Title: Analysis of Water and Pure Steam Samples

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1.0 Objective

To lay down a procedure for analysis of water samples.

2.0 Scope

This Standard Operating Procedure is applicable at Quality Control department.

3.0 Responsibility

Executive/Sr. Executive-QC : Shall be responsible to follow the procedure of analysis of water.

Head - QC/Designee : Shall be responsible for the compliance of this SOP.

4.0 Abbreviations and Definitions

SOP	: Standard Operating Procedure
QC	: Quality Control
IPA	: Isopropyl Alcohol
No	: Number
%	: Percentage
G	: Gram
Ml	: Milliliter
°C	: Degree Centigrade
TAMC	: Total aerobic microbial count
cfu	: Colony Forming Unit

5.0 Procedures for Microbiological analysis of Water

5.1 Carry out the determination under conditions designed to avoid extrinsic microbial contamination of the sample to be examined. The precautions taken to avoid contamination must be such that they do not affect any microorganisms that are to be revealed in the test.

5.2 Test Negative control: To verify testing conditions perform a negative control using the chosen diluent in place of the test preparation. There must be no growth of microorganisms.

5.2.1 Use pre incubated media for all tests, where pre incubated media are not possible to use incubate a Plate/ tube/ flask as negative control along with the test samples.

5.2.2 Before carrying out these tests for enumeration of the aerobic microorganisms in the test specimens, it should be confirmed that:

5.2.2.1 The media used in the tests, promote the growth of the respective microorganisms for which they are used.

5.2.2.2 The test specimens to which the tests are applied do not themselves inhibit the microorganisms and the appropriateness of the methods used for enumeration of the microorganisms in the presence of the product is determined.

5.3 Test Positive control:

5.3.1 For positive control inoculate approx. 10 to 100 cells of E. coli, Salmonella, P. aeruginosa and Staphylococcus aureus.

5.3.2 Simultaneously Carry out the positive control by streaking/ Direct inoculation on media, which is used for testing for negative control incubate the plate/Tube without streaking or direct inoculation.

5.4 **Definitions:**

5.4.1 Media Negative control: Negative control performed to ensure the media sterility.

5.4.2 Test Positive control: Positive control performed to proper growth of microorganism in media.

5.5 **Recommended Solutions And Culture Media:**

[Note: This section is given for information.]

5.5.1 **R2A Agar:**

R2A Agar pH 7.2

Yeast Extract	0.5 g
Proteose Peptone	0.5 g
Casein hydrolysate	0.5 g
Glucose	0.5 g
Starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Magnesium sulfate, anhydrous	0.024 g
Sodium pyruvate	0.3 g

Agar	15 g
Purified Water.	1000 mL

Sterilize in an autoclave using a validated cycle.

5.5.2 **Buffered Sodium Chloride–Peptone Solution**

Buffered Sodium Chloride–Peptone Solution pH 7.0

Potassium Dihydrogen Phosphate	3.6 g
Disodium Hydrogen Phosphate Dihydrate	7.2 g
Sodium Chloride.	4.3 g
Peptone (meat or casein).	1.0 g
Purified Water.	1000 mL

Sterilize in an autoclave using a validated cycle.

5.5.3 **Casein Soybean Digest Broth:**

Casein Soybean Digest Broth

Pancreatic Digest of Casein	17.0 g
Papaic Digest of Soybean	3.0 g
Sodium Chloride	5.0 g
Dibasic Hydrogen Phosphate	2.5 g
Glucose Monohydrate	2.5 g
Purified Water	1000 mL

Check the pH if necessary adjust the pH, after sterilization the pH is to be 7.3 ± 0.2 at 25° C. Sterilize in an autoclave using a validated cycle.

5.5.4 **MacConkey Broth:**

MacConkey Broth

Pancreatic Digest of Gelatin	20.0 g
Lactose monohydrate	10.0 g
Dehydrated Ox Bile	5.0 g
Bromocresol Purple	10 mg
Purified Water	1000 mL

Check the pH if necessary adjust the pH, after sterilization the pH is to be 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

5.5.5 **MacConkey Agar:**

MacConkey Agar

Pancreatic Digest of Gelatin	17.0 g
Peptones (meat and casein)	3.0 g
Lactose monohydrate	10.0 g
Sodium Chloride	5.0 g
Bile Salts	1.5 g
Agar	13.5 g
Neutral Red	30.0 mg
Crystal Violet	1 mg
Purified Water	1000 mL

Check the pH If necessary adjust the pH, after sterilization the pH is to be 7.1 ± 0.2 at 25°C. Boil for 1 minute with constant shaking, then sterilize in an autoclave using a validated cycle.

