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Alcohol-based hand sanitizers — Specification

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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 454 was prepared by Technical Committee RSB/TC 024, *Organic and Inorganic Chemicals.*

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

1) RS EAS 789: Instant hand sanitizers — Specification

2) FDARS 1470: Hand sanitizer (alcohol-based) — Specification

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

Committee membership

The following organizations were represented on the Technical Committee on Organic and Inorganic Chemicals (RSB/TC 024) in the preparation of this standard.

Star Construction and Consultancy Ltd

Rwanda Inspectorate, Competition and Consumer Protection Authority

Rwanda Food and Drugs Authority

Rwanda Investigation Bureau

Rwanda Forensic Laboratory

Rwanda Social Security Board

Rwanda Environment Management Authority

BARANYUZWE Cosmetics Ltd

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Introduction

The alcohol contained within hand sanitizers, when rubbed on the surface of skin is effective in killing 99.9% of dangerous germs on the skin. The type of alcohol used in most hand sanitizers is ethyl alcohol. Ethyl alcohol is the active ingredient in most hand sanitizers. A concentration of 60% to 95% alcohol in hand sanitizing product is recommended. However, different scientific literatures have shown that alcohol, at concentration of above 70% is a potential virucidal agent inactivating all of the lipophilic viruses and many hydrophilic viruses (e.g., adenovirus, enterovirus, rhinovirus and ratoviruses).

There are also several other non-active ingredients in hand sanitizer, the second most concentrated ingredient is water.

Alcohol-based hand sanitizers — Specification

1 Scope

This Draft Rwanda Standard specifies the requirements, sampling and test methods for alcohol-based hand sanitizers.

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 EAS 377, *Cosmetic Products (all parts)*

AOAC 942.06, *Alcohol by Volume in Distilled Liquors Pycnometer Method*

RS ISO 10523, *Water quality — Determination of pH*

3 Terms and definitions

For the purposes of this standard, the following terms and definitions apply.

3.1

hand sanitizer

antiseptic agents used to cleanse the hands with the aim to protect and prevent the passage of bacteria, virus and other pathogens that can cause infections.

3.2

alcohol

ethanol (ethyl alcohol, C_2H_5OH), isopropanol (isopropyl alcohol, $CH_3CHOHCH_3$), n-propanol (1-propanol, $CH_3CH_2CH_2OH$) or the mixture of them.

3.3

antimicrobial efficacy

efficiency of the product to kill or reduce microorganisms, such as bacteria, fungi and viruses.

3.4

dermal irritation

production of reversible damage of the skin following the application of a test substance for up to 4 hours.

4 Requirements

4.1 General requirements

4.1.1 The product shall be in the form of liquid or gel.

4.1.2 The product shall be clear and free from visible impurities.

4.1.3 The product shall not have any disagreeable odour or smell. It may be coloured or not.

4.1.4 The substances used in the formulation shall conform to all parts of RS EAS 377.

4.2 Specific requirements

The product shall also comply with the specific requirements given in the table 1 when tested in accordance with the corresponding test method.

Table 1 — Specific requirements for alcohol-based hand sanitizers

S/N	Characteristic	Requirement	Test method
i)	Alcohol content (ethanol and/or isopropanol, n-propanol), % v/v, min. [a]	60.0	AOAC 942.06
ii)	pH, neat	6 – 8	RS ISO 10523
iii)	Antimicrobial efficacy	To pass test	Annex A
iv)	Dermal irritation	To pass test	Annex B
v)	Physical impurities	To pass test	Annex C
[a] The product that has been declared as potential virucidal agent shall contain more than 70% of alcohol content in order to be effective as a virucidal agent inactivating all of the lipophilic viruses and many hydrophilic viruses (e.g., adenovirus, enterovirus, rhinovirus and ratoviruses)			

5 Packaging and labelling

5.1 Packaging

5.1.1 The packaging shall ensure integrity of the product during handling, storage and transportation.

5.1.2 Bulk packaging: Only the sanitizer of the same type and the same batch shall be packed together in one bulk package

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

5.2 Labelling

The following information shall appear in legible and indelible labelling on each container or on a label securely attached to each container:

a) name of the product as “alcohol-based hand sanitizer”;

b) name and full address of the manufacturer;

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

c) percentage of alcohol used

NOTE The tolerance limit from the declared alcohol content shall be less than 1

d) list of ingredients in descending order.

e) net content

f) batch identification;

g) manufacture and expiry dates;

h) general instructions for use;

i) storage conditions;

j) the following cautionary warnings:

1) “do not allow the sanitizer to come into contact with eyes”;

2) “keep out reach of children”;

3) “if swallowed contact a doctor”; and

4) “highly flammable, keep away from fire or flame”.

