# *भारतीय मानक मसौदा*

# फिनोलिक टाईप रोगाणुनाशी द्रव — विशिष्टि

(*छठा पुनरीक्षण*)

# *Draft Indian Standard*

# Disinfectant Fluids, Phenolic Type — Specification

(*Sixth Revision*)



ICS 71.080.90; 11.080

| | |
|---|---|
| CHD 25 — Soaps, Detergents and Surface Active Agents Sectional Committee | Last date of comments: 15th October, 2024 |

FOREWORD

*(formal clauses to be added later*)

Disinfectant fluids are applied to various surfaces for eliminating the harmful microorganisms, vermin or insects which cause disease in human beings or animals. Generally, these fluids are used for cleaning kitchen sinks, floors and bathroom wall tiles, etc. With the urbanization, there has been a growing demand for such disinfectant fluids.

As per the "Schedule O – Standard for Disinfectant fluids" of *Drugs and Cosmetic Act*, 1940, the fluids can be of two types:

a) Part 1 refers to Provisions applicable to black fluids & white fluids; and

b) Part 2 refers Provisions applicable to other disinfectant fluids.

BIS has formulated IS 14364 : 1996 "Quarternary ammonium compound-based surface cleaner, liquid" which covers products consist essentially of quaternary ammonium compound and non-ionic surfactants in aqueous medium. For phenolic type of disinfectant fluids, BIS has formulated IS 1061 "Disinfectant fluids-Phenolic Type" and IS 10758 "De-odourising-cum-disinfectant fluids".

The standard IS 1061 was first published in 1957 and subsequently revised in 1964, 1975 and 1982. During the fourth revision in 1997, the title was modified in order to harmonize with the scope of the standard. The grades were redesignated with only RW co-efficient to align with the grades given in the schedule 'O' of Drugs and Cosmetic Rules, 1945. Provision was made for six grades for white fluids also on lines with black fluid. Under the text 'Stability on storage' parameters to be tested were included. Further requirement for absence of mercury compounds along with

the test methods and all the five amendments issued were incorporated for ease of implementation was incorporated. Also, referred standards have been updated

The standard IS 10758 was first published in 1983 and was covering the de-odourising-cum-disinfectant fluids which were phenolic fluids.

In this revision, the committee decided to amalgamate IS 1061 and IS 10758 as both the products are essentially disinfectants containing phenolic components. Further, the de-odourising-cum-disinfectant fluids have additional property of de-ordourising to suppress the foul smell emitted due to decay of bacteria and fungi. Pine oil, Castor oil, Coaltar acid, Creosate oil etc., are extensively used for disinfectant-cum-de-odourizing purposes. Further, amendments issued to these standards have also been incorporated. Further, provisions have been made to incorporate phenolic coloured disinfectant fluids,

The non-phenolic type of disinfectant fluids is not covered in this standard.

Due consideration has been given to the provisions of schedule ‘O’ of Drugs and Cosmetic Rules, 1945. However, this standard is subject to the restrictions imposed under these rules wherever applicable.

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 IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard

*Draft Indian Standard*

# DISINFECTANT FLUIDS, PHENOLIC TYPE — SPECIFICATION

(*Sixth Revision*)

## 1 SCOPE

This standard prescribes the requirements and methods of sampling and test for disinfectant fluids of the phenolic type.

## 2 REFERENCES

The following standards contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

| *IS No.* | *Title* |
|---|---|
| IS 1448 (Part 20) : 2019/ ISO 13736 : 2013 | Methods of test for petroleum and its products: Part 20 Determination of flash point — Abel closed-cup method (*third revision*) |
| IS 4905 : 2015/ ISO 24153 : 2009 | Random sampling and randomization procedures (*first revision*) |
| IS 10758 : 1983 | Specification for De-Odourizing-Cum-Disinfectant fluids |
| IS 14364 : 2024 | Quaternary ammonium compound based surface cleaner, liquid — Specification (*first revision*) |

## 3 CLASS, GRADE AND TYPE

### 3.1 Class

Disinfectant fluids shall be of four classes namely:

a) Black;
b) White;
c) Other colour, and
d) Disinfectant-cum-deodouring fluids.

NOTE — The classification is based on the colour and de-odouring properties of disinfectants fluids.

The black fluid may be used with majority of water supplies whereas the white fluid may be used with all kinds of water and should mix easily with saline or abnormally hard water.

### 3.2 Grade

Each class of disinfectant fluid shall be of six grades depending upon Rideal Walker (RW) and Staphylococcal (SA) co-efficient by rules as given below:

| **Class** | **Grade** | **Rideal Walker (RW) Co-efficient, *Min*** | **Staphylococcal (SA) Co-efficient, *Min*** |
|---|---|---|---|
| All | 1 | 18 | – |
| | 2 | 10 | – |
| | 3 | 5 | – |

| | | |
|---|---|---|
| 1A | 18 | 8 |
| 2A | 10 | 5 |
| 3A | 5 | 2.5 |

NOTE — Requirement of Staphylococcal (SA) co-efficient has been given to ensure that disinfectant fluids are not unduly selective in their germicidal properties.

### 3.3 Type

Each class and grade of disinfectant fluids shall be of two types depending on the stability to temperature variations as given below:

| Type | stability at lower and upper temperature of |
|---|---|
| Normal | 15 °C and 45 °C |
| Winter | 5 °C and 30 °C |

NOTE — In the case of black disinfectant fluid at 15 °C slight sediment of naphthalene may appear.