5.5.6 **Rappaport Vassiliadis Salmonella Enrichment Broth:**

Rappaport Vassiliadis Salmonella Enrichment Broth

Soya Peptone	4.5 g
Magnesium Chloride Hexahydrate	29.0 g
Sodium Chloride	8.0 g
Dipotassium Phosphate	0.4 g
Potassium Dihydrogen Phosphate	0.6 g
Malachite Green	0.036 g
Purified Water	1000 mL

Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding 115°C. The pH is to be 5.2 ± 0.2 at 25°C after heating and autoclaving.

5.5.7 **Xylose Lysine Deoxycholate Agar:**

Xylose Lysine Deoxycholate Agar

Xylose	3.5 g
L-Lysine	5.0 g

Xylose Lysine Deoxycholate Agar

Lactose Monohydrate	7.5 g
Sucrose	7.5 g
Sodium Chloride	5.0 g
Yeast Extract	3.0 g
Phenol Red	80 mg
Agar	13.5 g
Sodium Deoxycholate	2.5 g
Sodium Thiosulfate	6.8 g
Ferric Ammonium Citrate	0.8 g
Purified Water	1000 mL

Check the pH if necessary adjust the pH after heating it is 7.4 ± 0.2 at 25 °C. Heat to boiling, cool to 50°C, and pour into Petri dishes. Do not heat in an autoclave.

5.5.8 **Cetrimide Agar:**

Cetrimide Agar

Pancreatic Digest of Gelatin	20.0 g
Magnesium Chloride	1.4 g
Dipotassium Sulfate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Purified Water	1000 mL
Glycerol	10.0 mL

Heat to boiling for 1 minute with shaking. Adjust the pH, so that after sterilization it is 7.2 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

5.5.9 **Mannitol Salt Agar:**

Mannitol Salt Agar

Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Beef Extract	1.0 g
D-Mannitol	10.0 g
Sodium Chloride	75.0 g
Agar	15.0 g
Phenol Red	0.025 g

Purified Water 1000 mL

Heat to boiling for 1 minute with shaking. Check the pH if necessary adjust the pH, after sterilization the pH is to be 7.4 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

5.6 Microbial enumeration tests: Test for Total Aerobic Microbial Count

5.6.1 Membrane Filtration: Use a filtration apparatus designed to allow the transfer of the filter to the medium.

5.6.2 Use membrane filters having a nominal pore size not greater than 0.45 µm.

5.6.3 Use the sterile filtration unit (previously sterilized as per the validated autoclave cycle).

5.6.4 Assemble the filtration unit under Laminar air/Biosafety hood flow by keeping the sterile 0.45 µm membrane filter on the holder of the filtration unit moisten the membrane with selected diluent.

5.6.5 Transfer a suitable quantity (10 ml for purified water and 200 ml for water for injection) of the sample on membranes filter, filter immediately.

5.6.6 For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of the R2A Agar and incubate the plates at 30°–35°C for the 5 days in inverted position.

5.6.7 After incubation period count the number of colonies and report the cfu/ml/100ml.

5.6.8 Test Negative Control

5.6.8.1 Transfer a suitable quantity of diluent on the membrane filters and filter immediately, transfer the membrane on to the R2A Agar and incubate the plate at 30°–35° C for 5 days.

5.6.8.2 After incubation period negative control plates should not show any growth. If growth occurs in the negative control plates test is invalid. Failed negative control needs investigation.

5.7 Plate-Count Methods: Perform plate-count methods at least in duplicate for each medium, and use the mean count of the result.

5.7.1 Pour-Plate Method

5.7.1.1 For TAMC: Transfer aseptically 1 mL of the sample to each of two Petri dishes about 9 cm in diameter, add 15 to 20 mL of sterile R2A Agar previously maintained at not more than 45° C (If larger Petri dishes are used, the amount of agar medium is increased accordingly) and gentley mix the plates for the equally distrubution of the sample and allow for solidification.

