برنامج المسار الوظيفي للعاملين بقطاع مياه الشرب والصرف الصحي

دليل المتدرب

البرنامج التدريبي كيميائي مياه الصرف الصحي – الدرجة الثالثة

Biological analysis

تم اعداد المادة بواسطة الشركة القابضة لمياه الشرب والصرف الصحي
قطاع تنمية الموارد البشرية - الادارة العامة لتخطيط المسار الوظيفي V1 1-7-2015

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Introduction

The tests required to detect specific pathogens in wastewater are very difficult and expensive so it is impractical for sewage utilities to routinely test for specific types of organisms or pathogens. A more practical approach is to examine the wastewater for indicator organisms associated with contamination. The coliform group of bacteria meets all of the criteria for an ideal indicator. These bacteria are generally not pathogenic, yet they are usually present when pathogens are present. As a rule, where coliforms are found in water or sludge it is assumed that pathogens may also be present, and if coliforms are absent, the water or sludge is assumed to be safe. Total coliform can be used as a general measure of the quality of the sludge as well as evaluating the efficiency of various stabilization processes.

Test methods

Total coliform bacterial densities may be determined either by the multiple-tube procedure or by the Membrane Filter (MF) technique. The Total coliform MF procedure uses an enriched lactose medium and incubation temperature of 35 ± 0.5 °C for selectivity. Because incubation temperature is critical, submerge waterproofed (plastic bag enclosures) MF cultures in a water bath for incubation at the elevated temperature or use an appropriate solid heat sink incubator or other incubator that is documented to hold the 35 °C temperature within 0.5 °C throughout the chamber, over a 24 h period.

Containers for culture medium:

Use clean borosilicate glass flasks. Any size or shape of flask may be used

Filtration units

Autoclave

Laminar-Flow Hoods/Biological Safety Cabinets

Laboratory Apparatus

For MF analyses use glassware and other apparatus composed of material free from agents that may affect bacterial growth.

a. Sample bottles:

For bacteriological samples, use sterilizable bottles of glass or plastic of any suitable size and shape. Use bottles capable of holding a sufficient volume of sample for all required tests and an adequate air space, permitting proper washing, and maintaining samples uncontaminated until examinations are completed. Ground-glass-stoppered bottles, preferably wide-mouthed and of resistant glass, are recommended. Plastic bottles of suitable size, wide-mouthed, and made of nontoxic materials such as polypropylene that can be sterilized repeatedly are satisfactory as sample containers. Presterilized plastic bags, with or without dechlorinating agent, are available commercially and may be used. Plastic containers eliminate the possibility of breakage during shipment and reduce shipping weight.

b. Dilution bottles:

Use bottles or tubes of resistant glass, preferably borosilicate glass, closed with glass stoppers or screw caps equipped with liners that do not produce toxic or bacteriostatic compounds on sterilization. Do not use cotton plugs as closures. Mark graduation levels indelibly on side of dilution bottle or tube. Plastic bottles of nontoxic material and acceptable size may be substituted for glass provided that they can be sterilized properly.

C. Pipets and graduated cylinders:

Before sterilization, loosely cover opening of graduated cylinders with metal foil or a suitable heavy wrapping-paper substitute. Immediately after sterilization secure cover to prevent contamination.

d Culture dishes:

Ttight-fitting plastic dishes are preferred because the membrane filter cultures are submerged in a water bath during incubation. Place Total coliform cultures in plastic bags or seal individual dishes with waterproof (freezer) tape to prevent leakage during submersion. Presterilized disposable plastic dishes with tight-fitting lids that meet the specifications above are available commercially and are used widely. Reseal opened packages of disposable dish supplies for storage.

e. Incubator:

The specificity of the Total coliform test is related directly to the incubation temperature. Static air incubation may be a problem in some types of incubators because of potential heat layering within the chamber, slower heat transfer from air to the medium, and the slow recovery of temperature each time the incubator is opened during daily operations. To meet the need for greater temperature control uses a water bath, a heat-sink incubator, or a properly designed and constructed incubator shown to give equivalent results. A temperature tolerance of 35 ± 0.5 °C can be obtained with most types of water baths that also are equipped with a gable top for the reduction of water and heat losses.

f. Containers for culture medium:

Use clean borosilicate glass flasks. Any size or shape of flask may be used, but erlenmeyer flasks with metal caps, metal foil covers, or screw caps provide for adequate mixing of the medium contained and are convenient for storage.

g. Filtration units:

The filter-holding assembly (constructed of glass, autoclavable plastic, porcelain, or stainless steel) consists of a seamless funnel fastened to a base by a locking device or by magnetic force. The design should permit the membrane filter to be held securely on the porous plate of the receptacle without mechanical damage and allow all fluid to pass through the membrane during filtration. Discard plastic funnels with deep scratches on inner surface or glass funnels with chipped surfaces.

Wrap the assembly (as whole or separate parts) in heavy wrapping paper or aluminum foil, sterilize by autoclaving, and store until use. Alternatively expose all surfaces of the previously cleaned assembly to ultraviolet radiation (2 mm exposure) for the initial sanitization before use in the test procedure, or before reusing units between successive filtration series. Field units may be sanitized by dipping or spraying with alcohol and then igniting or immersing in boiling water for 2 mm. After submerging unit in boiling water, cool it to room temperature before reuse. Do not ignite plastic parts. Sterile, disposable field units may be used.

Materials and Culture Medium

A. M-ENDO medium:

The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration the commercially prepared media (sterile ampoules) or powder.

Rehydrate product in 1 L water. Heat to near boiling, promptly remove from heat, and cool to below 50 °C. Do not sterilize by autoclaving. Final pH should be 7.4 ± 0.2. Refrigerate finished medium, preferably in sealed plastic bags or other containers to reduce moisture loss, and discard unused broth after 96 h.

B. Buffered dilution water:

Prepare a stock solution by dissolving 34 grams of KH_2PO_4 in 500 ml distilled water, adjusting the pH to 7.2 with 1 N NaOH and dilute to one liter. Prepare dilution water by adding 1.25 ml of the stock phosphate buffer solution and 5.0 ml magnesium sulfate (50 grams $MgSO_4.7H_2O$ dissolved in one liter of water) to 1 liter distilled water. This solution can be dispersed into various size dilution blanks or used as a sterile rinse for the membrane filter test.

Procedure

A. Selection of sample size:

Select a volume of the wastewater sample to be examined about 50-100 ml. Use sample volume and dilution that will yield counts between 20 and 60 Total coliform colonies per membrane.

When the bacterial density of the sample is unknown, filter several volumes or dilutions to achieve a countable density. Estimate volume and/or dilution expected to yield a countable membrane and select two additional quantities representing one-tenth and ten times this volume, respectively.

B. Filtration of sample:

Using sterile forceps, place a sterile membrane filter (grid side up) over porous plate of receptacle. Carefully place matched funnel unit over receptacle and lock it in place. Filter sample under partial vacuum. With filter still in place, rinse the interior surface of the funnel by filtering three 20 to 30 ml portions of sterile dilution water. Alternatively, rinse funnel by a flow of sterile dilution water from a squeeze bottle. This is satisfactory only if the squeeze bottle and its contents do not become contaminated during use. Rinsing between samples prevents carryover contamination. Upon completion of final rinse and the filtration process disengage vacuum, unlock and remove funnel, immediately remove membrane filter with sterile forceps, and place a pad in the culture dish and saturate with at least 2.0 ml M-ENDO medium and carefully remove excess medium by decanting the plate. Place prepared filter directly on pad, invert dish, and incubate for 22 to 24 h at 35 ± 0.5 °C.

Differentiation of some colonies from liquid medium substrates may be lost if cultures are incubated beyond 24 h.

Insert a sterile rinse water sample (100 ml) after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water

c. Incubation:

Place prepared dishes in waterproof plastic bags or seal, invert, and submerge petri dishes in water bath, and incubate for 24 ± 2 h at 35 ± 0.5 °C. Anchor dishes below water surface to maintain critical temperature requirements. Place all prepared cultures in the water bath within 30 min after filtration. Alternatively, use an appropriate, accurate solid heat sink or equivalent incubator.

d. Counting:

Colonies produced by Total coliform bacteria on M-ENDO medium are of metallic sheen. Non-Total coliform colonies are dark brown. Normally, few non-Total coliform colonies will be observed on M-ENDO medium because of selective action of the elevated temperature and the selectivity of the media. Count colonies with a low-power (10 to 15 magnifications) binocular wide-field dissecting microscope or other optical device.

Calculation of Total Coliform Density

a. General: Compute the density from the sample quantities that produced MF counts within the desired range of 20 to 60 Total coliform colonies. This colony density range is more restrictive than the 20 to 80 total coliform range because of larger colony size on M-ENDOmedium. Calculate Total coliform density and record densities as Total coliforms per 100 ml.

$$\text{Bacteria/100 ml} = \frac{\text{No. of Colonies Counted}}{\text{Dilution factor}}$$

OR

$$\text{Bacteria/100 ml} = \frac{\text{No. of Colonies Counted} \times 100 \text{ ml}}{\text{Sample Volume Filtered, ml in 100 ml}}$$

