

PS: 3509-2018

UDC: 668.585

**PAKISTAN STANDARD SPECIFICATION
FOR
SHAMPOO (1ST REVISION)**



**PAKISTAN STANDARDS AND QUALITY CONTROL AUTHORITY,
STANDARDS DEVELOPMENT CENTRE,
PSQCA Complex Plot # ST-7/A, Block-3, Scheme No: 36, Gulistan-e-Johar,
Karachi.**

PAKISTAN STANDARDS AND QUALITY CONTROL AUTHORITY
STANDARDS DEVELOPMENT CENTRE
(CHEMICAL DIVISION)

MEMBERS LIST OF COSMETICS AND TOILET GOODS TECHNICAL COMMITTEE (CTC – 18)

CHAIRMAN

- 1. Prof Dr. Abdul Malik**
Senior Professor
HEJ, Research Institute of Chemistry,
University of Karachi
Cell # 0300-2007250
abdul.malik@iccs.edu,

MEMBERS

- 2. Dr. Nighat Afza**
PCSIR
Cell No. 03008218862
hocprc@yahoo.com
- 3. Mr. Muhammad Arif**
Sanofi Pakistan Ltd,
Cell: 0333-3320706
Email: arif.kafray@sanofi.com
- 4. Mr. Ammar Akram Qureshi**
Unilever
Cell: 0300-2612313
Email: ammar.queshi@unilever.com
- 5. Dr. Rahat Sultana**
Department of Chemistry
University of Karachi
Cell: 0333-2189859
Email: rahat_sultana@yahoo.com
- 6. Mr. Hasan Fawad**
P&G Pakistan Karachi
Cell: 0332-8241350
Email: fawad.h@pg.com
- 7. Dr. Syed Haider Ali**
Head of Regulatory Affair
Reckitt Benckiser Pakistan
Karachi
0335-2333469
syedhaider.ali@rb.com
- 8. Dr. Shazia Yasmeen**
Sr. Scientific Officer
PCSIR Labs Complex
K.L.C.Karachi.
Cell: 0334-3244668
shazrasheed@yahoo.com
- 9. Dr. Sadia Farheen**
PCSIR Labs Complex
Karachi
Cell # 0306-2766945

E-mail: sadiahej@yahoo.com

- 10. Mr. Altaf Ashraf**
Basra Soap Factory
Karachi
Cell: 0300-8291188
E-mail: basrasoap@yahoo.com
- 11. Dr. Tahira Zaheer**
Director Standardization wing-ii
SDC, PSQCA, Karachi.
Cell: 0333229356
dr.tahira@hotmail.com
- 12. Ms. Sana Khan**
Reckitt Benckiser Pakistan
Karachi
Cell: 0335-2332250
sana.khan@rb.com
- 13. Dr. Uzma Tiwana**
Skin Specialist
Transforms Clinic
C1/1, Block 2 Clifton
Karachi
Cell # 0300-8901357
druzmatiwana@gmail.com

SECRETARIAT

- 1. Mr. Jawaid.H. Siddiqui**
Deputy Director Chemical
SDC/PSQCA Karachi
- 2. Mr. Latif-ur-Rehman**
Assistant Director (chemical)
Cell: 0320-2207392
Email: committeesecretary.sdc@gmail.com
- 3. Mr. M. Abid Khan**
Examiner (Chemical)
SDC/PSQCA
Karachi.
Cell: 0333-3283579
abidmashoodkhan@gmail.com
- 4. Mr. Mujeeb-ur-Rehman**
Assistant Director Chemical
SDC/PSQCA
Karachi