5.7.1.2 After solidification incubate the plate at 30°–35°C for 5 days.

5.7.1.3 After incubation period check the plates for the growth and count the number of CFU and take the arithmetic mean of the counts per medium, calculate the total number of CFU/ mL.

5.7.2 Media Negative Control

5.7.2.1 For TAMC: Perform the negative control by adding the 15 to 20 mL of R2A Agar previously maintained at not more than 45° C to a sterile Petri dish and allow for solidification. After soldification incubates plates in inverted position at 30°–35°C for 5 days.

5.7.2.2 After incubation period negative control plates should not show the growth. If growth occurs in the negative control plates test is invalid. Failed negative control needs the investigation.

5.7.2.3 Test Negative control: Perform a negative control using the chosen diluent in place of the test preparation. There must be no growth of microorganisms. Failed negative control needs investigation.

5.8 Testing of Products for Specified Pathogens:

5.8.1 Sample Enrichment:

5.8.1.1 Add 100 ml of water sample in 100 ml of double strength soyabean casein digest medium.

OR

Filter 100 ml of water sample and immerse the membrane filter in 100 ml soyabean casein digest medium.

5.8.1.2 Mix well, and incubate the sample at 30° to 35 ° C for 18 to 24 hrs

5.8.2 Tests for *Escherichia coli*:

5.8.2.1 After incubation period shake the container, transfer 1 mL of Soybean–Casein Digest Broth to 100 mL of MacConkey Broth.

5.8.2.2 Incubate at 42° to 44°C for 24 to 48 hours.

5.8.2.3 After incubation period Subculture on a plate of MacConkey Agar and incubate at 30° to 35°C for 18 to 72 hours.

5.8.2.4 Interpretation: Growth of colonies indicates the possible presence of *Escherichia coli*. This is confirmed by identification tests.

5.8.2.5 The product complies with the test if no colonies are present or if the identification tests are negative.

5.8.2.6 Test Negative control: To verify testing conditions perform a negative control using the chosen diluent or media in place of the test preparation. There must be no growth of microorganisms. Failed negative control needs investigation.

5.8.3 Identification test:

Note: *Biochemical test or identification by automated methods can be used for confirmatory identification.*

5.8.3.1 Transfer well-isolated suspected colonies from MacConkey agar plate to 5 mL of 5% Peptone water or MacConkey Broth or a suitable medium contained in a test tube.

5.8.3.2 Incubate the tube at 42°- 44°C for 24 h.

5.8.3.3 Add 0.5 mL of Kovac's reagent to the tube, shake well and allow to stand for one minute. If a red colour is observed in the reagent layer, indole is present.

5.8.3.4 The preparation being examined passes the test if such colonies are not seen or if the confirmatory biochemical tests are negative.

5.8.3.5 Test Negative control: To verify testing conditions perform a negative control using the chosen diluent in place of the test preparation. There must be no growth of microorganisms.

5.8.4 Test for *Salmonella*:

5.8.4.1 After incubation period transfer 0.1 mL of Soybean–Casein Digest Broth to 10 ml of Rappaport Vassiliadis *Salmonella* Enrichment Broth.

5.8.4.2 Incubate at 30° to 35°C for 18 to 24 hours. Sub culture on a plate of Xylose Lysine Deoxycholate Agar, and incubate at 30° to 35°C for 18 to 48 hours.

5.8.4.3 Interpretation

5.8.4.3.1 The possible presence of *Salmonella* is indicated by the growth of well- developed, red colonies, with or without black centers on Xylose Lysine Deoxycholate Agar: This is confirmed by identification tests.

5.8.4.4 The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

5.8.4.5 Test Negative control: To verify testing conditions perform a negative control using the chosen diluent or media in place of the test preparation. There must be no growth of microorganisms. Failed negative control needs investigation.

5.8.4.6 Identification test:

Note: *Biochemical test or identification by automated methods can be used for confirmatory identification.*

5.8.4.6.1 Transfer separately a few of the suspect colonies to Triple sugar iron agar in tubes, using surface and deep inoculation. (This can be done by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle and incubating at 30 - 35 °C temperature for 24 hours).

