product test concentrations	1:6 Dilution (1 part product: 6 parts water)
Appearance of product dilutions	Clear pale blue liquid
Contact time	15 minutes ± 10s
Test temperature range	20°C ± 2°C
Interfering substance	0.3g/l Bovine albumin
Stability and appearance of the mixture	Precipitate absent throughout test
Temperature of incubation	30°C ± 2°C
Identification of the fungal strains used	*Aspergillus brasiliensis* ATCC 16404 *Candida albicans* ATCC 10231

f) **Results:**

Test results	1) Controls and validation 2) Evaluation of fungicidal or Yeasticidal activity
g) **Conclusion:**	Based on EN 1650:2008 + A1:2013, the product CondenCide, when tested 1 part product: 6 parts water, possesses fungicidal activity in 15 minutes at 20°C under clean conditions for the referenced strains of *C. albicans* and *A. brasiliensis*
h) **Deviations:**	None
i) **Comments:**	This report replaces TRB-2016-122-01

Re-issued by: Linda James

Name: Linda James

Position: Laboratory Manager

Date: 22 SEP 16

Approved by:

Name: Kim Morwood

Position: Technical Director

Date: 22 SEP16

The MGS procedure WIN-1000.051 is a laboratory method based on the EN 1650:2008 + A1:2013 standard; the minor deviations from the standard, which do not affect the overall results, are detailed below:

- Mould spores have been validated for storage at 2-8°C beyond the date of preparation
- Yeast suspensions are prepared by swabbing plates and adding to 9ml diluent to form a suspension, rather than adding loopfuls of organism to 10ml diluent with beads, shaking for 3 minutes, aspirating and adding to a new container. Swabbing forms a smooth suspension removing the need to shake with beads.
- EN 1650 states an allowed tolerance of 30°C ±1°C, MGS laboratories equipment is validated to ±2°C therefore MGS procedures state ±2°C.
- The laboratory is regulated at 20°C; therefore for testing at 20°C a water bath is not used.
- Preparation of hard water is based on the most recently issued standard rather than taking into account minor sterilisation differences between methods
- The incubation period may be extended to a maximum of 4 days (6 for mould) due to business hours
- All tests performed include validation of neutralisation, but the neutraliser is not always pre-proved.
- Any part of the method may be altered to meet customer requirements; MGS does not insist on testing the standard conditions or three concentrations of product with replicates of the limiting organism

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate [ ]
Number of plates: 1 / ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 23.2°C
Test organism: *A. brasiliensis* ATCC 16404
Incubation temperature: 30°C ± 2°C
Interfering substances: 0.3g/l Bovine albumin
Date of Test: 09 Sep 16
Person responsible: Edward Webber Signature: E.Webber
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation and Controls

Presence of a high concentration of *A. brasiliensis* spiny spores in the spore suspension Yes [X] No [ ]

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutraliser Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	41	χ =45	Vc1	38	χ =42	Vc1	42	χ =42	Vc1	51	χ =49
Vc2	49		Vc2	46		Vc2	42		Vc2	47	
30 ≤ χ of Nv_0 ≤ 160? Yes [X] No [ ]			χ of A is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of B is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of C is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]		

Test suspension and test

Test suspension (N and N_0):	**N**	**Vc1**	**Vc2**	χ = 29 x 10^6; lgN= 7.46
	10^{-5}	>165	>165	N_0 = N/10; lgN_0 = 6.46
	10^{-6}	31	26	6.17 ≤ lg N_0 ≤ 6.70? Yes [X] No [ ]

Conc of the product	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	<14	<14	<140	<2.15	>4.31	15 minutes

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate
Number of plates: 1 / ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 23.2°C
Test organism: *C. albicans* ATCC 10231
Incubation temperature: 30°C ± 2°C
Interfering substances: 0.3g/l Bovine albumin
Date of Test: 09 Sep 16
Person responsible: Edward Webber Signature: E. Webber
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation and Controls

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutraliser Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	71	χ =71	Vc1	55	χ =63	Vc1	74	χ =78	Vc1	63	χ =68
Vc2	70		Vc2	70		Vc2	81		Vc2	72	
30 ≤ χ of Nv_0 ≤ 160? Yes [X] No [ ]			χ of A is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of B is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]			χ of C is ≥ 0.5 x χ of Nv_0? Yes [X] No [ ]		

