# Test Report: EN 1276:2019

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2, step 1)

**Identification of the test laboratory:** Abbott Analytical Ltd
Unit 2, Hickmans Road, Birkenhead, CH41 1JH, United Kingdom

**Identification of the client:** Centrego Ltd
The Coach House, Newbury, Frome, BA11 3RG, United Kingdom

**Identification of the sample:** 20B/170

| | |
|---|---|
| Name of the product: | Toucan ECA Solutions |
| Batch number/reference and expiry date (if available): | N/A |
| Date of delivery: | 11 February 2020 |
| Storage conditions: | Room temperature in darkness |
| Product diluent recommended by the manufacturer for use: | Not disclosed |
| Active substance(s) and their concentrations (s) (optional): | A solution of Hypochlorous acid generated using a single activation of Centrego's Toucan Electrochemical Activation (ECA) system.<br>Active substances: HOCl and NaOCl. |
| Appearance of the product: | Clear colourless liquid |

**Notes:**

1) The test results in this report relate only to the sample(s) tested.
2) This test report may not be reproduced except in full, adapted, altered or used to create a derivative work, without written approval from Abbott Analytical Ltd.
3) Re-issue of report CEG.20B170.IB, dated 06 March 2020.
Reason: Addition of active substances and their method of production.

**Test method and its validation:**

| | |
|---|---|
| Method: | Dilution-neutralisation |
| Neutraliser: | 30.0 g/l Polysorbate 80 + 5.0 g/l Lecithin + 1.0 g/l L-histidine + 1.0 g/l L-cysteine (Neutraliser A) |
| Neutraliser validation: | Validated in accordance with EN 1276:2019 (5.5.2) |

**Experimental conditions:**

| | |
|---|---|
| Period of analysis: | 03 March 2020 to 05 March 2020 |
| Product test concentration(s): | Neat |
| Diluent used for product test solution(s): | N/A |
| Contact time(s): | 1 min ± 10 s |
| Test temperature(s): | 20°C ± 1°C |
| Interfering substance: | 0.3 g/l bovine albumin (clean conditions) |
| Temperature of incubation: | 36°C ± 1°C |
| Identification of the bacterial strain(s) used: | *Pseudomonas aeruginosa* (NCIMB 10421)<br>*Escherichia coli* (NCTC 10418)<br>*Staphylococcus aureus* (NCTC 10788)<br>*Enterococcus hirae* (NCIMB 8192) |

**Deviations:** None

**Remarks:**

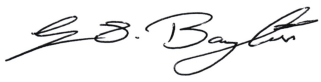
1) All test conditions are as requested by the client, irrespective of whether these are in accordance with EN 1276:2019 (5.4.2) or EN 1276:2019 (5.5.1.1).
2) Products can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

**Requirements:**

The product shall demonstrate at least a 5 decimal log (lg) reduction against every test organism.

**Conclusion:**

According to EN 1276:2019, this sample of Toucan ECA Solutions possesses bactericidal activity against all of the referenced strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae*, when tested neat with a contact time of 1 minute at 20°C under clean conditions.

| **Report re-issued by:** | | **Approved by:** | |
|---|---|---|---|
| Signed: |  | Signed: |  |
| Name: | Tony Watson | Name: | Gareth Bayliss |
| Position: | General Manager | Position: | Laboratory Manager |
| Date: | 13 March 2020 | Date: | 13 March 2020 |

**Results:** EN 1276:2019

RST 002 (Issue 4)

| | | | |
|---|---|---|---|
| Test organism: | *Pseudomonas aeruginosa* | | (NCIMB 10421) |
| Date of test: | 03 March 2020 | | |
| Test temperature: | 20°C ± 1°C | Incubation temperature: | 36°C ± 1°C |
| Dilution-neutralisation method: | Pour plate | Number of plates: | 1 / ml |
| Neutraliser: | A | Test conditions: | Clean conditions |

**Validation and controls:**

| Validation suspension ($Nv_o$) | | | Experimental conditions control (*A*) | | | Neutraliser or filtration control (*B*) | | | Method validation (*C*) Product conc.: *Neat* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Vc*1 | *87* | $\overline{n}$ = | *Vc*1 | *90* | $\overline{n}$ = | *Vc*1 | *91* | $\overline{n}$ = | *Vc*1 | *101* | $\overline{n}$ = |
| *Vc*2 | *94* | 90.5 | *Vc*2 | *87* | 88.5 | *Vc*2 | *93* | 92 | *Vc*2 | *94* | 97.5 |
| $30 \le \overline{n}$ of $Nv_o \le 160$ ? | | | $\overline{n}$ of $A \ge 0.5 \times \overline{n}$ of $Nv_o$ ? | | | $\overline{n}$ of $B \ge 0.5 \times \overline{n}$ of $Nv_o$ ? | | | $\overline{n}$ of $C \ge 0.5 \times \overline{n}$ of $Nv_o$ ? | | |
| ☒ yes ☐ no | | | ☒ yes ☐ no | | | ☒ yes ☐ no | | | ☒ yes ☐ no | | |