5.8.4.6.2 The presence of *Salmonella* is provisionally confirmed if, in the deep inoculation but not in the surface culture, there is a change of color from red to yellow and usually a formation of gas, with or without production of Hydrogen sulphide in the agar.

5.8.4.6.3 Precise confirmation may be carried out by appropriate biochemical test such as Urea broth.

5.8.4.6.4 Transfer separately a few of the suspect colonies to 10 mL of Urea broth contained in test tube and incubate at 30 - 35 °C temperature for 24 hours.

5.8.4.6.5 Upon incubation absence of red colour in Urea broth tube indicates presence of *Salmonellae*.

5.8.4.6.6 The preparation being examined passes the test if, colonies of the type described do not appear or if the confirmatory biochemical tests are negative.

5.8.4.6.7 Test Negative control: To verify testing conditions perform a negative control using the chosen diluent or media in place of the test preparation. There must be no growth of microorganisms. Failed negative control needs investigation.

5.8.5 Test for *Pseudomonas aeruginosa*:

5.8.5.1 After incubation period Subculture on a plate of Cetrimide Agar, and incubate at 30° to 35°C for 18 to 72 hours.

5.8.5.2 Interpretation: Growth of colony indicates the possible presence of *P. aeruginosa.* This is confirmed by identification tests.

5.8.5.3 The product complies with the test if colonies are not present or if the confirmatory identification tests are negative.

5.8.5.4 Test Negative control: To verify testing conditions perform a negative control using the chosen diluent or media in place of the test preparation. There must be no growth of microorganisms. Failed negative control needs investigation.

5.8.5.5 Identification test:

Note*: Biochemical test or identification by automated methods can be used for confirmatory identification.*

5.8.5.5.1 Oxidase test - Place 2 or 3 drops of a freshly prepared 1 % w/v solution of N, N, N'N'-Tetramethyl-4-phenylene diamine dihydrochloride on filter paper and smear with the suspect colony; if a purple color is produced, the test is positive. Alternatively readymade impregnated discs can also be used.

5.8.6 Test for *Staphylococcus aureus*:

5.8.6.1 After incubation period Subculture on a plate of Mannitol Salt Agar, and incubate at 30° to 35°C for 18 to 72 hours.

5.8.6.2 Interpretation: The possible presence of *S. aureus* is indicated by the growth of yellow or white colonies surrounded by a yellow zone. This is confirmed by identification tests.

5.8.6.3 The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

5.8.6.4 Test Negative control: To verify testing conditions perform a negative control using the chosen diluent or media in place of the test preparation. There must be no growth of microorganisms. Failed negative control needs investigation.

5.8.6.5 Identification Test: Coagulase Test:

Note: *Biochemical test or identification by automated methods can be used for confirmatory identification.*

5.8.6.5.1 With the aid of an inoculation loop, transfer representative suspected colonies from surfaces of Mannitol Salt Agar to individual tubes, each containing 0.5 mL of mammalian, preferably rabbit or horse plasma with or without suitable additives.

5.8.6.5.2 Incubate in a water bath at 37°C temperature, examining the tubes after 3 hours and subsequently at suitable intervals up to 24 hours.

5.8.6.5.3 If coagulation in any degree is observed, the test is positive.

5.8.6.5.4 Carry out a positive control by adding a volume of broth containing 10 to 100 cells of *Staphylococcus aureus*, prepared from a 24 hours. Culture in soyabean casein digest medium of *Staphylococcus aureus* (ATCC 6538 / NCTC 10788) to a tube containing 0.5 mL of plasma.

5.8.6.5.5 Carry out a negative control by incubating an un-inoculated tube containing 0.5 mL of plasma.

5.8.6.5.6 For the test to be valid the positive control tube should show coagulation within 3 hours of incubation and there should be no coagulation in negative control tube even after 24 hours of incubation.

5.8.6.5.7 Perform the negative control.

5.8.7 Test for coliforms:

5.8.7.1 Filtered 100 ml samples and transfer the membrane filter to pre incubated plate of M-Endo agar and incubate at 30-35°C for 24 hrs.