Test suspension and test

Test suspension (N and N_0):	**N**	**Vc1**	**Vc2**	χ wm = 274 x 10^5; lgN= 7.44
	10^{-5}	282	275	N_0 = N/10; lgN_0 = 6.44
	10^{-6}	24	21	6.17 ≤ lg N_0 ≤ 6.70? Yes [X] No [ ]

Conc of the product	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	<14	<14	<140	<2.15	>4.29	15 minutes

Explanations:
Vc = count per plate (one plate or more)
χ = average of Vc1 and Vc2 (1. + 2. duplicate)
χ wm = weighted mean of χ
R = reduction ($lgR = lgN_0 - lgNa$)
Na = number of survivors in the test mixture
Nv = number of cells in the validation suspension
Nv_0 = Nv/10

All test results have an associated uncertainty of measurement; for this test the expanded uncertainty is based on the estimated uncertainty multiplied by a coverage factor K=2 providing a level of confidence of approximately 95%. The uncertainty evaluation has been assessed in accordance with MGS laboratories' UKAS Accreditation and is available on request.

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

mgsLABORATORIES Microbiological Services and Consultancy					Doc No.	TRA-2016-081-01
Title	**Microbiological Analysis Based on EN 13697 (2015) 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 (Phase 2 / Step 2)**					
Product	**CondenCide**	**MGS No**	**24136**	**SO No**	**5786**	

UKAS TESTING 4393

a) **Identification of test laboratory:**

Test laboratory	MGS Laboratories Ltd Unit 20 Hoeford Point Barwell Lane Gosport Hampshire PO13 0AU

b) **Identification of the Customer:**

Customer Name	Advanced Engineering Ltd
Customer Address	Guardian House Stroudley Rd Basingstoke Hampshire RG24 8NL

c) **Identification of the sample:**

Name of product	CondenCide
Batch number	N/A
Manufacturer	Advanced Engineering Ltd
Date of delivery	01 Sep 16
Storage conditions	Room temperature and darkness
Active substance(s) and their concentration(s) (optional)	Not stated

d) **Test method and its validation:**	Didecyl dimethyl ammonium chloride - Pre-diluted: 6.25g/100g (0.173mol/L).
MGS procedure reference	WIN-1000.058-08
Neutraliser	Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Details of validation of the neutraliser	Neutraliser validation performed according to 5.5.2.3 and 5.5.2.4 of EN 13697: 2015

e) **Experimental conditions:**

Period of analysis	08 Sep 16 to 19 Sep 16
Product diluent used during the test	Hard water ≤375mg/l $CaCO_3$

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Product test concentrations	
Appearance of product dilutions	Clear colourless solution
Contact time	Bacteria: 5 minutes ± 10s Fungi: 15 minutes ± 10s
Test temperature range	18-25°C
Actual test temperatures	*Escherichia coli* 23.1°C *Enterococcus hirae* 23.1°C *Staphylococcus aureus* 23.1°C *Pseudomonas aeruginosa* 23.1°C *Candida albicans* 23.0°C *Aspergillus brasiliensis* 23.1°C
Interfering substance	3.0g/l Bovine albumin
Stability of the mixture	Precipitate absent throughout test
Temperature of incubation	Bacteria: 36°C ± 2°C Fungi: 30°C ± 2°C
Identification of the bacterial and fungal strains used	*Escherichia coli* ATCC 10536 *Enterococcus hirae* ATCC 10541 *Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 15442 *Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404
Identification of the test surface	Stainless steel Grade 304
f) **Results:**	
Test results	See tables: 1 & 2
g) **Conclusion:**	Based on EN 13697 (2015), the batch supplied of the product CondenCide, when tested 1 part product: 6 parts water, possesses bactericidal activity on surfaces in 5 minutes at 25°C under dirty conditions for the referenced strains of *E. coli*, *E. hirae*, *S. aureus* and *P. aeruginosa;* and possesses fungicidal activity in 15 minutes at 20°C under dirty conditions for the referenced strains of *C. albicans* and *A. brasilensis*
h) **Deviations:**	None
i) **Comments:**	None

Prepared by: Linda James

Name: Linda James

Position: Laboratory Manager

Date: 22 Sep 16

Locality: Hampshire, United Kingdom.

