DRAFT TANZANIA STANDARD

Sewage effluents – Sampling and test methods

0 Foreword

0.1 The sewage and waste effluent from the urban centers have to be treated either for re-use or for safe disposal instead of being left to pollute our surface and sub-surface water.

0.2 The kind and extent of treatment required depends on the composition of the effluents themselves, and the use to which the effluent is to be put.

0.3 It is, therefore, necessary that the factors normally accepted as being indicative of pollution such as dissolved oxygen, biochemical oxygen demand, and the presence of coliform bacteria be carefully determined by the appropriate test methods.

0.4 In the preparation of this standard, assistance has been derived from:

> IS 4733: 1972 – *Methods of sampling and test for sewage effluents,* published by the Indian standards Institution.

0.5 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with TZS 4 – *Rounding off numerical values.*

1 Scope

1.1 This Tanzania Standard prescribes the methods of sampling and test for sewage effluents.

2 Normative Reference

2.1 For the purpose of this standard the following reference shall apply:

TZS 59 Water-*Distilled Quality – Specification.*

3 Sampling

3.1 Point of sampling

In those cases, where the effluent at a specific point is to be tested, the question of choosing the point of sampling does not arise. However, where the composition of an effluent as finally discharged by a sewage treatment plant is to be ascertained, the point of sampling shall be the final outlet of the treatment plant.

3.2 Frequency of sampling

When it is required to find out variations in the composition of the effluent during a specified period, such as that of peak discharge, the samples shall be taken at short and appropriate intervals, say, every 5, 10, 15 or 30 min and analyzed. To study the average conditions over a cycle of operations or a period (usually 24 h or during the daily working period of the treatment plant, the collection of composite sample shall be adopted. The composite sample shall be made by collecting at appropriate intervals samples form the common channel or drain at a point where the flow of the effluent is likely to be most representative of the entire volume, and mixing. The volume of the individual samples shall be a fixed proportion of the volume of the effluent flowing at that time. The interval should depend upon the frequency of variation in the nature of the effluent flowing at the time. The interval should depend upon the frequency of variation in the nature of the effluent and the volume of flow. Care shall be taken to take the samples in such a way as to maintain the true proportion of suspended solids. Samples shall not be taken by skimming the top or scraping the bottom. A point about one-third of the way from the bottom shall normally be selected. The samples shall be drawn gently without unnecessary aeration. In most cases, collection of samples every hour would be sufficient.

3.3 Sapling instrument

Only clean glass vessels should be used as sampling instruments. The vessels used for taking the samples shall be wide-mouthed and small enough for the contents to be transferred quickly to the sample container without leaving behind any deposit or scum. Automatic sampling devices, if available, may be used.

3.3.1 Each individual small shall be deposited in a receptacle of sufficient size to hold the entire composite sample. Clean, dry and large glass containers with lids without chipping may be used for pooling the sample.

NOTE – These samples are to the used for testing parameters other than D.O.

3.4 Sample containers

3.4.1 The quantity of sample required for analysis shall be taken form the composite sample after thorough mixing in order to keep the solids sin suspension.

3.4.2 The sample for analysis shall be drawn in clean glass stoppered bottles, which shall be rinsed with a portion of the sample. New bottles shall be washed with acid and thoroughly rinsed with distilled water before being brought into use. About two to three litres of the sample will be required for analysis. The bottle containing the final sample shall be filled so that a small air bubble is present after closure to prevent leakage or even breakage arising from any subsequent changes in temperature. The stopper shall be firmly inserted and, if the sample is to be transported some distance, tied down to keep in position.

NOTE – Plastic bottles may be used as sample containers except when testing for heavy metals.

3.4.3 The label of the bottles shall bear the name of the sampling authority, details of the type of sample, place date and time of sampling.

3.5 Preservation of samples

3.5.1 The samples shall be kept at low temperature (about 4°C) during the collection and thereafter.

3.5.2 No single method of preservation is applicable for the sample for all the tests. The analysis shall be carried out, preferably, immediately after collection. Storage at 3 to 4°C in a well-insulated ice box or refrigerator is the best way to preserve most samples till the next day. Where chemical preservatives are used as specified for individual tests, these shall be added to each portion of samples taken for the particular test and not the entire sample.

4 General precautions and directions for tests

4.1 Quality of reagents

Unless specified otherwise, chemicals of analytical grade and distilled water in accordance with TZS 59:1980 (See clause 2) shall be used in tests.

4.2 It is important to obtain a representative sample. Appropriate methods of sampling are given in Clause 3. However, in tests where specific sampling procedures are prescribed these shall be followed.

4.3 For determinations for which calibrated glass discs are available, these may be used for routine examination provided instructions of the manufacturer are followed. But in case of dispute, test methods as prescribed in this standard shall be followed.

5 Total suspended solids

Suspended solids are determined by filtering a well-mixed sample through a standard glass fiber filter, retaining the residue, and drying it to a constant mass at 103°C to 105°C. The increase in the mass of the filter represents the total suspended solids.

5.1 Apparatus

5.1.1 Filtration Apparatus

A filtration apparatus suitable for use with glass fiber filters, consisting of:

- A filter holder (membrane filter funnel or Gooch crucible adapter) with a fritted glass or stainless steel support base.

