RWANDA STANDARD

DRS 383

First edition

2018-mm-dd

Antiseptics based on chlorhexidine gluconate — Specification

Rwanda Standards Board

Reference number

DRS 383: 2018

In order to match with technological development and to keep continuous progress in industries, standards are subject to periodic review. Users shall ascertain that they are in possession of the latest edition

Rwanda Standards Board

P.O Box 7099 Kigali-Rwanda

KK 15 Rd, 49

Tel. +250 788303492

Toll Free: 3250

E-mail: info@rsb.gov.rw

Website: www.rsb.gov.rw

ePortal: www.portal.rsb.gov.rw

Contents

Page

Foreword

Rwanda Standards are prepared by Technical Committees and approved by Rwanda Standards Board (RSB) Board of Directors in accordance with the procedures of RSB, in compliance with Annex 3 of the WTO/TBT agreement on the preparation, adoption and application of standards.

The main task of technical committees is to prepare national standards. Final Draft Rwanda Standards adopted by Technical committees are ratified by members of RSB Board of Directors for publication and gazettment as Rwanda Standards.

DRS 383 was prepared by Technical Committee RSB/TC 042, *Surface active Agents*.

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

SANS 1597, *Antiseptics based on chlorhexidine gluconate*

The assistance derived from the above source is hereby acknowledged with thanks.

Committee membership

The following organizations were represented on the Technical Committee on Surface Active agents (RSB/TC 042) in the preparation of this standard.

Paragraph of participants

University of Rwanda – College of Sciences and Technology

Rwanda Biomedical Center – Medical Procurement and Production Division

Trust Industries Limited

SULFO Rwanda Industries

Private Sector Federation – Beauty Makers Association

Mount Kenya University

IKIZERE Initiative Hope Shines

Rwanda Standards Board (RSB) – Secretariat

Antiseptic based on chlorhexidine gluconate — Specification

1 Scope

This Draft Rwanda Standard specifies requirements, sampling, test methods and inspection for antiseptic products based on chlorhexidine gluconate that may contain detergent (s) and other compatible chemical agents, and that are water-miscible and intended for use on live tissue that is free from excessive dirt.

This document does not cover the antiseptic products with a chlorhexidine gluconate content of less than 0.5% by volume.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

RS 278, *Cosmetics – Method of Sampling*

RS ISO 862, *Surface active agents – Vocabulary*

ISO 7218, *Microbiology of food and animal feeding stuffs -- General requirements and guidance for microbiological examinations*

ISO 10523, *Water quality -- Determination of pH*

RS EAS 346, *Labelling of cosmetic products — General requirements*

ISO 11133, *Microbiology of food, animal feed and water -- Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

For the purposes of this standard, the terms and definitions given in RS ISO 862 and the following apply.

3.1

chlorhexidine gluconate

antibacterial compound $C_{22}H_{30}Cl_2N_{10}$ used as a local antiseptic and disinfectant especially in the form of its hydrochloride, gluconate, or acetate

3.2

antiseptic

chemical agent without added detergent(s) that kills most vegetative forms of pathogenic and other micro-organisms (but not necessarily all resistant micro-organisms) and that is suitable, when used as directed, for application to live tissue.

3.3

antiseptic skin cleanser

antiseptic that contains a detergent(s) and that cleans lightly soiled skin surfaces, kills most vegetative forms of pathogenic and other micro-organisms (but not necessarily all resistant microorganisms) and that is suitable, when used as directed, for application to live tissue.

3.4

batch

that quantity of sealed containers of antiseptic that have been filled from one homogeneous blend or, in the case of a continuous production process, that have been filled from one day's production.

4 Requirements

4.1 General requirements

4.1.1 The product shall be a clear or slightly opalescent, viscous and homogeneous water-miscible liquid and shall be one of the following types:

Type 1: an antiseptic skin cleanser; or

Type 2: an antiseptic without added detergents.

4.1.2 The product shall not be irritating to the normal skin and shall not contain any ingredients in a quantity that is toxic to human beings.

4.1.3 During storage at ambient temperature, the odour of the product shall remain such as to be acceptable, and when perfumed, the fragrance shall not change.

4.1.4 The product shall, remain stable up to its best before date in the original container under normal storage conditions, still comply with all the requirements of this standard.