5.8.7.2 Interpretation of results: Golden Green colonies with metallic surface sheen.

5.8.7.3 Perform the negative control.

5.9 Procedures for Chemical analysis of Water

5.9.1 Description:

5.9.1.1 Check by physical examination. Clear, Colourless, odourless and tasteless liquid.

5.9.2 pH:

5.12.2.1 Refer SOP for Operation and calibration of pH meter and measure the pH of water by adding 0.3 mL of saturated KCl in 100 mL of water.

5.9.3 Total Dissolved Solids:

5.9.3.1 Take 100 ml sample and filter with 41 filter paper in 250 ml preweighed dry beaker. Evaporate it on hot plate completely and after that dry on 105ºc in oven for 2 hours, then cool on desiccator and weight the beaker and calculate the result (ppm).

$$\text{Calculation} = \frac{(W2 - W1) \times 100 \times 10000}{100} =$$

W1 = Weight of empty beaker

W2 = Weight of beaker + residue (after drying)

5.9.4 Total Hardness:

5.9.4.1 Take 100 ml sample to a 250 ml conical flask, add 10ml ammonia-ammonium chloride buffer and 5 mg mordant black indicator and shake well. Titrate with 0.01M EDTA to a permanent blue colour persists.

Calculation (ppm):

$$\frac{\text{T.V X Strength of EDTA X 0.100 X 100 X 10000}}{\text{Volume of sample}} =$$

T.V = Volume of EDTA consumed

5.9.5 Chlorides:

5.9.5.1 Take 50 ml Sample in 250 ml conical flask and titrate with 0.1 N Silver nitrate solution using potassium chromate as indicator. The colour change yellow to brick red is end point.

5.9.5.2 Each ml of 0.01N Silver nitrate corresponds to 0.003546g of chloride.

Calculation (ppm):

$$\frac{\text{T.V X Strength of 0.1 N } AgNo_3 \text{ X 0.03546 X 100 X 10000}}{\text{Volume of sample}} =$$

T.V = Volume of 0.1 N $AgNo_3$ consumed

Or

To 10 mL of sample, add 1 mL of 2M Nitric Acid and 0.2 mL of 0.1 M Silver Nitrate, the appearance of the solution does not change for at least 15 minutes.

5.9.6 Sulphates:

5.9.6.1 Place 100ml of 0.0908% w/v solution of potassium sulphate to form 500-ppm sulphate. Pipette out 1 ml of dilute solution in a nessler cylinder, add 2 ml of dilute HCL and dilute to 45 ml water and 5ml of barium sulphate reagent, stir and allow to stand for 5 minutes.

5.9.6.2 Test: Place 1ml of sample in a nessler cylinder, add 2ml of dilute HCL sol. and dilute to 45 ml with water, add 5ml barium chloride reagent, stir immediately and allow to stand for 5 minutes. The turbidity produced in test solution should not be greater than standard solution.

Or

To 10 mL of sample, add 0.1 mL of 2M Hydrochloric Acid and 0.1 mL of Barium Chloride Solution. The appearance of the solution does not change for at least 1 hour.

5.9.7 Nitrate:

5.9.7.1 To 5 mL of sample in a test tube immersed in ice, add 0.4 mL of a 10% w/v solution of Potassium Chloride, 0.1 mL of Diphenylamine Solution add drop wise with shaking, 5 mL of Sulphuric Acid. Transfer the tube to a water bath at 50°C and allow to stand for 15 minutes. Any blue colour in the solution is not more intense than that in a solution prepared at the same time and in the same manner using a mixture of 4.5 mL of nitrate free water and 0.5 mL of nitrate standard solution (2 ppm NO_3).

5.9.8 Total Alkalinity:

5.9.8.1 Take 100 ml Sample in 250 ml conical flask, and titrate with 0.1 N HCl solution using 0.1 ml methyl orange solution as indicator. The colour change yellow to red is end point.

Calculation(ppm):

$$\frac{\text{T.V X Strength of 0.1N HCl X 0.040 X 100 X 10000}}{\text{Volume of sample}} =$$

T.V. = Volume of 0.1N HCl

5.9.9 Ammonium:

5.9.9.1 To 20 mL of sample add 1 mL of alkaline potassium tetraiodomercurate solution and allow to stand for 5 minutes, when viewed vertically the solution is not more intensely coloured than a solution prepared at the same time by adding 1 mL of alkaline potassium tetraiodomercurate solution to a solution containing 4 mL of Ammonium Standard Solution (1 ppm NH_4) and 16 mL of Ammonia free water (0.2 ppm).

