KENYA STANDARD

KS 927: 2016
ICS 11.080; 7.100.25

Disinfecting general purpose liquid synthetic detergents – Specification

FEBRUARY 2016

Second Edition 2016

TECHNICAL COMMITTEE REPRESENTATION

The following organizations were represented on the Technical Committee:

PZ Cussons E.A.
Unilever Kenya
Colgate Palmolive
Kenya Industrial Research and Development Institute
Government Chemist's Department
University of Nairobi
Reckitt Beckinser
Orbit Chemical Industries
Kapa Oil Refineries
Consumer Information Network
Henkel Chemicals
Soilex Presolve Ltd.
MEP Chemicals
Bidco Oil Refineries
Diversey East & Central Africa
Kenya Bureau of Standards — Secretariat

REVISION OF KENYA STANDARDS

In order to keep abreast of progress in industry, Kenya standards shall be regularly reviewed. Suggestions for improvement to published standards, addressed to the Managing Director, Kenya Bureau of Standards, are welcome.

KENYA STANDARD

KS 927: 2016
ICS 11.080; 7.100.25

Disinfecting general purpose liquid synthetic detergents – Specification

KENYA BUREAU OF STANDARDS (KEBS)

Head Office: P.O. Box 54974, Nairobi-00200, Tel.: (+254 020) 605490, 602350, Fax: (+254 020) 604031
E-Mail: info@kebs.org, Web:http://www.kebs.org

Coast Region
P.O. Box 99376, Mombasa-80100
Tel.: (+254 041) 229563, 230939/40
Fax: (+254 041) 229448

Lake Region
P.O. Box 2949, Kisumu-40100
Tel.: (+254 057) 23549, 22396
Fax: (+254 057) 21814

North Rift Region
P.O. Box 2138, Nakuru-20100
Tel.: (+254 051) 210553, 210555

Foreword

This Kenya Standard was developed by the Technical Committee on Surface Active Agents under the guideline of the Standards Project Committee, and is in accordance with the procedures of the Kenya Bureau of Standards.

Disinfecting detergents are detergents that contain chemicals capable of destroying or inhibiting the growth of harmful micro-organisms. They contain synthetic detergent in a liquid form that contains a phenolic quaternary ammonium or other type germicide and which is intended primarily for general cleaning and disinfecting floors, walls and other hard surfaces. They are suitable for application on resilient flooring, porcelain, glass, tile, stainless steel, chrome and painted or varnished surface.

They are used for general cleaning and disinfecting of floors, walls and hard surfaces in institutions where the destruction of bacteria is important, such as hospitals, hotels and food processing areas.

This standard shall specify, among other requirements, the cleaning efficiency and disinfecting activities of the product.

In the preparation of this standard, reference was made to the following standard:

GSO 1948: Germicidal liquid detergent for general purposes.

Acknowledgement is made for the assistance received from this source.

KENYA STANDARD **KS 927:2016**

Disinfecting general purpose liquid synthetic detergents – Specification

1. SCOPE

This standard specifies requirements and methods of test for liquid synthetic detergent that contains a phenolic, quaternary ammonium or other type of germicide and which is intended primarily for general cleaning and disinfecting floors, walls and other hard surfaces.

2. Normative references

This Kenya Standard incorporates by dated and undated reference, provisions from other publications. These normative references are cited at the appropriate place in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this Kenya Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

KS EAS 814; *Kenya Standard — Determination of biodegradability of surfactants — Test method, Second Edition*

ISO 2870, *Surface active agents — Detergents — Determination of anionic-active matter hydrolysable and non-hydrolysable under acid conditions*

ISO 2871-1, *Surface active agents — Detergents — Determination of cationic-active matter content — Part 1: High-molecular-mass cationic-active matter*

ISO 2871-2, *Surface active agents — Detergents — Determination of cationic-active matter content — Part 2: Cationic-active matter of low molecular mass (between 200 and 500)*

3. CLASSIFICATION

Disinfecting liquid synthetic detergents shall be of the following types:

Type 1 – Phenolic
Type 2 – Quarternary ammonium compounds
Type 3 – Others

4. Definitions

For the purpose of this standard the definitions below shall apply.

4.1 Germicide

A substance that destroys microorganisms on inanimate environmental surfaces in the growing form but not necessarily the resistant spore form.