4.2 Specific requirements

4.2.1 When the product is tested in accordance with Annex A, it shall, within 1 min, obtain a log reduction of at least three (3 $\log_{10}$) of the organisms listed in A.4.1, in cases where:

a) the supplied product contains 5 % chlorhexidine gluconate or more; or
b) the supplied product contains less than 5 % chlorhexidine gluconate, at the prescribed concentration.

4.2.2 The product shall also comply with the requirements given in Table 1, when tested in accordance with the methods described therein.

Table 1 – Specific requirements

S/N	Parameters	Requirements	Test methods
(i)	Water insoluble matter, g/l, max	2	Annex B
(ii)	Corrosiveness to aluminium	pass the test	Annex C
(iii)	pH	5.5 – 6.5	RS ISO 10523
(iv)	Total plate count, cfu/ml, max.	10	RS ISO 7218
(v)	Foaming properties	Pass test	Annex E

4.2.3 Consistency and storage stability

4.2.3.1 In accordance to Annex D, the product shall remain homogeneous and free-flowing. After standing at a temperature of 20 °C ± 5 °C for a further 24 h, the antiseptic shall show no sign of precipitation or separation.

4.2.3.2 The product shall show no sign of precipitation or separation and shall comply with all the relevant requirements of this standard.

5 Packaging and labelling

5.1 Packaging

5.1.1 The containers (and their closures) in which the product is packaged shall not interact chemically or physically with the antiseptic. They shall be of an acceptable design and shall be strong enough to protect the antiseptic during normal handling, transportation and storage.

5.1.2 The closure shall not be made of cork or of any material that contains cork.

5.1.3 Only containers of the same size and bearing the same batch identification shall be packed together in a bulk container.

5.2 Labelling

In addition to the labeling requirement in accordance with RS EAS 346, the following information shall appear in legible and indelible marking on each container or on a label securely attached to each container (if containers are too small to accommodate the instructions regarding dilution rate):

a) the manufacturer's name and trade name or trademark;
b) a statement that the product is an antiseptic or an antiseptic skin cleanser (whichever is applicable) based on chlorhexidine gluconate;
c) the type of antiseptic (see 4.1);
d) the concentration, as a percentage of the total volume, of the chlorhexidine gluconate content of the antiseptic skin cleanser;
e) the nominal volume of the contents, in plain type and in a colour that contrasts distinctly with that of the container or label;
f) the batch identification or the production date (or both);
g) the expiry date of the batch;

h) general instructions for use, including the prescribed concentration (the dilution rates), for the various purposes for which the antiseptic is suitable;
i) the pH value at the prescribed concentration (see 6.2 (h)); and
j) the following warnings:
   1 unless recommended by the manufacturer, do not mix the antiseptic with other substances; and
   2 the antiseptic is sensitive to temperature and light and should be stored in closed containers in a dry place at a temperature of 23 °C ± 2 °C/50 %RH, and protected from intense light.

6 Inspection

Visually, or otherwise, inspect each container, taken in accordance with RS 278 for compliance with the requirements of the standard.

Annex A
(normative)

Bactericidal efficacy

A.1 Apparatus

The apparatus and equipment shall comply with the requirements given in ISO 7218.

A.2 Accuracy

Except where otherwise specified, allow the following tolerances:

a) on temperatures..... ± 2 °C
b) on masses..... ± 1,0 %
c) on volumes..... ± 1,0 %
d) on pH value..... ± 0,1 pH units

A.3 Test media

A.3.1 General

Media and reagents shall comply with the requirements given in ISO 7218 and ISO 11133. Many of the media required are obtainable commercially in dehydrated form and, for uniformity of results, the use of such media is recommended. If these are used, follow the manufacturer's instructions strictly regarding the reconstitution and sterilization. The water used in the preparation of media shall be distilled water.

A.3.2 Tryptone soy broth

A.3.2.1 Ingredients

Tryptone ..... 17,0 g
Sodium chloride..... 5,0 g
Soy peptone ..... 3,0 g
Potassium hydrogen dibasic phosphate..... 2,5 g
Dextrose ..... 2,5 g
Water ..... 1 000 mL

NOTE Nutrient broth may be used instead of tryptone soy broth.

A.3.2.2 Preparation

Dissolve the ingredients in water. Heat, if necessary, to ensure that the ingredients are completely dissolved. Adjust the pH value to 7.3. Dispense 10 mL volumes into tubes and sterilize by autoclaving at (120_{0}^{+3}) °C for 15 min.