- A vacuum source (filter pump or vacuum pump) capable of maintaining a pressure difference of approximately 70 kPa.
- Suitably sized filtering flasks (e.g., 500 ml or 1 litre).

5.1.2 Glass Fiber Filters

Circular glass fiber filters, without organic binder, with a nominal pore size of 0.7 µm to 1.5 µm (e.g., Whatman GF/C, Whatman GF/A, or equivalent). The filter size shall be chosen to fit the filtration apparatus (typically 25 mm, 47 mm, or 55 mm diameter).

5.1.3 Drying Oven

A well-ventilated oven capable of maintaining a temperature of 103°C to 105°C.

5.1.4 Desiccator

Provided with a desiccant (e.g., silica gel) containing a moisture indicator.

5.1.5 Analytical Balance

Capable of weighing to the nearest 0.1 mg.

5.1.6 Measuring Volumetric flask or Cylinder

Of appropriate volume (e.g., 25 ml, 50 ml, 100 ml) with a wide tip to prevent clogging.

5.1.7 Wash Bottle

Containing distilled water.

5.2 Procedure

5.2.1 Preparation of the Filter

i). Place the glass fiber filter (7.1.2) on the support base of the filtration apparatus (7.1.1).

ii). Apply a small amount of vacuum and wash the filter by drawing through three successive 20 ml portions of distilled water. This helps to remove any loose fibers or soluble material.

iii). Carefully remove the filter from the apparatus and place it in a clean, labeled aluminum weighing dish or on a watch glass.

iv). Dry the filter in the oven at 103°C to 105°C for one hour.

v). Remove the filter from the oven, cool to room temperature in a desiccator, and accurately weigh it to the nearest 0.1 mg.

vi). Repeat the drying (for 30-minute periods), cooling, and weighing until a constant mass is achieved, or until the mass change is less than 0.1 mg . Record this as the initial mass (M_1).

5.2.2 Sample Filtration

i). Assemble the clean, dry filtration apparatus with the pre-weighed glass fiber filter (from 7.2.1) in place. Ensure a good seal to prevent leakage.

ii). Shake the sample vigorously to ensure a homogeneous suspension. Immediately measure a measured volume of the well-mixed sample using a graduated cylinder or pipette. Select a sample volume that will yield a residue of between **2.5 mg and 200 mg** . A volume of 50 ml to 100 ml is often suitable for sewage and industrial effluents, but this may need adjustment based on the solids concentration.

iii). With the vacuum on, wet the filter with a small amount of distilled water.

iv). Immediately filter the measured volume of the sample through the filter. Rinse the graduated cylinder with two 10 ml portions of distilled water and pass the rinsings through the filter to ensure complete transfer of solids.

v). Wash the filter and residue with three successive 10 ml portions of distilled water to remove soluble salts. Allow complete drainage between washings.

vi). Continue to apply vacuum until the filter is visibly dry.

vii). Carefully remove the filter from the filtration apparatus using forceps.

5.2.3 Drying and Weighing

i). Transfer the filter containing the residue to the same aluminum weighing dish or watch glass used in 7.2.1.

ii). Dry in the oven at 103°C to 105°C for one hour.

iii). Cool to room temperature in a desiccator.

iv). Weigh the filter and residue to the nearest 0.1 mg. Record this as the final mass (M_2) .

v). Repeat the drying (for 30-minute periods), cooling, and weighing until a constant mass is achieved (mass change less than 0.1 mg).

5.3 Blank Determination

A blank determination shall be carried out in parallel with the sample analysis. Using the same apparatus and procedure, filter a measured volume of distilled water (equal to the volume of sample used). The mass change of the blank filter (if any) should be noted. If the blank mass change exceeds 0.5 mg, the source of contamination shall be investigated.

5.4 Calculation

Calculate the total suspended solids concentration using the following formula:

$$\text{Total Suspended Solids, mg/l} = \frac{(M_2 - M_1 - B) \times 1000}{V}$$

where:

- M_2 = final mass of the filter + residue, in milligrams (mg);
- M_1 = initial mass of the clean filter, in milligrams (mg);

- B = mass change in the blank filter, in milligrams (mg) (if significant; otherwise, $B = 0$);
- V = volume of the sample filtered, in millilitres (ml);
- 1000 = conversion factor from millilitres to litres.

5.5 Expression of Results

Report the result in milligrams per litre (mg/l) to the nearest 1 mg/l for values less than 100 mg/l, and to the nearest 5 mg/l for values of 100 mg/l or greater.

6 Dissolved oxygen

6. 0 Outline of the method

All the methods for the determination of dissolved oxygen are based on the original Winkler procedure. The principle is that of precipitation of manganous the principle is that of precipitation of manganous hydroxide, in a bottle filled with the sample, brought about by the addition of a solution of manganous sulphate followed by alkaline potassium iodine solution. The oxygen present in the sample quickly reacts with the manganous hydroxide. On subsequent acidification, iodine liberated is estimated by titration against a standard solution of sodium thiosulphate, using starch as indicator.

6.0.1 The Winkler method is subjected to interference due to various ions and compounds contained in the sample Suitable modifications of the method are adopted to correct for this interference. The choice of the exact modified procedure will depend upon the nature of the sample and the interference present. The application of these procedures and the methods are given below.