## 4 REQUIREMENTS

### 4.1 Composition

The compositions of the solution shall contain mixture of the following ingredients:

a) Coaltar acids or similar acids derived from petroleum;
b) Phenolic compounds; and
c) A suitable emulsifier.

In addition, the compositions of the solution may consist of the following ingredients:

a) Hydrocarbons, and
b) Substituted phenolic compounds.

NOTE — "Quaternary ammonium compounds are not compatible with the composition given above because of their cationic characteristic and hence should not be incorporated in such formulations."

The disinfectant-cum-deodouring fluids shall also contain essential oil like pine or lemon grass or any other essential oil or any other synthetic perfumes having pleasing odour.

### 4.2 Description

**4.2.1** *Black Fluids* **—** These shall be homogeneous solutions dark brown in colour and on dilution with water it shall give translucent off white to white colour.

**4.2.2** *White Fluids* **—** These shall be finely dispersed stabilized emulsions white in colour and on dilution with water it shall remain white to off-white.

**4.2.3** *Other colour* **—** These shall be homogeneous and uniform colour and on dilution with water the colour may become light. However, it shall remain unchanged.

**4.2.4** *Disinfectant-cum-De-odourizing fluids* — These shall be a clear homogeneous and transparent solution with stable colour.

### 4.3 Stability after Dilution

**4.3.1** When tested by the method prescribed in Annex A in proportion of 1 percent and 5 percent by volume the disinfectant fluids shall:

a) Be miscible with artificial hard water (for black, colour and disinfectant-cum-de-odourizing fluid) or with artificial sea water (for white fluid); and
b) Not show separation at the top and bottom.

NOTE — Negligible separation at the top and bottom in case of black fluid may take place, therefore, it shall not be considered as failure of the product. In case of the white fluid a small amount of creaming, which may be restored to a homogeneous condition on shaking. In other cases, negligible separation may be seen which may restored to homogenous condition on shaking.

**4.4 Germicidal Value**

Germicidal values of disinfectant fluids shall be ascertained in terms of phenol coefficient (Rideal Walker as well as Staphylococcal) when tested by these methods prescribed in Annex B and Annex C and the grade of the material shall be determined in accordance with **3.2.**

**4.5 Mercury Compounds**

When tested by the method prescribed in Annex D, mercury compound shall not be present in product.

**4.6 Stability on Storage**

The test mentioned under **4.2, 4.3, 4.4, 4.5, 4.7** and **4.8** where **4.8** is applicable for the de-ordourizing fluids only, shall be repeated by the methods referred in **4.2, 4.3, 4.4, 4.5, 4.7** and **4.8** just before the expiry period declared by the manufacturer (*see* **6.1**). The results obtained during this test shall satisfy the requirements prescribed.

**4.7 Detection of Phenolic Compounds**

The material when tested according to the method prescribed in Annex E, shall conform to the test for detection of phenolic compounds.

**4.8** Additional Requirements for Disinfectant-cum-deodourizing Fluids

**4.8.1** *Volatile Matter* — It shall not be less than 70 percent (m/m) of volatile-matter when tested by the method prescribed in Annex F.

**4.8.2** The material shall not be less than 40 percent (*v/v*) steam volatile oil when determined by the steam distillation as the method prescribed in Annex G.

**4.8.3** *Odour* — The material shall be with pleasant odour.

**4.8.4** *Persistance of odour* — The odour used shall persist for a minimum of 24 h when:

a) a strip of filter paper soaked in liquid is hung at room temperature; and

b) a mixture of 1 ml of liquid and 1 ml of water is kept in a small petridish at room temperature.

**4.8.5** *Flash Point (Abel)* — When determined by the method prescribed in IS 1448 (Part 20), the flash point of the material shall not be less than 32.2 °C.

**5 PACKAGING AND MARKING**

**5.1 PACKAGING**

Disinfectant fluids of all classes shall be packed in suitable containers in such a way that corrosion or reaction would not take place during storage. Galvanized iron sheet containers shall not be used.

**6 MARKING**

**6.1** The containers shall be marked legibly and indelibly with the following information:

a) Name of the product;
b) Name and address of the manufacturer and trade-mark, if any;
c) Class, grade and type of the material and the phenol coefficient (Rideal Walker or Rideal Walker and Staphylococcal);

d) Batch or Code number;
e) Month and year of the manufacture;
f) Date up to which the product can be used as agreed to between the manufacturer and the buyer subject to minimum of one year from the date of manufacture;
g) Net volume in ml or l;
h) Any specific instructions for use;
j) A statement that mercury compounds have not been added to the product; and
k) Any other marking required under the *Legal Metrology Regulations*.

### 6.2 BIS Certification Marking

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

## 7 SAMPLING FOR LOT ACCEPTANCE

**7.1** Unless otherwise agreed to between the manufacturer and the purchaser the sampling procedure and criteria for conformity shall be as given in Annex H.

## ANNEX A

(*Clause* 4.3.1)

## METHODS FOR DETERMINATION OF STABILITY AFTER DILUTION

### A-1 PREPARATION OF SAMPLE

The sample of the disinfectant fluid to be tested should be mixed thoroughly taking care that no air is beaten into the fluid immediately before withdrawing any Portion for testing. The test portion should be taken from the middle of the sample.