A.3.3 Nutrient agar

A.3.3.1 Ingredients

Agar ..... 15,0 g
Peptone ..... 5,0 g
Sodium chloride..... 5,0 g
Yeast extract..... 2,0 g
Beef extract ..... 1,0 g
Water ..... 1 000 mL

A.3.3.2 Preparation

Dissolve the ingredients in the water and bring to boil. Adjust the pH value to 7.1. Dispense 10 mL and 15 mL volumes into suitable bottles and sterilize by autoclaving at (120_{0}^{+3})°C for 15 min. Allow the 10 mL volumes to solidify in a sloped position.

A.3.4 Neutralizer media

A.3.4.1 tryptone soy azolecitn inactivator

A.3.4.1.1 Ingredients

Tryptone soy broth (see 5.2.3.2) ..... 100 mL
Soy lecithin (azolectin)..... 3.0 g
Polyoxyethylene sorbitan mono-oleate ..... 20 g

A.3.4.1.2 Preparation

Boil the ingredients with constant stirring until the ingredients are completely dissolved. Cool down to room temperature. Adjust the pH value to between 7.0 and 7.4. Dispense 9 mL volumes into suitable bottles and sterilize by autoclaving at (120_{0}^{+3})°C for 15 min.

NOTE Sometimes the azolectin/polyoxyethylene emulsion can settle to the bottom of the container after sterilization. If this happens, storage of approximately one week at room temperature usually allows the solids to redissolve. If the neutralizer is required sooner, heating in a water bath at (45_{0}^{+2})°C followed by occasional swirling during cooling will redissolve the solids.

A.3.4.2 Cetrimide inactivator

A.3.4.2.1 Ingredients

Polyoxyethylene sorbitan mono-oleate ..... 8,0 g
Sodium taurocholate..... 8,0 g
Sodium thiosulfate ..... 1,5 g
Potassium phosphate monobasic ..... 0,5 g
Sodium citrate..... 0,5 g
Water ..... 1 000 mL

A.3.4.2.2 Preparation

Dissolve the ingredients in the water by heating. Dispense 20 mL volumes into suitable bottles and sterilize by autoclaving at (120_{0}^{+3})°C for 15 min.

A.4 Reference cultures

A.4.1 Test organisms

Use the following test organisms:

a) *Pseudomonas aeruginosa*: Pse 16, ATCC 15442; and
b) *Staphylococcus aureus*: Sta 10, ATCC 6538.

A.4.2 Maintenance of test organisms

A.4.2.1 From a newly opened freeze-dried culture, or recently received agar culture, subculture each test organism into tubes of tryptone soy broth (see A.3.2).

A.4.2.2 Incubate the tubes at 37 °C for 24 h. Subculture from the tubes onto nutrient agar (see A.3.3) for each organism. Incubate the slopes at 37 °C for 24 h.

A.4.2.3 From each of these slope cultures, prepare four subcultures (stock cultures) of each test organism onto 10 mL nutrient agar slopes (see A.3.3). Incubate the stock cultures at 37 °C for 24 h and then store them in a refrigerator at 4 °C, except for *Pseudomonas aeruginosa*, which is stored at ambient temperature.

A.4.3 Preparation of the reference cultures for test suspensions

A.4.3.1 From a stock culture (see A.4.2.3), inoculate each of the test organisms into tubes of tryptone soy broth (see A.3.2) and incubate at 37 °C for 24 h.

A.4.3.2 For the test, use a 24 h culture that has been subcultured for two successive days.

A.4.3.3 Use a haemocytometer, Petroff-Häusser counting chamber or any other suitable means to ensure that the concentration of the test organisms is 1.0×10^7 to 1.0×10^8 organisms per millilitre.

A.5 Test procedure

NOTE Before the test for bactericidal efficacy is carried out, it is recommended that the efficacy of the inactivator be checked to ensure that the antiseptic to be tested is effectively neutralized.

A.5.1 Using sterile distilled water, prepare a dilution in accordance with the instructions on the label, where relevant.

A.5.2 Dispense 9 mL volumes of the sample aseptically into two sterile test tubes and place them in a water bath maintained at 37 °C for 10 min.

A.5.3 Repeat the procedure in A.5.2, but use 9 mL of sterile distilled water for the control.