4.2 Disinfectant

A substance or treatment that prevents the transmission or survival of undesirable microorganisms (usually in the growing form) on inanimate environmental surfaces by causing the complete destruction or irreversible inactivation of such microorganisms.

5. Requirements

5.1 General

5.1.1 The product shall be a clear homogenous solution at room (25 °C ± 2°C) temperature.

5.1.2 It shall not contain visible foreign matter and shall be free from objectionable odour (e.g., rancidity or odour of decomposition) both when received and when in solution.

5.1.3 The product shall clean and disinfect in one operation in the presence of hard water as specified.

5.1.4 All the ingredients used in the detergent shall be biodegradable when tested against KS EAS 814.

5.1.5 When stored under normal conditions in sealed containers for a period equal to its shelf-life, the material shall remain stable and homogeneous, and shall remain free from objectionable odour.

5.1.6 The product under conditions of use shall not cause deterioration of vinyl, asphalt, terrazzo , rubber or concrete cement flooring surfaces. When used on metallic or other hard surfaces the products shall cause no greater corrosion or harmful effects than water in the conditions.

5.2 Specific quality requirements

The product shall also comply with the requirements given in Table 1 when tested against the methods prescribed therein.

Table 1: Specific quality requirements

Sl. No	Characteristic		Requirement	Test method
i)	pH of 1 part solution to 9 parts of water		4.0 to 10.5	Annex A
ii)	Stability to hard water		Not more than 0.5 mL of curd shall be generated	Annex B
iii)	Stability at low and high temperature		To pass test	Annex C
iv)	Cleaning efficiency at 1:20 dilution with synthetic hard water , %,min		70	Annex D
v)	Active detergent level, % m/m, min	Phenolic & others	8.0	KS ISO 2870
		QAC	2.0	KS ISO 2871-1 KS ISO 2871-2
vi)	Disinfecting activity		To pass test	Annex E

6. Packaging and labeling

6.1 Packaging

The product shall be packaged in suitable containers that are strong enough to withstand normal usage and transportation and that will prevent leaking, drying out and contamination of the product.

6.2 Labelling

Each container and each bulk package shall be legibly and indelibly marked with the following information:

a) description of the product as “disinfecting detergent”;

b) manufacturer’s name and physical address;

NOTE:The name, physical address of the distributor/supplier and trade mark may be added as required.

c) batch or lot number;

d) net contents;

e) type of germicide used and it’s level;

f) Instructions for use, given in imperative tense, including at least the dilution recommended for particular applications and an instruction not to mix with other chemicals that the manufacturer knows may affect the efficacy of the disinfecting compound;

g) date of manufacture;

h) best before date;

i) list of ingredients in descending order of quantity;

j) instructions for remedy in case of accidental ingestion; and

k) country of origin.

7. Product information

7.1 The product is intended for general cleaning of surfaces such as floors, walls and woodwork where the destruction of bacteria is important.

7.2 The usual precaution against freezing should be taken. When the product is cooled to below 10ºC, germicide may crystallize out. The effectiveness of the product is not adversely affected provided it is warmed to room temperature and well shaken or agitated to re-dissolve any germicidal that precipitated out.

7.3 The products are designed to be effective for use with water of up to 200 mg/L $CaCO_3$ hardness. In areas where water hardness exceeds this level, either correspondingly more product, or softened water, should be used.

ANNEX A

pH

A1. APPARATUS AND REAGENTS

A1.1 pH meter

A1.2 Freshly boiled and cooled distilled water.

A2. PROCEDURE

Dilute a portion of the material to be tested in the proportions specified with distilled water. Measure the pH of the dilute solution electro-metrically at 25^0 C. For pH's over 10, the pH instrument shall be standardized against a buffer solution of at least pH 10 in order to minimize any error due to the unsuitability of the glass electrode for the pH range encountered.

A3. REPORT

Report the pH of the solution.

ANNEX B

STABILITY OF HARD WATER

B1. APPARATUS AND REAGENTS

B1.1 Double-strength artificial hard water — This is water with the following composition in grams per litre:

Calcium acetate ($Ca(CH_3COO)_2H_2$) – 2.0 g/L

Magnesium sulphate (mg SO_2. $7H_2O$) – 1.4 g/L

B1.2 Centrifuge tubes — 100 mL graduated pear-shaped tubes having 1 mL tips graduated in 0.05 mL divisions.