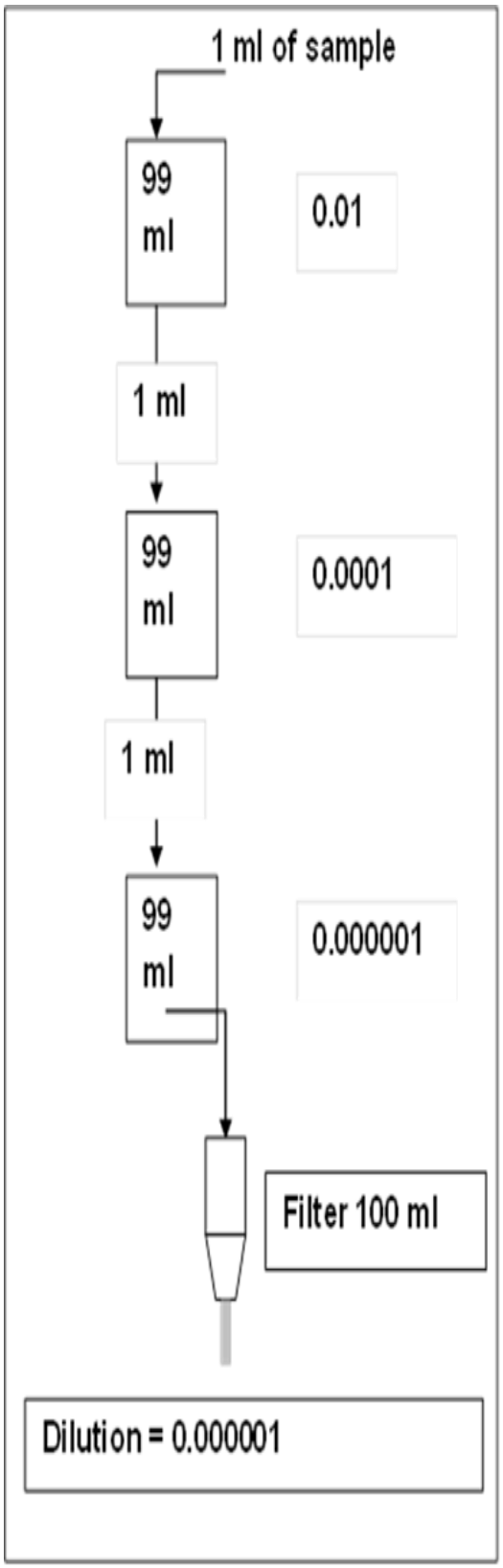
Note:

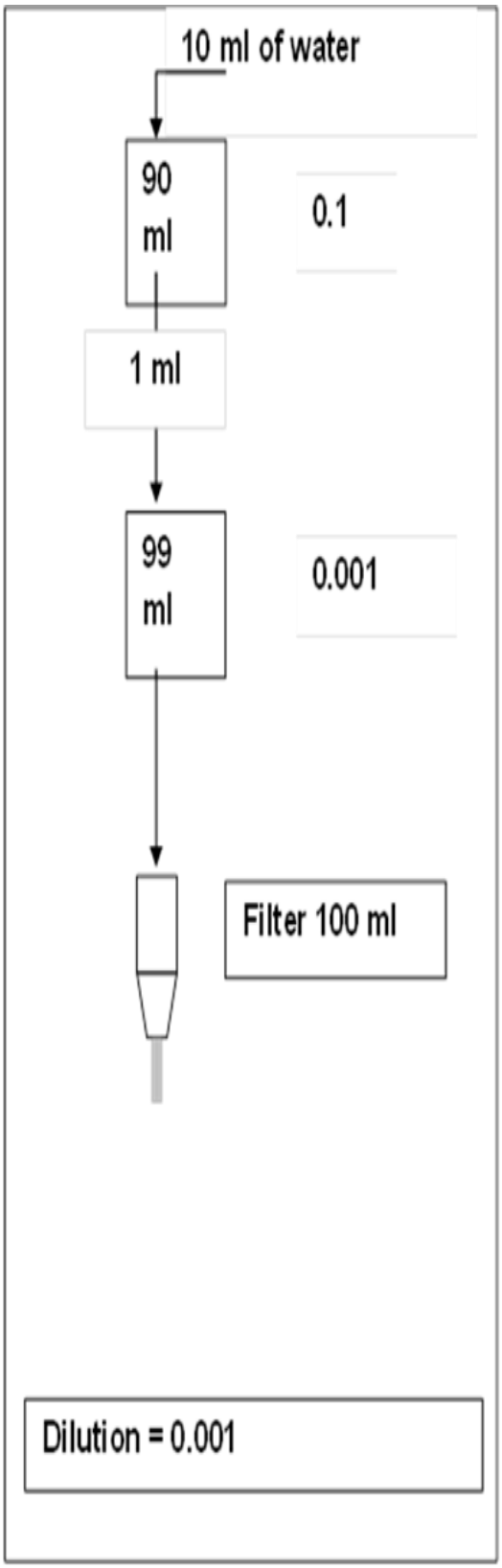
To aid in quality assurance, analyze samples in duplicate.

Suggested samples volumes for membrane filter total coliform test

	Volume (X) To Be Filtered ml							
Water Source	100	50	10	1	0.1	0.01	0.001	0.0001
Lakes, reservoirs	X	X						
Wells, springs	X	X						
Water supply intake		X	X	X				
Natural bathing waters		X	X	X				
Sewage treatment plant			X	X	X			
Farm ponds, rivers				X	X	X		
Stormwater runoff				X	X	X		
Raw municipal sewage					X	X	X	
Feedlot runoff					X	X	X	
Sewage sludge						X	X	X

Dilution scheme

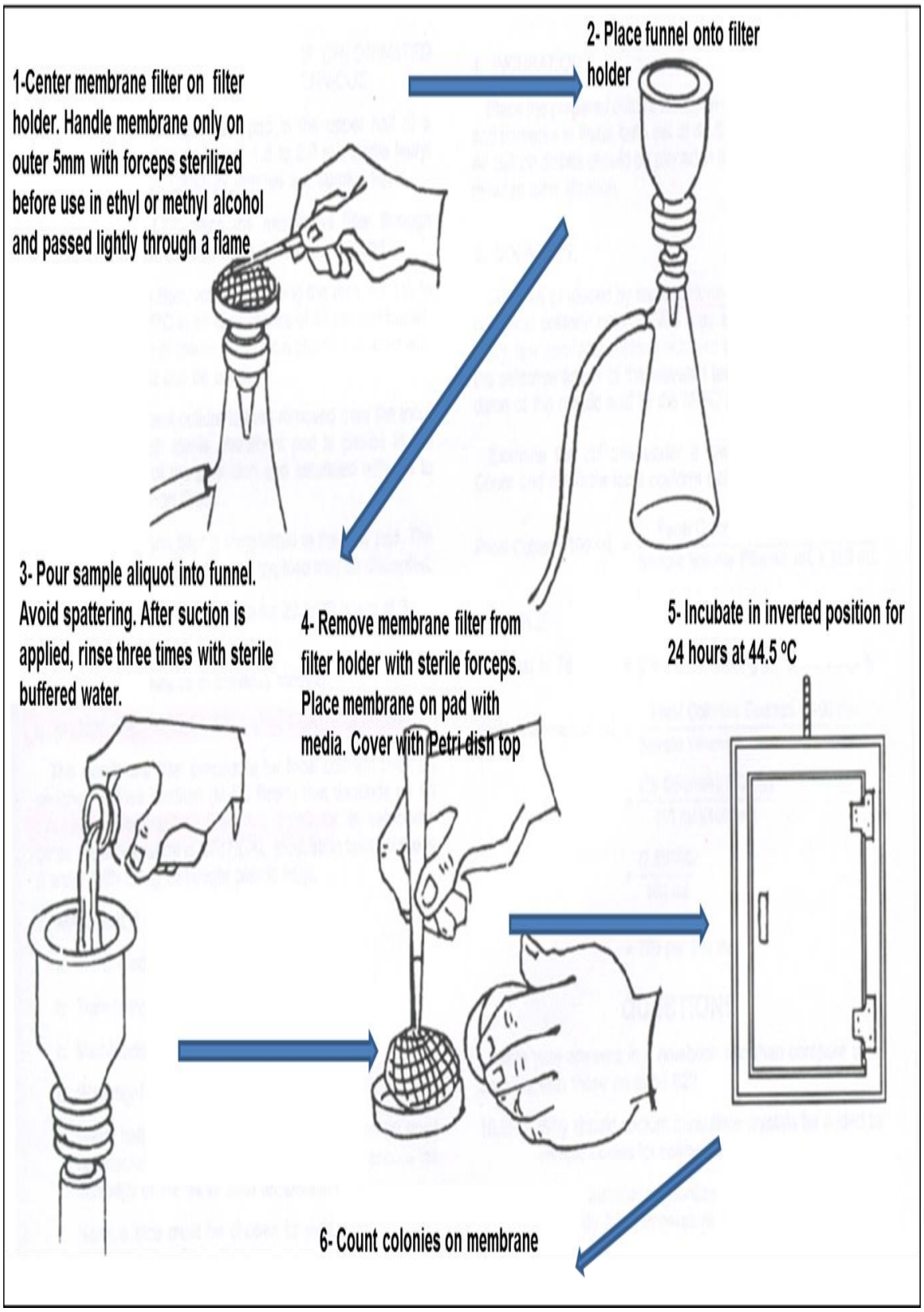




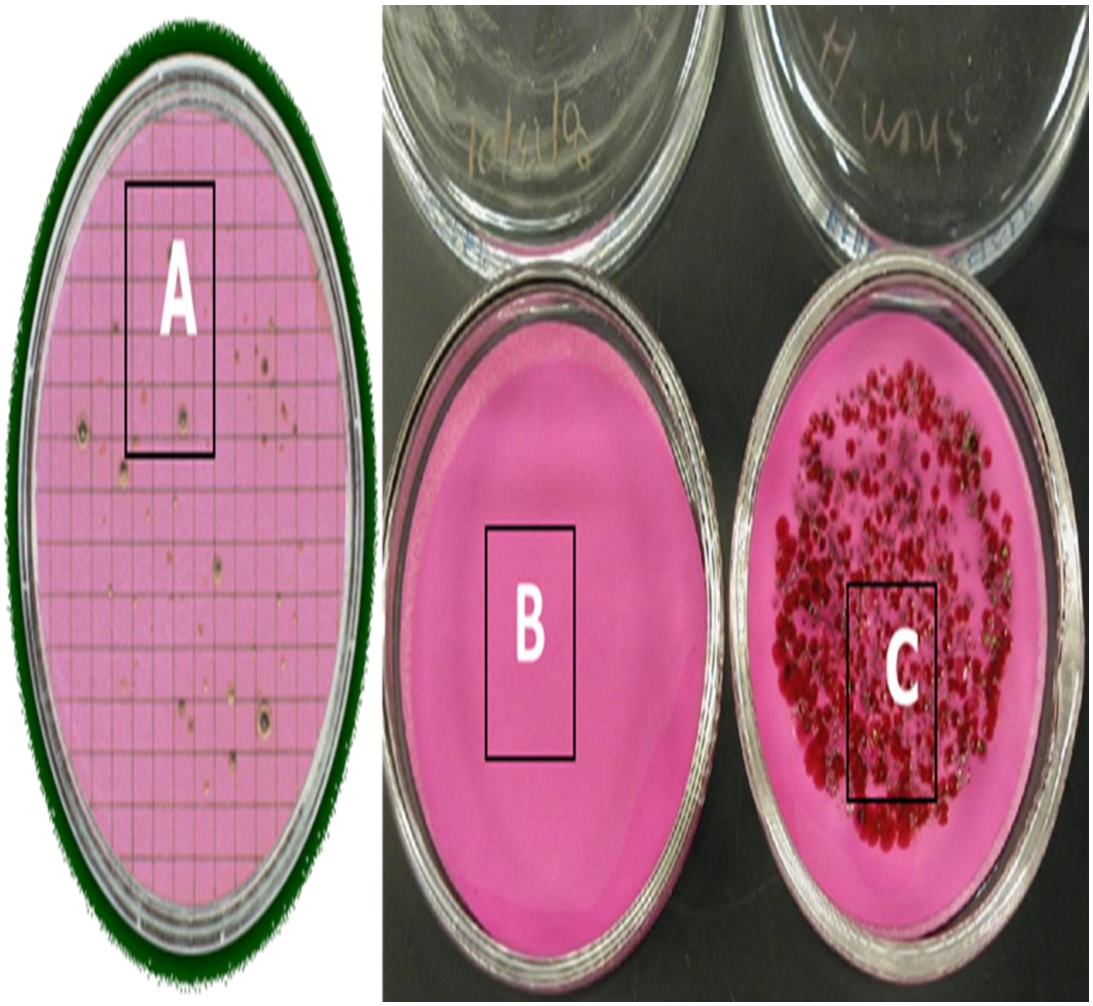
Dilution applied for the raw sewage samples.