**PAKISTAN STANDARD SPECIFICATION
FOR
SHAMPOO (1st Revision)**

0. FORWARD

- 0.1 This Pakistan Standard was adopted by the Pakistan Standards Quality Control Authority on **30-April-2018** after the draft finalized by the Cosmetic & Toilet Goods Technical Committee had been approved by the National Standard Committee.
- 0.2 This Pakistan Standard was first established in 1994, second revision has been prepared on the basis of views /Recommendations of consumers, manufacturers, Toilet Goods specialist, Chemists, Chemical Engineers, Scientists and other stakeholders have been given full consideration.
- 0.3 The primary function of a shampoo is to clean the hair and scalp. In addition to accomplishing this cleaning action, it should not remove an excessive amount of natural oil from the scalp and should leave the hair soft, lustrous and manageable.
- 0.4 It is necessary that the raw materials used are such that in the concentrations in which they are present in the shampoo and after interaction with other raw materials, they are free from harmful effects. It shall be the responsibility of the manufacturer of shampoo to satisfy himself of his physiological and dermatological safety of his product.
- 0.5 Ingredients which may be used in the formulation of shampoo are given in Appendix B.
- 0.6 This standard is intended chiefly to cover the technical provisions relating to the supply of material & it does not include all the necessary provisions of a contract.
- 0.7 For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the result of a test or analysis shall be rounded off in accordance with PS: 103-1991, Rules for rounding of Numerical values. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

- 1.1 This specification prescribes the requirements and methods of sampling and test for shampoo based on synthetic detergents.
- 1.2 It does not cover baby shampoo.

2. REQUIREMENTS

2.1 General requirements.

The shampoo shall be in the form of a liquid or emulsion and may be coloured and perfumed. It shall be of uniform consistency and free from sediments and suspended particles. It shall spread easily and possess good lathering and rising properties and shall be non toxic and non irritant.

2.2 RAW MATERIAL

Unless specified otherwise, all the raw materials used in the manufacture of shampoo shall be dermatologically safe.

2.2.1 The dyes if used, in the manufacture of shampoo shall comply with the provisions of PS: _____

2.2.2 Raw materials other than dyes shall comply with the provision of PS: _____

2.3 Other Requirements

The shampoo shall also comply with the requirements given in Table 1 when tested according to the relevant methods given in Column 4 of the table.

Table 1-Requirement for Shampoo

S.#	Characteristic	Requirement	Method of test Ref. to
1	2	3	4
i	Active ingredient content, as TELS * of its equivalent, percent by mass, min	10	ASTM D 3049
ii	Volatile matter at 105 °C percent by mass, max	90	Appendix C.2
iii	pH at 27 ± 2 °C	4 to 9	Appendix C.3
iv	Inorganic salts, percent by mass, max	7.0	Appendix C.4

2.4 **Microbiological Limits**

The shampoo shall also comply with the microbiological limits given in Table 2, when tested according to the relevant method given in Column 4 of the table.

TABLE 2- Microbiological limits

S.#	Characteristic	Limit	Method of test Ref. to clause No in Appendix
1	2	3	4
i.	Aerobic plate count per ml, max	300	C.5
ii.	Pathogenic bacteria	Nil	C.6

3. **PACKAGING AND MARKING**

- 3.1 The Material shall be packed in suitable containers. The containers shall be legibly and indelibly marked with the following:
- a. Name of the Product;
 - b. Name and address of the manufacturers, (including country of origin);
 - c. Registered trade mark, if any;
 - d. Brand name, if any;
 - e. Net volume, in milliliters;
 - f. batch or code number;
 - g. Instructions for use, where necessary; and
 - h. The WARNING contains formaldehyde if the concentration of formaldehyde in the finished product exceeds 0.05 percent.
- 3.2 A number of such containers, as agreed to between the purchaser and the supplier, shall be suitably packed. Each package shall be legibly and indelibly marked with the following.
- i. Name of the product;
 - ii. Name and address of the manufacturer, (including country of origin)
 - iii. Registered trade mark, if any; and
 - iv. Number of containers.

4. **SAMPLING**

Representative samples of the material for ascertaining conformity to the requirements of this specification shall be drawn as prescribed in Appendix A.

5. **METHODS OF TEST**

Tests shall be carried out as prescribed in Appendix C and ASTM D 3049: 1989

6. **CONFORMITY TO STANDARD**

A lot shall be declared as conforming to the requirements of this specification if the following conditions are satisfied.