Procedure	Applicability

Winkler Method	When no interfering substances are present.
Alsterberg (sodium aside) modification	When not more than 0.1 mg /l of nitrite nitrogen and not more than 1 mg/l of ferrous iron are present and in absence of other reducing or oxidizing agents. In the presence of 5 mg/ l or more of ferric iron, potassium fluoride is added. When potassium fluoride is added, the method is applicable in the presence of 100 to 200 mg /l of ferric iron.
Rideal-steward (permanganate) modification	In the presence of ferrous iron only. If ferrous or ferric iron is more than 10 mg/l potassium fluoride is added before acidifying.
Alkali hypochlorite modification	In the presence of sulphite, thiosulphate, polytionate, free chlorine and hypochlorite. However, the results obtained with this method connot be relied upon to give accurate results in the determination of dissolved oxygen as this procedure gives somewhat low results.
	In the presence of organic matter easily oxidized at the pH of the alkaline iodide treatment.
Short-Thereault modification	In the presence of high amounts of suspended solids.
Alum flocculation modification	

6.1 Winkler method

6.1.1 *Reagents*

6.1.1.1 *Manganous sulphate solution* – Dissolve 480 g of manganous sulphate tetrahydrate ($MnSO_4$ $4H_20$) in water; filter if not clear, and made up to one litre. The solution should not liberate more than a trace of iodine when added to an acidified potassium iodide solution. Dilute the mixture to one litre.

6.1.1.3 *Concentrated sulphuric acid*

6.1.1.4 Standard sodium thiosulphate working solution Exactly 0. 025 mol/l, freshly standardized against potassium dichromate. One millilitre of this solution is equivalent to 0.2 mg of oxygen.

6.1.1.5 Starch indictor solution – Triturate 5 g of starch and 0.01 g of mercuric oxide with 30 ml of cold water and slowly pour it with stirring into one litre of boiling water. Boil for here minutes. Allow the solution to cool and decant off the supernatant clear liquid.

6.1.2 Procedure

To the sample, collected in a 250 to 300 ml bottle, add two milliliters of maganous sulphate solution followed by tow milliliters of alkaline iodide solution well below the surface of the liquid. Stopper carefully to exclude air bubbles and mix by inverting the bottle at lest 15 times. When the precipitate settles, leaving clear supernatant above the manganous hydroxide floc, shake again. With sea water, allow at least two minute period of contact with the precipitate. After at least two minute settling has produced at least 100 ml of clear supernatant carefully remove the stopper and immediately add two millilitres conc. H_2SO_4 by allowing the acid to run down the neck of the bottle, stopper, and mix by gentle inversion until dissolution is complete. Distribute the iodine uniformly throughout the bottle before decanting the amount needed for titration. Use a volume corresponding to 200 ml of the original sample after correction for the loss of sample by displacement with the reagents. Thus for a total of four milliliters (2 ml each) of the magnous sulphate and alkaline iodide reagents in a 300 ml bottle, titrate 200 x 300 / 300-4) = 203 ml.

Titrate with 0.025 mol/l thiosulphate solution to pale straw colour. Add one to two milliliters of starch solution and continue titration to the first disappearance of the blue colour. Subsequent recolourations due to the catalytic effect of nitrites or to the presence of traces of ferric salts which have not formed fluoride complexes should be disregarded.

6.1.3 Calculation

For 200 ml of original sample, one milliliter 0.25 mol sodium thiosulphate = 1mg/lD.O.

6.2 Alsterberg sodium azide modification

6.2.1 *Reagents*

Use all the reagents used in clause 6.1.1 except alkaline iodide solution, which is to be replaced by alkaline iodide- sodium azide solution, and potassium fluoride reagent.

6.2.1.1 *Alkaline iodide-sodium azide solution* – Dissolve 10 g of sodium azide in 40 ml of water. Add this with constant stirring to the cool alkaline iodide solution prepared as in 6.1.1.2 but made up to 950 ml.

6.2.1.2 *Potassium fluoride solution* – Dissolve 40 g potassium fluoride (KF. $2H_2O$) in 100 ml of water

6.2.2 Procedure

The procedure given in 6.1.2 shall be followed but, instead of iodine solution, alkaline iodidesodium azide solution shall be used. When 5 mg/l of ferric iron is present, add one milliliter of potassium fluoride solution before acidifying the sample and titrate immediately after acid addition and mixing.

6.2.3 *Calculation* – Calculate as in 6.1.3.

6.3 Rideal – Steward (permanganate) modification)

6.3.1 *Reagents*
Use all the reagents given in 6.1.1, potassium fluoride solution given in 6.2.1 and, in addition, the following reagents.

6.3.1.1 Potassium permanganate solution – Dissolve 6.3 g of potassium permanganate in water and make up to one litre.

6.3.1.2 Potassium oxalate solution – Dissolve tow grams of potassium oxalate ($K_2C_2O_4H_2O$) in 100 ml of water.

6.3.2.2 *Procedure*
Remove the stopper of the bottle containing the sample, add below the surface, with a one milliliter graduate pipette, 0.70 ml of concentrated sulphuric acid followed by sufficient potassium permanganate solution (about one milliliter) to produce a violent tinge which persists for five minutes. Avoid large excess of permanganate. Stopper and mix by inversion. After five minutes remove the excess permanganate by adding 0.5 ml portions of potassium oxalate solution and mixing.

