SOP: LFA 00074
SOP for Analysis of Water

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1 Objective

To provide a guide on how to perform analysis of water

2 Scope

Applicable to all of the sampling point for water system

3 Responsibility

By Microbiologist

4 Accountability

Head of the Department

5 Procedure

Follow the guideline set as per the SOP for water sampling and Analysis for Chemical and Microbiological parameters in collecting the sample.

5.1. Chemical Analysis

- Prepare the reagent/solution for the chemical analysis

5.1.1. Description

- Examine the physical characteristics of the water such as colour and smell

5.1.2. Hardness

- Take 100ml sample, add 2ml of ammonia buffer pH 1-.0, 50mg mordant black 11 mixture, and add 0.01 M Disodium edetate. Wait until a pure colour blue is produced. Measure the disodium detate volume used and calculate the hardness using the below formula:

$$Hardness\,as\,mg/L = \frac{ml\,of\,EDTA\,used}{Sample\,volume} \times 1000mg/L$$

5.1.3. Total Suspended Solids (TSS)

- Place a clean and dry gouch crucible in the oven for 1 hour at 105°C. Place the gouch crucible in the desiccator to cool it. Take the empty weight of the crucible and then filter 30ml water sample from the crucible using a vacuum pump. Calculate the TSS using the formula provided:

$$TSS = \frac{W_2 - W_1 + 1000(mg/L)}{ml\,of\,solution\,taken}$$

$$W_1 : Weight\,of\,Gouch\,crucible\,Before\,filtration$$

$$W_2 : Weight\,of\,Gouch\,crucible\,After\,filtration$$

5.1.4. Total Dissolved Solids (TDS)

- Using a calibrated conductivity meter, measure the conductivity at 25°C. Convert the value in TDS using the formula below:

- TDS in mg/L = conductivity in mS x 0.667 (geographical factor of area)

5.1.5. Acidity

- Take 10ml sample (freshly boiled and cooled), add 0.05ml methyl red solution and mixed. Solution should not produce red colouring. Red colouring indicates the sample is acidic.

5.1.6. Alkalinity

- Take 10ml sample (freshly boiled and cooled), add 0.1ml bromothymol blue solution and mix. Solution must produce blue colouring to indicate that the sample is alkaline.

5.1.7. Ammonium

- Take 20ml sample water, drop 1ml of alkaline potassium mercuri-iodide to the sample, and allow standing for five minutes. Viewed vertically, solution must not be intensely coloured than a solution that is prepared at the same time adding 1ml of alkaline potassium mercuri-iodide to the solution that contains 2.5ml diluted ammonium chloride solution and 7.5ml of the liquid examined.

5.1.8. Calcium and Magnesium

- Take 100ml sample and add 2ml ammonia buffer pH 10.0, 50mg of mordant black 11 mixture and 0.5ml of 0.01 M disodium edetate. A blue colour will be produced

5.1.9. Heavy Metals

- Use a glass evaporating dish and using 150ml sample, evaporate it to 15ml of water bath

Standard Solution

- Using a small Nessler Cylinder, pipette 10ml of lead standard solution (1ppm Pb).

Test Solution

- Pipette 12ml sample to a small Nessler cylinder

Procedure

- Add 2.0ml of the test solution to the standard solution cylinder and mix. For each cylinder add 2ml acetate buffer pH 3.5, mix and then add 1.2ml thioacetamide reagent and allow standing for 2 minutes. View it downward over a white surface and observe if the colour produced from the test solution is not more intense than the one produced using the standard solution.

5.1.10. Chloride

- Take 10ml sample and add 1ml 2M nitric acid and 0.2ml of 0.1M silver nitrate. The solution appearance must not change for at least 15 minutes

5.1.11. Nitrate

- Take 5ml sample and place it in a test tube immersed in ice. Add 0.4ml of a 10% w/v potassium chloride, 0.1ml diphenylamine solution and drop wise with shaking 5ml of sulphuric acid. Move the test tube to a water bath at 50°C; allow standing for 15 minutes. Any blue colour in the solution not more intense than the prepared solution at the same time and the same manner, using a mix of 5.5ml nitrate free water and 0.5ml nitrate standard solution.

