

Test Report: Clinell® Universal Disinfectant Wipes EN13727 (Phase 2, Step 1) and three-stage wipe method.

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Test Laboratory: Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, King Edward VII Avenue, Cardiff CF10 3NB, UK

Client: GAMA Healthcare Ltd.  
Unit 2, the Exchange Brent Cross Gardens, London NW4 3RJ

Name of Product: Clinell® Universal Disinfectant Wipes

Test Organism: *Staphylococcus aureus* NCTC 10788  
*Enterococcus hirae* NCTC 13383  
*Enterococcus faecium* (VRE) ATCC 700221  
*Enterococcus faecalis* ATCC 29212  
*Escherichia coli* NCTC 10418  
*Klebsiella pneumoniae* (ESBL) ATCC 700603  
*Pseudomonas aeruginosa* NCTC 13359  
*Acinetobacter baumannii* NCIMB 9214

Test Method: EN13727 (Phase 2, Step 1) and three-stage wipe method

Contact Time: 10 seconds  
Diluent: PBS+0.1% Tween-80  
Test temperature: 22 ± 1°C  
Interfering substance: 3 g/L BSA (dirty conditions)

Neutralising solution: Saponin (Sigma) 30 g/L, polysorbate 80 (Sigma) 10 g/L, lecithin (Fisher), 3 g/L, L-Histidine (Sigma) 1 g/L and sodium dodecyl sulphate (Sigma) 5 g/L, sodium thiosulphate (Fisher) 10 g/L prepared in de-ionised water.

Reproducibility: Two biological repeats, duplicate counts

Test Suspension: All testing was conducted on fluid expressed from wipes. A single wipe was placed in a sterile 20 mL syringe; solution from the wipe was collected by applying pressure for 30-60 seconds. The process was repeated until sufficient fluid had been collected.

Test Surface: Steel disc (2 cm diameter with a grade 2B finish; Goodfellow Cambridge, Huntingdon, UK)

Mechanical action: 10 second wiping, 60 rpm, 500 ± 5 g of pressure, 10 second contact time.

Transfer: Five consecutive adpression onto BHI+10% neutraliser for 10 seconds, 500 ± 5 g of pressure.

Limit of Detection: Log<sub>10</sub> 3.00 ± 0.00

## **Background**

All tests were conducted in accordance with EN13727 (Phase 2, Step 1) suspension test with a 10 second contact time and the three-stage wipe method developed by Williams, G. J., Denyer, S. P., Hosein, I. K., Hill, D. W. & Maillard, J. Y. 2007. The development of a new three-step protocol to determine the efficacy of disinfectant wipes on surfaces contaminated with *Staphylococcus aureus*. *Journal of Hospital Infection*, 67, 329-335.

## **Bacterial Enumeration:**

Preparation of bacterial suspension: A single colony was inoculated to 10 mL Brain Heart Infusion broth (Oxoid, UK) and cultured overnight at 37°C statically under aerobic conditions. The broth culture was then centrifuged at 5,500 *g* for 15 min at room temperature. The supernatant was discarded and the pellet re-suspended in sterile de-ionised water. The bacterial suspension was standardised to between 10<sup>8</sup>-10<sup>9</sup> CFU/mL and combined with BSA, so that the final concentration of Bovine Serum Albumin in the test was 3 g/L. All counts were performed in duplicate from two biological repeats.

Controls included neutraliser toxicity, neutraliser efficacy and dry control recovery. All controls were assessed at the time of experimentation.

## **Carrier Preparation:**

Onto a clean sterile stainless steel disc, 20 µL of bacterial suspension+BSA was pipetted and allowed to dry at 37°C for 30 minutes. All carriers were visually inspected for wetness prior to use.

## **Stage 1:**

A wipe was attached to a sterile steel rod pressed onto the dry inoculated surface, for 10 seconds, 60 rpm exerting a weight of approximately 500 ± 5 g. The stainless steel disc was transferred inoculated side down into sterile glass bottles containing 10 mL neutraliser and 5 g glass beads, horizontally shaken for 5 minutes at 150 rpm and vortexed for 1 minute. The suspension was serially diluted in PBS+T and used to inoculate BHI agar plates. Bacterial colonies were counted after 24 h aerobic incubation at 37°C.