### A-2 DILUTION WATER

#### A-2.1 Artificial Hard Water

In case of black, colour and disinfectant cum-de-odourizing fluids the artificial hard water shall be used. The artificial hard water shall be prepared as given in **A-2.1.1.**

**A-2.1.1** 40 ml of 1 N hydrochloric acid (analytical reagent quality) is neutralized with a slight excess of calcium carbonate and filtered. The filtrate is diluted to 1 000 ml with reagent grade water (*see* IS 1070), 10 parts of this solution is further diluted to 100 parts with reagent grade water.

#### A-2.2 Artificial Sea Water

In case of white fluid the artificial sea water (*see* IS 8770) shall be used.

### A-3 PROCEDURE

**A-3.1** Take 1 ml and 5 ml portions of the sample (*see* **A-1**) in duplicate in 100 ml stoppered measuring cylinder (*see* IS 878) by means of pipettes.

**A-3.2** Dilute the sample with artificial hard water or artificial sea water (as the case may be) up to 100 ml mark.

**A-3.3** Mix thoroughly by inverting the cylinder 5 times.

**A-3.4** Keep the cylinders containing the diluted fluids for 6 h at the extremes of the temperatures as specified for the particular type of the fluid (*see* **3.3**).

**A-3.5** The cylinders shall be examined by reflected light for the requirements given under **4.3**.

## ANNEX B

(*Clause* 4.4)

## DETERMINATION OF RIDEAL WALKER (RW) COEFFICIENT

### B-1 GENERAL

It is a biological method for evaluating the germicidal property of disinfectant fluids. The disinfectant fluids are diluted with distilled water and tested against broth cultures of the prescribed organism. On the basis of response against this test the Rideal Walker coefficients of the disinfectant fluids are determined and the fluids are categorized into grades (*see* **3.2**).

### B-2 APPARATUS

#### B-2.1 Inoculating Loop

A loop, 4 mm internal diameter is formed at end of a 0.375 mm wire of platinum or platinum iridium alloy, 38 mm long from the loop to the holder. The loop is bent at such an angle to the length of the wire as will facilitate in removal vertically from the surface of the liquid, while keeping the plane of the loop horizontal.

**B-2.2 Incubator**

Capable to maintain a temperature of (37 ± 1) °C.

**B-2.3 Pipettes**

Graduated of capacity 10 ml, 5 ml and 1 ml.

**B-2.4 Dropping Pipette**

a) Sterile, made to deliver 0.2 ml in about 5 drops; and
b) Standardize to deliver 5 ml sample/reagents.

**B-2.5 Medication Tubes**

5 sterile, plugged, rimless test tubes of 125 mm × 20 mm size made of hard neutral glass.

NOTE — The culture shall be obtained from the Director, Central Drug Laboratory, Kolkata; Director, Haffkine Institute, Mumbai; Director, Central Research Institute, Kasauli; IMTECH Chandigarh or ATCC/ATCC registered vendors. In case of dispute, the test organisms supplied by Central Drug Laboratory, Kolkata shall be treated as reference test organisms

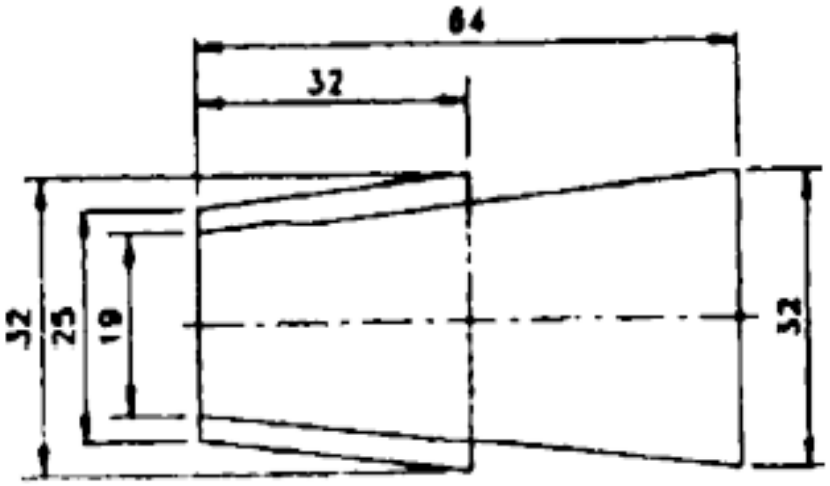
**B-2.6 Broth Tubes**

About 2 dozen of same description of medication tubes.

**B-2.7 Measuring Cylinders**

Stoppered and graduated — 500 ml, graduated in 10 ml; 100 ml; graduated in 1 ml.

NOTE — All apparatus shall be scrupulousy clean and sterile immediately before use.




All dimensions in mitimetres.

Fig.1 SPECIAL MEDICATION TUBE

**B-3 REAGENTS**

**B-3.1 Standard Rideal Walker Broth**

Take 20 g of meat extract, microbiological grade and 10 g of sodium chloride and dissolve in 1 000 ml of distilled water. Boil the solution for 30 min, cool and make up 1 000 ml with freshly boiled distilled water. Titrate 25 ml of the

broth at 37 °C with 0.1 N sodium hydroxide solution, using 0.1 ml of 0.5 percent phenolphthalein solution as indicator. By calculation from this titration, neutralize the bulk of broth 37 °C and with normal sodium hydroxide solution bring the mixture to boil, or steam for half an hour to bring down phosphates; remove the phosphates by filtration whilst the broth is hot. Adjust the broth to a *p*H value of 7.6 by the addition of normal hydrochloric acid, using a comparator with phenol red as indicator; the alkali and the acid being added slowly and with vigorous shaking. Finally, as sterilize the broth in bulk, either by autoclaving once for 20 at 121 degree and 15 psi or by steaming for 20 min on each of three successive days at the end of which filter through filter paper and place in quantities of 5 ml in the broth tubes which have been previously cleaned, plugged and sterilized. Sterilize the tubes with the contents at one atmosphere (121 °C) for 10 min. The final reaction of the medium should be between *p*H 7.3 to 7.5.