Allow five minutes interval after each addition of oxalate. Then proceed as in 6.1.2, using four millilitre alkaline iodide solution instead of two millers. When either ferrous or ferric iron is present in excess of 10 mg/ l, add one milliliter of potassium fluoride solution immediately after permanganate addition. The titration, after final acidification, should be carried our without delay.

6.3.3 *Calculation*

Calculate as in 6.1.3.

6.4 *Alkali Hypochloride modification*

6.4.1 *Reagents*

Use all the reagents five in 6.1.1, substituting alkaline iodide solution with alkaline iodide-sodium azide solution (see 6. 2.1.1). in addition, use the following reagents.

6.4.1.1 Alkaline hypochloride solution- Two mol/l prepared by passing chlorine gas trough a 2.1 mole/l sodium hydroxide solution, with cooling. One milliliter of this solution will require, on acidification in the presence of potassium iodide, about 20 ml of 0.1 mol / sodium thiosulphate solution for titration. Check the strength of the solution every week.

6.4.1.2 Potassium iodide solution – one mol/l. Dissolve 17 g of potassium iodide in water and make up to 100 ml. Preserve by adding one milliliter of one mol/l sodium hydroxide solution.

6.4.1.3 *Solphuric acid 1: (v/v)*

6.4.1.4 Sodium sulphite solution – 0.05 mol/l. Dissolve 6.3 g of anhydrous sodium sulphite or 12.6 g of sodium sulphite heptahydrate in water and make up to one litre. Do not use solution when its strength goes down to less than 80% of the original.

6.4.1.5 Potassium biniodate solution-o.1 mol/l. Dissolve 38.99 g of potassium bioniodate ($KH(10_3)_2$ in water and make up to one litre in a volumetric flask.

6.4.1 Procedure

Remove the stopper of the bottle containing the sample and add 0.2 ml or just sufficient quantity of alkali hypochlorite solution to oxidize the sulphite. Stopper and mix by inversion for 20 to 30 s. Add one milliliter of potassium iodide solution and acidify with one milliliter or more of sulphurc acid. Mix by inversion. Add 0.2 ml of starch indicator solution and destroy the iodine liberated with sodium sulphite solution. Restore the blue tinge with 0.1 ml portions of 0.1mol/l potassium biniodate solution. Proceed further as in 6.2.2 with the difference that four milliliters of alkali iodide-sodium azide solution has to be added instead of two milliliters.

6.4.3 *Calculation*
Calculate as in 6.1.3

6.5 Short – Theriault modification

6.5.1 *Reagents*

Use all the reagents given in 6.1.1, substituting alkaline iodide solution with alkaline iodidesodium azide solution (see 6.2.1.1).

6.5.2 Procedure

Remove the topper of the bottle containing the sample, add tow mol of manganous sulphate solution followed by two milliliters of alkali iodide- solution, stopper and mix by inversion for 20 s. Immediately add two milliliters of concentrated sulphuric acid before the precipitate settles, remix and titrate as prescribed in 6.1.2.

6.5.3 *Calculation* – Calculate as in 6.1.3

6.6 Alum Flocculation modification

6.6.1 *Reagents*

Use all the reagents given in 6.2.1 and, in addition the following reagents.

6.6.1.1 *Alum solution* – Dissolve 10 g of potassium aluminum sulphate in water and dilute to 100 ml

6.6.1.2 Ammonium hydroxide - Concentrated

6.6.2 Procedure

Collect the sample in a glass –stoppered bottle or 500 to 1 000 ml capacity, using the same precautions as are necessary for the 300 ml sample. Add 10 ml of alum solution followed by one to two milliliters of ammonium hydroxide. Stopper and invert gently for about one minute. Allow it to settle for about 10 minutes and then siphon the clear supernatant into a 250 to 300 ml dissolved oxygen bottle until it overflow. Avoid aeration and keep the siphon submerged at least 20 cm. Then follow the procedure and calculate the result as given in clauses 6.2, 6.4, or 6.5 as appropriate.

7 Biochemical oxygen demand

7.0 Outline the method

Biochemical oxygen demand (BOD) is the quantity of oxygen required by a definite volume of the liquid effluent for oxidizing the organic matter contained in it by micro-organisms under specified conditions. For its determination, the dissolved oxygen content of the sample, with or without dilution, is measured before and after incubation at 20°C for five days.

7.1 Apparatus

7.1.1 *Glass-stoppered bottles*

Narrow-neck bottles of about 200 mil capacity, with suitable water sealing.

7.1.2 Sodium hydroxide solution – Approximately one mol/l.

7.2.2 Hydrochloric acid – approximately 1 mol/l.

7.2.3 Sodium sulphite solution- dissolve, 1.5 g of anhydrous sodium sulphite in one litre of water. Prepare fresh solution daily for use.

7.2.4 *Dilution water*

Distilled water of good quality, free form metals particularly copper, and aerated.

7.2.5 Phosphate buffer solution dissolve 8.5 g of potassium dihydrogen phosphate (KH_2PO_4), 21.75 g of dispotassium hydrogen phosphate (K_2HPO_4), 33.4 g of disodium hydrogen phosphate ($Na_2 HPO_4. 7H_2O$) and 1.7 g of ammonium chloride in about 500 ml of water and dilute to one litre. The pH of this solution should be 7.2.

