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PREFACE

This Zimbabwe Standard ZWS 302:2011: Quaternary ammonium compound disinfectants, is the second revision of ZWS 302:2000.

First endorsed 1972 as SAZS K31,
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This standard was prepared by technical committee CH 24: Chemicals, under the general direction of the Chemicals and Textiles Standards Council.

This standard makes reference to the following publication:

ZWS 459 : Measurement of water pH value.

The following interests were represented on the Technical Committee entrusted with the preparation of this standard:

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ZIMBABWE STANDARD SPECIFICATION FOR QUATERNARY AMMONIUM COMPOUND DISINFECTANTS

(Second Revision of SAZS 302:2000)

1. SCOPE

This Zimbabwe Standard Specification covers formulations based on quaternary ammonium compounds in liquid or in powder form for disinfecting inanimate surfaces.

NOTE. The title of the publication referred to in this standard is indicated in the Preface.

2. DEFINITIONS

For the purpose of this Zimbabwe Standard, the following definitions shall apply:

2.1 Acceptable. Acceptable to the purchaser but in relation to the mark certification acceptable to the Standards Association of Zimbabwe.

2.2 Active Ingredient. Chemical in the formulation of a disinfectant or sanitizer that kills micro-organisms.

2.3 Aseptic. Descriptive of the avoidance of contamination by environmental micro-organisms.

2.4 Batch. Those sealed containers that have been filled from one homogenous blend and in the case of continuous production processes, that represent one day's production.

2.5 Defective. A container or a test sample that fails in one or more respects to comply with the appropriate requirements of the standard.

2.6 Detergent. Cleaning compound composed of mixtures of ingredients that interact with soils (and degrade specific food soil components) in different ways and facilities their removal from surfaces.

2.7 Disinfectant. An antibacterial agent that is applied to non-living objects and facilitates the elimination of disease causing organisms from surfaces.

2.8 Lot. That quantity of quaternary ammonium compound disinfectant in sealed containers of the same size, bearing the same batch identification, from one manufacturer, submitted at any one time for inspection and testing.

2.9 Sanitizer. A substance that simultaneously cleans and disinfects.

3. REQUIREMENTS

3.1 Disinfecting Efficacy. When tested in accordance with 6.2, the relevant dilution of the product {see 4.2.1 (g)} shall kill at least 99,9 % of the following organisms within 5 min:

a) *Staphylococcus aureus*;

b) *Escherichia coli* and

c) *Pseudomonas aeruginosa*.

3.2 Odour. When the product is used for the treatment of food utensils in the manner described on the label, it shall not leave any odour on the utensils.

3.3 pH Value. When determined in accordance with 6.3, the pH value of the strongest recommended solution in distilled water shall not exceed 10,0.

4. PACKAGING AND MARKING

4.1 Packaging. The containers (including the closures) in which the product is packed shall not interact chemically or physically with the product, shall be of acceptable design, and shall be strong enough to protect the product adequately during storage and transport. The closure shall not be made of cork or of any material containing cork. Only containers of the same size and batch identification shall be packed together in a bulk container.

4.2 Markings.

4.2.1 Individual containers. The following information shall appear prominently, legibly and durably on each container or on a label securely attached to each container.

a) a statement that the disinfectant contains quaternary ammonium compound(s);

b) a statement of the nominal volume or mass of the contents in metric units. The statement of volume or mass shall be in plain type and in a colour which affords a distinct contrast to the colour of the container or label;

c) the batch identification number;

d) the production date of the batch;

e) the expiry date;

f) general instructions for use for the various purposes for which the disinfectant is suitable (the instructions shall include the recommended dilutions and the minimum exposure period for each purpose);

g) the dilution that complies with the requirements of 3.1;

h) a statement that incompatible substances (such as soap and anionic detergents) should not be used with the product;

i) a statement that the product should preferably be used on previously cleaned surfaces rinsed with potable water;

j) a warning that contamination of food stuffs with the product should be avoided;

k) a statement that painted surfaces and linoleum, asphalt, rubber and plastic coverings must be thoroughly rinsed with water within 15 min of treatment.

l) a statement relating to the toxicity levels of the product;

m) a statement on safety precautions to be taken when using the product;

n) a statement on first aid in case of internal, external consumption or direct skin contact.