**B-3.1.1** The sterilized broth keeps indefinitely in bulk. Evaporation is liable to occur from the broth tubes if these are kept for a long period before use. Further re-sterilization, in bulk or in tubes, is not permissible.

**B-3.2 Test Organism**

This shall be Salmonella typhi (NCTC 786) cultures.

NOTE — The culture shall be obtained from the Director, Central Drug Laboratory, Kolkata; Director, Haffkine Institute, Mumbai; Director, Central Research Institute, Kasauli; IMTECH Chandigarh or ATCC/ATCC registered vendors. In case of dispute, the test organisms supplied by Central Drug Laboratory, Kolkata shall be treated as reference test organisms.

**B-3.3 Phenol (Carbolic Acid)**

With crystallizing point shall not be less than 40.5 °C.

**B-3.4** Preparation of Standard Phenol Control Dilutions Prepare a five percent stock solution in sterile distilled water, containing 5 g pure phenol in each 100 ml of solution, and use this for making the control dilutions, which are to be in the following proportions:

a) one gram of pure phenol in each 95 ml of solution made;
b) one gram of pure phenol in each 100 ml of solution made;
c) one gram of pure phenol in each 105 ml of solution made;
d) one gram of pure phenol in each 110 ml of solution made, and
e) one gram of pure phenol in each 115 ml of solution made.

**B-3.4.1** These dilutions shall not be kept for more than a week.

**B-4 PREPARATION OF CULTURES**

**B-4.1 Test Organism**

The freeze-dried cultures of the test organism may be obtained in tubes in vacuo (*see* Fig.2). The culture may also be obtained on cutrient agar slopes, or as “slab- culture’.

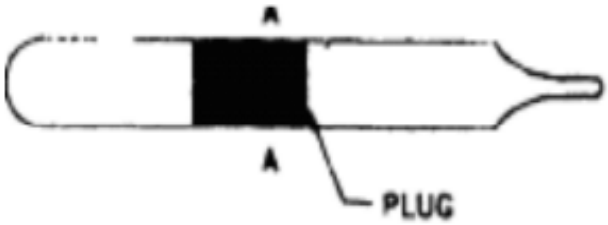



Fig. 2 Vacuum TUBE

**B-4.2 Preparation of Initial Culture**

When obtained as freeze-dried culture the tube containing the culture shall be handled as given in **B-4.2.1** and **B-4.2.2.**

**B-4.2.1** Make a file mark at a point about the middle of the plug (at A) and crack the tube along with mark with a red-hot glass rod, care being taken that air is admitted very slowly into the tube to avoid the risk of the plug being forced to the bottom of the tube. Remove the top of the tube when air has been admitted. Retain the numbered paper, as this was impregnated with the culture before drying. Withdraw the plug especially and discard it into a disinfectant. Replace the discarded plug by a new, previously sterilized plug of correct size.

**B-4.2.2** Add approximately 1 ml of sterile Rideal Walker broth (*see* **B-3.1**) to the contents of the tube by means of a sterilized pipette and incubate for 24 h at 37 °C. Prepare stock agar slope cultures and daily broth cultures from this initial culture as given in **B-4.3** and **B-4.4**.

**B-4.3 Stock Agar Slope Cultures**

Spread a standard loopful of the initial broth culture (*see* **B-4.2.2**) over the surface of an agar slope. Incubate for 24 h at 37 °C and then keep at a temperature not exceeding 22 °C until required.

**B-4.3.1** The agar medium is prepared by dissolving agar, microbiological grade in a suitable quantity of the bulk-sterilized Rideal Walker broth to give an agar content of 1.5 percent to 2.0 percent. Adjust *p*H, if necessary, to (7.4 ± 0.1) at 45 °C and distribute the medium into tubes or screw-cap bottles. Sterilize the tubes or bottles of the medium, either by autoclaving for 10 min at one atmosphere pressure (121 °C) or by steaming for 20 minutes on three successive days.

**B-4.4 Daily Broth Culture**

**B-4.4.1** *From Freeze-Dried Culture*

Transfer a standard loopful of the initial broth culture (*see* **B-4.2.2**) to 5 ml of Rideal Walker broth (*see* **B-3.1**) and incubate for 24 h at 37 °C. Start a second subculture from this culture by transferring a standard loopful or a second broth tube and incubate 24 h at 37 °C. Repeat this procedure on a day to day basis for a maximum period of 14 days after which discard the culture and replace by a fresh broth culture started from a stock agar slope culture.

**B-4.4.2** *From Stock Agar Slope Culture*

Transfer a small portion of growth from a stock agar slope culture to 5 ml of Rideal Walker broth (**B-3.1**) and incubate for 24 h at 37 °C. Start a second subculture from this culture by transferring a standard loopful to a second broth tube and incubating as before. Repeat this procedure on a day-to-day basis for a maximum period of 14 days, after which discard the culture and replace by a fresh broth culture.