- 6.1 Each container and each package examined as in A.6.1 satisfies the relevant requirements.
- 6.2 The composite samples tested as in A.6.2 and A.6.3 satisfies the relevant requirements.

SAMPLING

- A.1 **Lot**
In a consignment all the containers of same size and containing shampoo of one batch of manufacture shall constitute a lot.
- A.2 **General Requirements of Sampling**
In drawing, preparing, storing and handling samples the following precaution shall be observed.
- A.2.1 Samples shall be drawn in an environment not exposed to damp air, dust and soot.
- A.2.2 A sampling tube may be used for drawing the material from the containers. It shall be clean and dry when used.
- A.2.3 The samples shall be placed in clean, dry, glass or any other suitable containers. The sample containers shall be sealed air-tight after filling and shall be marked with necessary details of sampling.
- A.2.4 The material being sampled, the samples, the sampling instrument, and the sample containers shall be protected from adventitious contamination.
- A.2.5 Samples shall be stored, so that the conditions of storage do not affect the quality of the materials.
- A.2.6 When drawing samples for microbiological examination in addition to the requirements specified in A.2.1 to A.2.5 the following precautions shall be observed.
- A.2.6.1 Samples shall be drawn under aseptic conditions.
- A.2.6.2 The sampling instrument and sample containers shall be sterilized using an appropriate method.
- A.3. **SCALE OF SAMPLING**
- A.3.1 Samples shall be tested from each lot for ascertaining the conformity of the material to the requirements of this specification.
- A.3.2 The number of containers to be selected from a lot shall be in accordance with Table 3.
- A.3.3 If the containers are packed in packages, at least 5 percent of the packages shall be selected from the lot and as far as possible equal number of containers shall be drawn from each package so selected to form a sample size as given in column 2 or column 3 of the table.
- A.3.4 The containers and packages shall be selected at random in order to ensure randomness of selection random number tables as given in PS: _____ shall be used.

TABLE 3 Scale of Sampling

Number of Containers in the Lot	Number of containers to be selected	
	Net volume of the container is 40 ml or less	Net Volume o the container is more than 40 ml
Up to 50	9	3
51 to 200	12	4
201 to 400	15	5
401 to 650	18	6
651 to 900	21	7
901 to 1200	24	8
1201 to 1500	27	9
1501 and above	30	10

A.4 COMPOSITE SAMPLE

A.4.1 An equal quantity of material shall be drawn from each container selected as in A.3.3. Before drawing the sample, the contents of the containers shall be mixed thoroughly by suitable means. The material so obtained shall be mixed thoroughly to form a composite sample which shall be of a size sufficient to carry out the test for requirements specified in A.6.2.

A.4.2 A separate composite sample of about 20 ml shall be prepared under the conditions specified in A.2.6 for testing microbiological requirements before the preparation of the composite sample for testing the other requirements.

A.5 REFERENCE SAMPLE

If a reference sample is required for requirements other than the microbiological requirements, the size of the composite sample shall be three times the amount required in A.4.1. The composite sample so obtained shall be divided into three equal parts one for the purchaser, another for the supplier and the third as the reference sample which shall be used in case of dispute between the purchaser and the supplier.

A.6 NUMBER OF TESTS

A.6.1 Each containers and each package selected as in A.3.3 shall be examined for packaging and marking requirements (see 4).

A.6.2 Tests for requirements specified in 3.3 shall be carried out on the composite sample prepared as in A.4.1.

A.6.3 Tests for microbiological requirements (see 3.4) shall be carried out on the composite sample prepared as in A.4.2.

APPENDIX - B

List of Raw Materials which may be used in Formulation of Sample

B.1 Detergents

- a) Sodium, potassium, ethanolamine or ammonium salts of sulfonic acid;
- b) Alcohol ether sulfates;
- c) Sulfated monoglycerides
- d) Sodium alkyl sulfo acetate;
- e) Alkyl benzene polyoxyethylene sulfonates;
- f) Sodium n-lauryl sarcosinate; and
- g) Other synthetic detergents.