5. SAMPLING AND COMPLIANCE WITH THE STANDARD

NOTE. This clause applies to the sampling for inspection and testing before acceptance or rejection of single lots (consignments) in cases where no information about the implementation of quality control or testing during manufacture is available to help in assessing the quality of the lot. It is also used as the procedure for adjudicating in cases of dispute.

5.1 Sampling. The following sampling procedure shall be applied in determining whether a lot complies with the requirements of the standard. The samples so drawn shall be deemed to represent the lot.

5.1.1 Sample for inspection. From the lot, take at random and in relation to the appropriate lot size shown in Column 1 of Table 1 the number of containers shown in Column 2 of the same table.

TABLE 1 – SAMPLING FOR INSPECTION

Lot size, containers	Sample size, containers
1 – 10	1
11 – 300	2
301 – 800	3
801 – 2 000	4
2 001 – 8 000	5
more than 8 000	6

5.1.2 Sample for testing. After inspecting (see 6.1) the containers drawn in accordance with 5.1.1;

a) in the case of liquids, take from each container all the contents of ½ litre (whichever is less) and obtain a composite test sample by thoroughly combining and mixing these quantities; and

b) in the case of powders, take from each container all the contents or ½ kg (obtained by coning and quartering) whichever is less and use each sample so taken as a test sample, i.e. do not combine them to form a composite sample.

5.2 Compliance with the Standard. The lot shall be deemed to comply with the requirements of the standard if:

a) after inspection of the sample taken in accordance with 5.1.1 and

b) after testing of the sample(s) taken in accordance with 5.1.2 no defective is found.

6. INSPECTION AND METHODS OF TEST

NOTE. The tests shall be undertaken by persons experienced in microbiological techniques, using aseptic techniques.

6.1 Inspection. Inspect the containers taken in accordance with 5.1.1 for compliance with the requirements of Clause 4.

6.2 Disinfecting Efficacy

6.2.1 Apparatus

6.2.1.1 General. All glassware shall be clean and sterile. Wash in detergent solution, glassware that has not come into contact with the disinfectant, rinse thoroughly with tap water and finally with distilled water.

Wash in detergent solution, all glassware that has been in contact with quaternary ammonium compounds, rinse thoroughly in tap water and leave overnight in chromic-sulfuric acid solution. Then rinse the glassware thoroughly with tap water and finally with distilled water.

NOTE. Owing to the tendency for quaternary ammonium compounds to be adsorbed on to the surface of glassware, care must be taken to ensure that no residues are present on the glassware at the beginning of the test.

Dry all glassware at room temperature or in a hot-air oven maintained at 110 $\pm$ 5 ^{o}C, and seal with suitable closures.

Sterilize all glassware, preferably by the application of dry heat 170 $\pm$ 5 ^{o}C for 1 h. Where use of dry heat is not practicable, e.g. in the case of glassware fitted with rubber closures, autoclave at 121 $\pm$ 2 ^{o}C for 20 min.

The main major ingredients which constitute the major disinfectants and sterilizers are listed below. Their action on glass surfaces and whether they absorb on glass surfaces is summarized in Table 2.

6.2.1.2 Bottles. Glass bottles, 30 mℓ or 110 mℓ, fitted with metal screw caps are preferably to test tubes or flasks, but the use of the latter, if fitted with suitable closures, is permissible.

TABLE 2 -

Active ingredient	Adsorption on glass surfaces
Phenol and phenol compounds	Yes
Alcohols	No
Hologens and their compounds	
• Iodine	No
• Chloride and chlorine compounds	No
• Hypochlorites	No
• Chloramines	No
Heavy metals	Rarely used in detergent formulations
Heavy metal compounds	Rarely used in detergent formulations
Detergents	Yes
• Quaternary ammonium compounds	
Aldehydes	
• Gluteraldehyde	Slight
• Formaldehyde	Slight

6.2.1.3 Stop-watch. A stopwatch with an error not exceeding one second per hour.

6.2.2 Preparation of media, solutions and suspensions

6.2.2.1 Water. Unless otherwise stated, only glass-distilled water (or demineralized water of an equal purity) that is free from organic matter shall be used.

6.2.2.2 Ingredients. The ingredients used in the preparation of the media shall be suitable for microbiological test purposes.

6.2.2.3 Dehydrated media. Dehydrated culture media may be used instead of the media described, provided that these dehydrated preparations conform to the descriptions given and yielded equivalent results.