NOTES

1. Before use in a test, the culture shall have had at least three successive daily subculturings
2. In cases where, on a particular day, subculturing would be impossible, a 48 h culture may be used for subsequent subculturing, provided that during the 48 h period the culture has been kept in an incubator, but in such circumstances a further 24 h subculturing shall be carried out before performance of the test.

**B-5 DETERMINATION OF PHENOL, COEFFICIENT**

**B-5.1** Mix well the sample of disinfectant to be tested immediately before any portion is withdrawn for testing, if necessary, by transferring it to a dry vessel of sufficient size for the purpose.

**B-5.2** Withdraw 5 ml (which shall constitute the test portion) from the middle of the sample (*see* **B-5.1**) by means of a 5 ml delivery pipette which is filled to above the mark, wiped clean outside with sterile cotton wool and run down to the mark. Allow the contents to discharge into the 500 ml measuring cylinder previously filled to about the 480 ml mark with sterile distilled water at a temperature of 17 °C to 18 °C, with the nozzle of the pipette thrice, or ore in the case of viscous fluids, by drawing up and returning to the clear portion of the liquid. Make up the solution to 500 ml with sterile distilled water. Stopper the cylinder and thoroughly mix the contents by inverting with a corkscrew motion fifty times. Prepare suitable test dilution from the stock solution using sterile distilled water (*see* Table 2).

**B-5.3** Place 5 ml of the four dilution chosen in each of four plugged sterile medication tubes or bottles, starting with the weakest solution (when the coefficient is quite unknown, it is necessary to perform one or more ranging tests with broadly separated dilutions). Place medication tubes in a rack (provided with a water-bath maintained at a constant

temperature which shall be between 17 °C to 18 °C) with the strongest disinfectant on the left. Place the fifth medication tube, containing 5 ml of the particular phenol control on the right. Use a separate pipette for taking the 5 ml of standard phenol control solution.

NOTE — Before use, mix the broth culture thoroughly and allow to settle for half an hour at 17 °C to 18 °C.

**B-5.4** Starting at zero time, add 0.2 ml of the culture to the left-hand medication tube. Shake the tube well. After 30 s, inoculate the next tube on the right with 0.2 ml of culture in a similar manner. Repeat the process with each successive tube at intervals of 30 s, until finally the phenol control has been inoculated. 30 s after this last addition (that is, 2.5 min after zero) withdraw a loopful of the well-shaken contents of the tube on the extreme left and place in a tube containing 5 ml of the Rideal walker broth (see **B-3.1**), this tube having previously been marked '1'. Thirty seconds after this loopful has been withdrawn, perform a similar operation on the second medication tube, the loopful being transferred to a tube of broth marked '2'. Repeat the procedure at intervals of 30 s with each of the five medication tubes, working from left to right, until four sets of cultures have been made, that is, at 2.5 min, 5 min, 7.5 min and 10 min respectively after exposure. Shake each tube immediately after medication. In each withdrawal, precautions shall be taken to ensure that the loop is removed vertically from the surface of the liquid with its plane horizontal.

**B-5.4.1** Sterilize the loop by flaming between each operation, care being taken that the loop is cold before being used again.

**B-5.5** Incubate the 20 tubes for not less than 48 h and not more than 72 h at 37 °C, when tubes containing the organism will be recognized by the opalescence of the broth.

**B-6 CALCULATION**

**B-6.1** Obtain the Rideal Walker coefficient by dividing that dilution of the disinfectant which shows life in 2.5 min and 5 min but no life thereafter, by that dilution of phenol (1:95, 1:100, 1:110 or 1:115) which shows life in 2.5 min and 5 min, but no life thereafter.

**B-6.2** It is convenient to refer to a tube showing life of Salmonella typhi by a positive (+) sign, and a tube showing no life or no Salmonella typhi by a negative (-) sign.

**B-6.3** When no previous test has been carried out, so that the necessary to carry out a separate test with the five phenol dilutions only, in order to obtain the control dilution of phenol which satisfies the above requirements. However, when a number of tests have to be carried out at the same time, a different phenol dilution may be used for each test, thus avoiding the necessity for a separate phenol test to obtain the control dilution of phenol. A typical set of results is shown in Table 1.

**Table 1**

**Typical Set of Results — Rideal Walker Coefficient**

(*Clause* B-6.3)

| **Sl No** | **Sample Disinfectant** | **Dilution** | **Result After Exposing Culture to Action Disinfectant for Specified Time (in min)** | | | |
|---|---|---|---|---|---|---|
| | | | **2.5** | **5** | **7.5** | **10** |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| i) | A | 1 : 1 000 | — | — | — | — |
| ii) | A | 1 : 1 100 | + | — | — | — |
| iii) | A | 1 : 1 200 | + | + | — | — |
| iv) | A | 1 : 1 300 | + | + | + | — |

| | | | | | | |
|---|---|---|---|---|---|---|
| v) | Phenol | 1 : 1 00 | + | + | — | — |
| | | Rideal Walker coefficient = 1 200/100 = 12.0. | | | | |

**B-6.4** Rideal Walker Coefficient over the range of dilution of disinfectant from 1: 400 to 1: 2 500 are given in Table 2. A one-percent stock solution prepared as specified in **B-5.1** and **B-5.2** is used. The total volume to which 5 ml of stock solution is made for the purpose of the test is shown in col 1 of Table 2 and the proportion of original disinfectant in the final dilution is shown in col 2 of Table 2.