A.5.4 Add 1 mL of the *Staphylococcus aureus* suspension (see A.4.3.3) to one of the tubes (see A.5.2) and start a stopwatch immediately.

A.5.5 Using a vortex, mix the contents of the tube and replace it in the water bath.

A.5.6 Repeat the procedure in A.5.4 to A.5.5 (inclusive) for the control (see A.5.3).

A.5.7 Using a clean sterile pipette, draw up 1 mL of the mixture from the tube (see A.5.5). At exactly 1 min exposure time, expel the mixture into a 9 mL inactivator medium (see A.3.4.1).

A.5.8 Using a vortex, mix well. Allow the mixture to stand for at least 10 min before continuing with the test procedure.

A.5.9 Using sterile distilled water as diluent, prepare tenfold dilutions of the mixture in A.5.8.

A.5.10 Using a sterile pipette, dispense 1 mL of each dilution in A.5.9 into a labelled Petri dish. Add 15 mL of cooled nutrient agar (see A.3.3) to each Petri dish.

A.5.11 Mix thoroughly and allow the agar to solidify.

A.5.12 Repeat the procedure in A.5.7 to A.5.11 (inclusive) for the control (see A.5.3).

A.5.13 Invert the plates and incubate at 37 °C for at least 36 h.

A.5.14 After incubation, examine the plates for growth (the sample plates and the control plates). Count and record all the colonies on each plate that contains between 30 and 300 colonies. Ensure that the colonies that have been counted are derived from survivors of the test organisms and not from contamination.

A.5.15 Take the dilution factor (*DF*) for the sample and for the control as the inverse of the dilution, for example, if the sample has been diluted to 1:1 000, take *DF* as 1 000.

A.5.16 The log reduction (*RI*) shall be determined using the following formula:

$$RI = \log B - \log A$$

Where,

RI is the log reduction;

log B is the number of organisms counted in the control (see A.5.14); and

log A is the number of organisms counted in the sample (see A.5.14).

NOTE Results may also be given in percentage kill.

A.5.17 Repeat the procedure described in A.5.1 to A.5.16 (inclusive) using *Pseudomonas aeruginosa* as the test organism.

A.5.18 Repeat the whole test described in A.5.1 to A.5.16 (inclusive) once more, but not on the same day.

A.6 Interpretation of results

A.6.1 Deem the sample to comply with the requirements of 4.2 if, for each organism tested, a 3 $\log_{10}$ reduction is obtained.

A.6.2 If there is a discrepancy in results between the two tests (see A.5.16 and A.5.18), repeat the whole test once more.

A.6.3 If the test in A.5.16 shows a 3 $\log_{10}$ reduction, and if one of the results from A.5.18 or A.6.2 also shows a 3 $\log_{10}$ reduction, the sample shall be deemed to have passed.

Annex B
(normative)

Determination of matter insoluble in water

B.1 Standard hard water

B.1.1 Ingredients

Calcium chloride ($CaCl_2.2H_2O$) ..... 0.880 g

Magnesium sulphate ($MgSO_4.7H_2O$) ..... 0.986 g

Water ..... 5,000 mL

B.1.2 Preparation

Dissolve the calcium chloride and magnesium sulfate in distilled water and dilute to 5 L.

B.2 Procedure

B.2.1 Pipette 5.0 mL of the sample into a beaker and add 250 mL of the standard hard water (see B.1).

B.2.2 Heat in a steam bath with frequent stirring until the sample is completely dispersed.

B.2.3 Filter the solution immediately, under suction, through a tared 1.6 μm glass fibre filter and ensure that the insoluble matter is quantitatively transferred to the filter.

B.2.4 Wash the beaker and the residue five times with 20 mL volumes of hot standard hard water (see B.1). Wash the filter with distilled water to remove salts from the hard water.

B.2.5 Allow the solution to drain completely and dry the residue at 105 °C ± 5 °C until constant mass is attained. Cool in a desiccator and weigh.

B.3 Calculation

Calculate the water-insoluble matter content (*S*) in the test solution, expressed in grams per litre, using the following formula:

$$S\ (g/l) = 200\ M\ (g)$$

Where,

S is the water-insoluble matter content, in grams per litre; and

M is the mass of the residue after it has been dried, in grams.