B.2 Foam Stabilizers

- a) Ethanolamides or isopropanolamides of fatty acids;
- b) Amine oxides;
- c) Fatty alcohols;
- d) Sarcosinates; and
- e) Phosphates

B.3 Chelating agents

- a) Tetra Sodium pyrophosphate and tripolyphosphate; and
- b) Sodium salts of ethylenediamine tetraacetic acid.

B.4 Solubilizing Agents.

- a) Urea;
- b) Butyl and isopropyl alcohols;
- c) Sodium toluene sulfonate;
- d) Sodium xylene sulfonate;
- e) Diethylene/propylene glycol; and
- f) Diethyl carbitol

B.5 Preservatives

- a) Alcohols;
- b) Formaldehyde; and
- c) Esters of p-hydroxybenzoic acid

B.6 Opacifying agents

- a) Higher fatty alcohols;
- b) Glyceryl/ethylene/propylene glycol stearates and palmitates;
- c) Mono and di stearates of glycol;
- d) Zinc, calcium magnesium stearates;
- e) Behenic acid;
- f) Magnesium silicate; and
- g) Amides and ethanolamices of fatty acids

B.7 Inorganic Salts

- a) Sodium/potassium chlorides.

B.8 Conditioning Agents

- a) Lanolin and its derivatives;
- b) Isopropyl myristate;
- c) Butyl palmitate;
- d) Glycerol;
- e) Propylene glycol;
- f) Lauroyl and cocoyl sarcosines;
- g) Phosphate esters; and
- h) Cationic polymers

B.9 Thickening Agents

- a) Sodium carboxymethyl cellulose;
- b) Methyl cellulose;
- c) Hydroxyethyl cellulose;
- d) Polyvinyl cellulose;
- e) Alginates;
- f) Polyvinyl pyrrolidone;
- g) Alkylolansides; and
- h) Glycol/glycerol stearates.

B.10 Other groups of ingredients

- a) Perfumes;
- b) Dyes;
- c) Antioxidants;
- d) pH control agents; and

e) Suspending agents.

APPENDIX C
METHODS OF TESTS FOR SHAMPOO

C.1 QUALITY OF REAGENTS

C.1.1 unless specified otherwise, chemicals of a recognized analytical grade and distilled water shall be employed in tests.

C.2 Volatile matter at 105 °C

C.2.1 Procedure

Weigh, to the nearest 1 mg, approximately 5 g of the material in a tared evaporating dish and heat on a stem-bath until most of the volatile matter had escaped. Continue heating at 105 ± 0°C in an oven for 2 hours. Cool in a desiccator and weigh. Repeat heating, cooling and weighing until the difference in mass between two successive weighings does not exceed 1 mg retain the residue in the dish for subsequent tests.

C.2.2 Calculation

$$\text{Volatile matter at 105 °C percent by mass} = \frac{(m_1 - m_2)}{(m_1 - m_3)} \times 100$$

Where,

m_1 = mass, in grams, of the dish with the sample;
 m_2 = mass, in grams, of the dish after heating; and
 m_3 = mass, in grams, of the empty dish.

C.3 pH at 27 ± 2°C

C.3.1 Apparatus

A pH meter preferably equipped with a glass electrode.

C.3.2 Procedure

Determine the pH at a temperature of 27 ± 2 °C. In the case of liquid shampoo, read the pH directly using the pH meter. In the case of shampoo in the form of an emulsion mix 5 g of the sample with 45 ml of water and determine the pH of the resulting solution.

C.4 Inorganic Salts

C.4.1 Reagent

C.4.1.1 Sulfuric acid, concentrated rel. den = 1.84

C.4.2 Procedure

Take the dish containing the material after evaporation as obtained in C.2.1. Heat it at about 450°C to destroy organic matter. Cool, add a few drops of concentrated sulfuric acid (C.4.1.1) and heat again to dryness. Cool and weigh. Repeat heating cooling and weighing until the difference in mass between two successive weighings does not exceed the one milligram.

