# Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas (Phase 2 Step 1)

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## Scope

The standard method BS EN 1276:2019 describes a suspension test method for establishing whether a chemical disinfectant or antiseptic has or does not have bactericidal activity in the fields described.
The test takes into account practical conditions of application of the product, including contact time, temperature, test organisms and interfering substance, i.e. conditions which may influence its action in practical situations.
The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant or antiseptic found by this test corresponds to defined experimental conditions. However, for some applications, the recommendations of use of a product may differ and therefore additional test conditions may need to be used.

## Outline of Test Method (Obligatory Test Conditions)

A sample of the test product is diluted in synthetic hard water for products diluted at point of use (or distilled water in the case of ready to use products). A test suspension of bacteria and interfering substance is then added to the dilutions and maintained at 20°C for 1-60 minutes (general purpose disinfection) or 30-60 seconds (hand hygiene products) At the end of the contact time an aliquot is taken, and the bacterial / bacteriostatic activity is immediately neutralised or suppressed by the validated method. The numbers of surviving bacteria in each sample are determined and the reduction is calculated.
The test is performed using *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae* as standard organisms.
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.

## Acceptance Criteria

The product when tested as above shall demonstrate at least a 5 $\log_{10}$ (3 $\log_{10}$ hand washes) reduction in viable bacterial counts. The test is deemed valid where all control requirements are met.

## UKAS Accreditation

A UKAS accredited testing laboratory No. 4045.

| Test information | | Deviation |
|---|---|---|
| **Name of Product** | Nanoxx Premium Surface Wipes | |
| **Batch Number & Expiry Date** | N/S | |
| **Date of Delivery** | 14/04/2021 | |
| **Period of Analysis** | 10/05/2021 | |
| **Manufacturer / Supplier** | Shimyatech Limited | |
| **Storage Conditions** | Ambient | |
| **Appearance of the Product** | Turbid liquid | |
| **Neutraliser** | Filtration | |
| **Neutralisation Method** | Filtration | |
| **Product Diluent** | Distilled water | |
| **Test Concentrations** | Neat (80%), Mid-range (50%), Non active (0.1%) | |
| **Experimental Conditions** | Clean | |
| **Interfering Substance** | Clean 0.3g/l Bovine Albumin | |
| **Test Temperature** | 20°C ± 1°C | |
| **Temperature of Incubation** | Bacteria – 37°C ±1°C for 24hr to 48hrs | |
| **Identification of the Bacterial Strains:** | *Pseudomonas aeruginosa* NCTC 13359 (ATCC 15442)<br>*Staphylococcus aureus* NCTC 10788 (ATCC 6538)<br>*Enterococcus hirae* NCTC 13383 (ATCC 10541)<br>*Escherichia coli* NCTC 10418 (ATCC 10536) | |
| **Contact Times** | 1 minute ± 10s | |
| **Stability and Appearance During Test** | No Change Observed | |

**Deviations from Standard Method**

There were no deviations from the standard method

**Test Result Summary**

The test product received has achieved a >5 log reduction against all bacterial test isolates, when tested under the condition stipulated in this report.