Annex C
(normative)

Determination of corrosiveness to aluminium

C.1 Principle

A test strip is completely immersed in a detergent or a cleaning agent of a specified concentration. After conditioning the immersed test strip for a specified time under controlled temperature, the severity of the resulting corrosion is assessed visually for signs of pitting and lost lustre, or by determining the loss in mass of the test strip per exposed surface area.

C.2 Apparatus and reagents

C.2.1 Apparatus

C.2.1.1 Normally available laboratory glassware.

C.2.1.2 Laboratory oven, maintained at temperature of 105 ºC ± 5 ºC

C.2.2 Apparatus and reagents

C.2.2.1 Ethanol

C.2.2.2 Acetone

C.3 Corrosiveness to steel

C.3.1 Test strip

One strip, of size approximately 80 mm x 10 mm x 1 mm, steel of a grade specified in the relevant national standard.

C.4 Procedure

C.4.1 Accurately determine the total area of the surfaces of the steel test strip and degrease it by washing in a mixture of equal volumes of ethanol and acetone. After allowing the strip to air-dry,heat it for 30 min in an oven maintained at 105 °C ± 5 °C, cool it in a desiccator and immediately determine its mass to the nearest 0.1 mg.

C.4.2 Prepare the test solution of the detergent or cleaning agent under test, at a concentration recommended by the manufacturer, and transfer 250 mL of the freshly prepared solution to a suitably stoppered glass bottle.

C.4.3 Completely immerse the steel test strip in the solution in the glass bottle. Stopper the bottle and heat it in an oven maintained at the temperature for the period as specified in the relevant national standard.

C.4.4 Remove the test strip from the test solution, rinse it thoroughly, first with water, then with acetone, and allow it to air-dry. Then heat the test strip for 30 min in an oven maintained at105 °C ± 5 °C, cool it in the desiccator, and immediately determine its mass to the nearest 0,1 mg.

C.5 Calculation

Calculate the loss in mass in mg/100 mm^2 of surface area as follows:

$$M = \frac{100}{A} X (m1 - m2)$$

Where,

M is the loss in mass of the steel test strip, in milligrams per 100 square millimetres;
A is the total surface area, in square millimetres;
m_1 is the mass of the test strip before the test, in milligrams;
m_2 is the mass of the test strip after the test, in milligrams.

C.6 Corrosiveness to aluminium

C.6.1 Test strip

One strip, of dimensions 75 mm × 19 mm × 1.5 mm, of bright finished uncoated aluminium of a grade specified in the relevant national standard.

C.6.2 Procedure

NOTE Should the mass loss evaluation method be required for the corrosiveness to aluminium, use the procedure given in C.4.

C.6.2.1 Degrease the aluminium test strip by washing it in a mixture of equal volumes of ethanol and acetone. Allow the strip to air-dry, then heat it for 15 min in an oven at 105 °C ± 5 °C and allow it to cool in a desiccator.

C.6.2.2 Prepare the test solution of the detergent or cleaning agent under test, at a concentration recommended by the manufacturer, and transfer 250 mL of the freshly prepared solution to a suitably stoppered glass bottle.

C.6.2.3 Completely immerse the aluminium test strip in the test solution in the bottle. Stopper the bottle and heat it in an oven maintained at the temperature for the period as specified in the relevant national standard.

C.6.2.4 Remove the test strip from the test solution, rinse it thoroughly with water and then acetone and allow it to air-dry. Then heat the test strip for 30 min in an oven at 105 °C ± 5 °C and cool it in a desiccator.

C.6.2.5 Remove the test strip, and then visually inspect it for evidence of pitting, etching or discoloration.

Annex D
(normative)

Consistency and storage stability

C.1 Separately store samples of the product in their original unopened containers at 5 °C ± 1 °C and at 43 °C ± 1 °C for 48 h. Test the contents of the containers for compliance with the requirements of 4.7.1.

C.2 Store samples of the product at 23 °C ± 2 °C/50 %RH for a period of 12 months from the date of manufacture and then test the content of the containers for compliance with 4.2 and 4.7.2.

Annex E
(normative)

Determination of foaming properties

E.1 Accurately transfer 10 mL of the product and 40 mL of the standard hard water to a 100 mL measuring cylinder with a stopper.

E.2 Shake the cylinder vigorously for 30 s.

E.3 After 60 s, examine the contents of the measuring cylinder for compliance with 4.8.

DRS 383: 2018

ICS 11.080.20