## **Stage 2:**

Following stage 1 the wipe was stamped onto a BHI+neutraliser agar plate for 10 seconds exerting a weight of approximately 500 ± 5 g. This was completed for a total of five consecutive agar plates. Bacterial colonies were counted after 24 h aerobic incubation at 37°C.

**Test Results:**

**Table 1: *Staphylococcus aureus* NCTC 10788, 3g/L BSA EN13727 and in-use simulated wipe test**

		<b>Repeat 1</b> (Log <sub>10</sub> ± SD)	<b>Repeat 2</b> (Log <sub>10</sub> ± SD)
Control Data	Starting inoculum	9.01 ± 0.12	8.97 ± 0.19
	Neutraliser Toxicity (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.00	0.00 ± 0.00
	Neutraliser Efficacy (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.00	0.00 ± 0.00
EN13727 (Phase 2, step 1)	Log <sub>10</sub> Reduction ± SD 10 second exposure to Clinell® Universal Wipes	≥5.01 ± 0.00	≥4.97 ± 0.00
Control Data	Test inoculum on disk (log <sub>10</sub> ±SD)	7.61 ± 0.10	7.59 ± 0.17
	Recovery from disk post drying (log <sub>10</sub> ±SD)	7.99 ± 0.10	8.03 ± 0.10
3-Stage Wipe Test	Stage 1: Removal from disk (Log <sub>10</sub> Reduction ± SD)	≥4.99 ± 0.00	≥5.03 ± 0.00
	Stage 2: Transfer onto surfaces	No transfer onto five consecutive surfaces	No transfer onto five consecutive surfaces

The neutraliser was found to be efficacious and displayed <1 log<sub>10</sub> toxicity. Following a 10 second exposure, an average of ≥4.99 log<sub>10</sub> reduction in *S. aureus* was achieved in suspension. With an in-use simulated wipe test Clinell® Universal Wipes removed an average of ≥5.01 log<sub>10</sub> *S. aureus* from a stainless surface and did not transfer *S. aureus* onto five consecutive surfaces.

**Table 2: *Enterococcus hirae* NCTC 13383, 3g/L BSA EN13727 and in-use simulated wipe test**

		<b>Repeat 1</b> (Log <sub>10</sub> ±SD)	<b>Repeat 2</b> (Log <sub>10</sub> ±SD)
Control Data	Starting inoculum	9.01 ± 0.18	9.10 ± 0.06
	Neutraliser Toxicity (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.14	0.03 ± 0.07
	Neutraliser Efficacy (Log <sub>10</sub> Reduction ± SD)	0.06 ± 0.14	0.00 ± 0.13
EN13727 (Phase 2, step 1)	Log <sub>10</sub> Reduction ± SD 10 second exposure to Clinell® Universal Wipes	≥5.01 ± 0.00	≥5.10 ± 0.00
Control Data	Test inoculum on disk (log <sub>10</sub> ±SD)	7.31 ± 0.18	7.40 ± 0.06
	Recovery from disk post drying (log <sub>10</sub> ±SD)	6.88 ± 0.09	6.99 ± 0.05
3-Stage Wipe Test	Stage 1: Removal from disk (Log <sub>10</sub> Reduction ± SD)	≥3.88 ± 0.00	≥3.99 ± 0.00
	Stage 2: Transfer onto surfaces	No transfer onto five consecutive surfaces	No transfer onto five consecutive surfaces

The neutraliser was found to be efficacious and displayed <1 log<sub>10</sub> toxicity. Following a 10 second exposure, an average of ≥5.06 log<sub>10</sub> reduction in *E. hirae* was achieved in suspension. With an in-use simulated wipe test Clinell® Universal Wipes removed an average of ≥3.94 log<sub>10</sub> *E. hirae* from a stainless steel surface and did not transfer *E. hirae* onto five consecutive surfaces.