B1.3 Centrifuge — Capable of producing a relative centrifuge force (ref) of 510 ± 30,

$$\text{rpm} = 246 \sqrt{\frac{rcf}{d}}$$

where,

rpm = revolutions per min, and

d = the diameter of swing in centimetres, measured between tips of opposite tubes when in rotating position.

NOTE: A centrifuge with a 47 cm tip-to-tip diameter of whirling tubes at 1500 rpm complies.

B2. PROCEDURE

Accurately determine the mass of 1.00 g of the material and dissolve in 100 mL distilled water, stirring and warming to 30^0 C if necessary. Pour 50 mL of this solution into each of two centrifuge tubes and add 50 mL artificial hard water to each. Stopper and shake to mix . Allow to stand for 10 min to 15 min and then centrifuge for 10 min at an rcf of 510. Estimate the volume of precipitate in millilitres in each tube.

B3. REPORT

Calculate the average of the values and report it as the volume of precipitate in millilitres.

ANNEX C

STABILITY AT LOW AND HIGH TEMPERATURE

C1. PROCEDURE

Put the product at 10^0C for low temperature, and 40^0C for high temperature for 24 h. After 24 h remove it and allow it to return to room temperature (20^0C approximately) for another 24 h during which time it shall be well shaken or agitated. Pour the sample in a clean test jar and check for any cloudiness or turbidity, visually.

C2. REPORT

If any cloudiness, turbidity, separation or laying develops when the product is held at 10^0 C for 24 h and is completely eliminated when the product is returned to room temperature for 24 h and well shaken or agitated, then the product will have passed the test. If the cloudiness, turbidity or layering does not completely disappear after the product is returned to room temperature, then the product is not stable at low temperature.

ANNEX D

CLEANING EFFICIENCY

D1. PRINCIPLE OF THE METHOD

A solution of working strength of the product is made up with water and used in a scrubbing machine to remove a synthetic soil from a test panel. The reflectance of the cleaned panel is measured and expressed before application of soil.

D2. APPARATUS

D2.1 Washability machine — A gardener model 105 straight line washability machine equipped with a sponge box. The total weight of the sponge box and sponge shall be 454 ± 14 g. If necessary, sheet lead may be fastened to the top of the sponge box to attain this weight.

D2.2 Reflectometer — Equipped with an amber tristimulus filter.

D2.3 Standard white plegue (reflectance approximately 80 per cent)

D2.4 Test panels — Amtico VPII white vinyl tile 230 x 230 x 3.2 mm cut to 115 x 230 mm. Only new panels shall be used for the test. Scrub the surface with a wet cloth and scouring powder, rinse with water, oven-dry at 100^0C for 15 min and cool to room temperature. Measure and record the reflectance(R_1) of each. Use the template described in **D2.11**.

D2.5 Flat pan — Approximately 50 x 150 x 300 mm.

D.2.6 Sponge — A photographic grade cellulose friegrain sponge, preshrunk and cut to a dry size of about
95 x 75 x 40 mm.

D2.7 50 mL burette

D2.8 Boston — Brandley adjustable blade, or fixed blade applicator 75 mm wide and 0.10 mm clearance.

D2.9 Water of 150 ppm hardness

D2.10 Two vinyl title squares, 115 x 115 mm.

D2.11 Template of vinyl title, 115 x 230 mm with four evenly spaced holes for making reflectance readings.

D2.12 Forced draft oven, ± 0.5^0 C

D2.13 Standard reference detergent sodium dodecylbenzene sulfonate (linear, 80 per cent active) 23.5 per cent.

Sodium Tripolyphosphete, anhydrons — 40.0 per cent
Sodium metasilicate, pentahydrate — 7.0 per cent
Sodium sulfate,anhydrons — 29.5 per cent.

D3. PREPARATION OF SOILED PANELS

D3.1 Soil

	Parts by weight
Metallic brown, ASTM D & 4, Class 1	20
Kerosene	12
Stoddard solvent	12
Liquid petroleum	1
Lubricating oil	1
Hydrogenated vegetable shortening	1

D3.2 Soiling procedure

Dissolve oils and shortening in kerosene-stoddard solvent and then disperse metallic brown in this solution. While the bulk lot of soil is continuously stirred to ensure uniformity, remove a sample of approximately 4 g and apply along the entire length of vinyl panel. Use a 'doctor' blade, of fixed-blade applicator, set at 0.10 mm to provide an even spread. Air dry soiled panel in forced draft oven set at 80^0C for 15 min.