7.2.6 Magnesium sulphate solution

Dissolve 22.5 g of magnesium sulphate ($MgSO_4. 7H_2O$) in about 500 ml of water and dilute to litre.

7.2.7 Calcium chloride solution.

Dissolve 27.5 g of anhydrous calcium chloride in water and dilute to one litre.

7.2.8 *Ferric chloride solution*

Dissolve 0.25 g of ferric chloride ($FeCl_3. 6H_2O$) in water and dilute to one litre.

7.2.9 Seeding material

Supernatant liquor of domestic sewage for 24 to 36 h at 20°C in the case of industrial effluents containing organic compounds which are not easily oxidized by sewage seed the receiving water, collected about 3.5 km below the discharged point, may be used.

7.3 Procedure

7.3.1 Sample containing acidity or caustic alkalinity should be neutralized to Ph about 7.0 with sodium hydroxide solution or hydrochloric acid respectively by adding a predetermined quantity.

7.3.2 Sample containing residual chlorine or chloramines should be dechlorinated if chlorine is not dissipated on standing for two hours. To dechlorinate, first determine the quantity of sodium sulphite solution required for a known aliquot of the sample by titration to sarch-iodide end point after acidifying the sample with acetic acid (1:1 or sulphuric acid (1: 50) followed by 10 mol of 10% potassium iodide solution. Then add to the requisite volume of the sample the predetermined quantity of sodium sulphite avoiding any excess, and check for the absence of chlorine after 20 min.

7.3.3 Samples containing toxic substances in large amounts would require special treatment. However, the effect of small amounts may be overcome by using the proper dilution so that toxicity is removed and the maximum BOD value is obtained. If increasing dilutions show increasing BOD, the dilution should be increased to a level where BOD levels off at a maximum.

7.3.4 To check the quality of the dilution water and the effectiveness of the seed, determine the BOD of a standard solution of 300 mg/l of either glucose or glutamic acid in the dilution water. Standard glucose solution should show a BOD of 224 ± 10 mg/l and glutamic acid 217 ± 10 mg/l

7.3.5 Store the dilution water at 20°C and use when near the temperature. Take the desired volume of dilution water required for the test sample and add, for every one litre of water, one milliliter each of phosphate buffer solution, magnesium sulphate solution. Seed the dilution with seeding material. The quantity of seeding material (o.1 to one per cent of settled sewage) added should be such that oxygen depletion in the dilution water control is between 0.2 and 0.8 mg /l after incubation at 20° C for five days.

7.3.6 Prepare, as follows, several dilutions of the sample (usually five to 25%) so as to obtain a depletion of at least 2 mg/ l of dissolved oxygen after incubation for five days. In the case of dilution greater than 1: 100, prepare a 10% primary dilution a volumetric flask and from this make the final dilutions.

7.3.7 Siphon carefully the prepared seeded dilution water into a graduated 1 000 m measuring cylinder and fill to the 500 ml mark. Add the requisite quantity of the carefully well mixed sample to make the particular dilution and fill with dilution water to one litre. Mix thoroughly but gently with a plunger type of rod without entraining air siphon the dilution into tow glass-stoppered bottles, fill completely and stopper. Prepare serial dilutions of lower concentration in the same manner determine the initial dissolved oxygen concentrations in one of the two bottles of each dilution by the appropriate method given in clause 6. Water-seal the other bottles and incubate at 20°C for five days. At the same time siphon the dilution water alone into two glass-stoppered bottles and determine the blank in one and incubate the other at 20°C for five days. After incubation for five days determine the dissolved oxygen in the dilutions and the blank in the same manner as the initial dissolved oxygen content.

7.4 Calculation

Biochemical oxygen demand (five days at 20° C), mg/l =

$$\frac{(D_1 - D_2) - (C_1 - C_2)\,F}{P}$$

Where

D_1 = initial dissolved oxygen content of the sample;

D_2 = dissolved oxygen content of the diluted sample after incubation;

C_1 = initial dissolved oxygen content of the seeded dilution water,

C_1 = initial dissolved oxygen content of the seeded dilution water after incubation;

F = ratio of the seed in the sample to that in the control, that is, per cent seed in D1 divided by per cent seed in C1, and

P = decimal fraction of the sample used.

7.4.1 Express the result t the nearest 0.2 mg/l.

8 Coliform test

8.0 General

The coliform test is carried out in three stages, comprising the presumptive test, the confirmed test, and the completed test. The presumptive test consists of inoculating measured amounts of the sample into tubes of MacConkey bile-salt lactose broth and incubating the cultures at 37°C for 24 to 48 h. if acid and gas are formed, the test is positive. If they are not formed, the test is negative. If all tubes are negative the conclusion is that coliform bacteria are not present in the sample. Positive results require further testing for confirming the presence of coliform bacteria in the sample. The Eijkman test is done either by subculturing in brilliant green lactose bile broth and incubating at 44 ± 0.5°C for h; (in order to obtain 44°C, use a thermostatically controlled water bath,) examining the production of acid and gas; or by striking plates of MacConkey agar or eosin methylene blue agar with culture form the positive broth tubes in the presumptive test and examining the plates for the growth of typical coliform colonies after incubation at 37°C for 24 to 48 h. several isolated colonies of the typical kind are sued in the third test known as the completed test. This consists of inoculating the selected colony into MacConkey broth and also on an agar slant. If gas develops in the broth, and the organisms grow on the agar slant, and if a staining gram-negative rods, the presence of coliform bacterial in the original sample is proved.