5.9.10 Calcium & Magnesium:

5.9.10.1 To 100 mL of sample, add 2 mL of ammonium chloride buffer pH 10.0, 50 mg of mordant black 11 triturate and 0.5 mL of 0.01 M Disodium Edetate, a pure blue colour is produced.

5.9.11 Residue on Evaporation

5.9.11.1 Evaporate 100 mL to dryness on a water bath and dry to constant weight at 105°C. The residue weight not more than 1 mg (0.001%).

5.9.12 Heavy Metals:

5.9.12.1 Determined by Method D on 12 mL of a solution prepared in the following manner. In a glass evaporating dish evaporate 150 mL to 15 mL on a water bath. Use Lead Standard Solution (0.1 ppm) to prepare the standard.

Method D

Take two cylinders, one with 12 mL of evaporated water and second with 10 mL of standard solution & 2 mL of the evaporated water and mix. To each of the cylinders add 2 mL of Acetate buffer pH 3.5, mix, add, 1.2 mL of Thioacetamide reagent, allow to stand for 2 minutes. Then view downwards over a white surface; the color produced with the test solution is not more intense than that produced with the standard solution.

5.9.13 Acidity or Alkalinity:

5.9.13.1 To 10 mL of sample, freshly boiled and cooled in a borosilicate glass flask, add 0.05 mL of methyl red solution, the resulting solution is not red. To 10 mL, add 0.1 mL of Bromothymol Blue solution, the resulting solution is not blue.

5.9.14 Silica:

5.9.14.1 To 50 ml sample add in rapid succession 1.0 ml 1+1 HCl and 2ml ammonium molybdate reagent. Mix by inverting at least 6 times and let stand for 5 to 10 minutes .Add 2.0 ml oxalic acid solution and mix thoroughly. Read colour after 2 minutes but before 15 minutes, measuring time from addition of

oxalic acid. Because the yellow colour obeys beer's law, visually compare with permanent artificial color standard using K_2CrO_4 and borax solution as given in the table:

Value in Silica µg	Potassium Chromate Solution ml	Borax Solution ml	Water ml
0	0.0	25	30
100	1.0	25	29
200	2.0	25	28
400	4.0	25	26
500	5.0	25	25
750	7.5	25	22
1000	10.0	25	20

5.12.14.1 To 25 ml sample add 25 ml of Calcium hydroxide TS: The mixture remains same.

5.9.15 Conductivity:

5.9.15.1 Refer SOP for Calibration and Operation of Conductivity meter and measure the conductivity of water at 25°C.

Stage – I Determine the temperature of the water and the conductivity of the water using a non-temperature compensated conductivity reading. The measurement may be performed in a suitable container or as an on-line measurement.

Using the Stage - I Temperature and Conductivity requirements table, find the temperature value that is not greater than the measured temperature. The corresponding conductivity value is the limit at that temperature.

If the measured conductivity is not greater than the table value, the water meets the requirements of the test for conductivity. If the conductivity is higher than the table value, proceed with Stage 2.

Stage - II Transfer a sufficient amount of water [100 ml or more] to a suitable container, and stir the test specimen. Adjust the temperature, if necessary and while maintaining it at $25^o \pm 1^o$, begin vigorously agitating the test specimen while periodically observing the conductivity.

When the change in conductivity [due to uptake of atmospheric carbon dioxide less than a net of 0.1 µS/cm per 5 minutes, note the conductivity. If

the conductivity is not greater than 2.1 µS/cm, the water meets the requirements of the test for conductivity. If the conductivity is greater than 2.1 µS/cm, proceed with Stage 3.

Stage – III Perform this test within approximately 5 minutes of the conductivity determination in Step 5, while maintaining the sample temperature at 25°±1°.

Add a saturated potassium chloride solution to the same water sample [0.3 ml per 100 mL of the test specimen], and determine the pH to the nearest 0.1 pH unit, as directed under pH [791] of USP 24.

Referring to the Stage 3 - pH and Conductivity Requirement table, determine the conductivity limit at the measured pH value.