Allow panels to cool to room temperature, use between 20 and 24 h after removal from oven.

D3.3 Synthetic hard water

Prepare a litre of synthetic hard water as described in KS 03-92, Clause G2.2.

D3.4 Checking soiled panels — Clean 2 sets of 3 soiled panels by the procedure detailed in **E4.** with the exceptions that , in the case of set N0. 1, the wash solution shall be distilled water, and in the case of No. 2 , the wash solution shall be a 1.0 per cent solution standard. Reference detergent in 150 ppm hard water. Measure the reflectance of the cleaned panels and calculate cleaning efficiencies. Cleaning efficiency with distilled water shall be less than 30 percent and with the standard detergent shall exceed 80 per cent. If the criteria given above are not met in at least 2 of the 3 determinations, the lot of soiled panels shall be reflected. This checking procedure shall be followed with each lot of soiled panels.

D4. PROCEDURE

D4.1 Make up 1 litre of a 2 percent solution of the cleaner or detergent in 150 ppm hard water, and use at 25°C.

D4.2 Fit sponge into washability apparatus housing box 95 x 75 mm.

D4.3 Soak test panel for 60 s in sufficient wash solution to cover entire panel.

D4.4 Wet sponge with 25°C tap water and squeeze damp-dry by hand.

D4.5 Add 50 mL of wash solution to damp-dry sponge and insert into sponge housing box.

D4.6 Centre test panel on washability apparatus by use of two 115 x 115 mm pieces of vinyl tile, one on either end of the test panel.

D4.7 Start washability apparatus, set at one stroke per second (a complete cycle) back and forth is two stroke while dripping wash solution from a burette onto the centre of test panel.

D4.8 Time wash operation so that 12 mL wash solution from burette onto the centre of test panel.

D4.9 Wash panel for 100 strokes (50 cycles).

D4.10 Stop apparatus.

D4.11 Remove washed panel and rinse under a light stream of 25°C tap water.

D4.12 Drain water from panel and immediately replace on apparatus in a reverse direction.

D4.13 Rinse sponge in 25°C tap water, squeeze damp-dry by hand, and replace sponge in box with unused side facing test panel.

D4.14 Repeat wash cycle and rinse panel as before.

D4.15 Allow washed, rinsed panel to air dry.

D4.16 Make triplicate runs for each cleaning solution tested. Use a fresh sponge for each different detergent tested, but the same sponge, may be used for replicator runs on any one product. The first test result obtained from using a new sponge for the first time shall be reflected.

D4.17 Measure the reflectance of the washed panels as described in **2.4**. Average the results obtained (4 readings on each of thee panels).

D5. CALCULATION AND REPORT

Cleaning efficiency (% CE) = $\frac{R_2}{R_1} \times 100$

where,

R_1 = reflectance of unsoiled, unwashed panel,

R_2 = reflectance of soiled, washed panel.

ANNEX E

DETERMINATION OF DISINFECTING EFFICACY

E1. OUTLINE OF THE METHOD

E1.1 The disinfectant detergent is tested at the recommended 'use'-dilution' and concurrently at 0.5 and 1.5 times that dilution. The test consists of challenging the diluted disinfectant with bacterial moculum, withdrawing a sample in a suit recovery medium. After the sampling, the mixture is again challenged by a second moculum and after a second interval, is again sampled for curruting. This process is then repeated to provide a third challenge.

E1.2 The sample is considered to have passed or failed the test according to the extent of growth shown in the first two cultured samples.

E2. APPARATUS

E2.1 Facility for incubation at 37 °C ± 1 °C.

E2.2 Facility for incubation at 27 °C ± 1 °C**.**

F2.3 Stopclock

E2.4 Facility for refrigeration at 4 ± 1°C.

E2.5 **Universal containers** — Made of glass and having metal tops with rubber liners. Plastic containers or glass containers with plastic tops shall not be used.

E2.6 **Test tubes** — 19 mm X 150 mm.

E2.7 **Filter paper**, No. 4 whatman (sterile) or equivalent.

E2.8 **Facility**, for autoclaving at 121 ± 1°C.

E2.9 **Pipette**, capable of dispensing 0.02 °C ± 1°C 0.005 mL.

E2.10 pH meter

E2.11 **Facility**, to sterilize by filtration.

E2.12 150 µm test sieve.

