CALAMBA WATER DISTRICT

TITLE: MULTIPLE TUBE FERMENTATION TECHNIQUE

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Form No.

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2016-12-019	00	N/A	Ethel O. Paderes	Initial Issue

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1.0 PURPOSE

1.1 This documented information is for the detection and enumeration of coliforms and fecal coliforms
1.2 CWD shall implement production and service provision under controlled conditions. Controlled conditions shall include, as applicable:
a) the availability of documented information that defines:
1) the characteristics of the products to be produced, the services to be provided, or the activities to be performed;
2) the results to be achieved;
b) the availability and use of suitable monitoring and measuring resources;
c) the implantation of monitoring and measurement activities at appropriate stages to verify that criteria for control of processes or outputs, and acceptance criteria for products and services, have been met;
d) the use of suitable infrastructure and environment for the operation of processes;
e) the appointment of competent persons, including any required qualification;
f) the validation, and periodic revalidation, of the ability to achieve planned results of the processes for production and service provision, where the resulting output cannot be verified by subsequent monitoring or measurement;
g) the implementation of actions to prevent human error;
h) the implementation of release, delivery and post-delivery activities.

1.3 To use suitable means to identify outputs when it is necessary to ensure the conformity of products and services.
1.4 To identify the status of outputs with respect to monitoring and measurement requirements throughout production and service provision.
1.5 To control the unique identification of the outputs when traceability is a requirement, and shall retain the documented information necessary to enable traceability.

2.0 SCOPE

2.1 This documented information applies to Service reservoirs, Water treatment works, Consumer's taps nearest the meter, Refilling Stations, Water Haulers and Water Vending Machines.

3.0 RESPONSIBILITY

3.1 The Head of Laboratory shall manage the administrative and technical operations of the laboratory.
3.2 The Laboratory Analyst must perform the microbiological test with minimum supervision, summarizes the data and prepares the report from the results.

4.0 DEFINITION OF TERMS

4.1 Total Coliforms – aerobic and facultative anaerobic, gram negative, non-spore forming, rod shaped bacteria that ferment lactose with gas formation within 24- 48 hours at 35°C.

4.2 Fecal Coliforms – subgroup of coliform bacteria that has high positive correlation with fecal contamination associated with all warm-blooded animals. These organisms can ferment lactose at 44.5°C and produce gas in multiple tube procedure.

4.2 Most Probable number (MPN) – a statistical method of determining microbial populations. A multiple dilution tube technique is utilized with a standard medium and observations are made for specific individual tube effect.

4.3 BGB – Brilliant Green Broth

4.4 EC – *Echerichia coli* Broth

5.0 PROCESS FLOW STEPS

STEP	INPUT	PROCESS FLOW	OUTPUT	RESPONSIBLE
		START		
1	Request for Analysis	Presumptive Test		Laboratory Analyst
2		Mix sample by making 25 complete up and down or back and forth movements or about 0.3m (1ft) in 7secs.		Laboratory Analyst
3		With a sterile 10 ml pipette inoculate 10 ml of water sample into each 10 tubes of double strength Lauryl		Laboratory Analyst
4		Incubate the tubes at 35 ± 1°C for 24 hrs. After 24 hrs examine each tube for gas production		Laboratory Analyst
5		If no gas production is observed rs – incubate tubes for another 24 hrs. Repeat step 6.1.3.		Laboratory Analyst
6		Include blank per batch of analysis		Laboratory Analyst
7		Confirmatory Test	Test Results	Laboratory Analyst
8		Gently shake presumptive positive tubes		Laboratory Analyst
9		Using a wooden applicator, transfer one to two drops each presumptive tube in EC medium and BGB 2% ,		Laboratory Analyst
10		Check for gas production. Record the results.	Test Results	Laboratory Analyst
11		Re- incubate negative tubes for another 24 hrs. Check for gas production. Record the results and refer to the MPN	Test Results	Laboratory Analyst
		A		

4.5 LSTB– Lauryl Sulphate Tryptose Broth

4.6 Inoculate – to introduce microorganism or suspension of microorganisms (e.g. bacteria) into a culture medium

4.7 Presumptive Test – is the analysis of the sample which establishes either a.) the sample does not contain the target organism , b.) the sample probably contains the target microorganism

4.8 Confirmatory Test – tests required to confirm the presence of the target microorganism

STEP	INPUT	PROCESS FLOW	OUTPUT	RESPONSIBLE
		A		
12		To confirm the presence of thermotolerant (fecal) coliform, incubate EC tube at 44.5 ± 1°C for 24 hrs		Laboratory Analyst
13		Check for gas production. Record the results and refer to the MPN Table		Laboratory Analyst
14		EXPRESSION OF RESULTS	Test Results	Laboratory Analyst
		END		

6.0 PROCESS DETAILS

6.1 Presumptive Test

6.1.1. Mix sample by making 25 complete up and down or back and forth movements or about 0.3m (1ft) in 7secs.

6.1.2. With a sterile 10 ml pipette inoculate 10 ml of water sample into each 10 tubes of double strength Lauryl Sulfate Broth. Shake gently to distribute the sample throughout the medium

6.1.3. Incubate the tubes at 35 ± 1°C for 24 hrs. After 24 hrs examine each tube for gas production. If none is visible gently shake the tubes, if any effervescence is observed then the tube is considered positive. Record results

6.1.4. If no gas production is observed re – incubate tubes for another 24 hrs. Repeat step 6.1.3.

6.1.5. Include blank per batch of analysis

6.2 Confirmatory Test

6.2.1. Gently shake presumptive positive tubes

6.2.2. Using a wooden applicator, transfer one to two drops each presumptive tube in EC medium and BGB 2% , mix gently

6.2.3. To confirm the presence of total coliforms, incubate BGB 2% from each presumptive positive tube for 24 hrs at 35 ± 1°C.

6.2.4. Check for gas production. Record the results.

6.2.5. Re- incubate negative tubes for another 24 hrs. Check for gas production. Record the results and refer to the MPN Table

6.2.6. To confirm the presence of thermotolerant (fecal) coliform, incubate EC tube at 44.5 ± 1°C for 24 hrs

6.2.7. Check for gas production. Record the results and refer to the MPN Table

6.3 Expression of Results

6.3.1 MPN Index and 95% Confidence Limits for Various Combinations of Positive and Negative Results when Ten - 10ml portions are used.

Number of positive tubes giving positive reaction out of 10 of 10 ml each	MPN Index / 100 ml	95% Confidence Limits	
		Upper	Lower
0	< 1.1	0	3
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12	4.3	27.1
8	16.1	5.9	36.8
9	23	8.1	59.5
10	>23	13.5	Infinite

7.0 RECORDS RETENTION

7.0 Active Retention – indefinite retention period for current or active documents for both electronic and hardcopy Master Copy.

7.2 Inactive/Archival Retention – shall be kept for active three (3) years or may request for an extension as deemed necessary (hardcopy); for electronic/soft file; it shall be kept in a separate folder named "Obsolete Master Copy/Original".

8.0 REFERENCE

8.1 ISO 9001:2015 QMS Standard
8.2 Philippine National Standards for Drinking Water 2007
8.3 Standard Methods for the Examination of Water and Wastewater 20th edition

9.0 ATTACHMENTS

9.1 Request for Analysis Form

10.0 DISTRIBUTION LIST

Note 1: Select Relevant Recipient to Appear in below List.

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8	Laboratory (Quality Control Division)

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