6 Sampling

6.1 General

The following sampling procedure shall be applied in determining whether a lot submitted for inspection and testing complies with the relevant requirements of this standard. The sample so drawn shall be deemed to represent the lot.

6.2 Sample for inspection

After inspecting the lot for compliance with Clause 4, take, at random, the number of containers, as relevant, shown in column 2 of Table 2, relative to the appropriate lot size shown in column 1.

Table 2 — Samples for inspection and testing

Lot size (number of containers)	**Sample size for physical examination** (number of containers)	**Sample size for microbiological examination** (number of containers)
0 to 5 000	3	3
5 001 to 12 500	6	3
12 501 to 25 000	9	3
25 001 to 50 000	16	3
50 001 upwards	30	3

6.3 Sample for testing

After inspection of the containers taken in accordance with 6.2,

a) take, at random, half the number of containers and use them for the storage stability test; and

b) thoroughly mix the contents of each of the remaining containers and, take from each container the lesser of the total volume and 250 mL, and obtain a composite test sample by combining and thoroughly mixing these quantities. Use these samples for testing for compliance with the requirements of Clause 4.

Annex A
(normative)

Determination of antimicrobial efficacy

A.1 Outline of the method

A.1.1 The sanitizer 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 sanitizer with bacterial inoculum, withdrawing a sample after a given time and culturing the sample in a suitable recovery medium. After this sampling, the mixture is again challenged by a second inoculum and after a second interval, is again sampled for culturing. This process is then repeated to provide a third challenge.

A.1.2 The sample is considered to have passed or failed the test according to the extent of growth shown in the first two cultured samples**.**

A.1 Apparatus

A.2.1 Facility, for incubation at 37 ± 1 °C.

A.2.2 Facility, for incubation at 27 ± 1 °C.

A.2.3 Stop clock, indicating in seconds.

A.2.4 Facility, for refrigeration at 4 ± 1°C.

A.2.5 Universal containers — Made of glass and having metal tops with rubber liners. Plastic containers or glass containers with plastic tops shall not be used.

A.2.6 Test tubes — 19 mm X 150 mm.

A.2.7 Filter paper, No. 4 whatman (sterile) or equivalent.

A.2.8 Facility, for autoclaving at 121 ± 1°C.

A.2.9 Pipette, capable of dispensing 0.02 ± 1°C 0.005 ml.

A.2.10 pH meter

A.2.11 Facility, to sterilize by filtration.

A.2.12 150 µm test sieve.

A.2.13 Oven, capable of maintaining temperature at 100 ± 1 °C.

A.2 Media

A.3.1 *Growth media for test organisms.* Wright and Mundy Broth with Dextrose (WMBD).

A.3.1.1 Dispense 10 ml and 6 ml quantities of the Wright and Mundy Broth into universal bottles, and autoclave at 121 ± 1 °C for 12 minutes.

A.3.1.2 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).

A.3.2 Recovery medium — A nutrient broth prepared as follows:

A.3.2.1 Composition

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

A.3.2.2 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**.**

A.3.3 Hard water — Standard hard water with 342 mg/L (ppm) hardness prepared as follows:

Dissolve 0.304 g of anhydrous calcium chloride hexahydrate ($MgCl2.6H_2O$) in distilled water and make up the volume to one litre. Sterilize the standard hard water by autoclaving at 121 ± 1° C for 15 minutes. Allow this to reach room temperature before use**.**

A.3.4 Yeast suspension

A.3.4.1 Weigh to the nearest gram about 65 g of active dry yeast. Cream by the gradual addition of sterile hard water (A.3.3) 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.

A.3.4.2 Shake the contents of the flask vigorously and strain-through a 150 μm sieve (A.2.12) breaking down any remaining lumps.

A.3.4.3 Add 500 mL sterile hard water, shake vigorously.

A.3.4.4 Transfer 50 ml or 100 mL portions into screw-capped bottles, screw the caps tightly and autoclave at 121 ± 1°C for 15 minutes. Allow the autoclave to cool without releasing the pressure. Store cold but not freezing.

A.3.4.5 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.

A.3.4.6 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.

A.3.4.7 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.

A.3.5 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 ± 1°C for 15 minutes**.**

A.3 Selection of the most resistant organism by the minimum inhibitory concentration test

A.4.1 The following organisms shall be used for the test:

- *Pseudomonas aeruginosa* (NCTC 6749 or equivalent)
- *Proteus vulgaris* (NCTC 4635 or equivalent)
- *Staphyloccus 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.