E2.13 **Oven**, capable of maintaining temperature at 100 °C ± 1 °C.

E3. MEDIA

E3.1 Growth media for test organisms.
Wright and Mundy Broth with Dextrose (WMBD).

E3.1.1 Dispense 10 mL and 6 mL quantities of the Wright and Mundy Broth into universal bottles, and autoclave at 121 °C ± 1°C for 12 minutes.

E3.1.2 Add to this medium, 10 per cent (M/v) dextrose solution sterilized by filtration, to give a final dextrose concentration of 0.1 per cent (m/v), (i.e. to 10 mL broth add 0.1 dextrose solution and to 6.0 mL broth add 0.06 mL dextrose solution).

E3.2 **Recovery medium** — A nutrient broth prepared as follows:

E3.2.1 *Composition*

Beef extract	10 g
Peptone	10 g
Sodium chloride	5 g
Polyoxyethylene sorbitan mono-oleate	30 g

E3.2.2 *Preparation* — Add the ingredients to 1000 mL of water. Mix well. Dispense 10 mL quantities into test tubes and autoclave at 121 ± 1°C for 15 minutes.

E3.3 **Hard water** — Standard hard water with 342 mg/L (ppm) hardness prepared as follows:

Dissolve 0.304 g of anhydrous calcium chloride hexahydrate ($mgcl_2$-$6H_20$) in distilled water and make up the volume to one litre. Sterilize the standard hard water by autoclaving at 121 °C ± 1° C for 15 **minutes**. Allow this to reach room temperature before use.

E3.4 Yeast suspension

E3.4.1 Weigh to the nearest gram about 65 g of active dry yeast. Cream by the gradual addition of sterile hard water using a heavy glass rod for stirring. Decant the creamed portion into a flask, add more hard water to any lumpy residue remaining and repeat the creaming and decantation until no residue remains, and 500 mL of hard water has been used.

E3.4.2 Shake the contents of the flask vigorously and strain through a 150 µm sieve (**F2.12**) breaking down any remaining lumps.

E3.4.3 Add 500 mL sterile hard water, shake vigorously.

E3.4.4 Transfer 50 mL or 100 mL portions into screw capped bottles, screw the caps tightly and autoclave at 121 °C ± 1°C for 15 minutes. Allow the autoclave to cool without releasing the pressure. Store cold but not freezing.

E3.4.5 Dry two glass petri-dishes to constant mass. Into each of these dishes, pipette 25 mL of sterilized yeast suspension and dry to constant mass at 100°C.
Calculate the average solids content of the suspension.

E3.4.6 Before use, pipette 25 mL of the sterilized yeast suspension into a beaker. Determine the pH using a glass electrode, and determine the volume of 40 g/L sodium hydroxide solution needed to adjust the pH to 7.0 ± 0.1.

E3.4.7 Immediately before use, add to each bottle of sterilized yeast suspension a volume of sterile hard water and a volume of 40 g/L sodium hydroxide calculated to adjust the concentration of dry yeast to 5 per cent (m/v) and the pH to 7.0 ± 0.1. Discard prepared yeast, two weeks after preparation.

E3.5 **Ringers solution, 25 per cent (v/v)** — Dissolve 9.00 g of sodium chloride, 0.42 g of potassium chloride, 0.24 g of anhydrous calcium chloride and 0.20 g of sodium bicarbonate in water and dilute to 1000 mL.

Add 1 volume of this solution to 3 volumes of water to give a 25 per cent solution. Dispense into test tubes fitted with suitable closures and sterilized by auto-claving at 121 °C ± 1°C for 15 minutes.

E4. SELECTION OF THE MOST RESISTANT ORGANISM BY THE MINIMUM INHIBITORY CONCENTRATION TEST

E4.1 The following organisms shall be used for the test:

Pseudomonas aeruginosa (NCTC 6749 or equivalent)
Proteus vulgaris (NCTC 4635 or equivalent)
Stophyloccus aureus (NCTC 4163 or equivalent)

These organisms may be obtained as freeze dried cultures. Once sub-cultured, the organisms shall be maintained on agar slopes of suitable nutrient medium at 4 ± 1°C.

E4.2 Subculture each organism daily into a universal bottle containing 6 mL of growth medium (**E3.1**) and Incubate for 24 ± 2 h at 37 °C ± 1°C.