Dilution applied for the treated samples.

Outline of Procedure for inoculation of Membrane Filter



a EXAPMLES OF TOTAL COLIFORM PLATES



A – plate shows the metalic sheen colonies of total coliforms growing on M-ENDO media. The number of colonies is perfect for counting. The sample portion and dilution used is correct.

B – plate shows no colonies growing on M-ENDO media. The sample collected from filtered chlorinated water.

C – plate shows a huge number of total coliform colonies due to incorrect dilution

Calculation

$$\text{Bacteria/100 ml} = \frac{\text{No. of Colonies Counted}}{\text{Dilution factor}}$$

OR

$$\text{Bacteria/100 ml} = \frac{\text{No. of Colonies Counted} \times 100\ \text{ml}}{\text{Sample Volume Filtered, ml in 100 ml}}$$

Example (1):

A total of 42 colonies grew after filtering 100 ml of a sample with dilution factor 1: 10.

$$\text{Bacteria}/100\text{ ml} = \frac{42}{0.1} = 420/100\,\text{ml}$$

Example (2):

A total of 22 colonies grew after filtering a 10 ml sample.

$$= \frac{(22\ \text{Colonies}) \times (100\ \text{ml})}{10\ \text{ml}}$$

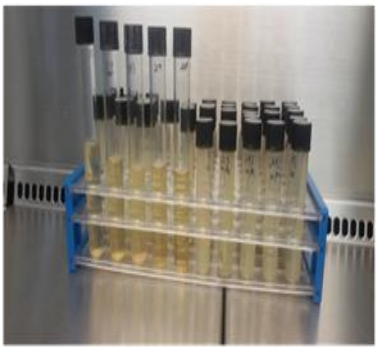
= 220 per 100 ml

(B) Total Coliform Bacteria (MPN)
Introduction

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. this group has been based on the method used for detection (lactose fermentation) when the fermentation technique is used, this group is defined as all facultative anaerobic, gram-negative, non-spore-forming, rod shaped bacteria that ferment lactose with gas and acid formation within 48 h at 35 °C. If the medium has been refrigerated after sterilization, incubate overnight at room temperature (20 °C) before use; discard tubes showing growth and/or bubbles.

a. Laboratory Apparatus

Apparatus



Fermentation tubes with an inverted vial.

Autoclave

Laminar-Flow Hoods/Biological Safety Cabinets

A Sample collection:

- See sampling part (sheet)

B Equipments:

1- Incubation

2- Fermentation tubes with an inverted vial.

3- Autoclave.

1.1. Presumptive Phase

Use lauryl tryptose broth in the presumptive portion of the multiple-tube test.

A preparation:

a- Add 35.6 g dehydrated prepared media to 1litre distilled water, mix through, and warm slightly to dissolve (pH 6.8 ±.2) after sterilization.

b- Before sterilization dispense sufficient medium (10 ml media), in Fermentation tubes with an inverted vial, add .01 g/l bro-cresol purple to Presumptive medium to determine acid production .close the tubes with metal or heat resistant plastic caps.

c- The media sterilized on the autoclave at temperature 121 ºC for 15 min.

Notes:

If the medium has been refrigerated after sterilization, incubate overnight at room temperature (20 °C) before use discards tubes showing growth and/or bubbles.

B. Procedure:

1- Arrange fermentation tubes in rows of five or ten tubes each in a test tube rack. The number of rows and the sample volumes selected depend upon the quality and character of the water to be examined. For potable water use five 20 ml portions, ten 10 ml portions, or a single bottle of 100 ml portion; for non potable water use five tubes per dilution (of 10, 1, 0.1 ml, etc.).

2- Incubate inoculated tubes or bottles at 35 ± 0.5 °C. After 24 ± 2 h. Swirl each tube or bottle gently and examine it for growth, gas, and acidic reaction (shades of yellow color) and, if no gas or acidic reaction is evident, re incubate and reexamine at the end of 48 ± 3 h.

Record presence or absence of growth, gas, and acid production. If the inner vial is omitted, growth with acidity signifies a positive presumptive reaction.

C Interpretation:

- Production of an acidic reaction or gas in the tubes or bottles within 48 ± 3 h constitutes a positive presumptive reaction. Submit tubes with a positive presumptive reaction to the confirmed phase.

-The absence of acidic reaction or gas formation at the end of 48 ± 3 h. of incubation constitutes a negative test.

- Submit drinking water samples demonstrating growth without a positive gas or acid reaction to the confirmed phase.

1.2. Confirmed Phase

Use brilliant green lactose bile broth fermentation tubes for the confirmed phase.

A. preparation:

a- Add 40 gram dehydrated ingredients to distilled water, mix thoroughly, and warm slightly to dissolve. pH should be7.2 ± 0.2 after sterilization.

b- Before sterilization, dispense, in fermentation tubes with an inverted vial, sufficient medium to cover inverted vial at least one-half to two-thirds after sterilization. Close tubes with metal or heat-resistant plastic caps.

c-The media sterilized on the autoclave at temperature 115 ºC for 15 min.

B. Procedure:

1- Submit all presumptive tubes or bottles showing growth, any amount of gas, or acidic reaction within 24 ± 2 h of incubation to the confirmed phase. If active fermentation or acidic reaction appears in the presumptive tube earlier than 24 ± 2 h, transfer to the confirmatory medium; preferably examine tubes at 18 ± 1 h. If additional presumptive tubes or bottles show active fermentation or acidic reaction at the end of a 48 ± 3 h incubation period submit these to the confirmed phase.
2-.Gently shakes or rotates presumptive tubes or bottles showing gas or acidic growth to resuspend the organisms.

3- With a sterile loop 3.0 to 3.5 mm in diameter, transfer one or more loopfuls of culture to a fermentation tube containing brilliant green lactose bile broth. Repeat for all other positive presumptive tubes.

4- Incubate the inoculated brilliant green lactose bile broth tube at 35 ± 0.5 °C.

c. Interpretation:

Formation of gas in any amount in the inverted vial of the brilliant green lactose bile broth fermentation tube at any time (e.g., 6 ± 1 h, 24 ± 2 h) within 4 8 ± 3 h constitutes a positive confirmed phase. Calculate the MPN value from the number of positive brilliant green lactose bile tubes as described in calculation.

D. Estimation of bacterial density:

Table reading and recording of most probable number.

- Record coliform concentration as MPN/100 ml values of positive and negative tube combination and the sample volumes indicated as in table below which illustrates the MPN values for combination of positive and negatives results when five 1 ml and five 0.1 ml sample portion volumes of non potable water

- Select MPN value from the table for the combination of positive and negative results and calculate according to the following formula

$$\mathrm{MPN/100ml} = \frac{(\text{ table MPN / 100ml }) \times 10}{\mathrm{V}}$$

Where:

V: volume of sample portion at lowest collected dilution

Unit: The result is recorded as MPN/100ml.

Selection rules for different dilution:

When more than three dilutions are used in a decimal series of dilutions, use the following guidelines to select the three most appropriate dilutions and refer to Table 9221: IV. Several illustrative examples (A throughG) of combinations of positives are shown in Table 9221: V.

First, remove the highest dilution (smallest sample volume) if it has all negative tubes and at least one remaining dilution has a negative tube.

Next, remove the lowest dilution (largest sample volume) if it has all positive tubes and at least one remaining dilution has a positive tube. According to these guidelines.

1-The three dilutions in (Example A) are selected by removal of the highest (0.001 ml) and the lowest (10 ml) dilutions.

2-If the lowest dilution does not have all positive tubes, and several of the highest dilutions have all negative tubes, and then remove the highest negative dilutions (Example B).

3-More than three dilutions may remain after removal of the lowest dilution with all positive tubes and high dilutions with all negative tubes. In this case, if the highest dilution with all positive tubes is within two dilutions of the highest dilution with any positive tubes, then use the highest dilution with any positive tubes and the two immediately lower dilutions. In (Example C), the highest dilution with all positive tubes is 0.1 ml, which is within two dilutions of 0.001 ml, which has one positive tube.

In Example D, the highest dilution with all positive tubes is 0.01 ml, which is within two decimal dilutions of 0.001 ml.

4-If, after removal of the lowest dilution with all positive tubes, no dilution with all positive reactions remains, then select the lowest two dilutions and assign the sum of any remaining dilutions to the third dilution. (Example E).

5-If no dilution has all positive tubes (Example F), select the lowest two dilutions, corresponding to 10 and 1 ml sample. For the third dilution, add the number of positive tubes in the remaining dilutions (0.1, 0.01, and 0.001 ml sample), to yield a final combination of 4-3-2. If the third dilution is assigned more than five positive tubes, then the selected combination will not be in Table 9221: IV.