A.4.2 Subculture each organism daily into a universal bottle containing 6 ml of growth medium (A.3.1) and incubate for 24 ± 2 h at 37 ± 1°C.

A.4.3 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 (A.3.1) before dilution, the P. aeruginosa, culture shall be filtered using a whatman No.4 filter paper.

A.4.4 Prepared three sets of ten, doubling dilutions of the sanitizer in universal containers (A.2.5). For this purpose dilute the neat sanitizer in the growth medium (A.3.1) or the recovery medium (A.3.2) to give a final volume of 5 ml of the diluted sanitizer for each dilution.

A.4.5 Inoculate each dilution in one set with 0.02 mL of a diluted culture of one organism (see A.4.3).

A.4.6 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 sanitizer, that is the organism for which the minimum inhibitory concentration is highest.

A.4 Preparation of inoculum

A.5.1 Daily sub-cultures of the test organism selected as in A.4.6 shall be grown in 6 ml quantities of the growth medium (A.3.1) and incubated at 37 ± 1°C for 24 ± 2 hours.

A.5.2 The day before the test, inoculate 10 ml of the growth medium (A3.1) with the test organism from a daily sub-culture and not more than a fourteenth. Incubate the inoculated, broth at 37 ± 1°C for 24 ± 2 hours.

A.5.3 Add 6 ml of the test organism culture (A.5.1) and (A.5.2) to 4 ml of the yeast suspension (A.3.4) 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**.**

A.5.4 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 A.3.5) 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**.**

A.5 Preparation of the sanitizer dilutions

Prepare three dilutions of the sanitizer in hard water (A.3.3) based on the recommended 'use dilution' of the sanitizer, 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 sanitizer dilutions shall be prepared and tested on the same day.

A.6 Test procedure

A.7.1 The test shall be carried out at 27 ± 1°C.

A.7.2 Dispense 3 mL of each dilution of sanitizer (A.6) into separate universal bottles labelled A, B, and C, then allow to equilibrate to 27 ± 1°C.

A.7.3 Add 1 mL of the inoculum to A, B and C at 0, 1 and 5 minutes respectively and mix by swirling gently.

A.7.4 Eight minutes after the addition of the inoculum, remove a sample of the inoculum/sanitizer 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**.**

A.7.5 Ten minutes after the first addition of the inoculum, add another 1 ml of the inoculum to each of the sanitizer dilutions and mix by swirling gently.

A.7.6 Eight minutes later, remove a sample of the mixture as put before (A.7.4) and put 0.02 mL into each of the second group of five tubes of recovery broths.

A.7.7 Twenty minutes after the first addition of the inoculum, add a further 1 mL of inoculum to each of the sanitizer dilutions and mix by swirling gently.

A.7.8 Eight minutes later, remove a sample of the mixture as before and place 0.02 ml into each of the third group of five tubes of recovery broths.

A.7.9 Swirl the recovery broths and incubate at 37 ± 1°C for 48 ± 2 h. Examine the growth and record the results**.**

A.7 Interpretation of results

A.8.1 The sanitizer, 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.

A.8.2 To be acceptable, an instant hand sanitizer shall pass the test on three separate occasions using freshly prepared sanitizer and freshly prepared inoculum on each occasion**.**

Annex B
(normative)

Determination of dermal irritation

B.1 Test panel

A test panel that consists of three men and three women, none of whom is known to have an abnormally sensitive skin or has an injury or abrasion on the hands.

B.2 Procedure

Place approximately 5 mL of the test sample onto the cupped palm of one hand of each member of the panel, and get him or her to spread the Hand Sanitizer over the back and between the fingers of the other hand, and rub it thoroughly into the skin for 2 min. Repeat this procedure twice, with 30 min intervals between applications. Do not allow a treated hand to be washed until 2 h after the last application of the test sample.

Immediately after the tests, and again 2 h, 24 h and 48 h later, examine the treated hand of each member of the panel for any signs of irritation or inflammation, using the untreated hand as a control.

Annex C
(normative)

Freedom from visible impurities

C.1 A sample of approximately 50 mL of each test sample is spread over the bottom of a 150 mm diameter Petri dish.

C.2 When viewed at a range of approximately 600 mm, the number of visible specks of impurities shall not exceed five.

Bibliography

[1] World Health Organization (WHO), *Guidelines on Hand Hygiene in Health Care*, 2009

[2] World Health Organization (WHO), *Guide to local production — WHO-recommended Handrub Formulations,* 2009.

[3] Rwanda Foods and Drugs Authority (FDA), *Guidelines on Preparation of Alcoholic Based Hand sanitizers,* 2020.

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