Approved by:

Name: Kim Morwood

Position: Technical Director

Date: 22 SEP 16

Locality: Hampshire, United Kingdom.

The MGS procedure WIN-1000.058 is a laboratory method based on the EN 13697 (2015) standard; the minor deviations from the standard, which do not affect the overall results, are detailed below:

- Temperature tolerance of ±2°C rather than ±1°C. Validations are performed in tandem with the test, if the temperature has an adverse effect this would be reflected in the validations and the test would be invalid.
- Since the laboratory is maintained at 20°C a water bath is not used for testing at this temperature
- For mould a cryovial bead is added to broth and stored at 2-8°C for a maximum of 7 days; streaks are made from that broth, rather than streaking from stored slopes for 6-9 weeks.
- Organisms are prepared by swabbing plates and adding to diluent which gives a homogenous suspension; rather than adding loopfuls to diluent with beads and vortexing to get a homogenous suspension
- Mould spores have been validated for storage at 2-8°C beyond the date of preparation
- The incubation period may be extended to a maximum of 4 days (6 for mould) due to business hours
- All tests performed include validation of neutralisation; however the neutraliser is not always pre-proved
- Any part of the method may be altered to meet customer requirements; MGS does not insist on testing the standard conditions or three concentrations of product with replicates of the limiting organism

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Table 1: Bacterial surface testing results

Test organisms	Bacterial test suspension: N	Validation test: NT	Validation test: NC	Water control Nc	Test procedure at concentrations	1:6		
Escherichia coli ATCC 10536	10^{-6}: >330, >330 10^{-7}: 107, 116 N: 7.45	10^{-3}: 213, 217 10^{-4}: 20, 23 10^{-5}: 3, 3 NT: 6.33	10^{-3}: 202, 228 10^{-4}: 26, 28 10^{-5}: 2, <1 NC: 6.34	10^{-3}: >330, >330 10^{-4}: 38, 28 10^{-5}: 8, 3 Nc: 6.52	10^{-0}: 10^{-1}: 10^{-2}: Nd : Nts : R :	<1, <1 <1, <1 <1, <1 <1.00 <1 >5.52		
Enterococcus hirae ATCC 10541	10^{-6}: 257, 296 10^{-7}: 24, 28 N: 6.84	10^{-3}: >330, >330 10^{-4}: 84, 64 10^{-5}: 7, 9 NT: 6.87	10^{-3}: >330, >330 10^{-4}: 68, 64 10^{-5}: 9, 3 NC: 6.82	10^{-3}: >330, >330 10^{-4}: 73, 68 10^{-5}: 7, 3 Nc: 6.85	10^{-0}: 10^{-1}: 10^{-2}: Nd : Nts : R :	<1, <1 <1, <1 <1, <1 <1.00 <1 >5.85		
Staphylococcus aureus ATCC 6538	10^{-6}: 329, 287 10^{-7}: 29, 30 N: 6.89	10^{-3}: >330, >330 10^{-4}: 72, 78 10^{-5}: 6, 9 NT: 6.88	10^{-3}: >330, >330 10^{-4}: 102, 105 10^{-5}: 10, 12 NC: 7.02	10^{-3}: >330, >330 10^{-4}: 70, 90 10^{-5}: 14, 13 Nc: 6.92	10^{-0}: 10^{-1}: 10^{-2}: Nd : Nts : R :	<1, <1 <1, <1 <1, <1 <1.00 <1 >5.92		
Pseudomonas aeruginosa ATCC 15442	10^{-6}: 295, 285 10^{-7}: 20, 37 N: 6.86	10^{-3}: 39, 52 10^{-4}: 5, 5 10^{-5}: <1, <1 NT: 5.66	10^{-3}: 93, 101 10^{-4}: 7, 14 10^{-5}: <1, <1 NC: 6.00	10^{-3}: 201, 198 10^{-4}: 27, 18 10^{-5}: 1, 3 Nc: 6.31	10^{-0}: 10^{-1}: 10^{-2}: Nd : Nts : R :	<1, <1 <1, <1 <1, <1 <1.00 <1 >5.31		

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Table 2: Fungal surface testing results