6.2.2.4 Limits of accuracy. Unless otherwise specified, the following limits of accuracy shall apply in the preparation of media, solutions and suspensions:

Measurements of mass and volume : $\pm$ 5 %
Measurement of pH values : $\pm$ 0,1 units

6.2.2.5 Adjustment of pH value of media. Unless otherwise specified, use a 0,1 M solution of hydrochloric acid or sodium hydroxide, as relevant. The reagent shall be of analytical reagent quality.

6.2.2.6 Filtration of media. Whenever it is necessary to filter a medium in the course of its preparation, use the following procedure:

a) Filter media which do not contain solidifying agents (i.e. liquid media) through a medium-speed filter paper.

b) Filter media containing solidifying agents (e.g. gelatin or agar) through a layer of pre-wetted paper pulp 10 to 15 mm thick. To prevent solidification of the medium during filtration, use a stem-jacketed funnel. Alternatively, carry out the filtration in a steam chamber.

6.2.2.7 Sterilization by autoclaving. Where sterilization by autoclaving is specified, autoclave the medium at 121 ± 2 °C for 15 min, unless otherwise stated.

NOTE. This temperature corresponds to a pressure of 1, 036 bar above atmospheric pressure at sea level.

6.2.2.8 Storage of media. Ensure that prepared media are not exposed to heat and to sunlight.

6.2.3 Media

6.2.3.1 Nutrient medium

Ingredients	:	beef extract ……..	1 g
		peptone …….	5 g
		sodium chloride ……	5 g
		yeast extract ……..	2 g

Dissolve the ingredients in water by warming and dilute to 1 00 mℓ. So adjust the pH value that after autoclaving it will be 7.1. Dispense in 10 mℓ volumes. Sterilize by autoclaving.

6.2.3.2 Nutrient agar

Ingredients	:	agar ……..	15 g
		beef extract ……..	1 g
		peptone …….	5 g
		sodium chloride ……	5 g
		yeast extract ……..	2 g

Dissolve the ingredients in water by boiling and dilute to 1 000 mℓ. So adjust the pH value that after autoclaving it will be 7.1. Dispense in volumes of 5 and 15 mℓ. Sterilize by autoclaving. Allow the smaller (5 mℓ) volumes to set in a sloped position.

6.2.3.3 MacConkey medium (purple)

Ingredients	:	bile salts……..	5 g
		bromocresol purple…… …..	0,01 g
		lactose …….	10 g
		peptone…… ……	20 g
		sodium chloride ……..	5 g

Dissolve the ingredients in water by warming and dilute to 1 000 mℓ. So adjust the pH value that after autoclaving it will be 7.4. Dispense 10 mℓ volumes into containers fitted with inverted Durham tubes. Sterilize by autoclaving.

6.2.3.4 *Staphylococcus* medium

Ingredients	:	dipotassium phosphate (K_2HPO_4)..	5 g
		lactose …….	2 g
		mannitol…… ……	10 g
		sodium chloride ……..	5 g
		tryptone …….	75 g
		yeast extract ……...	2,5 g

Dissolve the ingredients in water by warming and dilute to 1 000 mℓ. So adjust the pH value that after autoclaving it will be 7.2. Dispense 10 mℓ volumes. Sterilize by autoclaving.

6.2.4 Hard water. Dissolve 280 mg of anhydrous calcium chloride in 100 mℓ of water and dilute to 1 000 mℓ. Dispense 97 mℓ volumes into suitable containers, preferably 110 mℓ bottles with screw caps and hermetically seal the containers. Sterilize by autoclaving (when 97 mℓ is diluted to 100 mℓ, this water has a hardness of approximately 250 p.p.m, calculated as calcium carbonate).

6.2.5 Sterile skimmed milk

6.2.5.1 Prepare a 10 % solution of fat free skimmed milk in deionized water. Dispense 5 mℓ aliquots into clean test tubes and sterilize by boiling at 100 °C for 10 min (Sterilizing the milk solution at 121°C at 15 psi for 15 min will result in the milk coagulating and it will not be suitable for the sterility test). If the milk solution is not to be used immediately, it can be stored in a refrigerator at 4 ^{o}C.