E4.3 Dilute one part of freshly grown sub-culture of each organism, which is at least a fifth sub-culture and not more than a fourteenth, with ten parts of the growth medium (**E3.1**) before dilution, the *P. aeruginosa*, culture shall be filtered using a whatman No.4 filter paper.

E4.4 Prepared three sets of ten, doubling dilutions of the disinfectant in universal containers (**E2.5**). For this purpose dilute the neat disinfectant in the growth medium (**E3.1**) or the recovery medium (**E3.2**) to give a final volume of 5 mL of the diluted disinfectant for each dilution.

E4.5 Inoculate each dilution in one set with 0.02 mL of a diluted culture of one organism (see **E4.3**)).

E4.6 Incubate all the three sets of inoculate dilutions at 37 ± 1°C for 72 hours, and examine to determine the organism most resistant to the disinfectant, that is the organism for which the minimum inhibitory concentration is highest.

E5. PREPARATION OF INOCULUM

E5.1 Daily sub-cultures of the test organism selected as in **E4.6** shall be grown in 6 mL quantities of the growth medium (**E3.1**) and incubated at 37 ± 1°C for 24 ± 2 hours.

E5.2 The day before the test, inoculate 10 mL of the growth medium (**E3.1**) with the test organism from a daily sub-culture and not more than a fourteenth. Incubate the inoculated, broth at 37 °C ± 1°C for 24 ± 2 hours.

E5.3 Add 6 mL of the test organism culture (**E5.1**) and (**E5.2**) to 4 mL of the yeast suspension (**E3.4**) thus making a final concentration of 2 per cent (m/v) of yeast in the yeast/organism suspension. If a culture of P. aeruginosa is used, it shall be filtered using a whatman No.4 filter paper before addition.

E5.4 Shake the yeast/organism suspension for one minute with a few sterile glass beads. Immediately before the test, count the number of viable organisms in the inoculum by decimal dilutions in 25 per cent Ringers solution (see **E3.5**) and by the drop plate method. The viable count shall be not less than 10^8 organisms/mL or more than 10^{10} organisms/mL or the test results are considered invalid.

E6. PREPARATION OF THE DISINFECTANT DILUTIONS

Prepare three dilutions of the disinfectant in hard water (**E3.3**) based on the recommended 'use dilution' of the disinfectant, as follows

A = 0.5 times the recommended 'use-dilution'
B = 1.0 times the recommended 'use-dilution'
C = 1.5 times the recommended 'use-dilution'

The disinfectant dilutions shall be prepared and tested on the same day.

E7. TEST PROCEDURE

E7.1 The test shall be carried out at 27 °C ± 1°C.

E7.2 Dispense 3 mL of each dilution of disinfectant (**E6**) into separate universal bottles labelled A, B, and C, then allow to equilibrate to 27 °C ± 1°C.

E7.3 Add 1 mL of the inoculum to A, B and C at 0, 1 and 5 minutes respectively and mix by swirling gently.

NOTE: Although **E7.4** to **E7.7** inclusive relate to a single sample, in practice all three dilutions are tested concurrently and a protocol for this is given in **E7.7**. It is recommended that before commencing the test, universal containers holding the disinfectant dilutions are arranged in a rack together with a fourth container holding the inoculum. The tubes of the recovery broth, labeled A1, A2 and A3, B1, B2 and B3; C1, C2 and C3 are placed nearby and using a copy of the test protocol, each step is ticked off as it is completed.

E7.4 Eight minutes after the addition of the inoculum, remove a sample of the inoculum/disinfectant mixture and put 0.02 mL into each of the first group of five tubes of recovery broths. Return the remainder of the mixture in the pipette to the universal container.

E7.5 Ten minutes after the first addition of the inoculum, add another 1 mL of the inoculum to each of the disinfectant dilutions and mix by swirling gently.

E7.6 Eight minutes later, remove a sample of the mixture as put before (**E7.4**) and put 0.02 mL into each of the second group of five tubes of recovery broths.

E7.7 Swirl the recovery broths and incubate at 37 °C ± 1°C for 48 ± 2 h. Examine the growth and record the results.

E8. INTERPRETATION OF RESULTS

E8.1 The disinfectant, shall be regarded as having passed the test at the recommended 'use dilution' if there is no growth in at least two of the five recovery broths for the first and second additions of the inoculum.

E8.2 To be acceptable, a disinfectant shall pass the test on three separate occasions using freshly prepared inoculum on each occasion.