Test organisms	Fungal test suspension: N	Validation test: NT	Validation test: NC	Water control Nc	Test procedure at concentrations	1:6		
Candida albicans ATCC 10231	10^{-5}: >330, >330 10^{-6}: 42, 45 N : 6.04	10^{-2}: 172, 220 10^{-3}: 16, 15 10^{-4}: 4, 3 NT: 5.28	10^{-2}: 147, 175 10^{-3}: 15, 22 10^{-4}: 4, 1 NC: 5.21	10^{-2}: 266, 274 10^{-3}: 24, 24 10^{-4}: 3, 2 Nc: 5.43	10^{-0}: 10^{-1}: 10^{-2}: Nd : Nts : R :	<1, <1 <1, <1 <1, <1 <1.00 <1 >3.28		
Aspergillus brasiliensis ATCC 16404	10^{-5}: 154, 156 10^{-6}: 24, 25 N : 5.61	10^{-2}: >165, >165 10^{-3}: 41, 36 10^{-4}: 14, 10 NT: 5.64	10^{-2}: >165, >165 10^{-3}: 73, 51 10^{-4}: 14, 13 NC: 5.82	10^{-2}: >165, >165 10^{-3}: 58, 63 10^{-4}: 13, 15 Nc: 5.81	10^{-0}: 10^{-1}: 10^{-2}: Nd : Nts : R :	3, 2 <1, <1 <1, <1 1.48 <1 4.33		

Weighted means are used in all calculations where appropriate.

N = $\log_{10}$ number of cfu applied to test surface;
NT = $\log_{10}$ number of cfu per test surface of the neutralisation test;
NC = $\log_{10}$ number of cfu per test surface of the neutralisation control;
Nc = $\log_{10}$ number of cfu per test surface of the water control;
Nd = $\log_{10}$ number of cfu per test surface of the disinfectant test;
Nts = number of residual colony forming units remaining on the test surface;
R = Log Reduction achieved.

All test results have an associated uncertainty of measurement; for this test the expanded uncertainty is based on the estimated uncertainty multiplied by a coverage factor K=2 providing a level of confidence of approximately 95%. The uncertainty evaluation has been assessed in accordance with MGS laboratories' UKAS Accreditation and is available on request.

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

a) **Identification of test laboratory:**	
Test laboratory	MGS Laboratories Ltd Unit 20 Hoeford Point Barwell Lane Gosport Hampshire PO13 0AU
b) **Identification of the Customer:**	
Customer Name	Advanced Engineering Ltd
Customer Address	Guardian House Stroudley Rd Basingstoke Hampshire RG24 8NL
c) **Identification of the sample:**	
Name of product	CondenCide
Batch number (and expiry date if available)	N/A
Manufacturer	Advanced Engineering Ltd
Date of delivery	01 Sep 16
Storage conditions	Room temperature and darkness
Product diluent recommended by the manufacturer for use	Not stated
Active substance(s) and their concentration(s) (optional)	Didecyl dimethyl ammonium chloride - Pre-diluted: 6.25g/100g (0.173mol/L).
Appearance of the product	Clear pale blue liquid
d) **Test method and its validation:**	
MGS procedure reference	WIN-1000.053-05
Method	Dilution neutralisation
Neutraliser	Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Details of validation of the neutraliser	Neutraliser validation performed according to 5.5.2 of EN 13727:2012.

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

e) **Experimental conditions:**

Period of analysis	05 Sep 16 16 to 15 Sep 16
Product diluent used during the test	Standard Hardness Water
Product test concentrations	1:6 Dilution (1-part product:6 parts water)
Appearance of product dilutions	Clear pale blue liquid
Contact time	1 minute ± 10s
Test temperature range	20°C ± 2°C
Interfering substance	3g/l Bovine albumin + 3ml/l blood erythrocytes
Stability and appearance of the mixture	Precipitate absent throughout test
Temperature of incubation	36°C ± 2°C
Identification of the bacterial strains used	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541

f) **Results:**

Test results	1) Controls and validation 2) Evaluation of bactericidal activity
g) **Conclusion:**	Based on EN 13727 (2012), the product CondenCide, when tested 1 part product:6 parts water, possesses bactericidal activity in 1 minute at 20°C under dirty conditions for the referenced strains of *P. aeruginosa, S. aureus* and *E. hirae.*
h) **Deviations:**	This report replaces TRB-2016-126-01

Re-issued by: Linda James

Name: Linda James

Position: Laboratory Manager

Date: 22 SEP 16

Approved by:

Name: Kim Morwood

Position: Technical Director

Date: 22 SEP 16

The MGS procedure WIN-1000.53 is a laboratory method based on the EN 13727 (2012) standard; the minor deviations from the standard, which do not affect the overall results, are detailed below:

- Any part of the method may be altered to meet customer requirements; MGS does not insist on testing the standard conditions or three concentrations of product with replicates of the limiting organism.
- EN 13727states an allowed tolerance of 36°C ±1°C or 37°C±1°C, MGS laboratories equipment is validated to ±2°C therefore MGS procedures state ±2°C.
- Organisms are prepared by swabbing plates and adding to 9ml diluent to form a suspension, rather than adding loopfuls of organism to 10ml diluent with beads, shaking for 3 minutes, aspirating and adding to a new container. Swabbing forms a smooth suspension removing the need to shake with beads.
- The laboratory is regulated at 20°C; therefore for testing at 20°C a water bath is not used.
- Preparation of hard water is based on the most recently issued standard rather than taking into account minor sterilisation differences between methods.
- Plates are incubated for the full time rather than performing an interim read; in addition the incubation period may be extended to a maximum of 4 days due to business hours.
- All tests performed include validation of neutralisation, but the neutraliser is not always pre-proved

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate [ ]
Number of plates: 1 / 0.1 ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 23.5°C
Test organism: *P. aeruginosa* ATCC 15442
Incubation temperature: 36°C ± 2°C
Interfering substances: 3.0ml/l sheep erythrocytes and 3.0g/l Bovine albumin
Date of Test: 05 Sep 16
Person responsible: Edward Webber Signature:
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation suspension Nv_{R0}, Nv_B and Test suspension (N_{R0})

Validation suspension (Nv_{R0})			**Validation suspension (Nv_B)**	**N_R**	10^{-7}	10^{-8}	$\chi = 39 \times 10^8$ $\text{Log } N_R = 9.59$ $N_{0R} = N_R/100$; $\lg N_{0R} = 7.59$ $7.17 \leq \lg N_{0R} \leq 7.70$?
Vc1	94	$\chi = 96$	$Nv_B = 9.6 \times x10^4$ $(Nv_B = Nv_{R0} \times 1000)$	Vc1	>330	38	
Vc2	97						
$30 \leq \chi$ of $Nv_{R0} \leq 160$ Yes [X] No [ ]			$3 \times 10^4 \leq Nv_B \leq 1.6 \times 10^5$ Yes [X] No [ ]	Vc2	>330	39	Yes [X] No [ ]

Controls

Experimental Conditions Control (A)			**Neutraliser Control / Filtration Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	55	χ =58	Vc1	76	χ =75	Vc1	88	χ =90
Vc2	61		Vc2	74		Vc2	92	
χ of A is ≥ 0.5 x χ of Nv_{R0}? Yes [X] No [ ]			χ of B is ≥ 0.5 x χ of Nv_{R0}? Yes [X] No [ ]			χ of C is ≥ 0.5 x χ of Nv_{R0}? Yes [X] No [ ]		

Test

Conc of the product	**Dilution**	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	10^0	<14	<14	<140	<2.15	>5.44	1 minute
	10^{-1}	<14	<14				

mgsLABORATORIES Microbiological Services and Consultancy					Doc No.	TRB-2016-126-02
Title	**Microbiological Analysis Based on EN 13727 (2012) Chemical Disinfectant and Antiseptics - Quantitative Suspension Test for the Evaluation of Bactericidal Activity in the Medical Area - Test Method and Requirements (phase 2 / step 1)**					
Product	**CondenCide**	**MGS No**	**24136**	**SO No**	**5786**	

UKAS TESTING 4393

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate [ ]
Number of plates: 1 / 0.1 ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 21.9°C
Test organism: *S. aureus* ATCC 6538
Incubation temperature: 36°C ± 2°C
Interfering substances: 3.0ml/l sheep erythrocytes and 3.0g/l Bovine albumin
Date of Test: 13 Sep 16
Person responsible: Marek Warlinksi Signature: M. Warlinski
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation suspension Nv_{R0}, Nv_B and Test suspension (N_{R0})