6.2.5.2 Sterity test of the skimmed milk. To check the sterility of the skimmed milk solution as well as that it does not contain any other growth inhibiting factors, the following test is performed.

a) Prepare a 10 mℓ overnight broth culture *S.aureus* in sterile nutrient broth. Transfer 0,1 mℓ of the bacterial suspension into a sterile Petri dish. Pour 10 mℓ of sterile nutrient agar which has been precooled to 45 °C into the Petri dish. Swirl gently the contents to ensure thorough mixing. Allow the plate to set before proceeding with the test.

b) In the meantime, prepare some filter paper discs by punching holes on a filter paper using an office puncher. Wrap the discs in aluminium foil and sterilize them in a hot oven at 100 °C for 10 min.

c) After sterilization, use a sterile pair of fine forceps to dip one of the sterile discs into the sterilie skimmed milk. Carefully placed the wetted disc on the surface of the set nutrient gar plate and incubate overnight. Repeat the procedure above with overnight cultures of *E.coli* and *P.aereuginosa*.

d) After incubation, check for zones of growth inhibition around the filter paper discs. If no zones of growth inhabitation are visible on the plate, milk may be used for the test.

6.2.6 Inactivator. Prepare a sterile solution of a suitable inactivating agent. This inactivating agent shall when used as in 6.2.9.3;

a) be non-toxic to the test organisms;

b) be capable of instantaneously inactivating the quaternary ammonium compound under test;

c) be stable;

d) not render the agar plates opaque; and

e) not form lumps in the agar.

NOTE. The following inactivator has been found suitable for most quaternary ammonium compounds:

Ingredients	:	mono-potassium phosphate (KH_2PO_4) ….	0,5 g
		sodium citrate ($Na_3C_6H_5O_7.3H_2O$) ……	0,5 g
		sodium taurocholate ……...	8,0 g
		sodium thiosulfate ($Na_2S_2O_3.5H_2O$) ……..	1,5 g
		sorbitan mono-oleate complex ……	8,0 g

Dissolve the ingredients in water by heating and dilute to 1 000mℓ. Dispense in 20 mℓ volumes. Sterilize by autoclaving. This inactivator may also be used with some halogen containing disinfectants.

6.2.7 Test organism

6.2.7.1 Organisms to be used. The test organisms shall be:

Staphylococcus aureus	:	SATCC Sta 53
Escherichia coli	:	SATCC Esc 25
Pseudononas aeruginosa	:	SATCC Pse 2

NOTE. If any strain other than those mentioned in this standard is used the strain should be obtained from an approved culture collection.

6.2.7.2 Maintenance of test organisms. At intervals of 8 weeks prepare, from a newly opened freeze-dried culture or recently received agar culture of the test organisms, subculture as follows:

Subculture *E.coli* into a bottle of MacConkey medium (6.2.3.3), *S.aureus* into a bottle of 6.2.3.1 *Staphylococcus* medium (6.2.3.4) and *P.aeruginosa* into a bottle of nutrient medium (6.2.3.1). Incubate the bottles at 37 ± 2 °C for 24 to 48 h. Make subcultures from the cultures in the bottles on to slopes of nutrient agar (6.2.3.2). Incubate the slopes at 37 ± 2 °C for 24 h. From each of these slope cultures prepare simultaneously 4 subcultures (stock cultures) of each test organism on nutrient agar (6.2.3.2). Incubate the stock cultures at 37 ± 2 °C for 24 h and then store them in a refrigerator maintained at 4 °C.

6.2.7.3 Preparation of cultures for test suspensions. For each of the test organisms (6.2.7.1) proceed as follows:

Inoculate a nutrient agar slope (6.2.3.2) from a stock culture kept at 4 °C (6.2.7.2) and incubate it at 37 ± 2 °C for 24 h.

Continue subculturing on to fresh slopes at daily intervals, use for the test a subculture made on any day between the third and the fifteenth day (inclusive). Omission of one daily subculturing operation requires no special re-organization of procedure but, it subculturing cannot take place on two successive days, three successive daily subculturing operations must be carried out before organisms suitable for the test are obtained. After two weeks of subculturing as described, begin the process again with a fresh culture.

6.2.7.4 Preparation of test suspension. Using 10 mℓ of sterile water, wash from a slope (see 6.2.7.3) the bacterial growth resulting from 24 ± 1 h incubation, shake the suspension with a few sterile glass beads or scrap the surface of the solvent with a sterile loop, filter to remove clumps and dilute the suspension so obtained until it contains 100 000 ± 10 000 organisms per mℓ. Place it in a water bath maintained at 22 ± 0,5 °C before using it for the test. Use the suspension within 3 h of preparation.