If the three dilutions selected are not found in Table 9221: IV, then something in the serial dilution was unusual. In this case, the usual methods for calculating the MPN, presented here, may not apply. If a new sample cannot be collected and an MPN value is still desired, use the highest dilution with at least one positive tube and the two dilutions immediately lower as the three selected dilutions. In (Example G), the first selection, 4-3-6 (the outcome from the highest three dilutions), is not in Table 9221: IV because 6 is greater than 5. The second selection, according to the above guidelines, would be 3-2-1. If this second set of selected dilutions is not in Table 9221: IV

Reference:

(Standard methods for the examination of water and wastewater TM, 22 nd edition. 9221B)

TABLE 9221:IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 mL, 1.0 mL, 0.1 mL)*

Combination of Positives	MPN Index/100 mL	Confidence Limits		Combination of Positives	MPN Index/100 mL	Confidence Limits	
		Low	High			Low	High
0-0-0	<1.8	—	6.8	4-0-3	25	9.8	70
0-0-1	1.8	0.090	6.8	4-1-0	17	6.0	40
0-1-0	1.8	0.090	6.9	4-1-1	21	6.8	42
0-1-1	3.6	0.70	10	4-1-2	26	9.8	70
0-2-0	3.7	0.70	10	4-1-3	31	10	70
0-2-1	5.5	1.8	15	4-2-0	22	6.8	50
0-3-0	5.6	1.8	15	4-2-1	26	9.8	70
1-0-0	2.0	0.10	10	4-2-2	32	10	70
1-0-1	4.0	0.70	10	4-2-3	38	14	100
1-0-2	6.0	1.8	15	4-3-0	27	9.9	70
1-1-0	4.0	0.71	12	4-3-1	33	10	70
1-1-1	6.1	1.8	15	4-3-2	39	14	100
1-1-2	8.1	3.4	22	4-4-0	34	14	100
1-2-0	6.1	1.8	15	4-4-1	40	14	100
1-2-1	8.2	3.4	22	4-4-2	47	15	120
1-3-0	8.3	3.4	22	4-5-0	41	14	100
1-3-1	10	3.5	22	4-5-1	48	15	120
1-4-0	10	3.5	22	5-0-0	23	6.8	70
2-0-0	4.5	0.79	15	5-0-1	31	10	70
2-0-1	6.8	1.8	15	5-0-2	43	14	100
2-0-2	9.1	3.4	22	5-0-3	58	22	150
2-1-0	6.8	1.8	17	5-1-0	33	10	100
2-1-1	9.2	3.4	22	5-1-1	46	14	120
2-1-2	12	4.1	26	5-1-2	63	22	150
2-2-0	9.3	3.4	22	5-1-3	84	34	220
2-2-1	12	4.1	26	5-2-0	49	15	150
2-2-2	14	5.9	36	5-2-1	70	22	170
2-3-0	12	4.1	26	5-2-2	94	34	230
2-3-1	14	5.9	36	5-2-3	120	36	250
2-4-0	15	5.9	36	5-2-4	150	58	400
3-0-0	7.8	2.1	22	5-3-0	79	22	220
3-0-1	11	3.5	23	5-3-1	110	34	250
3-0-2	13	5.6	35	5-3-2	140	52	400
3-1-0	11	3.5	26	5-3-3	170	70	400
3-1-1	14	5.6	36	5-3-4	210	70	400
3-1-2	17	6.0	36	5-4-0	130	36	400
3-2-0	14	5.7	36	5-4-1	170	58	400
3-2-1	17	6.8	40	5-4-2	220	70	440
3-2-2	20	6.8	40	5-4-3	280	100	710
3-3-0	17	6.8	40	5-4-4	350	100	710
3-3-1	21	6.8	40	5-4-5	430	150	1100
3-3-2	24	9.8	70	5-5-0	240	70	710
3-4-0	21	6.8	40	5-5-1	350	100	1100
3-4-1	24	9.8	70	5-5-2	540	150	1700
3-5-0	25	9.8	70	5-5-3	920	220	2600
4-0-0	13	4.1	35	5-5-4	1600	400	4600
4-0-1	17	5.9	36	5-5-5	>1600	700	—
4-0-2	21	6.8	40				

Schematic outline of presumptive and confirmed phases for total coliform detection.

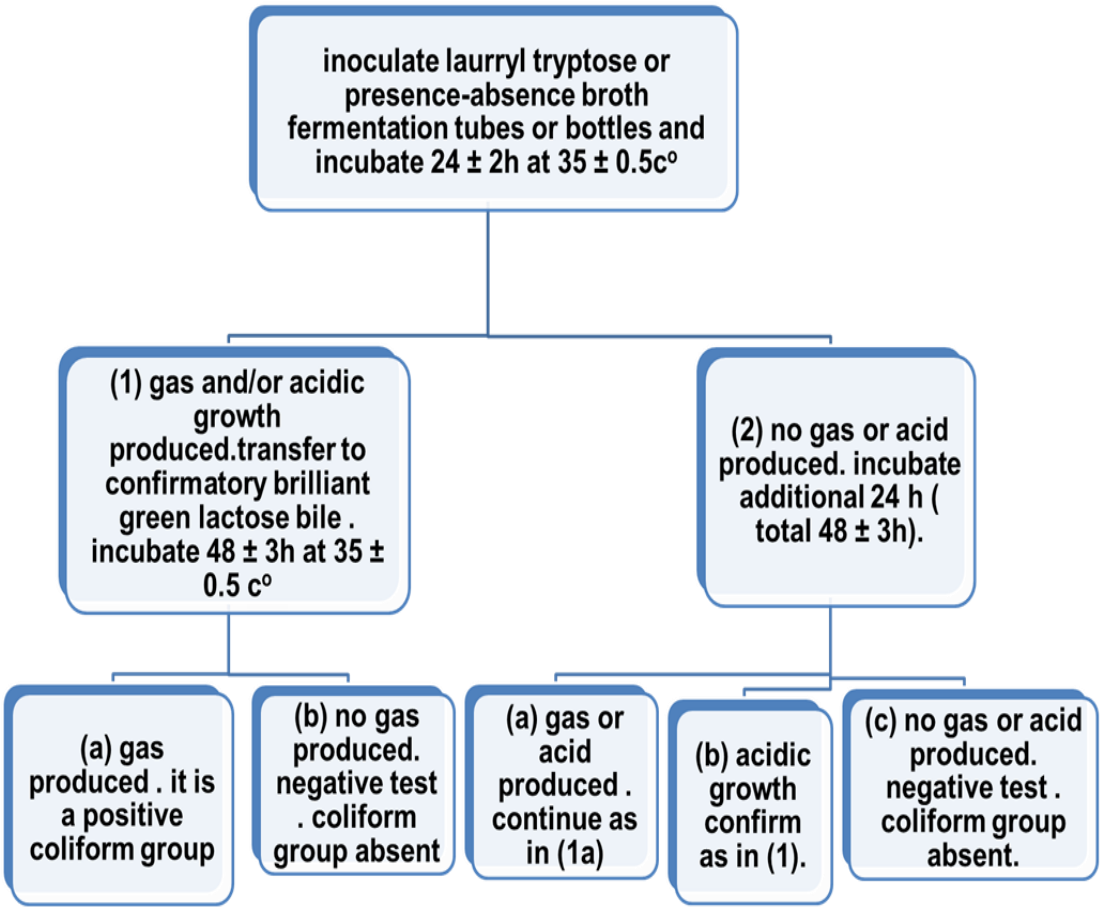


TABLE 9221:V. EXAMPLES FOR CHOICE OF THREE COMBINATIONS OF POSITIVES FROM FIVE DILUTIONS

Example	Volume mL 10	1	0.1	0.01	0.001	Combination of Positives	MPN Index No./100 mL
A	5	5	1	0	0	x-5-1-0-x	330
B	4	5	1	0	0	4-5-1-x-x	48
C	5	2	5	2	1	x-x-5-2-1	7000
D	4	5	4	5	1	x-x-4-5-1	4800
E	5	4	4	0	1	x-4-4-1-x	400
F	4	3	0	1	1	4-3-2-x-x	39
G	4	3	3	2	1	x-x-3-2-1	1700

Note: if gas or acid growth occurs before maximum incubation time (ex. 6 ±1 h), transfer to next appropriate medium.

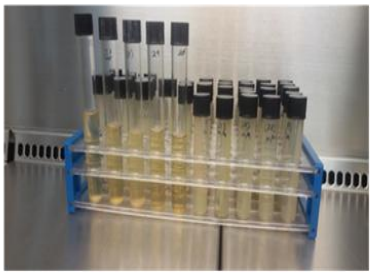
2- Fecal Coliform
Membrane Filter Procedure
Introduction

The tests required detecting specific pathogens in water or wastewater are very difficult and expensive so it is impractical for sewage utilities to routinely test for specific types of organisms or pathogens. A more practical approach is to examine the wastewater for indicator organisms associated with contamination. The coliform group of bacteria meets all of the criteria for an ideal indicator. These bacteria are generally not pathogenic, yet they are usually present when pathogens are present. As a rule, where coliforms are found in water or sludge it is assumed that pathogens may also be present, and if coliforms are absent, the water or sludge is assumed to be safe. Total coliform can be used as a general measure of the quality of the sludge as well as evaluating the efficiency of various stabilization processes.

Test methods

Fecal coliform bacterial densities may be determined either by the multiple-tube procedure or by the Membrane Filter (MF) technique. The fecal coliform MF procedure uses an enriched lactose medium and incubation temperature of 44.5 ± 0.2 °C for selectivity. Because incubation temperature is critical, submerge waterproofed (plastic bag enclosures) MF cultures in a water bath for incubation at the elevated temperature or use an appropriate solid heat sink incubator or other incubator that is documented to hold the 44.5 °C temperature within 0.2 °C throughout the chamber, over a 24 h period.

Apparatus



Fermentation tubes with an inverted vial.