5.1.12. Sulphate

- Take 10ml sample and add 0.1ml 2M Hydrochloric acid and 0.1ml Barium chloride solution. Sample should not change its appearance for an hour.

5.1.13. Oxidisable Substances

- Take 100ml sample and add 10ml 2M Sulphuric acid and 0.1ml of 0.02M potassium permanganate. Boil for 5 minutes, solution should remain faintly pink in colouring.

5.1.14. Residue on Evaporation

- Evaporate 10ml sample to dryness using hot plate and dry to constant weight at 105°C. Residue weight should not be more than 1mg (0.001%)
- Computation:

$$Residue\,on\,evaporation = \frac{W_2 - W_1 \times 100(ml/L)}{ml\,of\,solutiontaken}$$

$$W_1 : Weight\ of\ Evaporating\ dish$$

$$W_2 : Weight\ of\ Evaporating\ dish + Residue$$

5.1.15. Total Organic Carbon

- Analysing the sample for TOC using a calibrated TOC Analyser as per the SOP

5.1.15.1. Alert and Action Limit for Total Organic Carbon (TOC) of Water System

S.No.	Type of Water	Alert Limit (ppb)	Action Limit (ppb)
1	Purified water	300.0	500
2	Water for injection	250.0	500
3	Pure steam	250.0	500

5.1.15.2. If the TOC is above the limit provided above, follow the SOP

- Take 100ml sample using an appropriate container; stir the test sample while maintaining $25°C \pm 1°C$. Measure the conductivity using a calibrated conductivity meter.

5.1.16. Conductivity

Temperature (°C)	Condictivity $\mu S\, cm^{-1}$
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

5.1.17. pH

- Take 100ml sample and add 0.3ml saturated KCL solution and mix. Measure the pH using a calibrated pH meter. Note: if the results observed are out of limit in chemical analysis of water, follow the SOP.

5.2. Microbiological Analysis

- Analysis of the water sample for microbiological as per specification

5.2.1. Pour Plate Method

- Dispense one ml sample to two petri dishes. Add approximately 15 to 20ml R_2A, plate count agar into each of the petri dishes. Cool the dishes approximately 45°C. (Test the sides, holding it should be bearable.) Cover the petri dishes. Mix the sample with the agar using a tilting or rotating motion allowing the contents to solidify at room temperature. Invert the petri dishes and incubate for 5 days at 30 to 35°C. After the incubation period, examine for any growth and count the colonies. Express the average for the two plates in terms of the colony forming per ml.

5.2.2. Membrane Filtration Technique

- This technique will use a single disposable filtration funnel and filter holder using the MILLIFLEX system.
- Preparing the Filtration Apparatus
  - Follow the SOP as per Milliflex operating process. Use the specified sample size for filtration through the 0.45m filter. After completing the filtration of the sample, wash and rinse the filter using 100ml sterile water. Remove the filter using a sterilized forceps and transfer it to the prepared petri-dish with the appropriate medium. (R2A agar/plate count agar)
  - Carefully place the membrane filter to prevent any air trapped in the filter. This is to prevent the nutrient medium from reaching the entire surface of the membrane. Cover the lid.