**Test suspension (*N* and $N_o$):**

| *N* | *Vc*1 | *Vc*2 | | | |
|---|---|---|---|---|---|
| $10^{-6}$ | *>330* | *>330* | $\overline{n}$ wm = $4.15 \times 10^8$ ; | lg *N* = | 8.62 |
| $10^{-7}$ | *39* | *44* | $N_o = N / 10$ ; | lg $N_o$ = | 7.62 |
| | | | $7.17 \le \lg N_o \le 7.70$ ? | ☒ yes | ☐ no |

**Test:**

| Conc. of the product | Contact time | *Vc*1 | *Vc*2 | *Na* ($\overline{n} \times 10$) | lg *Na* | lg *R* (lg $N_o$ - lg *Na*) |
|---|---|---|---|---|---|---|
| *Neat* | *1 min* | *0* | *0* | <140 | <2.15 | >5.47 |

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**Results:** EN 1276:2019

RST 002 (Issue 4)

| | | | |
|---|---|---|---|
| Test organism: | *Escherichia coli* | | (NCTC 10418) |
| Date of test: | 03 March 2020 | | |
| Test temperature: | 20°C ± 1°C | Incubation temperature: | 36°C ± 1°C |
| Dilution-neutralisation method: | Pour plate | Number of plates: | 1 / ml |
| Neutraliser: | A | Test conditions: | Clean conditions |

**Validation and controls:**

| Validation suspension ($Nv_o$) | | | Experimental conditions control (*A*) | | | Neutraliser or filtration control (*B*) | | | Method validation (*C*) Product conc.: *Neat* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Vc*1 | *109* | $\bar{n}$ = | *Vc*1 | *104* | $\bar{n}$ = | *Vc*1 | *120* | $\bar{n}$ = | *Vc*1 | *113* | $\bar{n}$ = |
| *Vc*2 | *117* | 113 | *Vc*2 | *111* | 107.5 | *Vc*2 | *110* | 115 | *Vc*2 | *107* | 110 |
| $30 \le \bar{n}$ of $Nv_o \le 160$ ? | | | $\bar{n}$ of $A \ge 0.5 \times \bar{n}$ of $Nv_o$ ? | | | $\bar{n}$ of $B \ge 0.5 \times \bar{n}$ of $Nv_o$ ? | | | $\bar{n}$ of $C \ge 0.5 \times \bar{n}$ of $Nv_o$ ? | | |
| ☒ yes ☐ no | | | ☒ yes ☐ no | | | ☒ yes ☐ no | | | ☒ yes ☐ no | | |

**Test suspension (*N* and $N_o$):**

| *N* | *Vc*1 | *Vc*2 | | | |
|---|---|---|---|---|---|
| $10^{-6}$ | *276* | *274* | $\bar{n}$ wm = $2.74 \times 10^8$ ; | lg *N* = | 8.44 |
| $10^{-7}$ | *27* | *25* | $N_o = N / 10$ ; | lg $N_o$ = | 7.44 |
| | | | $7.17 \le \lg N_o \le 7.70$ ? | ☒ yes | ☐ no |

**Test:**

| Conc. of the product | Contact time | *Vc*1 | *Vc*2 | *Na* ($\bar{n} \times 10$) | lg *Na* | lg *R* (lg $N_o$ - lg *Na*) |
|---|---|---|---|---|---|---|
| *Neat* | *1 min* | *0* | *0* | <140 | <2.15 | >5.29 |

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RST 002 (Issue 4)

| | | | |
|---|---|---|---|
| Test organism: | *Staphylococcus aureus* | | (NCTC 10788) |
| Date of test: | 03 March 2020 | | |
| Test temperature: | 20°C ± 1°C | Incubation temperature: | 36°C ± 1°C |
| Dilution-neutralisation method: | Pour plate | Number of plates: | 1 / ml |
| Neutraliser: | A | Test conditions: | Clean conditions |

**Validation and controls:**

| Validation suspension ($Nv_o$) | | | Experimental conditions control (*A*) | | | Neutraliser or filtration control (*B*) | | | Method validation (*C*) Product conc.: *Neat* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Vc*1 | *54* | $\bar{n}$ = | *Vc*1 | *61* | $\bar{n}$ = | *Vc*1 | *47* | $\bar{n}$ = | *Vc*1 | *51* | $\bar{n}$ = |
| *Vc*2 | *50* | 52 | *Vc*2 | *50* | 55.5 | *Vc*2 | *53* | 50 | *Vc*2 | *50* | 50.5 |
| $30 \le \bar{n}$ of $Nv_o \le 160$ ? | | | $\bar{n}$ of $A \ge 0.5 \times \bar{n}$ of $Nv_o$ ? | | | $\bar{n}$ of $B \ge 0.5 \times \bar{n}$ of $Nv_o$ ? | | | $\bar{n}$ of $C \ge 0.5 \times \bar{n}$ of $Nv_o$ ? | | |
| ☒ yes ☐ no | | | ☒ yes ☐ no | | | ☒ yes ☐ no | | | ☒ yes ☐ no | | |

