**TEMPLATE FOR SENDING COMMENTS ON BIS DOCUMENTS**

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| Date: | 02.09.2024 | Document No.: | | **CHD 36 (26063)** | Title of the Document: | Water Quality Sampling Part 1 Guidance on the Design of Sampling Programmes and Sampling Techniques (First Revision) | | |
| Name of the Commentator/  Organization: | | |  | | | | Abbreviation of the Commentator/Organization: |  |

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| **Abbreviation of the Commentator/Organization** | | **Clause/ Subclause No.**  (e.g. 3.1) | | **Paragraph No. /**  **Figure No. /**  **Table No.**  (e.g. Table 1) | | **Type of Comment1)** | | **Comments/Suggestions along with Justification for the Proposed Change** | | **Proposed Change/Modified Wordings** | | **Committee Decision** | |
| **(1)** | | **(2)** | | **(3)** | | **(4)** | | **(5)** | | **(6)** | | **(7)** | |

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| Y L PARUCHURI | 1 | 1 | Technical | Scope to include applicability of method to wastewater also.  Justification: These methods can also be used for wastewater. Currently, no Indian Standards are separately published for microbiological examination of wastewater. APHA-Standard methods for examination of water and wastewater is applicable for both water and wastewater. | This method prescribes methods of sampling and microbiological examination of water and Wastewater. | The Panel reviewed the comment and decided to incorporate the sample dilution under clause **4.3.1.2.1.1** as this document covers the wastewater also. The Panel also recommended to incorporate dilution chart and calculation methodology as per Method no. 9221 B under procedure presumptive test from 24th edition APHA  Method No. 9221 C under estimation of bacterial density  Presumptive test. |
| Y L PARUCHURI | 3  Reference document given in Annex D) | 1 | Technical | IS 1622 needs modification as per highlighted comments in the attached file |  |  |
| Y L PARUCHURI | 3.1 | 1 | Technical | Sampling for bacteriological examination: Sampling procedure to be harmonized with existing procedure on sampling for microbiological examination : IS 17614 Part 25:2022 (ISO 19458:2006) Water quality - Sampling for microbiological analysis. | 3.1 Sampling for Microbiological examination : Perform sampling as per IS 17614 Part 25:2022 (ISO 19458:2006) Water quality - Sampling for microbiological analysis. |  |
| Shri N. Murali Mohan | 3.1.2 | 1 | General | 5th line  touched instead of touches | 5th line  touched instead of touches |  |
| Muralikrishna Pinninti | 3.3  (Reference document given in Annex A) | 1 | General | Enzyme substrate method IS 17819:2022/ISO 9308-2: 2012/ APHA 9223B) Water Quality- Enumeration of E.coli and Coliform bacteria – Most Probable Number”). | Enzyme substrate method IS 17819:2022/ISO 9308-2: 2012/ APHA 9223B) Water Quality- Enumeration of E.coli and Coliform bacteria – Most Probable Number”). |  |
| Muralikrishna Pinninti | 3.3.4.1  (Reference document given in Annex B) | 1 | Technical | 3.3.4.2 E. Coli: The fluorogenic substrate 4-methyl-umbelliferyl-beta-D-glucuronide (MUG) is used to detect the enzyme beta-D glucuronidase, which is produced by most strains of E. coli. The beta-D-glucuronidase enzyme hydrolyzes the fluorogenic substrate that produces bluish fluorescence when viewed under long-wavelength (365–366 nm) ultraviolet (UV) light. Together, the colour change (due to beta-D-galactosidase) and the fluorescence (due to beta-D-glucuronidase) indicate that a sample contains E. coli. | 3.3.4.2 E. Coli: The fluorogenic substrate 4-methyl-umbelliferyl-beta-D-glucuronide (MUG) is used to detect the enzyme beta-D glucuronidase, which is produced by most strains of E. coli. The beta-D-glucuronidase enzyme hydrolyzes the fluorogenic substrate that produces bluish fluorescence when viewed under long-wavelength (365–366 nm) ultraviolet (UV) light. Together, the colour change (due to beta-D-galactosidase) and the fluorescence (due to beta-D-glucuronidase) indicate that a sample contains E. coli. |  |
| Muralikrishna Pinninti | 3.4  (Reference document given in Annex C) | 1 | Technical | 3.4 Test for Faecal Streptococci  The test terms faecal streptococci and enterococci have been used somewhat synonymously by many in recent years. The faecal streptococci group are indicators of faecal pollution of water Because the general habitat of these organisms IS the intestine of man and animals. They are gram positive cocci and ferment glucose with the production of acid only and are capable of growing in the presence of 40 percent bile and at 45 degrees C, On the basis of newer concepts of speciation of faecal streptococci, it is suggested that the terms faecal streptococci and Lancefield’s group D streptococcus be considered synonymous. The standard test for the estimation of number