Autoclave

Laminar-Flow Hoods/Biological Safety Cabinets

Laboratory Apparatus

For MF analyses use glassware and other apparatus composed of material free from agents that may affect bacterial growth.

a. Sample bottles*:*

For bacteriological samples, use sterilizable bottles of glass or plastic of any suitable size and shape. Use bottles capable of holding a sufficient volume of sample for all required tests and an adequate air space, permitting proper washing, and maintaining samples uncontaminated until examinations are completed. Ground-glass-stoppered bottles, preferably wide mouthed and of resistant glass, are recommended. Plastic bottles of suitable size, wide mouthed, and made of nontoxic materials such as polypropylene that can be sterilized repeatedly are satisfactory as sample containers. Presterilized plastic bags, with or without dechlorinating agent, are available commercially and may be used. Plastic containers eliminate the possibility of breakage during shipment and reduce shipping weight.

b. Dilution bottles:

Use bottles or tubes of resistant glass, preferably borosilicate glass, closed with glass stoppers or screw caps equipped with liners that do not produce toxic or bacteriostatic compounds on sterilization. Do not use cotton plugs as closures. Mark graduation levels indelibly on side of dilution bottle or tube. Plastic bottles of nontoxic material and acceptable size may be substituted for glass provided that they can be sterilized properly.

c Pipets and graduated cylinders:

Before sterilization, loosely cover opening of graduated cylinders with metal foil or a suitable heavy wrapping-paper substitute. Immediately after sterilization secure cover to prevent contamination.

d. Incubator:

The specificity of the fecal coliform test is related directly to the incubation temperature. Static air incubation may be a problem in some types of incubators because of potential heat layering within the chamber, slower heat transfer from air to the medium, and the slow recovery of temperature each time the incubator is opened during daily operations. To meet the need for greater temperature control uses a water bath, a heat-sink incubator, or a properly designed and constructed incubator shown to give equivalent results. A temperature tolerance of 44.5 ± 0.2 °C can be obtained with most types of water baths that also are equipped with a gable top for the reduction of water and heat losses.

e. Containers for culture medium:

Use clean borosilicate glass flasks. Any size or shape of flask may be used, but erlenmeyer flasks with metal caps, metal foil covers, or screw caps provide for adequate mixing of the medium contained and are convenient for storage.

f. Filtration units:

The filter-holding assembly (constructed of glass, autoclavable plastic, porcelain, or stainless steel) consists of a seamless funnel fastened to a base by a locking device or by magnetic force. The design should permit the membrane filter to be held securely on the porous plate of the receptacle without mechanical damage and allow all fluid to pass through the membrane during filtration. Discard plastic funnels with deep scratches on inner surface or glass funnels with chipped surfaces.

Wrap the assembly (as whole or separate parts) in heavy wrapping paper or aluminum foil, sterilize by autoclaving, and store until use. Alternatively expose all surfaces of the previously cleaned assembly to ultraviolet radiation (2 mm exposure) for the initial sanitization before use in the test procedure, or before reusing units between successive filtration series. Field units may be sanitized by dipping or spraying with alcohol and then igniting or immersing in boiling water for 2 mm.

After submerging unit in boiling water, cool it to room temperature before reuse. Do not ignite plastic parts. Sterile, disposable field units may be used.

b. Materials and Culture Medium

A. M-FC medium:

The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration the commercially prepared media (sterile ampoules) or powder.

Rehydrate product in 1 L water containing 10 ml (1%) rosolic acid in 0.2 N NaOH. Heat to near the boiling point, promptly remove from heat and cool to below 50 °C. Do not sterilize by autoclaving. Final pH should be 7.4 ± 0.2. Refrigerate finished medium, preferably in sealed plastic bags or other containers to reduce moisture loss, and discard unused broth after 96 h or unused agar after 2 weeks.

B. Buffered dilution water*:*

Prepare a stock solution by dissolving 34 g of KH_2PO_4 in 500 ml distilled water, adjusting the pH to 7.2 with 1 N NaOH and dilute to one liter. Prepare dilution water by adding 1.25 ml of the stock phosphate buffer solution and 5.0 ml magnesium sulfate (50 grams $MgSO_4$ $7H_2O$ dissolved in one liter of water) to 1 liter distilled water. This solution can be dispersed into various size dilution blanks or used as a sterile rinse for the membrane filter test.

Procedure

a Selection of sample size:

Select a volume of the wastewater sample to be examined about 50-100 ml. Use sample volume and dilution that will yield counts between 20 and 60 fecal coliform colonies per membrane.

When the bacterial density of the sample is unknown, filter several volumes or dilutions to achieve a countable density. Estimate volume and/or dilution expected to yield a countable membrane and select two additional quantities representing one-tenth and ten times this volume, respectively.

Filtration of sample:

Using sterile forceps, place a sterile membrane filter (grid side up) over porous plate of receptacle. Carefully place matched funnel unit over receptacle and lock it in place. Filter sample under partial vacuum. With filter still in place, rinse the interior surface of the funnel by filtering three 20- to 30-ml portions of sterile dilution water. Alternatively, rinse funnel by a flow of sterile dilution water from a squeeze bottle. This is satisfactory only if the squeeze bottle and its contents do not become contaminated during use. Rinsing between samples prevents carryover contamination. Upon completion of final rinse and the filtration process disengage vacuum, unlock and remove funnel, immediately remove membrane filter with sterile forceps, and place a pad in the culture dish and saturate with at least 2.0 ml MFC medium and carefully remove excess medium by decanting the plate. Place prepared filter directly on pad, invert dish, and incubate for 22 to 24 h at 44 ± 0.5 °C.

Differentiation of some colonies from liquid medium substrates may be lost if cultures are incubated beyond 24 h.

Insert a sterile rinse water sample (100 ml) after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water.

Incubation:

Place prepared dishes in waterproof plastic bags or seal, invert, and submerge petri dishes in water bath, and incubate for 24 ± 2 h at 44.5 ± 0.2 °C. Anchor dishes below water surface to maintain critical temperature requirements. Place all prepared cultures in the water bath within 30 min after filtration.

Counting:

Colonies produced by fecal coliform bacteria on MFC medium are various shades of blue. Non-fecal coliform colonies are gray to cream colored. Normally, few non-fecal coliform colonies will be observed on MFC medium because of selective action of the elevated temperature and addition of rosolic acid salt reagent. Count colonies with a low-power (x10 to x15 magnifications) binocular wide-field dissecting microscope or other optical device.

Calculation of Fecal Coliform Density

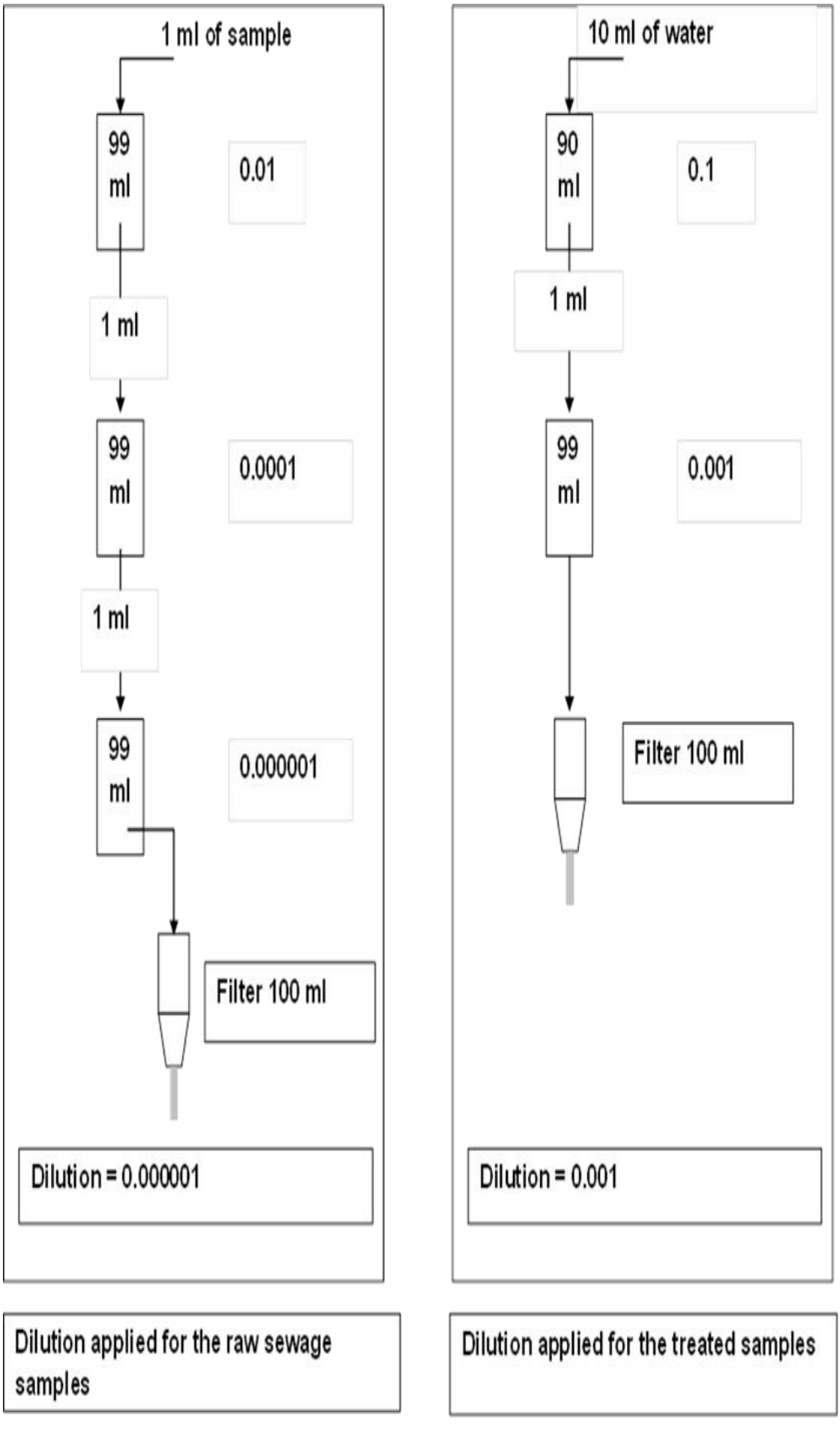
a. General: Compute the density from the sample quantities that produced MF counts within the desired range of 20 to 60 fecal coliform colonies. This colony density range is more restrictive than the 20 to 80 total coliform ranges because of larger colony size on MFC medium. Calculate fecal coliform density and record densities as fecal coliforms per 100 ml.