If the measured conductivity in Step 4 is not greater than the conductivity requirements for the pH determined in Step 6, the water meets the requirements of the test for conductivity.

STAGE - I Temperature ad Conductivity Requirements

[For Non-Temperature-Compensated Conductivity Measurement Only]

Temperature	Conductivity Requirements [µS/cm]
0	0.6
5	0.8
10	0.9
15	1.0
20	1.1
25	1.3
30	1.4
35	1.5
40	1.7
45	1.8
50	1.9
55	2.1
60	2.2
65	2.4
70	2.5
75	2.7
80	2.7
85	2.7
90	2.7

95	2.9
100	3.1

STAGE - III pH and Conductivity Requirements

[For Atmosphere and Temperature Equilibrated Samples Only]

pH	Conductivity Requirements [µS/cm]
5.0	4.7
5.1	4.1
5.2	3.6
5.3	3.3
5.4	3.0
5.5	2.8
5.6	2.6
5.7	2.5
5.8	2.4
5.9	2.4
6.0	2.4
6.1	2.4
6.2	2.5
6.3	2.4
6.4	2.3
6.5	2.2
6.6	2.1
6.7	2.6
6.8	3.1
6.9	3.8
7.0	4.6

5.9.16 Oxidisable Substances:

5.12.16.1 To 100 mL of sample, add 10 mL of dilute Sulphuric Acid and 0.1 ml of 0.02 M Potassium Permanganate and boil for 5 minutes, the solution remains faintly pink.

5.9.17 Sulfide:

5.9.17.1 Measure from a burette in to a 500 ml flask an amount of iodine solution estimated to be an excess over the amount of sulfide present. Add distilled water, if necessary, to bring volume to about 20 ml. pipette 200 ml sample

into flask, discharging sample under solution surface. If iodine colour disappears, add more iodine until colour remains. Back- titrate with 0.01 N $Na_2S_2O_3$ solution adding a few drops of starch solution as end point is approached, and counting until blue colour disappears.

Calculation (ppm):

$$\frac{(\text{Blank T.V - T.V}) \text{ X Strength of 0.01N } Na_2S_2O_3 \text{ X 0.016 X 100 X 10000}}{\text{Volume of Sample}} =$$

5.9.18 Total Suspended Solids:

5.9.18.1 Assemble filtering apparatus and filter and being suction. Wet filter with a small volume of reagent grade water to seat it. Stir sample with a magnetic stirrer at a speed to shear larger particles, if practical, to obtain a more uniform (preferably homogenous) particle size. Centrifuge force separate particle by size and density, resulting in a poor precision when point of sample withdrawal is varied. While stirring pipette a measured volume on to the seated glass fiber filter for homogeneous sample pipette from the approximate mid point of container but not in vortex. Choose a point both mid depth and midway between wall and vortex. Wash filter through three successive 10 ml volumes of reagent grade water allowing complete drainage between washings, and continue suctions for about three minutes after filtration is complete. Sample with high dissolved solids may require additional washings. Carefully remove filter from filtration apparatus add to an aluminum weighing dish as a support. Alternatively remove the crucible and filter combination from the crucible adapter if a Gooch crucible is used. Dry for at least 1 hour at 103 to 105 °C in an oven, cool in desiccators to balance temperature and weigh. Repeat the cycle of drying, cooling and desiccating and weighing until a constant weight is obtained or until a weight change is less than 4 % of the previous weight or 0.5 mg whichever is less.

Calculation:

$$\text{Total suspended Solids mg/L} = \frac{(A - B) \text{ X } 10000}{\text{Volume of sample mL}}$$

Where: A= Weight of filter + dried residue (mg)

B= Weight of filter (mg)

5.9.19 **Carbon Dioxide**

5.9.20.1 To 25 ml sample add 25 ml of Calcium hydroxide TS: The mixture remains same.

5.9.20 Total Organic Carbon (TOC)

5.9.20.1 Refer to SOP "Operating and calibration procedure of TOC Analyser."

5.10 **Apparatus Requirements.**

5.10.1 This test method is performed using a calibrated instrument. The suitability of the apparatus must be periodically demonstrated as described below. In addition it must have a manufacturer's specified limit of detection of 0.05mg of carbon per liter (0.05 ppm of carbon) or lower.