of the faecal streptococci may be came out either by the multiple tube dilution technique or by the membrane filter technique and Enzyme substrate method (Reference APHA 9230D Fluorogenic Substrate Enterococcus test) | 3.4 Test for Faecal Streptococci  The test terms faecal streptococci and enterococci have been used somewhat synonymously by many in recent years. The faecal streptococci group are indicators of faecal pollution of water Because the general habitat of these organisms IS the intestine of man and animals. They are gram positive cocci and ferment glucose with the production of acid only and are capable of growing in the presence of 40 percent bile and at 45 degrees C, On the basis of newer concepts of speciation of faecal streptococci, it is suggested that the terms faecal streptococci and Lancefield’s group D streptococcus be considered synonymous. The standard test for the estimation of number of the faecal streptococci may be came out either by the multiple tube dilution technique or by the membrane filter technique and Enzyme substrate method (Reference APHA 9230D Fluorogenic Substrate Enterococcus test) | Add this method along with convention method (existing method) prescribed in the document |
| Dr Roopadevi | 4.2.3.2.1.1 |  | Technical | As adjustment of pH for media is set for exactly 7.2 | Adjustment of pH to 7.2 suggested to be changed as pH 7.2±0.5 |  |
| Dr Roopadevi | Page 40 | Table 1,2,3,4 | General | 4.3.1.1.2.3 line 1 pH suggested to be changed as pH 7.4±0.2  4.3.1.1.3.2 line 1 pH suggested to be changed as pH 7.4±0.2  4.3.1.1.4.2 line 1 pH suggested to be changed as pH 7.4±0.2  4.3.1.1.5.2 line 1 pH suggested to be changed as pH 7.4±0.2  4.3.1.1.2.1 Addition of 0.01 gm Bromocresol purple or Neutral red /lt is suggested to be added to composition of Macconkey broth for effective colour identification which indicates purple to yellow for positive , If no colour changes its –ve (APHA24th edition)  4.3.1.2.1.2 line 3 Record the presence of or absence of gas and acidic reaction (turbidness/yellow colour) to be incorporated for effective clarity.  4.3.1.2.1.3 line 2 The absence of gas or acidic reaction or yellow colour formation at the end of (48±3)h of incubation constitutes a negative test.  Page 40:Tables of Most probable Numbers  It is suggested to follow APHA 24th edition Table for MPN chart or the following points may be considered.  Table 1 : As only 1-4 tubes mentioned in the method, number of positive tubes 1-5 to be considered for MPN  Table 2 : As only 1-5-4 tubes mentioned , number of positive tubes 1-5-5 to be considered for MPN  Table 3 : As only 5-5-2 tubes for 542 MPN mentioned . MPN for 5-5-5 tubes to be arrived  Table41 : As only 5-5-4 tubes considered for 1600 . 5-5-5 tubes to be considered for > 1600 not for 1600MPN(as per APHA 24th edition) | 4.3.1.1.2.3 line 1 pH suggested to be changed as pH 7.4±0.2  4.3.1.1.3.2 line 1 pH suggested to be changed as pH 7.4±0.2  4.3.1.1.4.2 line 1 pH suggested to be changed as pH 7.4±0.2  4.3.1.1.5.2 line 1 pH suggested to be changed as pH 7.4±0.2  4.3.1.1.2.1 Addition of 0.01 gm Bromocresol purple or Neutral red /lt is suggested to be added to composition of Macconkey broth for effective colour identification which indicates purple to yellow for positive , If no colour changes its –ve  4.3.1.2.1.2 line 3 Record the presence of or absence of gas and acidic reaction (turbidness/yellow colour) to be incorporated for effective clarity.  4.3.1.2.1.3 line 2 The absence of gas or acidic reaction or yellow colour formation at the end of (48±3)h of incubation constitutes a negative test.  Page 40:Tables of Most probable Numbers  It is suggested to follow APHA 24th edition Table for MPN chart or the following points may be considered.  Table 1 : As only 1-4 tubes mentioned in the method, number of positive tubes 1-5 to be considered for MPN  Table 2 : As only 1-5-4 tubes mentioned , number of positive tubes 1-5-5 to be considered for MPN  Table 3 : As only 5-5-2 tubes for 542 MPN mentioned . MPN for 5-5-5 tubes to be arrived  Table41 : As only 5-5-4 tubes considered for 1600 . 5-5-5 tubes to be considered for > 1600 not for 1600MPN |  |
|  | 1 |  | Scope | Scope to include applicability of method to wastewater also | These methods can also be used for wastewater. Currently, no Indian Standards are separately published for microbiological examination of wastewater. APHA-Standard methods for examination of water and wastewater is applicable for both water and wastewater. |  |
|  | 2 |  | Reference | Specific ISO standard available for Water quality- General requirements and guidance for microbiological examinations by culture. | ISO reference to be added IS 15188:2022 /ISO 8199:2018, Water quality- General requirements and guidance for microbiological examinations by culture. |  |