$$\text{F. C Bacteria}/100\text{ ml} = \frac{\text{No. of Colonies Counted} \times 100}{\text{sample volume filtered, ml in 100 ml}}$$

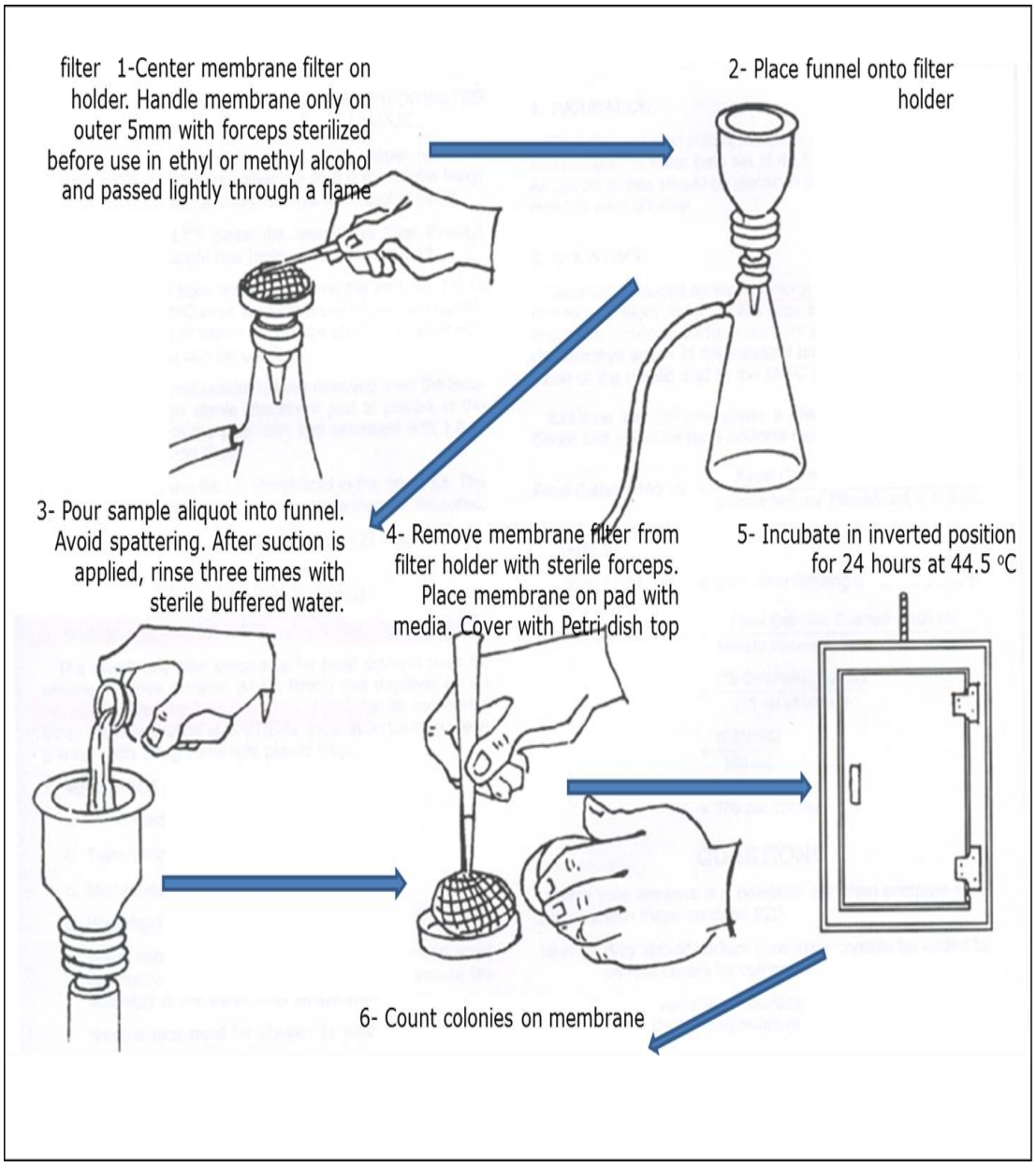
Suggested samples volumes for membrane filter fecal coliform test

	Volume (X) To Be Filtered ml							
Water Source	100	50	10	1	0.1	0.01	0.001	0.0001
Lakes, reservoirs	X	X						
Wells, springs	X	X						
Water supply intake		X	X	X				
Natural bathing waters		X	X	X				
Sewage treatment plant			X	X	X			
Farm ponds, rivers				X	X	X		
Stormwater runoff				X	X	X		
Raw municipal sewage					X	X	X	
Feedlot runoff					X	X	X	
Sewage sludge						X	X	X

Dilution scheme



Outline of Procedure for inoculation of Membrane Filter



Calculation

$$\text{Bacteria}/100\text{ ml} = \frac{\text{No. of Colonies Counted} \times 100\text{ ml}}{\text{sample volume filtered, ml in 100 ml}}$$

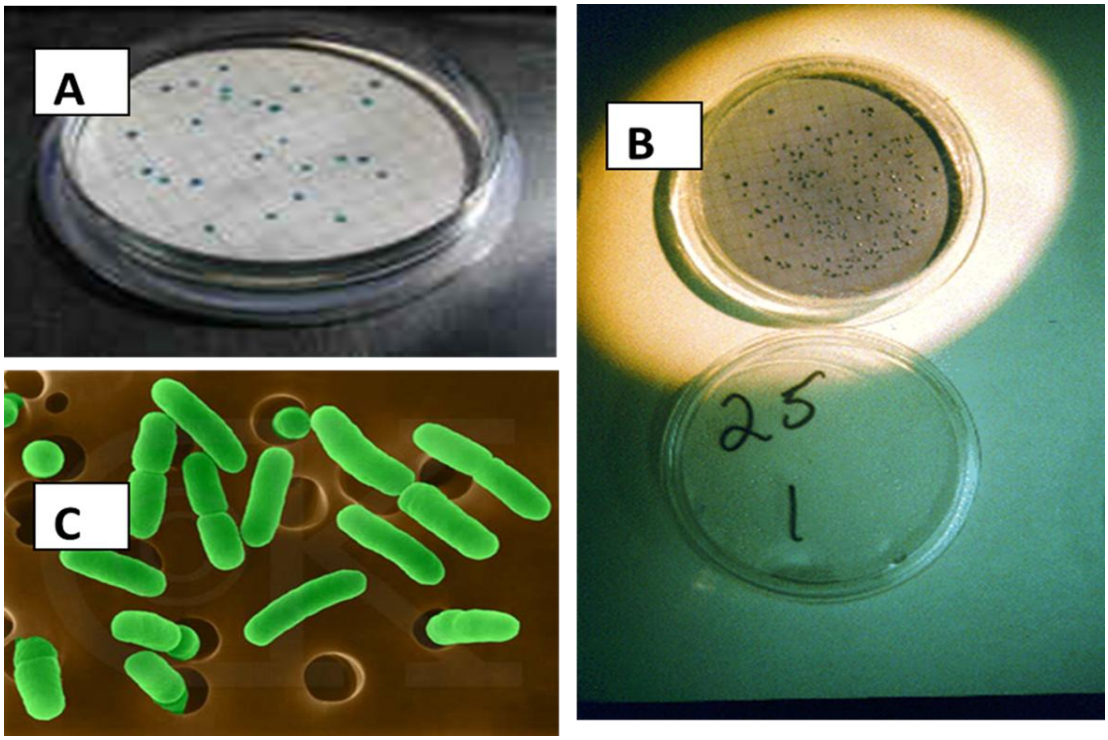
Example:

A total of 42 colonies grew after filtering 10 ml sample

$$= \frac{(42\text{ Colonies})(100\text{ ml})}{10\text{ ml}}$$

= 420 /100 ml

Examples of fecal coliform plates

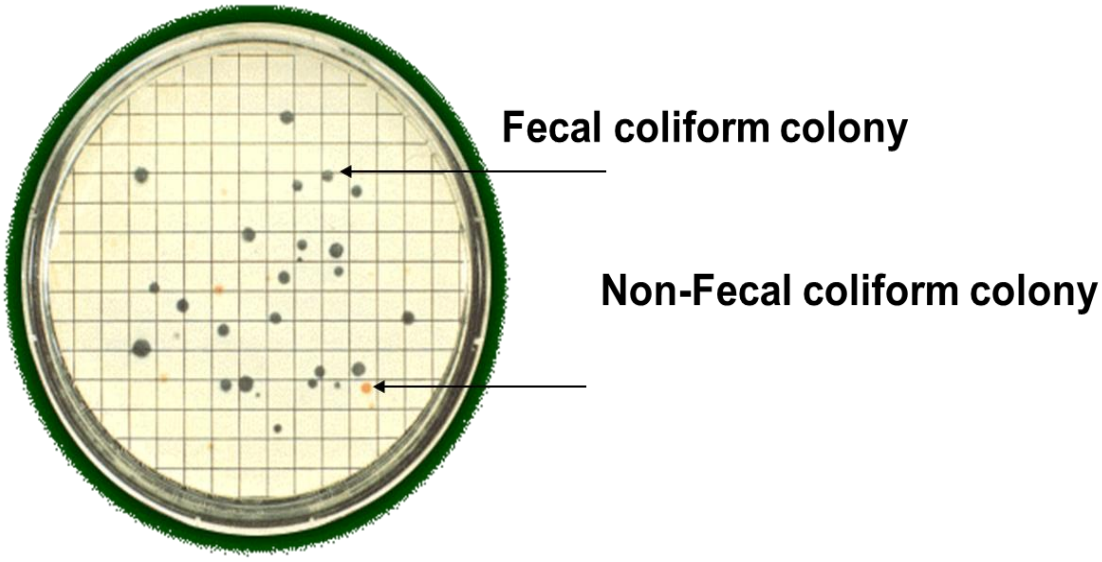


A – plate shows the blue colonies of fecal coliforms growing on MFC media. The number of colonies is perfect for counting. The sample portion and dilution used is correct.