C.4.3 Calculation

$$\text{Inorganic salts, percent by mass} = \frac{m_1}{m_2} \times 100$$

Where,

m_1 = mass, in grams, of the residue; and

m_2 = mass, in grams, of the material taken for the test in C.2.1.

C.5 MICROBIOLOGICAL EXAMINATION

C.5.1 Apparatus

C.5.1.1 Sterile instruments for preparation of sample – forceps, scissors, spatula and scalpel.

C.5.1.2 Gauze pads, sterile 100 mm x mm.

C.5.1.3 balance with weights, sensitivity of 0.01 g

C.5.1.4 Water baths, controlled at $42 \pm 1^\circ\text{C}$ and $45 \pm 2^\circ\text{C}$.

C.5.1.5 Incubators, controlled at $36 \pm 1^\circ\text{C}$ and $42 \pm 1^\circ\text{C}$.

C.5.1.6 Petridishes, sterile, 15 mm x 100 mm

C.5.1.7 Pipettes sterile, graduated, capacity 1 ml, 5 ml and 10 ml

C.5.1.8 Wide mouth dilution bottles

C.5.1.9 Glass spreaders, sterile.

C.5.2 Culture media and reagents

Dehydrated media of any brand equivalent to formulations may be used.

C.5.2.1 Diluent

C.5.2.1.1 Peptone, 0.1 percent – water diluent

Peitone 1.0 g

Distilled water 1000 ml

Dissolve the ingredients in distilled water. Sterilize at 121°C for 15 minutes. Adjust the final pH to 7.3 ± 0.2

C.5.2.1.2 Modified lethten broth

Tryptone	10.0 g
Beef extract	5.0 g
Lecithin	0.7 g
Tween 80	5.0 g
Trypticase peptone	5.0 g
Thiotone peptone	10.0 g
Yeast extract	2.0 g
Sodium Chloride	5.0 g
Sodium bisulfate	0.1 g
Distilled water	1000 ml

Dissolve ingredients by boiling. Autoclave for 15 minutes at 121°C . Adjust the final pH to 7.0 ± 0.2 . Dispense in appropriate amounts into dilution Bottles.\\

C.5.2.2 Plate count agar (PCA)

Dehydrated yeast extract	2.5 g
Tryptone	5.0 g
Anhydrous D-glucose (anhydrous dextrose)	1.0 g
Agar	9 g to 10 g
Distilled water	1000 ml

Dissolve all ingredients in water by boiling. Sterilize by autoclaving at 121 °C for 20 minutes. Adjust the final pH to 7.0 ± 0.2 .

C.5.2.3 Trypticase soy agar (TSA)

Trypticase or tryptone	15 g
Phytone	5 g
Sodium chloride	5 g
Agar	15 g
Distilled water	1000 g

Suspend and mix thoroughly. Heat with frequent agitation and boil for about one minute to dissolve completely. Autoclave for 15 minutes at 121 °C. Adjust the final pH to 7.3 ± 0.1 .

C.5.2.4 Vogel – Jonhson (VJ) agar.

Tryptone	10.0 g
Yeast extract	5.0 g
Mannitol	10.0 g
Dipotassium phosphate	5.0 g
Lithium chloride	5.0 g
Glycine	10.0 g
Phenol red	0.025 g
Agar	15.0 g
Distilled water	1000 ml

Suspend ingredients in water and heat to boiling to dissolve completely. Sterilize by autoclaving at 121 °C for 15 minutes. Cool to between 45 °C and add 20 ml of 1.0 percent tellurite solution. Adjust the final pH 7.2 ± 0.2 . Mix thoroughly and dispense in Petridishes. Plates may be stored at 4 °C for upto 7 days.