**Table 2**

**Rideal Walker Coefficients for Different Dilutions**

(*Clauses* B-5.2 and B-6.4)

| *Total Volume Containing 5 mi of Stock Solution of Sample* | *Dilution of Sample* | *Coefficient when Growths in Disinfectant Dilution Equal to Growths in Phenol Dilution of One Part in* | | | | |
|---|---|---|---|---|---|---|
| | | 95 | 100 | 105 | 110 | 115 |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| 125 | 1:25 00 | 26.3 | 25.0 | 23.8 | 22.7 | 21.7 |
| 120 | 1:24 00 | 25.3 | 24.0 | 22.9 | 21.8 | 20.9 |
| 115 | 1:23 00 | 24.2 | 23..0 | 21.0 | 20.9 | 20.0 |
| 110 | 1:22 00 | 23.2 | 22.0 | 21.0 | 20.0 | 19.1 |
| 105 | 1:21 00 | 22.1 | 21.0 | 20.0 | 19.1 | 18.3 |
| 100 | 1:20 00 | 21.1 | 20.0 | 19.0 | 18.2 | 17.4 |
| 95 | 1:19 00 | 20.0 | 19.0 | 18.1 | 17.3 | 16.5 |
| 90 | 1:18 00 | 18.9 | 18.0 | 17.1 | 16.4 | 15.7 |
| 85 | 1:17 00 | 17.9 | 17.0 | 16.2 | 15.3 | 14.8 |
| 80 | 1:16 00 | 16.8 | 16.0 | 15.2 | 14.3 | 13.9 |
| 75 | 1.15 00 | 15.8 | 15.0 | 14.3 | 13.6 | 13.0 |
| 70 | 1:14 00 | 14.7 | 14.0 | 13.3 | 12.7 | 12.2 |
| 65 | 1:13 00 | 13.7 | 13.0 | 12.4 | 11.3 | 113 |
| 60 | 1:12 00 | 12.6 | 12.0 | 11.4 | 10.9 | 10.4 |
| 55 | 1:11 00 | 11.6 | 11.0 | 10.5 | 10.0 | 9.6 |
| 50 | 1:10 00 | 10.5 | 10.0 | 9.5 | 9.1 | 8.7 |
| 45 | 1:9 00 | 9.3 | 9.0 | 8.6 | 83 | 7.3 |
| 40 | 1:8 00 | 8.4 | 8.0 | 7.6 | 73 | 7.0 |
| 35 | 1:7 00 | 7.4 | 7.0 | 6.7 | 6.4 | 6.1 |
| 30 | 1:6 00 | 6.3 | 6.0 | 5.7 | 5.5 | 5.2 |
| 25 | 1:5 00 | 5.3 | 5.0 | 4.8 | 4.5 | 4.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20 | 1:4 00 | 4.2 | 4.0 | 3.8 | 3.6 | 3.5 |

NOTE — Rideal Walker test, specified above, is applicable only to water soluble or water miscible substances

## ANNEX C

(*Clause* 4.4)

## DETERMINATION OF STAPHYLOCOCCAL COEFFICIENT

### C-1 APPARATUS

The apparatus shall be the same as described in **B-2**.

### C-2 REAGENTS

#### C-2.1 Standard Staphylococcal Broth

Take 5 g of meat extract, microbiological grade, 10 g of peptone, microbiological grade and 5 g of sodium chloride and dissolve in 1 000 ml of distilled water. Add sufficient 1 N sodium hydroxide solution to bring the *p*H value at 20°C to approximately 7.0. As sterilize the broth in bulk, either by autoclaving once for 20 at 121 degree and 15 psi, at the end of which filter through a filter through a filter paper and place in quantities of 10 ml in broth tubes which have been previously cleaned, plugged and sterilized. Sterilize the tubes with the contents at one atmosphere (121 °C) for 10 min. The final reaction of the medium shall be between *p*H 6.8 to 7.0.

**C-2.1.1** The sterilized broth keeps indefinitely in bulk. Evaporation is likely to occur from the broth tubes if these are kept for a long period before use. Re-sterilization in bulk, or in tubes, is not permissible.

#### C-2.2 Test Organism

This shall be Staphylococcus aureus (MTCC 3160 or NCTC 3750) cultures.

NOTE — The culture shall be obtained from the Director, Central Drug Laboratory, Kolkatta, Director, Haffkine Institute, Mumbai or Director, Central Research Institute, Kasauli, IMTECH, Chandigarh. In case of dispute, the test organism supplied by Central Drug Laboratory, Kolkatta shall be treated as reference test organisms.

#### C-2.3 Phenol (Carbolic Acid)

The crystallizing point shall not be less than 40.5 °C.

#### C-2.4 Preparation of Standard Phenol Control Dilutions

Prepare a 5 percent stock solution in sterile distilled water, containing 5 g of pure phenol in each 100 ml of solution and use this for making the control dilutions, which are to be in the following proportions:

a) one gram of pure phenol in each 80 ml of solution made
b) one gram of pure phenol in each 85 ml of solution made,
c) one gram of pure phenol in each 90 ml of solution made,
d) one gram of pure phenol in each 95 ml of solution made, and
e) one gram of pure phenol in each 100 ml of solution made

**C-2.4.1** These dilutions shall not be kept for more than a week.

### C-3 PREPARATION OF CULTURE

**C-3.1** As prescribed for Rideal Walker coefficient (*see* **B-4**).

### C-4 DETERMINATION OF COEFFICIENT PHENOL

#### C-4.1 Procedure

As prescribed for Rideal Walker coefficient (*see* **B-5**).