8.1 Presumptive colifrom test

8.1.1 *Apparatus*

8.1.1.1 *Dilution bottles and tubes* – The dilution bottles shall each contain 90.0 ml of sterile quarter strength Ringer's solution and the tubes 9.0 ml of sterile quarter strength Ringer's solution (see 8.1.2.3).

8.1.1.2 *Fermentation bottles* – Approximately 150 to 170 ml capacity provided with a stopper or screw cap with liners that do not produce toxic or bacteriostatic compounds on sterilization and Durban tubes approximately 75 x 11 mm) for use with 50 ml of double strength MacConkey broth (see 8.1.2.2).

8.1.1.3 *Fermentation tubes* – of approximately 150 x 18 mm, provided with Durham tubes (approximately 35 x 8 mm) for use with 10 ml of double strength broth, and tubes of 150 x 15 mm provided with Durham tubes for use with five milliliter of the media.

8.1.1.4 *Petri dishes* – of 100 mm diameter and 15 mm depth.

8.1.1.5 *Pipettes* – 10 ml and one milliliters straight-sided delivered pipettes. The tips shall be unbroken.

8.1.1.6 *Wire loops and needles* – The loops shall have an internal diameter of three millilimetres and shall be made form 0.800 m (or 21 gage platinum of nichrome wire).

8.1.2 *Media and reagents*

8.1.2.1 *Single strength MacConkey broth* – Dissolve five grams of sodium taurocholate, 20 g of peptone and five grams of sodium chloride in one litre of water place in the steam sterilizer for two hours, cool and keep in the refrigerator overnight. Add 10 g of lactose, and, when dissolved, filter through a good grade of filter paper while still cold. Adjust the solution pH 7.4 using phenol red as indicator. Add one milliliter of one per cent aqueous solution of neutral red. Distribute in five millilitre quantities into fermentation tubes (150 x 15 mm) and in 50 ml quantities into fermentation bottles and sterilize in the autoclave at pressure of 0.7 kg cm2 for 15 min. In the absence of an autoclave, steam sterilization for 30 min on three successive days may be done.

8.1.2.2 *Double strength MacConkey broth* - This shall be prepared in the same way as the single strength MacConkey broth, using double the quantities of the ingredients (except water) Distribute in 10 ml quantities into fermentation tubes (150 x 18 mm) and sterilize at a pressure of 0.7kg /cm2 for 15 min. Steam sterilization for 30 min on three successive days may be used in the absence of an autoclave.

8.1.2.3 *Quarter strength Ringer's solution* – Dissolve nine grams of sodium chloride, 0.42 g of potassium chloride, 0.48 g of calcium chloride and 0.20 g of sodium bicarbonate in one litre of water. This solution is known as Ringer's solution. Dilute 500 ml of this solution. I.e. one part of Ringer's solution ad three parts of distilled water. Alternatively quarter all the ingredients and dissolve in a litre of distilled water.

8.1.2.4 *Phosphate buffer solution* – Dissolve 34.0 g of potassium dihydrogen phosphate (KH2PO4) in 500 ml of water, adjust pH to 7.2 with sodium hydroxide solution (1mol/l) and dilute with water to one litre. Pipette out 1. 25 ml of the above stock solution into one litre of water. Autoclave the dilute solution for 20 min and distribute in amounts of 99 ± 2 ml or 9.0 ±0.2 ml as prescribed.

8.1.3 Procedure

8.1.3.1 Shake the sample bottle vigorously 25 times. Remove the stopper and flame the mouth of the bottle. Pour away about a quarter of the water. Replace the stopper and shake again 25 times. The amount of water sample used in test will depend on the anticipated bacteria purity of water. For very good samples, transfer 50 ml of the well-shaken sample with a sterile pipette into one fermentation bottle containing 50 ml of double strength McConkey broth and, separately, 10 ml of the sample to each of five fermentation tubes containing 10 ml of double strength MacConkey broth. If the density of coliform bacteria is expected to be higher, transfer 50 ml of the sample into the fermentation bottle and five 10 ml and five one milliliter portions of the sample, each into five tubes containing appropriate quantities of MacConkey borth (see note). With samples of poor bacteria quality inoculate each of a series of five or three tubes with 10 ml, one milliliter, and 0.1 ml, of one in 10 dilutions of the sample. Very bad samples may require one in 100 or higher dilutions of the sample.

NOTE – Volumes of one milliliter of the original sample or of the dilutions should be inoculated into five milliliters of single strength MacConkey broth and 10 ml or more of the sample should be added to the same volume of double strength medium.

8.1.3.2 Prepare one in 10 dilutions of the sample by adding, with a sterile pipette, 10 ml of the sample to 90 ml of sterile quarter strength Ringer's solution, or one milliliter of the sample to nine milliliters of the Ringer's solution and mixing vigorously. Higher decimal dilutions shall be prepared in the same manner, by adding 10 ml of the one in 100 dilutions to 90 ml of the dilution, and so on.

8.1.3.3 Incubate the inoculated fermentation bottles and tubes at 37°C and examine each bottle and tube for the production of acid and gas after 24 to 48 h or earlier.