B – plate shows the blue colonies of fecal coliforms growing on MFC media. The number of colonies is too much for counting. The sample portion and dilution used is incorrect.

C – Fecal coliform bacteria under electronic microscope.

Example of fecal coliform colonies (blue) and nonfecal coliform colonies (Gray, pale browen or creamy) do not count this colonies as fecal coliform.



3- Ascaris Examination

Introduction:

The Ascaris lumbricoides, commonsaying “round worm of man”, is the largest of the intestinal nematodes parasitizing humans. It is the most common worm found in human.

It is worldwide in distribution and most prevalent throughout the tropics, sub-tropics and more prevalent in the countryside than in the city.

The incidence is over 1500 million infections annually. Of these cases, about 210 million are symptomatic. In some rural settings with poor sanitation, perhaps half the children of 2-12 years have ascariasis.Then many of them will also have trichuriasis and various of other chronic illnesses.

Morphology

Adult: The adults are cylindrical in shape, cream white or pinkish in color.

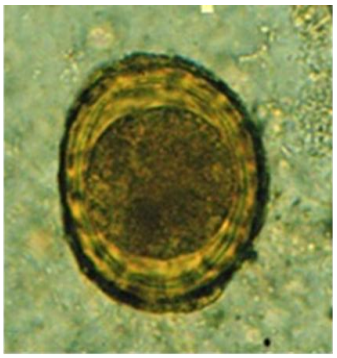
- The female averages 20-35cm in length, the largest 49 cm.
- The male is smaller, averaging 15-31 cm in length and distinctly more slender than the female.
- The typical curled tail with a pair sickle like copulatory spines. On the tip of the head there are three lips, arranged as a Chinese word.
- They have a complete digestive tract.
- Reproductive organs are tubular. Male has a single reproductive tubule. The female has two reproductive tubules and the vulva is ventrally located at the posterior part of the anterior 1/3 of the body.

Egg: There are three kinds of the eggs. They are fertilized eggs, unfertilized eggs and decorticated eggs. We usually describe an egg in 5 aspects: size, color, shape, shell and content.

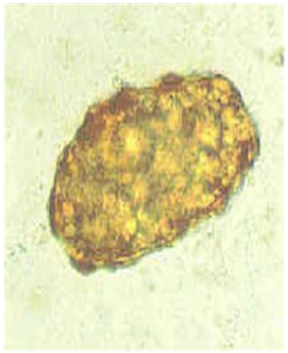
1. Fertilized eggs: broad oval in shape, brown in color, an average size 60× 45 µm. The shell is thicker and consists of ascaroside, chitinous layer, fertilizing membrane and mammillated albuminous coat stained brown by bile. The content is a fertilized ovum. There is a new moon (crescent) shaped clear space at the each end inside the shell as in shape (1).

2.Unfertilized egg: Longer and slender than a fertilized egg. The chitinous layer and albuminous coat are thinner than those of the fertilized eggs without ascaroside and fertilizing membrane. The content is made of many refractable granules various in size as in shape (2).

3-Decorticated eggs: Both fertilized and unfertilized eggs sometimes may lack their outer albuminous coats and are colorless as in shape (3).

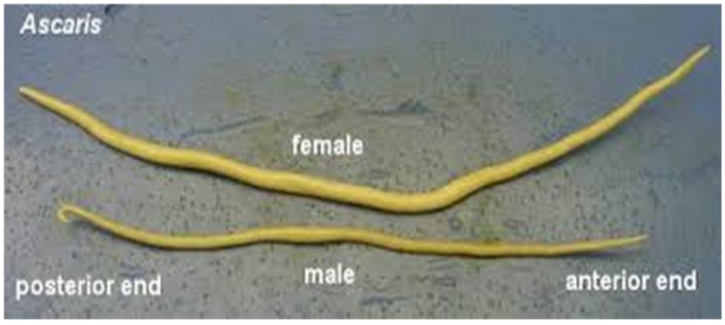


Fertilized eggs



Unfertilized egg

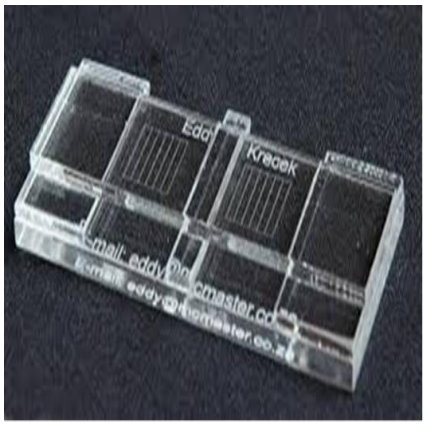
Decorticated eggs



Ascaris

B-Sample Collection, Preservation, and Storage:

For ascaris examination, collect a sample of wastewater of known volume (V litres), usually 1 litre for raw or partially treated wastewaters and 10 litres for final treated effluents.



McMaster counting slides

plastic containers

Measuring cylinder

-Membrane filtration equipment.

centrifuge capable of generating 1000g

C- Requirements:

Equipment (Apparatus):

a. plastic containers for sample collection
b. centrifuge capable of generating 1000 g
c. centrifuge tubes with lids
d. pasteur pipetts and teats
e. McMaster counting slides
f. Vortex mixer (not absolutely essential).
g. A siphon
h. Measuring cylinder or grduated pipette

Reagents:

The reagents required are the following:

a. zinc sulfate solution (33 %, relative density 1.18)
b. ether or ethyl acetate
c. acetoacetic buffer (pH = 4.5)(15 g sodium acetate trihydrate, 3.6 ml glacial acetic acid, made up to 1 litre with distilled water)
d. detergent solution (1 ml triton x-100 or tween 80, made up to 1 litre with tap water)

D- Procedure:

1. Collect a sample of wastewater of known volume (V liters), usually 1 liter for raw or partially treated wastewaters and 10 liters for final treated effluents.

2. Allow the sample to sediment for 1-2 hours, depending on the size of the container. It is recommended that an open topped, straight sided container should be used for sedimentation, since this makes removal of the supernatant easier and permits thorough rinsing of the container.

3. Remove 90% of the supernatant using a suction pump or siphon.

4. Carefully transfer the sediment to one or more centrifuge tubes, depending on the volume, and centrifuge at 1000 g for 15 min. Remember to rinse the container well with detergent solution, and add the rinsing to the sediment.

5. Remove the supernatant. If more than one centrifuge tube has been used in step 4, transfer all the sediments to one tube (remember to rinse thoroughly with detergent solution to ensure that no sediment is discarded), and recentrifuge at 1000 g for 15 min.

6. Suspend the pellet in an equal volume of acetoacetic buffer, pH 4.5 (if the volume of the pellet is 2 ml, add 2 ml of buffer). If the pellet is less than 2 ml, add buffer up to 4 ml to ensure that, after extraction with ethyl acetate (steps 7 and 8), there is sufficient volume of buffer above the pellet to allow the ethyl acetate layer to be poured off without resuspension of the pellet.

7. Add two volumes of ethyl acetate or ether (i.e. 4 ml in the above example) and mix the solution thoroughly in a vortex mixer. The

sample can also be shaken by hand. This is quite acceptable if a mechanical mixer is not available.

8. Centrifuge the sample at 1000 g for 15 min. The sample will now have separated into three distinct phases (layer).

All the nonfatty, heavier debris, including helminthes eggs, larvae and protozoa, will be in the bottom layer.

Above this will be the buffer, which should be clear.

The fatty and other material moves into the ethyl acetate or ether and forms a thick dark plug at the top of the sample.

9. Record the volume of the pellet containing the eggs, and then pour off the rest of the supernatant in one smooth action. It may be necessary to loosen the fatty plug first by running a fine needle around the side of the centrifuge tube.

10. Resuspend the pellet in five volumes of zinc sulfate solution, (i.e. if the volume of the pellet is 1 ml, add 5 ml of $ZnSO_4$). Record the volume of the final product (X ml).

Mix the sample thoroughly, preferably using a vortex mixer. Note that a minimum of 1.5 ml is required to fill a two chambered McMaster slide.

11. Quickly remove an aliquot with a Pasteur pipette and transfer to a McMaster slide for final examination.

12. Leave the full McMaster slide to stand on a flat surface for 5 min before examination. This allows all the eggs to float to the surface.

13. Place the McMaster slide on the microscope stage and examine less than 10× or 40× magnification. Count all the eggs seen within the grid in both chambers of the McMaster slide.

For greater accuracy, the mean of two slides, or preferably three, should be recorded.

E- Calculation:

Calculate the number of eggs per litre from the equation:

$$N = \frac{AX}{PV}$$

Where:

N = number of eggs per litre of sample

A = number of eggs counted in the McMaster slide or the mean of counts from two or three slides

X = volume of the final product (ml)

P = volume of the McMaster slide (0.3 ml)

V = original sample volume (litres)

Note:

Mix the final flotation suspension thoroughly, preferably in a vortex mixer to ensure homogeneous mix. Quickly fill a Pasteur pipette and carefully run the solution into one compartment of the McMaster slide. Fill the whole compartment completely even though it is only the section under the grid that is to be counted. Work quickly and smoothly at this stage so that eggs do not start to float in the test-tube or in the pipette. Ensure that there are no air bubbles under the grid.

f- Use of centrifuges:

Most published methods that involve the use of centrifuge quote centrifuge speed in terms of relative centrifuge force, however, in some papers; speed is expressed in revolutions per minute (rpm). To convert rpm to force, the following formula is used:

$$\mathbf{RCF} = \frac{\mathbf{r}^{\mathbf{2}}(\mathbf{rpm})}{K}$$

Where

RCF= relative centrifuge force (g).

r = radius of the centrifuge from the spindle to the center of the bucket (cm).