5.11 **Reference Standards:**

5.11.1 1,4-Benzoquinone RS, Sucrose RS.

5.11.2 Reagent water: Use water having a TOC level of not more than 0.10mg per liter.

5.12 **Glassware preparation:**

5.12.1 Organic contamination of glassware results in higher TOC values. Therefore, use glassware and sample containers that have been scrupulously cleaned of organic residues. Any method that is effective in removing organic matter can be used. Use reagent water for final rinse.

5.13 **Standard Solution:**

5.13.1 Dissolve in the reagent water an accurately weighed quantity of USP Sucrose RA, previously dried at 105°C for 3 horst obtain a solution having a concentration of 1.19mg of Sucrose per liter (0.50mg of carbon per liter)

5.14 **Test solution:**

Note: *Use extreme caution when obtaining samples for TOC analysis. Then can be contaminated during process of sampling and transportation.*

5.14.1 Collect the test solution in a tight container with minimal headspace, and test in a timely manner to minimize the impact of organic contamination from the closure and container.

5.15 **System suitability Solution:**

5.15.1 Dissolve in Reagent water an accurately weighed quantity of USP 1, 4 - Benzoquinone RS to obtain a solution having a concentration of 0.75mg per liter

5.16 **Reagent water control:**

5.16.1 Use a Suitable quantity of reagent water obtained at the same time as that used in the preparation of the standard solution and the system suitability solution.

5.17 **Other control solutions:**

5.17.1 Prepare appropriate reagent blank solutions or other specified solutions needed for establishing the apparatus base line or for calibration adjustments following the manufacturer's instructions, and run the appropriate blanks to zero the instrument.

5.18 **System Suitability:**

5.18.1 Test the reagent water control in the apparatus and record the response r_w Repeat the test using the standard solution, and record the response, r_s. Calculate the corrected standard solution response, which is also the limit response, by subtracting the reagent water control response from the response of the standard solution. The theoretical limit of 0.50 mg of carbon per liter is equal to the corrected standard solution response, r_{ss}. Calculate the corrected system suitability solution response of the system suitability solution, r_{ss} - r_w calculate the response efficiency for the system suitability solution by the formula.

$$100 ((r_{ss} - r_w) / (r_s - r_w)).$$

5.18.2 The system is suitable if the response efficiency is not less than 85% and not more than 115% of the theoretical response.

5.18.3 **Procedure:**

5.18.3.1 Perform the test on the test solution, and record the response, r_u. The test solution meets the requirements if r_u is not more than the limit response, r_s - r_w.This method also can be performed alternatively using on-line instrumentation that has been appropriate calibrated,

standardized, and has demonstrated acceptable system suitability. The acceptability of such on line instrumentation for quality attribute testing is dependent on its location in the water system. These instrument locations and responses must reflect the quality of the water used.

6.0 Procedures for Endotoxin of Water

6.1 Refer to the SOP for Bacterial Endotoxin Test by gel clot Method.

7.0 Reporting of Results

7.1.1 Results of different type of waters and pure steam condensate shall be recorded in Analytical datasheets as per Annexure-1 and Annexure -2.

7.1.2 Certificate of Analysis shall be issued as per Annexure-3.

7.1.3 Trend analysis shall be done monthly, by mentioning alert and action limits for all critical parameters like pH, conductivity, microbial counts and Total Organic Carbon for Purified water (Storage Tank).

7.1.4 All other user points of Purified Water, Potable water, water for injection and pure steam condensate shall be covered for Trend analysis of Microbial counts at the end of every month.

7.1.5 At the end of year Annual Summary Report and trends shall be prepared by QC as per Annexure-4 and submitted to QA.

8.0 Forms and Records

6.1	Data Sheet for Microbiological Analysis of Water	: Annexure-1
6.2	Analytical Data Sheet format for Water	: Annexure-2
6.3	Certificate of Analysis	: Annexure-3
6.4	Annual Summary Report of Microbiological Results of Water	: Annexure-4

9.0 References

Nil

10.0 Distribution

8.1 Master Copy : Documentation Cell (Quality Assurance)

8.2 Controlled Copies : Quality Control, Quality Assurance

11.0 History

Date	Revision Number	Reason for Revision