8.1.3.4 Record all tubes showing acid and sufficient gas to fill the concavity at the top of the Durham tube as 'presumptive positive'. The absence of formation of acid and gas at the end of 48 hours incubation constitutes a negative test. The most probable number (MPN) coliform organisms in 100 ml of the sample shall be determined form the probability tables given in Annex B.

8.2 Confirmed test

8.2.0 General

Presumptive positive tubes form any sample of chlorinated water shall be confirmed for the actual presence of coliform organisms. Such information may not be necessary in the case of unchlorinated samples. Conformation shall be done using a solid medium or a liquid medium.

8.2.1 Confirmation using solid medium

8.2.1.1 *Media and reagents*

MacConkey agar - mix five grams sodium taurocholate , 20 g of peptone, five grams of sodium chloride and 20 g of shredded agar with 1 00 ml of water. Steam until the solids are dissolved. Add egg white, using the white of one egg for three litres of the medium. Autoclave at 0.7 kg /cm^2 pressure for 15 min and filter while hot through a good grade of filter paper, or a plug of cotton wrapped in gauze and placed in the funnel. Adjust reaction of the filtrate to pH 7.3 at 50°C or pH 7.5 at room temperature. Add 100 ml of 10% aqueous solution of lactose and 3.5 ml of two per cent solution of neutral red in 50% ethanol. Mix thoroughly, distribute into flasks and sterilize in the autoclave at 0.7 kg/cm^2 pressure for 15min. For use, melt in the steam oven and pour into sterile petri dishes (12 to 15 ml in each) and allow setting. Dry the surface of the medium in the incubator before use.

Eosin methylene blue agar – Place in a flask 10 g of the peptone, tow prams of dipotassium phosphate (K_2HPO_4) 20 g of agar and 1 00 ml of water. Boil until dissolved and make up losses due to evaporation with water place measured quantities (100 or 200 ml) of the solution in flasks or bottles and sterilize in the autoclave at 1 kg/ cm^2 pressure for 15 min. Just before use, add; to each 100 ml of the melted agar prepare as above, the following:

Lactose, sterile, 20% solution	5 ml
Eosin, tow percent aqueous solution	2 ml
Methylene blue, 0.5% aqueous solution	1.3 ml

Mix thoroughly and put into petri dishes and allow to harden. There is no adjustment of reaction. Filtration of the medium is also not necessary.

Endo agar – Take in a flask five grams of beef extract, 10 g of peptone, 30 g of agar and 1 00 ml of water and boil until dissolved. Make up losses due to evaporation with water and adjust reaction so the pH after sterilization shall be 7.4. Clarify if required by adding 10 g of egg albumen, mixing and steaming for one to two hours and filtering while hot. Add 10 g of lactose to one litre of the medium and place the medium so prepared in small flasks or bottle, 100 ml in each and sterilize

in an autoclave at a pressure of 1 kg /cm^2 for 15 min. Prepared a three per cent solution of basic fuschin in rectified spirit and allow to stand for 24 h and filter. To each 100 ml of the molten lactose agar solution add one milliliter of the basic duschin soluton and 0.125 g of anhydrous sodium sulphite solution shall be freshly prepared). Mix thoroughly, pour into petri dishes and allow to harden. The medium shall be light pink when hot and almost colourless when cool. If it is highly coloured before incubating or does not give proper reaction when seeded with coliform organisms, depending on the dye content of basic fuschin, ray the strength of the basic fuschin solution suitably.

8.2.1.2 *Procedure* – Streak a plate of MacConkey agar on eosin methylien blue agar or Endo agar form each of the primary fermentation tubes showing acid and gas. An inoculation needle slightly bent at the tip or a bent glass rod may be used fro spreading the incoculum on the surface of the medium. It is essential that plates are so inoculated as to ensure the growth of discrete colonies. Incubate the plates (inverted if with cover) at 37°C for 24 ± 2 hours.

Coliform bacteria form deeply coloured nucleated colonies with or without metallic surface luster on eosin methylene blue agar. On Endo agar, coliform colonies are nucleated, dark red, with or without metallic sheen. Typical colonies are enucleated, opaque and pink in these medial. The colonies of coliform bacteria are pink in colour in MacConkey agar medium. The appearance of typical colonies on the plates within the incubation period constitutes a positive test.

8.2.2 *Confirmation using liquid medium*

8.2.2.1 *Medium*

Brilliant green bile lactose broth – Take 10 g of peptone and 500 ml of water in a flask and steam till dissolved. Also prepare a solution of 20 g of dehydrated ox-gall in 200 ml of water a ph between seven and 7.5. Mix the two solutions and add water to an appropriate volume of 975 ml. Add 10 g of lactose. Adjust Ph to 7.4. Add 13.3 ml of 0.1 aqueous solution of brilliant green in water and make up to 1ooo ml. Filter through cotton. Distribute in five milliliter quantities into 150 x 10 mm fermentation tubes and sterilize in the autoclave for 15 min at 0.7 kg /cm^2 pressure or in the steam sterililser for 30 min on three successive days. The pH after sterilization shall be not less than 7.1 or more than 7.4.

8.2.2.2 *Procedure* – Gently shake the presumptive tube or mix by rotating, and with a wire loop of not less than 3 mm diameter, transfer a small portion of the culture into a fermentation tube containing brilliant green bile lactose broth. Incubate the tubes at 44°C for 24 h. the formation of gas in any amount in the inverted tube of the fermentation tube constitutes a confirmed test for the coliform organisms.

