Laboratory Manager Approval: Mary K. Bowman / 08/19/2021

QA Manager Approval: Jeffrey Moore / 08/19/2021

Suitability Test for Bacteriological Quality of Reagent Water

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1 Scope and Application

Suitability is a test method used to test the quality of purified (distilled or reagent-grade) water used in microbiological testing. The test is based on the growth of *Enterobacter aerogenes* in a chemically defined minimal growth medium. The presence of a toxic agent or a growth-promoting substance will alter the 24-h population by an increase or decrease of 20% or more when compared to a control. The test should be performed at least annually and when the source of reagent water is changed.

The test is complex and requires skill and experience. It requires work over 4 days, ultrapure water from an independent source as a control, high-purity reagents, and extreme cleanliness of culture flasks, petri dishes, test tubes, pipettes, etc.

2 Definitions

Refer to Chapter 3 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions.

3 Interferences

3.1 Method interferences may be caused by contaminants in reagents, media, dilution water, bottles or glassware. To abstain from interferences, all reagents, dilution water, and media are tested for sterility prior to use. Also, all bottles and glassware are washed, sterilized and tested prior to use.

3.1.1 All glassware must be washed, sterilized, and put in the hot air oven at 180° C for 2 hours. A pH check is performed on all batches of glassware using a 0.04% solution of bromothymol blue. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants.

Note: Due to test sensitivity of Suitability, use borosilicate glassware and rinse in

water freshly redistilled from a glass still before sterilizing it with dry heat; steam sterilization will re-contaminate these specially cleaned items. Test sensibility and reproducibility depend in part on cleanliness of sample containers, flasks, tubes and pipettes.

3.1.2 Each batch of media is aseptically prepared according to manufacturers instructions and sterilized before being used. A control agar plate is used during testing to assure the quality of the agar. ***Note:*** *Due to the testing sensitivity of Suitability, use only freshly prepared media and reagents (less than two weeks old).*

3.1.3 Dilution water is asceptically prepared and sterilized according to prescribed methods. Each batch of water is tested for sterility.

4 Safety

Refer to Laboratory Chemical Hygiene Plan, online revision.

5 Apparatus and Equipment

5.1 35º ± 0.5ºC Incubator
5.2 3 Liter Water Still
5.3 Hot Plate
5.4 2 ml Pipettes (3 canisters)
5.5 5 ml Pipettes (1 canister)
5.6 10 ml Pipettes (1 canister)
5.7 11 ml Pipettes (1 canister)
5.8 Pipette Holder
5.9 100 x 15 mm Petri Dishes (96 plates)
5.10 Spatulas (8)
5.11 New foam plugs (20 small)
5.12 500 ml Erlenmeyer Flasks with caps (5)
5.13 1000 ml Erlenmeyer Flasks with caps or stoppers (6)
5.14 125 ml Erlenmeyer Flasks (12)
5.15 500 ml graduated cylinders (4)
5.16 1000 ml graduated cylinders (2)
5.17 Wire Baskets
5.18 Colony Counter
5.19 Pan Balance

6 Reagents

6.1 Plate Count Agar (15 bottles)
6.2 Buffered Dilution Water
6.3 Deionized (Distilled) Water
6.4 Nutrient Agar Slants

6.5 Phosphate Buffer
6.6 Sodium Citrate Solution
6.7 Ammonium Sulfate Solution
6.8 Salt Mixture Solution (Magnesium Sulfate, Calcium Chloride, Sodium Chloride, and Ferrous Sulfate)
6.9 Enterobacter aerogenes stock bacteria

7 Sample Collection

7.1 Refer to Chapter 5 of the Georgia EPD Laboratory Quality Assurance Manual for Sample Container, Sample Preservation and Sample Holding Times.

8 Calibration

8.1 There are no calibrations associated with this method. As with any test for bacteria, all equipment and reagents must be sterile to ensure accuracy. This includes the glass plates, pipettes, and dilution water.

9 Quality Control

Refer to Table 13.1 Quality Control Acceptance Criteria associated with this method.

10 Procedure

10.1 On **Monday**, one week before the test, prepare all glassware:

a. Wash and rinse all glassware two (2) times in washer. (Pipettes are washed and rinsed in pipette washer.)
b. Rinse all glassware in freshly REDISTILLED WATER from the glass still.
c. Let air dry.
d. Put in the hot air oven for two (2) hours.

10.2 Wash new foam plugs and dry as normal.

10.3 Keep all glassware, foam plugs, etc. covered until use.

10.4 Check to see if the following has been freshly made:
a. Sterile plate count agar.
b. Sterile buffered dilution water.
c. Phosphate buffer.
d. Agar slants.

10.5 On **Friday**, one week before the test:
a. Have all glassware ready for use.
b. Check the glass still. Let it run about halfway full – rinse and pour out.
c. Streak a slant with Enterobacter aerogenes (EA).