|  | 2 |  | Reference | Specific ISO standard available for Water quality - Sampling for microbiological analysis. | ISO reference to be added IS 17614 (Part 25) : 2022 /ISO 19458:2006, Water quality – Sampling for microbiological analysis |  |
|  | 2 |  | Reference | Alternate method for enumeration to detail procedures for Faecal coliforms and E.coli | ISO reference to be added IS 17819:2022/ISO 9308-2: 2012/ APHA 9223B, Water Quality- Enumeration of E.coli and Coliform bacteria – Most Probable Number method /Enzyme substrate method. |  |
|  | 3.1 |  | Sampling | Sampling for bacteriological examination: Sampling procedure to be harmonized with existing procedure on sampling for microbiological examination : IS 17614 Part 25:2022 (ISO 19458:2006) Water quality - Sampling for microbiological analysis. | Perform sampling as per IS 17614 Part 25:2022 (ISO 19458:2006) Water quality - Sampling for microbiological analysis. |  |
|  | 3.1.4 |  |  | To harmonize with ISO 19458 requirements | Samples should be kept at 5±3oC |  |
|  | 4.3 |  |  | Methodology for Faecal coliforms and E.coli not specified as per IS 17819. | The procedure to include MPN methodology for Total coliforms, Faecal coliforms and E.coli as per IS 1622 and also as per IS 17819. |  |
|  | 4.3.3.3 |  |  | While using IS 17819 – Enzyme substrate method, procedure for enumeration of Faecal coliforms and E.coli to be described, in addition to methodology for enumeration of Coliforms. | Include Procedure for Faecal coliforms as per IS 17819.  Testing Procedure for Faecal Coliforms:  1.Add contents of one snap pack to a 100 mL room temperature water sample in a sterile vessel.  2.Cap vessel and shake until dissolved.  3.Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer.  4.Place the sealed tray in a 44.5˚C ±0.2˚C incubator for 18 hours (prewarming to 35˚C is not required).  For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring.  5.Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the Quanti-Tray to obtain a Most Probable Number.  Result Interpretation:   |  |  | | --- | --- | | Appearance | Result | | Less yellow than the comparator when incubated at 44.5°C ±0.2°C | Negative for faecal coliforms | | Yellow equal to or greater than the comparator when incubated at 44.5°C ±0.2°C | Positive for faecal coliforms | |  |
|  | 4.3.4 |  |  |  | Test for fecal coliform is included with reference of EPA approved document.  Colilert18 is used for testing E.coli |  |
|  | 4.7 |  |  | Medium proposed for Sulphate reducing bacteria is: 10 g tryptone, 1 g sodium sulphite and 10 ml of 5 percent ferric citrate solution in 1000 ml of distilled water. Since the test is for Sulphate reducing (and not sulphite reducing), medium should contain sulphate source instead of sulphite source (sodium sulphite). | Medium for Sulphate reducing medium (Reference: APHA- Standard methods for examination of water and wastewater, 23rd edition, section 9240D, section 5) Sulfate-reducing medium: Sodium lactate 3.5 g; Beef extract 1.0 g; Peptone 2.0 g; Magnesium sulfate (MgSO4.7H2O) 2.0 g; Sodium sulfate (Na2SO4) 1.5 g ; Dipotassium hydrogen phosphate (K2HPO4) 0.5 g; Ferrous ammonium sulfate [Fe(NH4)2(SO4)2.6H2O] 0.392 g; Calcium chloride (CaCl2) 0.10 g; Sodium ascorbate 0.10 g; Reagentgrade water 1 L. Prepare medium (excluding ferrous ammonium sulfate and sodium ascorbate), dispense in screw-capped test tubes, and sterilize via autoclaving (121°C, 15 min). Final pH should be 7.5 ± 0.3. Use completely filled tubes. Separately sterilize extra medium to be added to tubes for filling. On day of use, prepare separate solutions of ferrous ammonium sulfate (3.92 g/100 mL) and sodium ascorbate (1.00 g/100 mL), filter through a 0.45µm membrane filter, and aseptically add 0.1 mL each solution/10 mL basal medium. |  |
|  | 4.10 |  |  | Procedure to be elaborated including procedure for culturing of slime forming organisms, media to be used, incubation conditions, and interpretation for slime forming organisms. | Method for examination of water for slime forming organisms and interpretation of results is not clear. |  |
| ANNEX B | | | | | |  |
|  | Clause 4.3.1, 4.3.1.3, 4.4.1.2.2 Tables 1,2,3,4 |  |  | MPN tables does not cover all feasible combinations for single and multiple dilution systems. | MPN tables 1,2,3,4 of IS 1622 does not cover all feasible combinations for single and multiple dilution systems. |  |
|  | Provide reference to MPN tables of APHA 9221B Table 9221:1,2,3,4 |  |  | A calculator for MPN determination is available at : http://standards.iso.org/iso/8199/ | Reference to Excel Calculator to be provided |  |
|  | Include reference to IS 15188:2022 / ISO 8199:2018 section 9.2.7.4, Determination of MPN values using an MPN calculator. |  |  |  |  |  |
|  | Include MPN tables for IS 17819:2022/ ISO 9308-2: 2012/ APHA 9223B |  |  |  |  |  |
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