**Table 3: *Enterococcus faecium* ATCC 700221(VRE), 3g/L BSA EN13727 and in-use simulated wipe test**

		<b>Repeat 1</b> (Log <sub>10</sub> ±SD)	<b>Repeat 2</b> (Log <sub>10</sub> ±SD)
Control Data	Starting inoculum	8.89 ± 0.10	8.93 ± 0.13
	Neutraliser Toxicity (Log <sub>10</sub> Reduction ± SD)	0.08 ± 0.06	0.02 ± 0.17
	Neutraliser Efficacy (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.11	0.00 ± 0.10
EN13727 (Phase 2, step 1)	Log <sub>10</sub> Reduction ± SD 10 second exposure to Clinell® Universal Wipes	≥4.89 ± 0.00	≥4.93 ± 0.00
Control Data	Test inoculum on disk (log <sub>10</sub> ±SD)	7.19 ± 0.10	7.23 ± 0.13
	Recovery from disk post drying (log <sub>10</sub> ±SD)	7.65 ± 0.18	6.95 ± 0.09
3-Stage Wipe Test	Stage 1: Removal from disk (Log <sub>10</sub> Reduction ± SD)	≥4.65 ± 0.00	≥3.95 ± 0.00
	Stage 2: Transfer onto surfaces	No transfer onto five consecutive surfaces	No transfer onto five consecutive surfaces

The neutraliser was found to be efficacious and displayed <1 log<sub>10</sub> toxicity. Following a 10 second exposure, an average of ≥4.91 log<sub>10</sub> reduction in *E. faecium* was achieved in suspension. With an in-use simulated wipe test Clinell® Universal Wipes removed an average of ≥4.30 log<sub>10</sub> *E. faecium* from a stainless steel surface and did not transfer *E. faecium* onto five consecutive surfaces.

**Table 4: *Enterococcus faecalis* ATCC 29212, 3g/L BSA EN13727 and in-use simulated wipe test**

		<b>Repeat 1</b> (Log <sub>10</sub> ±SD)	<b>Repeat 2</b> (Log <sub>10</sub> ±SD)
Control Data	Starting inoculum	8.89 ± 0.10	8.69 ± 0.20
	Neutraliser Toxicity (Log <sub>10</sub> Reduction ± SD)	0.03 ± 0.09	0.16 ± 0.09
	Neutraliser Efficacy (Log <sub>10</sub> Reduction ± SD)	0.32 ± 0.12	0.12 ± 0.17
EN13727 (Phase 2, step 1)	Log <sub>10</sub> Reduction ± SD 10 second exposure to Clinell® Universal Wipes	≥4.88 ± 0.00	≥4.70 ± 0.00
Control Data	Test inoculum on disk (log <sub>10</sub> ±SD)	6.51 ± 0.13	6.58 ± 0.07
	Recovery from disk post drying (log <sub>10</sub> ±SD)	5.91 ± 0.17	5.79 ± 0.14
3-Stage Wipe Test	Stage 1: Removal from disk (Log <sub>10</sub> Reduction ± SD)	≥3.01 ± 0.00	≥2.79 ± 0.00
	Stage 2: Transfer onto surfaces	No transfer onto five consecutive surfaces	No transfer onto five consecutive surfaces

The neutraliser was found to be efficacious and displayed <1 log<sub>10</sub> toxicity. Following a 10 second exposure, an average of ≥4.79 log<sub>10</sub> reduction in *E. faecalis* was achieved in suspension. With an in-use simulated wipe test Clinell® Universal Wipes removed an average of ≥2.90 log<sub>10</sub> *E. faecalis* from a stainless steel surface and did not transfer *E. faecalis* onto five consecutive surfaces.