**C-5 CALCULATION**

**C-5.1** Obtain the Staphylococcal coefficient by dividing that dilution of the disinfectant which shows life 2.5 and 5 min, but no life thereafter, by that dilution of phenol (1:80, 1:85, 1:90, 1:95 or 1:100) which shows life in 2.5 min and 5 min but no life thereafter.

**C-5.2** It is convenient to refer to a tube showing life of staphylococcus aureus by a positive (+) sign and a tube showing no life or no Staphylococcus aureus by a negative (-) sign.

**C-5.3** When no previous test has been carried out, so that the necessary phenol strength is quite unknown, it is necessary to Carry out a separate test with the five phenol dilutions only, in order to obtain the control dilution of phenol which satisfies the above requirements. However, when a number of tests have to be carried out at the same time, a different phenol dilution may be used for each test, thus avoiding the necessity for a separate phenol test to obtain the control dilution of phenol. A typical set of results is shown in Table 3.

**Table 3**

**Typical Set of Results - Staphylococcal**

(*Clause* C-5.3)

| **Sl No.** | **Sample Disinfectant** | **Dilution** | **Result After Exposing Culture to Action Disinfectant for Specified Time (in min)** | | | |
|---|---|---|---|---|---|---|
| | | | **2.5** | **5.0** | **7.5** | **10** |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| i) | A | 1 : 1 000 | — | — | — | — |
| ii) | A | 1 : 200 | + | — | — | — |
| iii) | A | 1 : 300 | + | + | — | — |
| iv) | A | 1 : 400 | + | + | + | — |
| v) | A | 1 : 500 | + | + | + | + |
| vi) | Phenol | 1 : 90 | + | + | — | — |
| | | Staphylococcal coefficient = 300/90 = 3.3 | | | | |

**ANNEX D**

(*Clause* 4.5)

**DETERMINATION OF MERCURY COMPOUNDS**

**D-1 PROCEDURE**

Take 20 ml sample in a 100 ml beaker. Add 5 ml of hydrochloric acid (1 part concentrated hydrochloric acid + 1-part water). Keep in the beaker a bright and clean copper rod (100 mm length x 3 mm diameter). Boil for 10 min, if mercury is present in the disinfectant fluid, the portion of the copper rod immersed in the boiling mixture will be coated with white or grey film.

**ANNEX E**

(*Clause* 4.7)

## METHOD FOR DETECTION OF PHENOLIC COMPOUNDS IN DISINFECTANT LIQUIDS

### E-1 GENERAL

It is a colorimetric method to determine the presence of phenolic compounds in any disinfectant liquid, phenolic type. The method involves the reaction of Folin Ciocalteu's reagent (Phosphomolybdic- Phosphotungstic phenol reagent) with phenols to form chromogens that can be detected by colour change from yellow to greenish blue. The colour development is due to the transfer of electrons at basic pH to reduce

the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals have lower valence.

### E-2 REAGENT

**E-2.1** 2 N Folin and Ciocalteu's reagent (Phosphomolybdic-Phosphotungstic phenol reagent).

**E-2.2** Sodium carbonate solution (7 percent solution).

**E-2.3** Standard phenol AR grade (1 percent solution)

**E-2.4** Distilled water.

### E-3 APPARATUS

**E-3.1** 250 ml conical flask.

**E-3.2** Graduated pipettes 5 ml and 10 ml.

**E-3.3** Measuring cylinder.

### E-4 PROCEDURE

Weigh accurately 1 gm of sample in a conical flask. Add 70 ml of distilled water and mix well. This solution is then mixed with 5 ml of 2N Folin and Ciocalteu's reagent. Add 10 ml of 7 percent sodium carbonate solution and mix well. Observe for colour change from yellow to deep bluish green. Prepare a blank set with distilled water and a positive control set with standard phenol reagent (AR grade).

### E-5 CRITERIA FOR CONFORMITY

The positive control will respond to this test and a deep bluish green coloured solution is obtained. The presence of phenolic compounds in the test sample will be indicated by change in colour from yellow to bluish green upon addition of Sodium carbonate solution. Whereas in the absence of phenolic compounds there will be no colour change.

## ANNEX F

(*Clause* 4.8.1)

## DETERMINATION OF VOLATILE MATTER

### F-1. APPARATUS

**F-1.1**. **Petri-Dish** — 75 mm diameter.

### F-2. PROCEDURE

**F-2.1** Weigh accurately about 10 g of the material in a tared petri-dish dry to constant weight at 105 °C.

### F-3. CALCULATION

$$\text{Volatile matter, percent by mass } = 100 - \frac{M_2 - M_1}{\text{M}} \times 100$$

where

$M_2$ = mass of petri-dish with residue;
$M_1$ = mass of empty petri-dish; and
$M$ = mass of sample taken for the test.

## ANNEX G

(*Clause* 4.8.2)

## DETERMINATION OF STEAM VOLATILE OIL

### G-1 GENERAL

The determination of volatile oil is made by distilling with water, collecting the distillate in a graduated tube/cylinder in which the aqueous portion of the distillate is automatically separated into two distinct layers, and measuring the volume of the oil. The content of volatile oil is expressed as a percentage (*v /v*).