C.5.2.5 Centrimide agar

Peptone	20.0 g
Magnesium Chloride	1.4 g
Potassium Sulphate	10.0 g
Centrimide (cetyl trimethyl ammonium bromide)	0.3 g
Agar	13.6 g
Distilled water	1000 ml

Suspend ingredients in water. Add 10 ml of glycerol and heat to boiling to dissolve. Sterilize by autoclaving at 121 °C for 15 minutes. Adjust the final pH to 7.2 ± 0.2 .

C.5.2.6 Fluid soya bean casein digest medium (SDC)

USP pancreatic digest of casein (Trypticase)	15.0 g
USP soya peptone	5.0 g
Sodium chloride	5.0 g
Lecithin	0.7 g
Tween 80	5.0 g
Distilled Water	1000 ml

Suspend ingredients in distilled water and dissolve by boiling. Sterilize by autoclaving at 118°C to 121°C for 15 minutes. Adjust the final pH to 7.3 ± 0.2 .

C.5.2.7 Soyabean casein digest (SDC) agar

- Composition and preparation as for fluid-soya bean casing digest medium (C.5.2.6), with the addition of 15.0 g of agar per liter.
- C.5.2.8 Motility test medium
- | | |
|-----------------|---------|
| Beef extract | 3.0 g |
| Peptone | 10.0 g |
| Sodium Chloride | 5.0 g |
| Agar | 4.0 g |
| Distilled water | 1000 ml |
- Suspend the ingredients in distilled water and boil for one to two minutes to dissolve. Dispense in 8 ml portions into test tubes. Sterilize by autoclaving at 121°C for 15 minutes. Final pH 7.4
- C.5.2.9 Oxidase reagent
- | | |
|---|--------|
| Tetramethyl-o-phenylene diamine dihydrochloride | 1.0 g |
| Distilled Water | 100 ml |
- Store in dark glass bottles at 5°C to 10°C. Storage life is 15 days.
- C.5.2.10 Nutrient broth
- | | |
|-----------------|---------|
| Beef extract | 3.0 g |
| Peotone | 5.0 g |
| Distilled water | 1000 ml |
- Suspend ingredients in distilled water and boil to dissolove. Dispense as required and autoclave at 121 °C for 15 minutes. Final pH 6.8
- C.5.2.11 Rabbit coagulase plasma with EDTA.
- Use a commercially available denyarated rabbit plasma and rahydrate in accordance with the manufacturer’s instructions.
- C.5.2.12 Tween 80
- C.5.2.13 Aqueous mixture of 80 percent ethanol (V/V) and 1 percent hydrochloric acid (V/V).
- C.5.3 **Procedure**
- C.5.3.1 Preparation of test sample
- C.5.3.1.1 Analyse the samples as soon as possible after arrival in the laboratory. If storage is necessary, the samples should be stored at room temperature.
- C.5.3.1.2 Disinfect the surface of the sample container with the aqueous mixture (C.5.3.13). Dry the surface with sterile guaze before opening and removing contents.
- C.5.3.1.3 Transfer by means of a pipette, 10 ml of the product to 90 ml of either of the diluent given in C.5.2.1 to obtain 1: 10 dilution.
- C.5.3.1.4 Shake the dilution bottle 50 times through a distance of 300 mm with one minute.
- C.5.3.1.5 Prepare serial dilutions to obtain a dilution series from 10^{-1} to 10^{-4} by adding one ml of previous dilution to nine ml of either of the dilutes given in C.5.2.1
- C.5.3.1.6 Shake all dilutions by rotating the tubes or with a mechanical shaker.
- C.5.4 Aerobic plate count
- C.5.4.1 Using a sterile pipette transfer one ml of each dilution into each of duplicate Petri dishes.