Validation suspension (Nv_{R0})			**Validation suspension (Nv_B)**	N_R	10^{-7}	10^{-8}	$\chi = 40 \times 10^8$ $\text{Log } N_R = 9.60$ $N_{0R} = N_R/100$; $\lg N_{0R} = 7.60$ $7.17 \leq \lg N_{0R} \leq 7.70$?
Vc1	86	$\chi = 79$	$Nv_B = 7.90 \times 10^4$ ($Nv_B = Nv_{R0} \times 1000$)	Vc1	>330	33	
Vc2	72						
$30 \leq \chi$ of $Nv_{R0} \leq 160$ Yes [X] No [ ]			$3 \times 10^4 \leq Nv_B \leq 1.6 \times 10^5$ Yes [X] No [ ]	Vc2	>330	46	Yes [X] No [ ]

Controls

Experimental Conditions Control (A)			**Neutraliser Control / Filtration Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	78	χ =75	Vc1	62	χ =78	Vc1	66	χ =60
Vc2	72		Vc2	94		Vc2	53	
χ of A is ≥ 0.5 x χ of Nv_{R0}? Yes [X] No [ ]			χ of B is ≥ 0.5 x χ of Nv_{R0}? Yes [X] No [ ]			χ of C is ≥ 0.5 x χ of Nv_{R0}? Yes [X] No [ ]		

Test

Conc of the product	**Dilution**	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	10^0	<14	<14	<140	<2.15	>5.45	1 minute
	10^{-1}	<14	<14				

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Product batch number: N/A
Dilution-neutralisation method Pour plate [X] Spread plate [ ]
Number of plates: 1 / 0.1 ml
Neutraliser: Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Actual test temperature: 21.9°C
Test organism: *E. hirae* ATCC 10541
Incubation temperature: 36°C ± 2°C
Interfering substances: 3.0ml/l sheep erythrocytes and 3.0g/l Bovine albumin
Date of Test: 13 Sep 16
Person responsible: Marek Warlinski Signature: M. Warlinski
Diluent used for product test solutions: Standard Hardness Water
Appearance of product test solutions: Clear pale blue liquid

Validation suspension Nv_{R0}, Nv_B and Test suspension (N_{R0})

Validation suspension (Nv_{R0})			**Validation suspension (Nv_B)**	N_R	10^{-7}	10^{-8}	χ wm = 169 x 10^7 Log N_R = 9.23 $N_{0R} = N_R/100$; lgN_{0R} =7.23 $7.17 \leq lgN_{0R} \leq 7.70$?
Vc1	31	χ = 32	$Nv_B = 3.20 \times 10^4$ ($Nv_B = Nv_{R0} \times 1000$)	Vc1	184	14	Yes [X] No [ ]
Vc2	33						
$30 \leq \chi$ of $Nv_{R0} \leq 160$ Yes [X] No [ ]			$3 \times 10^4 \leq Nv_B \leq 1.6 \times 10^5$ Yes [X] No [ ]	Vc2	160	14	

Controls

Experimental Conditions Control (A)			**Neutraliser Control / Filtration Control (B)**			**Method Validation (C)** Prod conc: 1:6		
Vc1	23	χ =26	Vc1	26	χ =24	Vc1	27	χ =26
Vc2	29		Vc2	21		Vc2	24	
χ of A is $\geq 0.5 \times \chi$ of Nv_{R0}? Yes [X] No [ ]			χ of B is $\geq 0.5 \times \chi$ of Nv_{R0}? Yes [X] No [ ]			χ of C is $\geq 0.5 \times \chi$ of Nv_{R0}? Yes [X] No [ ]		

Test

Conc of the product	**Dilution**	**Vc1**	**Vc2**	**Na = χ x10**	**lgNa**	**lgR**	**Contact time**
1:6	10^0	<14	<14	<140	<2.15	>5.08	1 minute
	10^{-1}	<14	<14				

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Explanations:
Vc = count per plate (one plate or more)
X = average of Vc1 and Vc2 (1. + 2. duplicate)
χ wm = weighed mean of χ
R = reduction ($lgR = lgN_{0R} - lgNa$)
Na = number of survivors in the test mixture

All test results have an associated uncertainty of measurement; for this test the expanded uncertainty is based on the estimated uncertainty, multiplied by a coverage factor K = 2, providing a level of confidence of approximately 95%. The uncertainty evaluation has been assessed in accordance with MGS laboratories' UKAS Accreditation and is available on request.