### G-2 REAGENT

**G-2.1 Distilled water**

### G-3 APPARATUS

**G-3.1 Distilling flask —** A round-bottomed distilling flask of 200 ml capacity and having a total length of 17 cm to 19 cm and an inside neck diameter of 20 mm to 22 mm. Attached about midway on the neck, approximately 12 cm from the bottom of the flask, is a side-arm 10 cm to 12 cm long and 5mmin internal diameter which is at an angle of 70 ° to 75° with the lower portion of the neck.

**G-3.2 Condenser** —A straight glass condenser 55 cm to 60 cm long with a water jacket about 40 cm long or any other type of condenser having equivalent condensing capacity. The lower end of the condenser may be bent to provide a delivery tube, or it may be connected to a bent adaptor that serves as a delivery tube.

**G-3.3 Receiver** — A 100 ml cylinder graduated in 1ml sub-divisions.

**G-3.4 Pipettes** — 50 ml

**G-3.5 Heating Arrangement** —Water Bath or Bunsen burner or electric heater or heating mantle with arrangement for adjustment of the applied heat.

**G-3.6 Thermometer** — An accurately standardized, partial immersion thermometer having the smallest practical sub-divisions (not greater than 0.2°). When placed in position, the stem is located in the center of the neck and the top of the bulb is just below the bottom of the outlet to the side arm

### G-4 PROCEDURE

Assemble the apparatus i.e. Distilling flask, Condenser, Receiver, thermometer, heating equipment and place 50 ml of the liquid being examined into the distilling flask by 50 ml pipette. Rinse thoroughly the pipette with distilled water and add rinse distil water into distilling flask. Insert the thermometer and seal the entire heating and flask assembly from external air currents. Add a few pieces of porous material and heat rapidly to boiling using a heating arrangement for adjustment of the applied heat and maintain distillation rate of 4 to 5 ml per minute. Remove heating equipment and cool at room temperature. Receiver should be kept constant for 30 minutes before taking reading. Aqueous and oil phase to be distinguished clearly and separately.

### G-5 CALCULATION

$$\text{Volatile Oil, percent by volume} = \frac{V_1 - V_2}{\text{V}} \times 100$$

where

$V_1$ = Upper Level reading of oil phase;
$V_2$ = Lower Level reading of oil phase; and
$V$ = Volume of sample taken for the test.

## ANNEX H

(*Clause* 7.1)

## SAMPLING OF DISINFECTANT FLUIDS, PHENOLIC TYPE

### H-1 GENERAL REQUIREMENTS

**H-1.1** In drawing, preparing, storing and handling samples, the following precautions shall be taken.

**H-1.2** Samples shall not be taken in an exposed place.

**H-1.3** The sampling instrument shall be clean and dry.

**H-1.4** To draw a representative sample, the contents of each container selected for sampling shall be mixed as thoroughly as possible by suitable means.

**H-1.5** Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the container for samples from adventitious contamination. The sampling instrument and the container for samples shall be rinsed with the material prior to drawing the sample.

**H-1.6** Samples shall be placed in suitable clean, dry and airtight containers.

**H-1.7** Sample containers shall be of such a size that an ullage of at least 10 percent is left after pouring in the sample.

**H-1.8** Each sample container shall be sealed air tight with a stopper after filling and marked with full details of sampling and the particulars given in **6.1.**

**H-1.9** Samples shall be stored in a cool and dry place.

### H-2 SCALE OF SAMPLING

**H-2.1 Lot**

All containers in a single consignment of the same class of material and drawn from a single batch of manufacture shall constitute a lot.

**H-2.2** For ascertaining conformity of the material to the requirements of the standard, samples shall be selected from each lot separately.

**H-2.3** The number of containers to be selected shall depend on the size of the lot and shall be in accordance with Table 4.

**Table 4 Scale of Sampling**

(*Clause* H-2.3)

| Sl No. | Number of Containers in the Lot | Sample Size |
|---|---|---|
| (1) | (2) | (3) |
| i) | Up to 100 | 2 |
| ii) | 101 to 300 | 3 |
| iii) | 301 to 1 000 | 4 |

| | | |
|---|---|---|
| iv) | 1 001 and above | 5 |

**H-2.3.1** The containers shall be chosen at random from the lot. In order to ensure the randomness of selection, procedures given in IS 4905 may be followed.

**H-3 TEST SAMPLES AND REFEREE SAMPLES**

**H-3.1** Draw with an appropriate sampling instrument small portions of the material from different parts of each container selected according to **H-2.3** and freshly

opened. Keep the samples from different containers separately. Mix approximately equal portions from different containers selected from sampling so as to obtain a composite sample. The quantity of material in the composite sample shall be sufficient for triplicate determination of all the requirements given in the standard.

**H-3.1.1** Divide the composite sample into three equal parts constituting three test samples and transfer each of the test samples to thoroughly dried bottles and seal the bottles alright. Each sample container shall be labeled with all the particulars of the sampling given in **H-1.8.**

**H-3.1.2** Send one sample to the purchaser and another to the supplier. The third test sample, bearing the seals of the purchaser and the supplier shall be kept as a referee sample. The referee sample shall be kept as a place agreed to between the purchaser and the supplier so as to be used in case of a dispute between the two.

**H-4 CRITERIA OF CONFORMITY**

**H-4.1** The lot shall be declared as conforming to the requirements of the standard if the composite samples meets all the requirements given in the standard except stability on storage (*see* **4.6**).

NOTE — For the requirements relating to stability on storage, the assurance shall be provided by the manufacturer as agreed with the purchaser.