MONDAY (week of test)

10.6 Turn on the glass still and let run until full. Collect water freshly redistilled from the still into two 1000 ml cylinders. Let cool to room temperature.

10.7 Prepare the following reagents each in a 1000 ml flask in this freshly redistilled water. (After all solutions have been prepared, **boil** each solution for 1-2 minutes. This will kill all vegetative bacteria.)

a. Sodium Citrate Solution	0.29 g in 500 ml of redistilled water
b. Ammonium Sulfate Solution	0.60 g in 500 ml of redistilled water
c. Phosphate Buffer	20 ml stock PO4 buffer in 480 ml redistilled water
d. Salt Mixture Solution	500 ml of redistilled water
*Magnesium Sulfate	0.26 g
*Calcium Chloride	0.17 g
*Sodium Chloride	2.50 g
**Ferrous Sulfate	0.23 g

****NOTE:** Add **Ferrous Sulfate** after boiling the salt mixture. Wait until mixture has cooled to room temperature.

10.8 After all solutions above have come to a room temperature, put screw caps on flasks and store in refrigerator overnight. ***Note:*** *the Salt Mixture Solution must be stored in the dark, therefore wrap aluminum foil completely around this flask before storing in the refrigerator.*

10.9 Streak a slant with EA from the one previously streaked on Friday.

TUESDAY

10.10 Streak the entire surface of at least two (2) slants with EA from the one previously streaked on Monday.

10.11 Do **plate counts** on reagents. (Melt 1 bottle of plate count agar.)

*Plate 2ml of each solution:

2ml – Citrate Solution	2ml – Salt Solution
2ml – Ammonium Solution	2ml – PO4 Buffer Solution
1ml - Agar Control	(Total of 5 Plates)

10.11.1 Add agar which has been tempered between 44ºC and 46ºC to each plate and swirl to mix. Allow plates to cool and solidify.

10.11.2 Turn plates upside down and place in a wire basket. Incubate plates at 35º C ±0.5º Cfor 24 ± 2 hours.

WEDNESDAY

10.12 Read and record Reagent Control Plates from Tuesday. All plates should have

no colonies present.

10.13 Turn on the still and let run until half full or less.

10.14 Collect 200 ml of Control Water (Redistilled) A and the various other unknown waters B, C, D, etc. in 500 ml screw cap flasks. **Boil** each for 1-2 minutes and let cool to room temperature.

10.15 Label small 125 ml flasks in duplicate for each water tested.
Ex. A1 A2 B1 B2 C1 C2 D1 D2 etc.

10.16 Label duplicate plates for each flask used. **Note:** Duplicate tests are done to get initial plate counts that are similar. Therefore, do **DUPLICATE PLATES FOR EACH FLASK USED** (i.e. two plates each for A1, A2, B1, B2, C1, C2, etc.)

10.17 Add reagents to the flasks as follows:

REAGENTS	**FLASKS**			
	A1A2	B1B2	C1C2	D1D2
Citrate Solution	2.5 ml	2.5 ml	2.5 ml	2.5 ml
Ammonium Sulfate Soln.	2.5 ml	2.5 ml	2.5 ml	2.5 ml
Salt Solution	2.5 ml	2.5 ml	2.5 ml	2.5 ml
Phosphate Buffer Solution	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Redistilled Water (A)	21.0 ml	---	---	---
Unknown Water (B)	---	21.0 ml	---	---
Unknown Water (C)	---	---	21.0 ml	---
Unknown Water (D)	---	---	---	21.0 ml

10.18 Prepare **Enterobacter aerogenes** bacterial suspension using four leveled dilution bottles A, B, C, and D. (Select EA slant with the most growth.)
a. Using dilution bottle "A", make your original bacterial suspension. (2 ml of sterile dilution water from bottle "A" onto the EA slant to make the suspension and then back into bottle "A" again. Follow the standard procedure for this task.)
b. From bottle "A", transfer 1 ml to bottle "B". Shake vigorously.
c. From bottle "B", transfer 1 ml to bottle "C". Shake vigorously.
d. From bottle "C", transfer **10 ml** to bottle "D". Shake vigorously.
NOTE: Bottle "D" is your working dilution (diluted inoculum).

10.19 Inoculate each flask with **1.0 ml** of the prepared inoculum from bottle "D". Swirl thoroughly to mix.

10.20 Plate **1.0 ml** from each flask in duplicate to determine the initial count per ml (i.e. two plates for A1, two for A2 etc.). Do **control plates** for dilution water and each bottle of agar used.

10.21 Incubate all plates and 125 ml flasks for 24 ± 2 hrs. at 35º ± 0.5º C to determine the initial count. (Plug the 125 ml flask with foam plugs.)