- Incubate the plates in an upright position for five days at 30 to 35°C.
- Count the colonies in the membrane and record the results as per the specification

5.2.3. Bacterial Endotoxin Test (Follow the SOP for Bacterial Endotoxin Test)

5.2.4. Pathogens

- Sample would be tested versus four specific pathogens: salmonella species, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.
- Used a 0.45 membrane filter fixed on Milliflex system to filter 100ml water sample. Once filtered, remove the filter aseptically and place a 100ml Soybean Casein Digest medium. Incubate for 24 to 48 hours at 20 to 35°C
- From the Soybean Casein Digest medium, inoculate sterile 10ml volume of the following broth using a 1ml of inoculated broth
  - Salmonella Species - (a) Selenite Cystine and (b) Tetrathinate broth
  - Escherichia Coli - MacConkey broth
  - Pseudomonas Aeruginosa - Cetrimide broth
  - Staphylococcus Aureus - Giolitti Cantoni broth (use a sterile liquid paraffin for anaerobic conditions). Incubate the tube at 30 to 35°C for 24 to 48 hours.
- (a) Salmonella Species Test

Medium	Description of Colony
Xylose-Lysine Deoxycholate agar medium	Red with or without Black Centre
Bismuth Sulphite agar medium	Black or Green colonies
Brilliant Green agar	Small, transparent colorless or pink to white Opaque (frequently surrounded by pink to red zone)

- If there is growth in the Selenite Cystine and Tetrahionate broth, inoculate the selected media plates for 24 to 48

hours at 30 to 35°C for a presumptive identification of the pathogen.

- Confirmatory Test
  - From the selected plates, pick the suspected colonies and perform the confirmatory test using biochemical/media and by gram reaction.
  - Transfer the suspected colony individually. Using an inoculating loop, streak the slant slop of Triple Sugar iron agar and then stab it with the inoculating straight wire well in the butt area. Incubate for 24-48 hours at 30 to 35°C.
  - After the incubation, examine the Triple Sugar Iron Agar tube to check for any microbial growth. Look for any changes in the physical appearance including:
    (a) Slant surface - alkaline reaction (appearance of red colouring)
    (b) Butt area - acidic reaction (appearance of yellow colouring) or gas bubble (w/ or w/o concomitant blackening)
    (c) If there, are any growth or change in appearance as per description shown above, the sample is tested positive for salmonella species.
- (b) Escherichia Coli
  - If there are any acid or gas formation in the inoculated MacConkey broth tube, inoculate the selective media and incubate for 24-48 hours at 30 to 35°C

Medium	Description of Colony
MacConkey's Agar	Brick red may have surrounding zone of precipitates bile
Eosin Methylene Blue Agar	Metallic sheen with dark green colonies

- Confirmatory Test
  - From the selected plates, pick the suspected colonies and perform the confirmatory test using biochemical/media and by gram reaction.

- Add 0.1ml of the tube content that shows gas and acid to a tube that contains 10ml peptone water
- Using the peptone water tube, perform the indole test
- Add 0.5ml of the Kovac?s reagent to the peptone water tube. Allow it to stand for one minute. If it changes colour to red, indole is present.
- The presence of the gas and acid in the broth, in the peptone water and in the indole indicates that the sample contains Escherichia coli.
- The presence of the E. coli can also be confirmed through gram staining where one would streak a loopful of the broth with the acid and gas on the plates of the MacConkey Agar and Levine Eosin Methylene Blue Agar. Incubate it for 24-48 hours at 30 to 35°C.
- If the incubation plate shows colonies (with the following characteristics) then E. coli is present.

(a) MacConkey's Agar - brick red colonies w/ or w/o surrounding precipitates

(b) Levine Eosin Methylene blue Agar - metallic sheen colour when place under reflected light. Blue black in appearance when place under transmitted light

- (c) Pseudomonas Aeruginosa Test

Medium	Description of Colony
Cetrimide Agar	Greenish colonies, which exhibit a greenish flourescence under a violet light
Pseudomonas Agar (for Pyocyanin)	Colourless to yellowish, yellowish under ultra voilet light
Pseudomonas Agar (for Flourescein)	Colourless to yellowish, yellowish under ultra voilet light

- If there is growth in the Cetrimide broth showing bluish/greenish pigmentation, inoculate the selected media plates for 24 to 48 hours at 30 to 35°C for a presumptive identification of the pathogen.