Study Title:
Quantitative suspension test for evaluation of virucidal activity in the medical area (Phase 2 Step1)

Microbiological Solutions Limited (MSL)
Gollinrod, Walmersley, Bury, BL9 5NB, UK

Angela Davies, CEO

Customer: Advanced Engineering Ltd
Contact name: Kajally Jobe
Email:
Address: Guaridian House, Stroudley Road, Basingstoke, RG24 8NL
PO/Quote number: Q003251
Report date: 25/08/2020
Issue number: 1

Megan Barrett
Laboratory Manager

Peter Thistlethwaite
Technical Projects Manager

Scope

The standard method BS EN 14476 describes a test method and the minimum requirements for virucidal activity of a chemical disinfectant and antiseptic products that form a homogenous physically stable preparation when diluted with hard water – or in the case of ready to use products that are not diluted when applied, - with water. Products can only be tested at a concentration of 80% (97% with a modified method for special cases) as some dilution is always produced by adding the test organisms and interfering substances. This European Standard applies to products that are used in the medical area in the fields of hygienic handrub, hygienic handwash, instrument disinfection by immersion, surface disinfection by wiping, spraying, flooding or other means and textile disinfection.

This European standard applies to areas and situations where disinfection is medically indicated. Such indication occurs in patient care, for example: In hospitals, in community medical facilities and in dental institutions or in clinics of schools, of kindergartens and of nursing homes, and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for patients.

Outline of Test Method (Obligatory Test Conditions)

A sample of the test product is diluted in synthetic hard water in products diluted at point of use or water in the case of ready to use products is added to a test suspension of viruses in a solution of interfering substance. The mixture is maintained at one of the temperatures and contact times specified in the standard. At the end of this contact time, an aliquot is taken; the virucidal action in this portion is immediately suppressed by a validated method (dilutions of the sample in ice-cold cell maintenance medium). The dilutions are transferred into cell culture units either using monolayer or cell suspension. Infectivity tests are done either by plaque test or quantal tests. After incubation, the titres of infectivity are calculated according to Spearman and Käber or by plaque counting. Reduction of virus infectivity is calculated from differences of lg virus titres before (virus control) and after treatment with the product. The standard minimum spectrum of test organisms is Poliovirus, Adenovirus and Murine Norovirus.

Acceptance Criteria

The product when tested as above shall demonstrate at least a 4 $\log_{10}$ reduction against the test virus. The test is deemed valid where all control requirements are met.

Test information		Deviation
Name of Product	CondenCide	
Batch Number & Expiry Date	29/06/2025 0082906	
Date of Delivery	13/07/2020	
Period of Analysis	28/07/2020-	
Manufacturer / Supplier	Advanced Engineering Ltd	
Storage Conditions	Ambient	
Appearance of the Product	Pale blue liquid	
Neutralisation Method	Dilution	
Product Diluent	Synthetic hard water	
Test Concentrations	Neat (56700ppm), 1:6 (8110ppm), 1:13 (4050ppm)	
Experimental Conditions	Clean	
Interfering Substance	Clean 0.3g/l Bovine Albumin	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	37°C ±1°C	
Identification of the Viral Strains:	Modified vaccinia virus Ankara (MVA), ATCC VR-1508	
Contact Times	15 minutes ± 10s	
Stability and Appearance During Test	No Change Observed (Homogenous)	

Deviations from Standard Method

There were no deviations from the standard method

Test Result Summary

The test product received has achieved to achieve a 4-log reduction against Vaccinia virus, when tested under the condition stipulated in this report.

See page 2 for acceptance criteria and raw data tables below for complete test results.

Summary

Controls					
Conditions	**Concentration**	**Contact time**	**log TCID50**	**log reduction**	**Control validation**
Virus control (water)	N/A	15 minutes	7.71	N/A	Validated
Cytotoxicity (product)	Neat	N/A	3.00	N/A	Validated
Product supression control	Neat	Neat	7.38	0.33	Validated
Reference virus inactivation (formaldyehyde)	1.4%	5 minutes	4.46	3.25	Validated
Reference virus inactivation (formaldyehyde)	1.4%	15 minutes	3.75	3.96	Validated
Cytotoxicity (formaldehyde)	1.4%	N/A	2.50	N/A	Validated