8.3 *Completed test*

8.3.0 *General*

The completed test shall be carried out in case typical colonies appear on the eosin methylene blue or Endo agar plates, and in those cases where the results of the coliform test on the samples of the water are to be used for the control of the quality of the treated water, in order to establish beyond reasonable doubt the value of the confirmed test.

8.3.1 *Media*

8.3.1.1 MacConkey broth – Single strength or double strength as described in 8.1.2 and 8.1.2.2.

8.3.1.2 Agar *slant* – Dissolve tree of yeastrel and five grams of peptone in 1 00 ml of water in the steam steriliser. Adjust the ph at room temperature to 7.4 using 0.1% alcoholic solution of phenol red as indicator. Take 15% of agar, chop it up and place it in a muslin bag. Wash it in the bag in running water for 15 min and after squeezing out excess water, add it to the yeastrel peptone solution. Autoclave for 20 min and filter through paper pup in a Buchner funnel or through a plug of cotton wrapped in gauze. Egg shall not be used for clearing. The agar shall be filtered hot, the whole apparatus being kept warm by steaming. The reaction of the filtrate shall be adjusted to pH 7.0 at 50°C. Place in tubes in 10 ml quantities and autoclave at a pressure of 1 kg /cm^2 for 20 min. For use, the tubes shall taken either directly from the autoclave or, if they have set, they shall be melted by heating in a warm bath and then cooled down to room temperate in a slanting

position till the agar has set. Unless they are to be used within one week of preparation, thy shall be placed in the cold store.

8.3.1.3 *Formate ricinoleate broth* - Add five grams each of peptone, lactose and sodium format and one gram of sodium ricinoleate to 1 00 ml of water and heat slowly in a water-bath with constant sitting to dissolve. Add water to make up to none litre. Adjust reaction so that ph after sterilisation is 7.3 to 7.5. Distribute into fermentation tubes and autoclave at 0.7 kg /cm^2 pressure for 15 min.

8.3.2 *Procedure* –
Pick two or more typical coliform colonies form each of the plates used in the confirmation test, or if not typical colonies are present, pick two or more colonies adjusted to consist of organisms of the coliform group, and transfer each of a MacConky broth tube and an agar slant. Select well isolated colonies and touch lightly with the needle to take an inoculum for transfer. Incubate the broth tubes at 37°C for 48 ± 3 h and examine for gas production. Record all tubes showing gas as positive. Incubate the agar slant at 37°C for 24 to 48 h and examine gram-stained preparation of the growth on slants corresponding to the broth tubes that show gas. The presence of gram-negative non-spore forming bacilli in the agar culture shall be recorded as positive.

The production of gas in MacConkey broth, and demonstration of gram negative rods on staining constutes a completed positive test. If spore bearing organisms are found on staining transfer the culture to formate recinoleate broth. Incubate at 37°C for 48 ± 3 h and examine. If no gas is produced, it is negative for coliform bacteria and only spore forming lactose fermenting babble presence of coliform organisms by:

a) inoculating a plate of confirmatory medium from the formate ricinoleate broth and incubating at 37°C for 48 ± 3h, and

b) picking typical colonies form the confirmatory medium and transferring to MacConkey broth and agar slant and repeating examination as above.

In after 48 h, gas is produced in the MacConkey broth and no spored are formed in the agar slant, the test shall be considered completed and the presence of coliform organisms established.

9 Quality Assurance and Quality Control

9.1 General

Laboratories performing determinations under this standard shall implement quality control procedures to ensure the accuracy, precision and reliability of analytical results.

Quality control measures shall apply to sampling, preservation, reagent preparation, calibration, incubation and reporting.

9.2 Calibration of Instruments

9.2.1 Measuring instruments, including balances, pH meters, dissolved oxygen meters, thermometers, incubators and spectrophotometers, shall be calibrated at appropriate intervals.

9.2.2 Calibration records shall be maintained.

9.2.3 Where calibration curves are required, their validity shall be verified prior to sample analysis.

9.3 Reagents and Standard Solutions

9.3.1 Only analytical grade reagents and distilled water conforming to Clause 2 shall be used.

9.3.2 Standard solutions shall be standardized where applicable.

9.3.3 Reagents shall be stored under conditions that prevent deterioration.

9.4 Blank Determinations

Where applicable, reagent blanks or dilution water blanks shall be analysed alongside samples.

If blank values are significant relative to sample concentrations, the cause shall be investigated before results are reported.

9.5 Replicate Analyses

At least one sample per analytical batch should be analysed in duplicate to assess repeatability.

Where duplicate results show unacceptable variation, the analysis shall be repeated.

9.6 Control of Test Conditions

Where results depend on environmental conditions such as temperature, dissolved oxygen or pH, these parameters shall be monitored and maintained within the limits specified in the relevant test clauses.

Incubators used for BOD and microbiological tests shall maintain the prescribed temperature within acceptable tolerance.

9.7 Microbiological Quality Control

For coliform determinations:

a) Culture media shall be prepared and sterilized as specified.

b) Sterility controls shall be included for each batch of prepared media.

c) Incubation temperatures shall be verified.

9.8 Corrective Action

If quality control requirements are not satisfied, results shall not be reported until corrective action has been taken and compliance restored.