THURSDAY

10.22 Read plates from Wednesday and record results. Should be 25 – 75 colonies per plate. **To Read Plates:** After 24 hours of incubation, remove plates and prepare to read under the colony counter. Examining each plate individually, count each colony and record the total number of colonies under the proper dilution. **Note:** Do not count surface colonies.

10.23 Determine the best initial counts and prepare dilutions from that flask:

Example: A1 – 30, 26 A2 – 31, 16 ***Use A1**
B1 – 31, 42 B2 – 33, 35 ***Use B2**

10.24 Pipette 1.0 ml and 0.1 ml from each individual **FLASK** used (A, B, C, D, etc.) into their respective labeled plates.

10.25 Make 1st Dilution:
1.0 ml from **FLASK** into first dilution bottle and label "A".
** Plate 1.0 ml and 0.1 from Bottle "A" into duplicate plates.
1.0 ml = 1_1
0.1 ml = 1_2

[In other words after the first dilution has been made, plate in **duplicate 1.0** and **0.1 ml** from the first dilution bottle for each water tested (A, B, C, D, etc.).]

10.26 Make 2nd Dilution:
1.0 ml from Bottle "A" into a second dilution bottle and label "B".
** Plate 1.0 ml and 0.1 ml from Bottle "B" into triplicate plates.
1.0 ml = 1_3
0.1 ml = 1_4

[In other words after the second dilution has been made, plate in **triplicate 1.0** and **0.1 ml** from the second dilution bottle for each water tested (A, B, C, D, etc.).]

Summary of Dilutions:

Plate 1.0 and 0.1 ml from each
Individual flask used:
FLASKS

```
   A          B
  / \        / \
1.0 0.1    1.0 0.1
```

Therefore 4 plates needed

Make 1st Dilution

Then do plates in Duplicate

A		B		
/	\	/	\	Therefore 8 plates needed
1.0	0.1	1.0	0.1	
(2)	(2)	(2)	(2)	

Make 2nd Dilution
Then do plates in Triplicate

A		B		
/	\	/	\	Therefore 12 plates needed
1.0	0.1	1.0	0.1	
(3)	(3)	(3)	(3)	

Dil. H_2O – 1
Agar Control –6

31 Plates 6 Bottles of Agar

10.27 Add agar which has been tempered 44ºC to 46ºC to each plate and swirl to mix. Allow plates to cool and solidify.

10.28 Incubate all plates upside down in a wire basket at 35º ±0.5º C for 24 ±2 hrs.

FRIDAY

10.29 Read plates from Thursday and record results.

10.30 Determine the suitability.

11 Calculations

RATIO B/A = Final Count for Suitable Water
0.8 – 1.2 Acceptable Limit (no toxic substances)
Ratio less than 0.8 ---------- growth inhibiting substances
Ratio greater than 1.2 ---------- growth stimulating substances

*The ratio could go as high as 3.0 without any undesirable results.

12. Waste Management

12.1 See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedure, SOP 6-015, online revision.

13. References

13.1 Section 9020B Quality Assurance, Standard Methods for the Examination of

Water and Wastewater, 20th Edition, American Public Health Association: Washington, D.C., 1998.

13.2 9215B Heterotrophic Plate Count (HPC)-Pour Plate Method, Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association: Washington, D.C., 1998.

13.3 GA EPD Laboratory SOP – Initial Demonstration of Capability SOP 6-001, online revision and/or Continuing Demonstration of Capability SOP 6-002, online revision.

13.4 GA EPD Laboratory SOP – EPD Laboratory Waste Management SOP, SOP 6-015, online revison.

13.5 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA/815-R-05-004, January 2005.

13.6 GA EPD Laboratory Quality Assurance Plan, online revision.

13.7 GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

14. Precision and Accuracy Criteria and Quality Control Approach

Table 14.1 Suitability Test Summary of Data Quality Objectives

Method	Parameter	QC Check	Min. Frequency	Accepted Criteria	Corrective Action
SM 9215B-Heterotrophic Plate Count (HPC)-Pour Plate Method and SM 9020B Quality Assurance	Citrate Solution	2 mls / agar plate	1 per test	No Growth	Discard and Remake
	Ammonium Solution	2 mls / agar plate	1 per test	No Growth	Discard and Remake
	Salt Solution	2 mls / agar plate	1 per test	No Growth	Discard and Remake
	Phosphate Buffer	2 mls / agar plate	1 per test	No Growth	Discard and Remake
	Agar Control	agar w/o inoculums	1 per test	No Growth	Discard and Remake
	Citrate, Ammonium, Salt, Phosphate Buffer Solution	21.0 mls of Redistilled Water	2 per set	0.8--1.2	Unacceptable Limits cannot use.
	Citrate, Ammonium, Salt, Phosphate Buffer Solution	21.0 mls of Sample	2 per set	0.8--1.2	Unacceptable Limits cannot use.
	Dilution Water	1.0 ml	1 per set	No Growth	Unacceptable cannot use.