Interference controls					
Condition	**Concentration**	**Contact time**	**log TCID50**	**Log difference**	**Control validation**
Interference control (untreated)	N/A	N/A	8.58	N/A	N/A
Interference control (treated)	Neat	N/A	8.50	0.08	Validated

Test Results					
Condition	**Concentration**	**Contact time**	**log TCID50**	**log reduction**	**Pass/Fail**
Test product	Neat	15 minutes	3.58	>4	Pass
Test product	1 in 6	15 minutes	3.50	>4	Pass
Test product	1 in 13	15 minutes	3.50	>4	Pass

Raw data

Virus control (water)				Contact time		15 minutes		
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	2	0.91666667	0.076389
-8	2	2	1	0	1	1	0.29166667	0.206597
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	2.21
n	8
SD50	-7.71
SE	0.20
xp	-6

Cytotoxicity (product)				Product concentration			Neat	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	2	2	2	2	2	2	0.5	0.25
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.50
n	8
SD50	-3.00
SE	0.19
xp	-2

Product supression control				Product concentration			Neat	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	2	3	3	4	4	0.75	0.1875
-8	1	1	1	0	0	0	0.125	0.109375
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.88
n	8
SD50	-7.38
SE	0.21
xp	-6

Interference control (untreated)				Product concentration			Neat	
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	4	1	0
-8	4	4	3	4	3	3	0.875	0.109375
-9	1	1	2	1	0	0	0.20833333	0.164931
-10	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	2.0833
n	10
SD50	-8.583
SE	0.1746
xp	-7

Raw data

Interference control (treated)				Product concentration			Neat	
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	4	1	0
-8	3	3	3	4	4	4	0.875	0.109375
-9	2	1	0	0	0	0	0.125	0.109375
-10	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	2
n	10
SD50	-8.5
SE	0.1559
xp	-7

Reference virus inactivation (formaldyehyde)					Contact time		5 minutes	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	3	3	3	3	3	3	0.75	0.1875
-5	2	2	1	0	0	0	0.20833333	0.164931
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.96
n	8
SD50	-4.46
SE	0.22
xp	-3

Reference virus inactivation (formaldyehyde)					Contact time		15 minutes	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	2	2	1	0	1	0	0.25	0.1875
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.25
n	8
SD50	-3.75
SE	0.16
xp	-3

Cytotoxicity (formaldehyde)								
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Raw data

Test product		Product concentration			Neat	Contact time		15 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	1	1	0	0	0	0	0.08333333	0.076389
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.08
n	8
SD50	-3.58
SE	0.10
xp	-3

Test product		Product concentration			1 in 6	Contact time		15 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.00
n	8
SD50	-3.50
SE	0.00
xp	-3

Test product		Product concentration			1 in 13	Contact time		15 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.00
n	8
SD50	-3.50
SE	0.00
xp	-3

KEY

CPE	Cytopathic effect		
Counts	0-4 indicating degree of cytopathic effect 0 = No effect, 1 = 25% CPE, 2 = 50% CPE, 3 = 75% CPE, 4 = 100% CPE		
d	Dilution factor (log)		
Sum px	Sum of % CPE from the highest dilution showing 100% CPE to the lowest dilution assessed.		
n	Number of dilutions		
SD50	Dilution showing 50% of the end point according to Spearman-Kärber method		
SE	Standard error		
xp	Lowest dilution showing 100% CPE		
TCID50	Titre causing 50% of the end point according to Spearman-Kärber		
PASS	= lg R greater than or equal to 4		
FAIL	= lg R less than 4		
>	greater than	≥	equal to or greater than
<	less than	≤	equal to or less than

Calculation notes

In cases where the highest dilution assessed has not shown 100% CPE, the value has been calculated assuming the dilution above this would give 100% CPE and the corresponding value has been assigned as <x.

The standard requires the product suppression control to show a <0.5 log reduction in viral titre. In cases where the product has failed to achieve the required 4 log reduction, but the product suppression control shows a >0.5 log reduction the result has been deemed as valid for fail as the consequence of inadequate suppression would be a partially extended contact time which would generate false positives, but not false negatives.

A similar approach has been taken in regards to the cytotoxicity controls. The standard requires a 4-log difference between the cytotoxicity level and the viral titre. In cases where this is not obtained, but the log reduction observed by the product is within the difference between the cytotoxicity levels and the viral titre the result is deemed acceptable for a fail as there will be no impact on the determination of efficacy.