**Table 5: *Escherichia coli* NCTC 10418, 3g/L BSA EN13727 and in-use simulated wipe test**

		<b>Repeat 1</b> (Log <sub>10</sub> ±SD)	<b>Repeat 2</b> (Log <sub>10</sub> ±SD)
Control Data	Starting inoculum	8.99 ± 0.07	9.08 ± 0.13
	Neutraliser Toxicity (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.10	0.00 ± 0.05
	Neutraliser Efficacy (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.09	0.00 ± 0.04
EN13727 (Phase 2, step 1)	Log <sub>10</sub> Reduction ± SD 20 second exposure to Clinell® Universal Wipes	5.23 ± 0.00	5.11 ± 0.00
Control Data	Test inoculum on disk (log <sub>10</sub> ±SD)	7.29 ± 0.07	7.38 ± 0.13
	Recovery from disk post drying (log <sub>10</sub> ±SD)	7.58 ± 0.12	7.68 ± 0.20
3-Stage Wipe Test	Stage 1: Removal from disk (Log <sub>10</sub> Reduction ± SD)	≥4.58 ± 0.00	≥4.68 ± 0.00
	Stage 2: Transfer onto surfaces	No transfer onto five consecutive surfaces	No transfer onto five consecutive surfaces

The neutraliser was found to be efficacious and displayed <1 log<sub>10</sub> toxicity. Following a 20 second exposure, an average of ≥5.17 log<sub>10</sub> reduction in *E. coli* was achieved in suspension. With an in-use simulated wipe test Clinell® Universal Wipes removed an average of ≥4.63 log<sub>10</sub>*E. coli* from a stainless steel surface and did not transfer *E. coli* onto five consecutive surfaces.

**Table 6: *Klebsiella pneumoniae* ATCC 700603 (ESBL), 3g/L BSA EN13727 and in-use simulated wipe test**

		<b>Repeat 1</b> (Log <sub>10</sub> ±SD)	<b>Repeat 2</b> (Log <sub>10</sub> ±SD)
Control Data	Starting inoculum	9.09 ± 0.05	9.10 ± 0.08
	Neutraliser Toxicity (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.00	0.00 ± 0.00
	Neutraliser Efficacy (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.07	0.06 ± 0.04
EN13727 (Phase 2, step 1)	Log <sub>10</sub> Reduction ± SD 10 second exposure to Clinell® Universal Wipes	≥5.09 ± 0.00	≥5.10 ± 0.00
Control Data	Test inoculum on disk (log <sub>10</sub> ±SD)	7.39 ± 0.05	7.40 ± 0.08
	Recovery from disk post drying (log <sub>10</sub> ±SD)	6.85 ± 0.12	5.90 ± 0.16
3-Stage Wipe Test	Stage 1: Removal from disk (Log <sub>10</sub> Reduction ± SD)	≥3.85 ± 0.00	≥2.90 ± 0.00
	Stage 2: Transfer onto surfaces	No transfer onto five consecutive surfaces	No transfer onto five consecutive surfaces

The neutraliser was found to be efficacious and displayed <1 log<sub>10</sub> toxicity. Following a 10 second exposure, an average of ≥5.10 log<sub>10</sub> reduction in *K. pneumoniae* was achieved in suspension. With an in-use simulated wipe test Clinell® Universal Wipes removed an average of ≥3.38 log<sub>10</sub> *K. pneumoniae* from a stainless steel surface and did not transfer *K. pneumoniae* onto five consecutive surfaces.