-
- C.5.4.2 Add about fifteen ml of plate count agar (C.5.2.2), trypticase soy bean (C.5.2.3) or soy bean casein digest agar (C.5.2.7) at 45°C into each petridish (C.5.4.1) and rotate the dish carefully to mix the inoculum with the medium. Allow to solidify.
- C.5.4.3 Invert the dishes and incubate at $36 \pm 1^\circ\text{C}$ for 48 hours.
- C.5.4.4 Count colonies on plates containing 30 to 300 colonies, and record results per dilution counted. If plates do not contain 30 to 300 colonies record the dilution counted and note the number of colonies found.
- C.5.4.5 Average the count obtained, multiply by the appropriate dilution factor and report the results as the aerobic plate count per gram of sample.
- C.5.5 Detection of *Staphylococcus aureus*
- C.5.5.1 Transfer 0.5-ml portions from the dilutions prepared in C.5.3.1.5 to duplicate plates of Vogal-Johnson agar (C.5.2.4).
- C.5.5.2 Distribute the inoculum over the surface of the agar with a sterile glass spreader.
- C.5.5.3 When inoculum has been completely absorbed, invert the plates and incubate at $36 \pm 1^\circ\text{C}$ for 48 hours.
- C.5.5.4 Count plates at the dilution having 30 to 300 well distributed colonies that are convex, shining black, with or without a yellow zone surrounding the colony.
- C.5.5.5 Plates having more than 300 colonies may be selected when plates at a greater dilution do not contain the colonial types described above. Plates from minimal dilutions having less than 30 colonies may also be used if necessary.
- C.5.5.6 Coagulase test.
From each plate demonstrating growth, pick one or more of the typical colonies into tubes containing 0.2 ml nutrient broth (C.5.2.10)
Incubate at $36 \pm 1^\circ\text{C}$ for 18 hours to 24 hours. Add 0.5 ml of reconstituted rabbit coagulase plasma with EDTA (C.5.2.11) and mix thoroughly.
Incubate at $36 \pm 1^\circ\text{C}$ and examine for clotting at 3 hour intervals for up to 24 hours.
A known coagulase positive and coagulase negative organism should be included with every set of samples.
- C.5.5.7 All strains that yield positive coagulase reactions may be considered as *Staphylococcus aureus*.
- C.5.5.8 Calculate the number of *Staphylococcus aureus* organisms present by determining the fraction of colonies tested that are coagulase positive. Multiply this fraction by the average number of characteristic colonies appearing on the VJ agar plates. Multiply the number obtained by the appropriate dilution factor and report as the number of *staphylococcus aureus* per gram of sample.
- C.5.6 Detection of *Pseudomonas aeruginosa*
- C.5.6.1 Select different type colonies from the agar plates used in C.5.4.4 and streak plates of centrimide agar (C.5.2.5).
- C.5.6.2 Incubate at $36 \pm 1^\circ\text{C}$ for 24 hours.
- C.5.6.3 Select colonies which show a greenish fluorescence as presumptive *Pseudomonas aeruginosa*.
- C.5.6.4 Cytochrome oxidase test
-

Add a loopful of oxidase reagent (C.5.2.9) to a filter paper in a Petri dish. Using a loop, semear the presumptive *Pseudomonas aeruginosa* colonies across the moistened paper. A purple colour appearing across the streak within 10 seconds indicates a positive reaction.

- C.5.6.5 Motility test
Stab motility, medium (C.5.2.8) with oxidase positive colonies and incubate at 36 ± 1 °C for 24 hours. Growth from the line of the stab constitutes a positive test.
- C.5.6.6 Growth at 42°C

Transfer oxidase positive and motility positive colonies to fluid soya bean casein digest medium (C.5.2.6) and incubate at 42 ± 1 °C for 24 hours to 48 hours.
- C.5.6.7 Oxidase positive, motility positive colonies which show growth at 42°C may be considered as *Pseudomonas aeruginosa*. Lack of characteristic pigmentation and failure to grow at 42°C indicates other pseudomonas.
- C.5.6.8 Count the typical colonies on agar plates (C.5.4.4) corresponding to the *Pseudomonas aeruginosa* colonies selected from that medium and plated on centrimide agar.
- C.5.6.9 Multiply by the appropriate dilution factor and report as the number of *Pseudomonas aeruginosa* per gram of sample.