**Test suspension (*N* and $N_o$):**

| *N* | *Vc*1 | *Vc*2 | $\bar{n}$ wm = $3.60 \times 10^8$ ; lg *N* = 8.56 |
|---|---|---|---|
| $10^{-6}$ | *>330* | *>330* | $N_o = N / 10$ ; $\lg N_o$ = 7.56 |
| $10^{-7}$ | *38* | *34* | $7.17 \le \lg N_o \le 7.70$ ? ☒ yes ☐ no |

**Test:**

| Conc. of the product | Contact time | *Vc*1 | *Vc*2 | *Na* ($\bar{n} \times 10$) | lg *Na* | lg *R* (lg $N_o$ - lg *Na*) |
|---|---|---|---|---|---|---|
| *Neat* | *1 min* | *0* | *0* | <140 | <2.15 | >5.41 |

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RST 002 (Issue 4)

| | | | |
|---|---|---|---|
| Test organism: | *Enterococcus hirae* | | (NCIMB 8192) |
| Date of test: | 03 March 2020 | | |
| Test temperature: | 20°C ± 1°C | Incubation temperature: | 36°C ± 1°C |
| Dilution-neutralisation method: | Pour plate | Number of plates: | 1 / ml |
| Neutraliser: | A | Test conditions: | Clean conditions |

**Validation and controls:**

| Validation suspension ($Nv_o$) | | | Experimental conditions control (*A*) | | | Neutraliser or filtration control (*B*) | | | Method validation (*C*) Product conc.: *Neat* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Vc*1 | *107* | $\overline{n}$ = | *Vc*1 | *109* | $\overline{n}$ = | *Vc*1 | *104* | $\overline{n}$ = | *Vc*1 | *103* | $\overline{n}$ = |
| *Vc*2 | *113* | 110 | *Vc*2 | *117* | 113 | *Vc*2 | *98* | 101 | *Vc*2 | *102* | 102.5 |
| $30 \leq \overline{n}$ of $Nv_o \leq 160$ ? | | | $\overline{n}$ of $A \geq 0.5 \times \overline{n}$ of $Nv_o$ ? | | | $\overline{n}$ of $B \geq 0.5 \times \overline{n}$ of $Nv_o$ ? | | | $\overline{n}$ of $C \geq 0.5 \times \overline{n}$ of $Nv_o$ ? | | |
| ☒ yes ☐ no | | | ☒ yes ☐ no | | | ☒ yes ☐ no | | | ☒ yes ☐ no | | |

**Test suspension (*N* and $N_o$):**

| *N* | *Vc*1 | *Vc*2 | | | |
|---|---|---|---|---|---|
| $10^{-6}$ | *>330* | *>330* | $\overline{n}$ wm = $4.75 \times 10^{8}$ ; | lg *N* = | 8.68 |
| $10^{-7}$ | *46* | *49* | $N_o = N / 10$ ; | lg $N_o$ = | 7.68 |
| | | | $7.17 \leq \lg N_o \leq 7.70$ ? | ☒ yes | ☐ no |

**Test:**

| Conc. of the product | Contact time | *Vc*1 | *Vc*2 | *Na* ($\overline{n} \times 10$) | lg *Na* | lg *R* (lg $N_o$ - lg *Na*) |
|---|---|---|---|---|---|---|
| *Neat* | *1 min* | *0* | *0* | <140 | <2.15 | >5.53 |

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**Explanations:**

| | |
|---|---|
| *Vc* | count per ml (one plate or more) |
| $\overline{n}$ | average of *Vc*1 and *Vc*2 (1 + 2 duplicate) |
| $\overline{n}$ wm | weighted mean of $\overline{n}$ |
| *N* | number of cells per ml in the test suspension |
| $N_o$ | number of cells in the test mixture at the beginning of the contact time ($N_o = N / 10$) |
| *Na* | number of survivors per ml in the test mixture at the end of the contact time (before neutralisation or filtration) |
| *R* | reduction ($\lg R = \lg N_o - \lg Na$) |
| *Nv* | number of cells per ml in the validation suspension |
| $Nv_o$ | number of cells in the validation mixtures at the beginning of the contact time ($Nv_o = Nv / 10$) |
| *A* | number of survivors per ml in the experimental conditions control mixture |
| *B* | number of survivors per ml in the neutraliser or filtration control mixture |
| *C* | number of survivors per ml in the method validation mixture |

All test results have an associated uncertainty of measurement, details of which are available on request.

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