**Table 7: *Pseudomonas aeruginosa* NCTC 13359, 3g/L BSA EN13727 and in-use simulated wipe test**

		<b>Repeat 1</b> (Log <sub>10</sub> ±SD)	<b>Repeat 2</b> (Log <sub>10</sub> ±SD)
Control Data	Starting inoculum	9.44 ± 0.05	9.25 ± 0.09
	Neutraliser Toxicity (Log <sub>10</sub> Reduction ± SD)	0.04 ± 0.23	0.00 ± 0.21
	Neutraliser Efficacy (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.00	0.00 ± 0.00
EN13727 (Phase 2, step 1)	Log <sub>10</sub> Reduction ± SD 10 second exposure to Clinell® Universal Wipes	≥5.44 ± 0.00	≥5.30 ± 0.00
Control Data	Test inoculum on disk (log <sub>10</sub> ±SD)	7.74 ± 0.05	7.55 ± 0.09
	Recovery from disk post drying (log <sub>10</sub> ±SD)	6.93 ± 0.20	5.79 ± 0.21
3-Stage Wipe Test	Stage 1: Removal from disk (Log <sub>10</sub> Reduction ± SD)	≥3.93 ± 0.00	≥2.79 ± 0.00
	Stage 2: Transfer onto surfaces	No transfer onto five consecutive surfaces	No transfer onto five consecutive surfaces

The neutraliser was found to be efficacious and displayed <1 log<sub>10</sub> toxicity. Following a 10 second exposure, an average of ≥5.37 log<sub>10</sub> reduction in *Ps. aeruginosa* was achieved in suspension. With an in-use simulated wipe test Clinell® Universal Wipes removed an average of ≥3.36 log<sub>10</sub> *Ps. aeruginosa* from a stainless steel surface and did not transfer *Ps. aeruginosa* onto five consecutive surfaces.

**Table 8: *Acinetobacter baumannii* NCIMB 9214, 3g/L BSA EN13727 and in-use simulated wipe test**

		<b>Repeat 1</b> (Log <sub>10</sub> ±SD)	<b>Repeat 2</b> (Log <sub>10</sub> ±SD)
Control Data	Starting inoculum	8.86 ± 0.12	8.95 ± 0.09
	Neutraliser Toxicity (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.17	0.13 ± 0.03
	Neutraliser Efficacy (Log <sub>10</sub> Reduction ± SD)	0.00 ± 0.14	0.00 ± 0.15
EN13727 (Phase 2, step 1)	Log <sub>10</sub> Reduction ± SD 10 second exposure to Clinell® Universal Wipes	≥4.86 ± 0.00	≥4.95 ± 0.00
Control Data	Test inoculum on disk (log <sub>10</sub> ±SD)	7.16 ± 0.13	7.26 ± 0.09
	Recovery from disk post drying (log <sub>10</sub> ±SD)	7.19 ± 0.09	6.78 ± 0.21
3-Stage Wipe Test	Stage 1: Removal from disk (Log <sub>10</sub> Reduction ± SD)	≥4.19 ± 0.00	≥3.78 ± 0.00
	Stage 2: Transfer onto surfaces	No transfer onto five consecutive surfaces	No transfer onto five consecutive surfaces

The neutraliser was found to be efficacious and displayed <1 log<sub>10</sub> toxicity. Following a 10 second exposure, an average of ≥4.91 log<sub>10</sub> reduction in *A. baumannii* was achieved in suspension. With an in-use simulated wipe test Clinell® Universal Wipes removed an average of ≥3.99 log<sub>10</sub> *A. baumannii* from a stainless steel surface and did not transfer *A. baumannii* onto five consecutive surfaces.

**Conclusion:**

The Clinell® Universal Disinfectant Wipes tested have demonstrated a >4 log<sub>10</sub> bactericidal activity as per EN13727 (Phase 2, Step 1), with a 10 second contact time, in the presence of 0.3% BSA against *S. aureus*, *E. faecium* (VRE), *E. faecalis*, and *A. baumannii*, and a >5 log<sub>10</sub> bactericidal activity as per EN13727 (Phase 2, Step 1), with a 10 second contact time, in the presence of 0.3% BSA against *E. hirae*, *K. pneumoniae* (ESBL) and *Ps. aeruginosa*. A 5 log<sub>10</sub> reduction of *E. coli* was achieved at a 20 second contact time.

Using an in-use simulated wipe test, no bacteria were detected on the stainless steel surface following the application of Clinell® Universal Disinfectant Wipes. Clinell® Universal Disinfectant Wipes did not transfer any of the tested microorganisms onto five consecutive surfaces.

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