6.2.8 Preparation of control and test solutions. For each test organism proceed as follows;

NOTE. For the purpose of checking the resistance of the test organisms and other test conditions, it is advisable to include a reference standard. It is essential that this is a quaternary ammonium compound, but, because it is difficult to select a universal standard, each laboratory should make its own choice of material.

6.2.8.1 Milk and hard water mixture. Immediately before testing, add aseptically 1 mℓ of sterile skimmed milk (6.2.5) to each of two bottles containing 97 mℓ of hard water (6.2.4). The pH value of the mixtures shall be 6,6 ± 0,2.

NOTE. When disinfectants in powder form are tested, only one such mixture is required.

6.2.8.2 Control solution. Add 1 mℓ of sterile water to the contents of one bottle of milk and hard water mixture.

6.2.8.3 Test solution.

6.2.8.3.1 Liquids. Prepare a solution of the test sample in sterile water of such concentration that when 1 volume is diluted to 100 volumes, the dilution will be that given on the label (see 3.1 g) and add 1 mℓ of this solution to the other milk and hard water mixture (see 6.2.8.1).

Alternatively, if the labelled dilution is equal to or less than 1 in 100, add the required quantity of sample directly to such a volume of the mixture as to produce a final volume of 99 mℓ.

6.2.8.3.2 Powders. Weigh directly into a dry, sterile 110 mℓ bottle a quantity of the sample that will, after the addition of 1 mℓ of sterile water, 1 mℓ of sterile skimmed milk (6.2.5), 1 mℓ of the suspension of the test organism and enough sterile hard water (6.2.4) to bring the total volume to 100 mℓ, produce the dilution given on the label (see 3.1 g). To the sample in the bottle add (in the order given) the appropriate volume of sterile hard water, 1 mℓ of sterile water, 1 mℓ of sterile skimmed milk.

NOTE. Each pipette used for the measurement of a solution containing a quatenary ammonium compound must, before use, be pre-rinsed at least twice with that solution.

6.2.8.4 Immediately after the dilution has been prepared, mix well but gently (to minimize foaming) and before testing, place the test and control solutions for 30 ± 0,5 min in a water bath maintained at 22 ± 0,5 °C.

6.2.9 Test procedure

6.2.9.1 Melt the contents of two bottles (containing 15 mℓ volumes) of nutrient agar (6.2.3.2), cool to 45 °C, and maintain them at this temperature.

6.2.9.2 When the solutions are ready for testing (see 6.2.8.4), add 1 mℓ of the *S.aureus* test suspension (6.2.7.4) to the test solution (without removing the bottle from the water bath), simultaneously start the stop-watch and, 30 sec later, add in the same way 1 mℓ of the same test suspension to the 99 mℓ of control solution. Close the bottles, remove them from the water bath, shake gently (ro minimize foaming) and immediately replace the bottles in the water bath maintained at 22 ± 0,5 °C.

6.2.9.3 Transfer aseptically 1 mℓ of inactivator solution (6.2.6) into a petri dish. About 20 sec before the end of the 5 min exposure period shake the test solution gently, draw up aseptically 1 mℓ of the test solution into a 1 mℓ pipette, graduated in 0,1 mℓ divisions, and at the exact end of the exposure period expel this into the inactivator solution in the petri dish and immediately mix the inactivator solution and the test solutions thoroughly. Add the melted agar from one bottle (see 6.2.9.1) to the mixture in the petri dish, mix thoroughly, allow to cool and invert.

6.2.9.4 Repeat the procedure given in 6.2.9.3 with the control solution.

6.2.9.5 Incubate the petri dishes at 32 ± 2 °C for 72 h.

6.2.9.6 After the incubation period has elapsed, count (with the aid of a colony counter) the colonies on each plate. Calculate the percentage kill from the result for the test solution and that for the control.

6.2.9.7 Repeat the test procedure described in 6.2.9.1 to 6.2.9.6 using successively, the *E.coli* and the *P.aeruginosa* test suspensions, but incubate the petri dishes for 48 h at 32 ± 2 °C.

6.2.9.8 Repeat the whole test (6.2.8 to 6.2.9) on two subsequent days.

6.2.10 Interpretation of results. Deem the sample to comply with the requirements of 3.1 if, for each organism tested, no result is below 99,9 % kill.

6.3 pH Value. Use ZWS 459.