- Confirmatory Test
  - From the selected plates, choose the suspected colonies and perform a confirmatory test
  - Streak the suspected colony of Pseudomonas Agar for Fluorescent Detection (PAF) and Psedomonas Agar for Pyocyanin (PAP) detection using an inoculated loop. Incubate in inverted condition for 24-48 hours at 30 to 35°C. At the same time, inoculate the suspected colony in 100ml of Soybean casein Digest medium for 18 to 24 hours at 41 to 43°C
  - Once incubation is up, check the plates and the tube for any presence of microbial growth of gram-negative rods showing the following conditions.

(a) Pseudomonas Agar (fluorescenin detection) - colourless to yellowish fluorescence when place under a UV light

(b) Pseudomonas Agar (pyocyanin detection) - greenish colouring changing to blue fluorescence when place under UV lights

(c) Soybean casein digest - medium growth

  - If the incubated colonies confirmed description above, one should perform oxidase test to determine what microbial is present.

(a) Transfer the suspected colonies using an inoculating loop to a strip of filter paper impregnated with N and N-dimethyl-p-phenylenediamine dihydrochloride

(b) A pink to purplish colouring within 5 to 10 seconds indicate the presence of Pseudomonas aeruginosa.

- (d) Staphylococcus Aureus

Medium	Description of Colony
Mannitol Salt Agar Medium	Yellow colonies with yelow zones
Volgel Johnson Agar Medium	Black surrounded by yellow zone
Baird Parker Agar Medium	Black, shiny, surrounded by clear zones 2-5mm

- If there is growth in the Giolitti Cantoni broth, characterized by black growth at the bottom of the broth due to anaerobic conditions, inoculate the selected media plates for 24 to 48 hours at 30 to 35°C for a presumptive identification of the pathogen.

- Confirmatory Test
  - From the selected plates, choose the suspected colonies and perform a confirmatory test. If colonies are present, (confirmed from the description above) perform a coagulase test.

  (a) Using an inoculating loop, transfer the colony individually to separate tubes that contains 0.5ml mammalian plasma (you can use rabbit or horse)

  (b) Incubate using water bath and place in incubator for 3 to 24 hours at 37°C in parallel with positive control using Staphylococcus aureus strain and negative control using plasma alone.

  (c) After the 3 hours, check and observe for any presence of coagulation and thereafter.

  (d) If coagulation is present, the sample is positive with Staphylococcus aureus. Perform gram staining to determine presence of gram-positive cocci.

5.2.5. Coli Forms

- Filter 100ml of the test sample and transfer to M-endo agar. Incubate it for 22 to 24 hours at 35°C.
- Count the pink colonies and those dark red with metallic green sheen. The sheen can vary from one pinpoint to another.
- Report the number of the Coliforms colonies per 100ml

5.2.6. After test is completed, prepare a test report

5.2.7. If the count result is higher than the limits specified, perform an investigation and take the necessary steps as per the SOP.

5.3. Alert and Action Limit for TAMC of Water System

If the microbial results are above the limit above, follow the SOP for Out of Specification.

S.No	Type of water	Alert limit	Action limit
1	Raw water	300	500
2	Soft water	200	500
3	Potable water	150	500
4	Drinking water	100	500
5	Purified water	50	100
6	Water for injection (100ml)	3	10
7	Pure steam (100ml)	3	10

6 Abbreviations

SOP Standard Operating Procedure

M Molarity

mg Milligram

TSS Total Suspended Solids

TDS Total Dissolved Solids

ppm Parts per million

TOC Total Organic Carbon

% Percent

° Degree Centigrade

PAF Pseudomonas agar for Fluorescenin

PAP Pseudomonas agar for Pyocyanin

G.C Giolitti Cantoni broth

TAMC Total Aerobic Microbial Count

ml Millilitre