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

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## Validation and Controls

| Validation suspension ($Nv_0$) | | | | Experimental condition controls (A) | | | | Neutraliser or Filtration Control (B) | | | | Method Validation (C ) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\bar{x}$ = | | | | $\bar{x}$ = | | | | $\bar{x}$ = | | | | $\bar{x}$ = |
| Vc1 | Ps. | 80 | | Vc1 | Ps. | 61 | | Vc1 | Ps. | 64 | | Vc1 | Ps. | 92 | |
| | Sa. | 51 | | | Sa. | 34 | | | Sa. | 36 | | | Sa. | 24 | |
| | Ec. | 36 | Ps. 77 | | Ec. | 34 | Ps. 56 | | Ec. | 31 | Ps. 68 | | Ec. | 24 | Ps. 87 |
| | Ent. | 39 | St. 38 | | Ent. | 31 | St. 34 | | Ent. | 49 | St. 33 | | Ent. | 31 | St. 24 |
| Vc2 | Ps. | 73 | Ec. 34 | Vc2 | Ps. | 50 | Ec. 38 | Vc2 | Ps. | 72 | Ec. 36 | Vc2 | Ps. | 81 | Ec. 26 |
| | Sa. | 25 | Ent. 39 | | Sa. | 34 | Ent. 37 | | Sa. | 30 | Ent. 49 | | Sa. | 24 | Ent. 33 |
| | Ec. | 32 | | | Ec. | 41 | | | Ec. | 40 | | | Ec. | 27 | |
| | Ent. | 38 | | | Ent. | 43 | | | Ent. | 48 | | | Ent. | 35 | |
| $30 \leq \bar{x}$ of $Nv_0 \leq 160$? | | | | $\bar{x}$ of A $\geq$ 0.5 Nv0 | | | | $\bar{x}$ of B $\geq$ 0.5 Nv0 | | | | $\bar{x}$ of C $\geq$ 0.5 Nv0 | | | |
| Yes | | | | Yes | | | | Yes | | | | Yes | | | |

## Test Results

| SOLUTION PROVIDERS | | Test Procedure at concentrations % (V/V) | | |
|---|---|---|---|---|
| **Test Organism** | **Suspension N** | **Neat** | **50** | **0.1** |
| ***Pseudomonas*** | 10^6 >330 ; >330 | 10^0 0 ; 0 | 10^0 0 ; 0 | 10^0 31 ; 54 |
| ***aeruginosa*** | 10^7 38 ; 32 | Na ; < 2.15 | Na ; < 2.15 | Na ; 2.63 |
| **ATCC 15442** | $N_0$ : 7.54 Valid | R > 5.40 | R > 5.40 | R 4.92 |
| ***Escherichia*** | 10^6 151 ; 159 | 10^0 0 ; 0 | 10^0 0 ; 0 | 10^0 16 ; 39 |
| ***coli*** | 10^7 22 ; 23 | Na ; < 2.15 | Na ; < 2.15 | Na ; 2.44 |
| **ATCC 10536** | $N_0$ : 7.21 Valid | R > 5.06 | R > 5.06 | R 4.77 |
| ***Staphylococcus*** | 10^6 >330 ; >330 | 10^0 0 ; 0 | 10^0 0 ; 0 | 10^0 234 ; 191 |
| ***aureus*** | 10^7 50 ; 48 | Na ; < 2.15 | Na ; < 2.15 | Na ; 3.33 |
| **ATCC 6538** | $N_0$ : 7.69 Valid | R > 5.54 | R > 5.54 | R 4.36 |
| ***Enterococcus*** | 10^6 165 ; 163 | 10^0 0 ; 0 | 10^0 0 ; 0 | 10^0 91 ; 91 |
| ***hirae*** | 10^7 18 ; 15 | Na ; < 2.15 | Na ; < 2.15 | Na ; 2.96 |
| **ATCC 10541** | $N_0$ : 7.22 Valid | R > 5.07 | R > 5.07 | R 4.26 |

**KEY**

| | |
|---|---|
| $N_0$ | $Log_{10}$ number of cfu/ml at the beginning of the contact time = $N/10$ |
| $Nv_0$ | is the number of cfu/ml in the validation test suspension at the beginning of the contact time |
| *A* | is the verification of experimental conditions control |
| *B* | is the neutraliser toxicity control |
| *C* | is method validation |
| Vc | is the colony forming units counted per 1ml of sample |
| $\bar{x}$ | is the average of $Vc_1$ & $Vc_2$ |
| $\bar{x}$ wm | is the weighted mean of *N* |
| *Na* | $Log_{10}$ number of surviving cfu/ml in the test mixture |
| *R* | ($lg N_0 – lg Na = lg R$) is the calculation for reduction in viability |
| > | Greater than |
| ≥ | Equal to or greater than |
| < | Less than |
| ≤ | Equal to or less than |

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