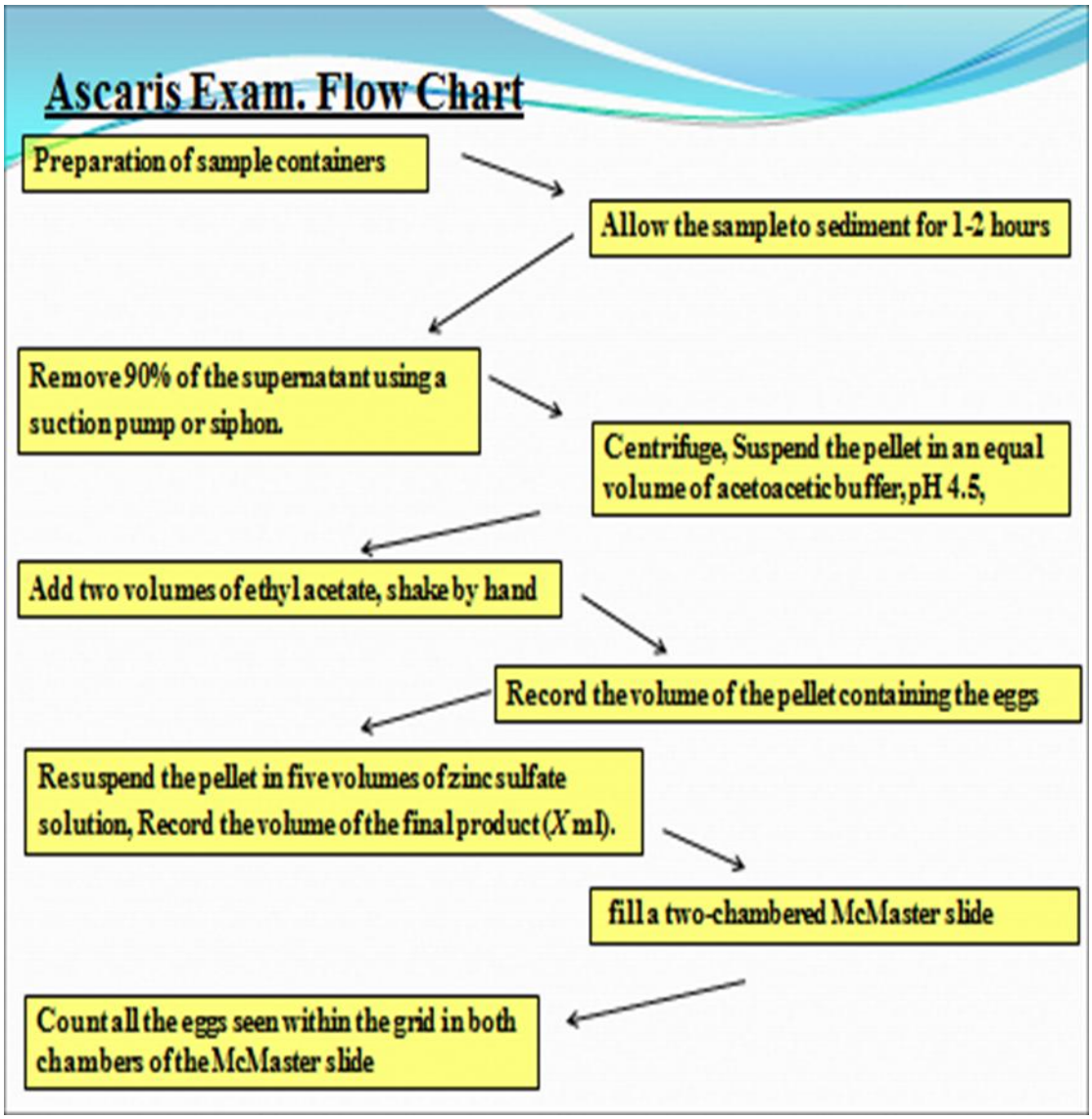
k= 89456

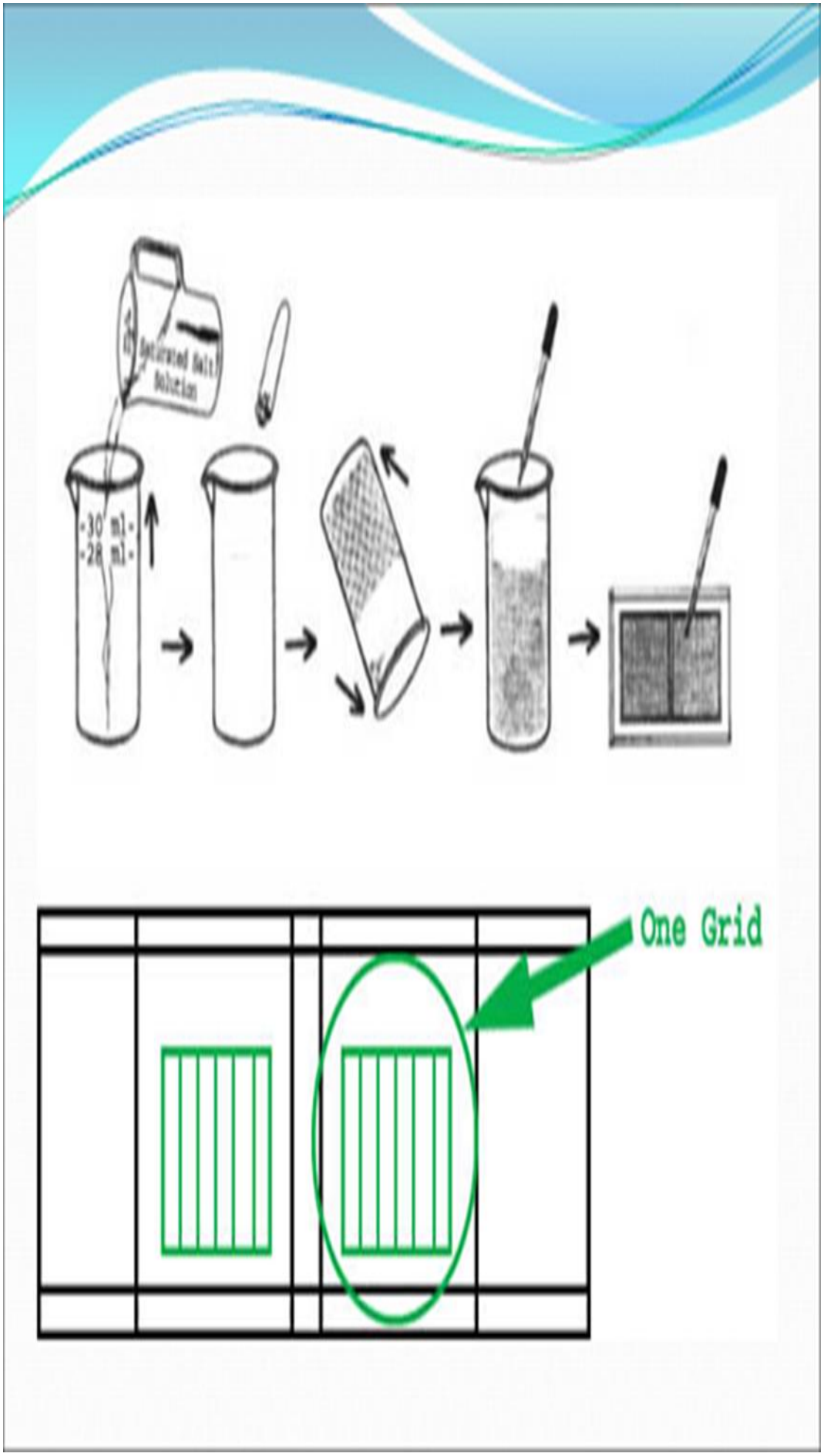
Unit: The result is recorded as number of eggs / liter

Reference:

1- (According to "world health organization, geneva ,1996")

2- "Analysis of wastewater for use in agriculture–A laboratory manual of parasitological and bacteriological techniques", Rachel M.Ayres & D.Duncan Mara,Department of civil engineering , universitey of leeds, leeds ,England




30 ml
28 ml
One Grid

4-Nematological Examination

Introduction

Nematodes are unsegmented worms with elongated, cylindrical bodies; they are present worldwide in fresh, brackish, and salt waters, and in soil.

A freshwater nematode has been defined as “any nematode species inhabiting either fresh water or non-brackish swampy soil below the water table; hence a species that will not drown in fresh water; a species fitted to utilize oxygen dissolved in fresh water.”1 Nematodes are a food source for other invertebrates, small fish, and fungi, and play a fundamental role in cycling carbon and nitrogen through the benthic ecosystem. Predaceous nematodes from the Aporcelaimidae, Diplogasteridae, Dorylaimidae, and Mononchidae families abound in fresh water, devouring other nematodes, oligochaetes, and other small invertebrates.

Their role in ingesting algae and diatoms is less clear; however, algae in the gut can occasionally cause dorylaimids to turn bright amber, yellow, or green.

In recent years, examinations of nematode communities in soil or aquatic sediments have been increasingly used to study many aspects of environmental health. The maturity index (MI) originally was developed to measure the ecological succession status of a terrestrial nematode community; the concept was later extended to marine, estuarine, and then freshwater nematodes. The role of nematodes in wastewater treatment systems is also increasingly being investigated.

Bactivorous freshwater nematodes can ingest human enteric pathogens, which then can survive chlorination inside nematode bodies.

These nematodes often appear in large numbers in secondary wastewater effluents and have been used as bioindicators of water quality.

Personal Responsible

Title	Chemist
Responsibilities	Responsible to measure Nematodes in efflue

Samples

Principal samples are tap or well water; free flowing or standing water without bottom sediment; bottom sediment; and aquatic plants and coarse detritus, such as stones, twigs, or leaves.

a. Sample collection:

1) Tap water Place a 20 cm, 325 mesh (45-m pore size) sieve at a 45° angle under the discharge. Adjust water flow to a moderately slow rate, with no splashing, striking upper one-third of sieve. Run for 4 h.

2) Free-flowing or standing water. Take samples from sites where bottom sediments are absent or too deep to be collected.

Collect five subsamples as follows: Hold 20 cm, 325 mesh sieves firmly at a 45° angle. Dip 3- to 4 L stainless steel pitcher in water and fill to 1 L mark. Pour contents slowly through top one-third of sieve. Repeat three more times. Collect additional 1 L to wash and concentrate detritus on sieve surface from top to bottom of sieve.

3) Bottom sediment sample. If bottom to surface depth is less than 20 cm, stir bottom with hand garden rake. Scoop up stirred sediments in stainless steel pitcher. Add water to pitcher to within 5 cm of top. Stir,then wait 30 s. Pour contents of pitcher onto an 18 mesh sieve (1-mm pore size) nested in a 325 mesh sieve (45 µm pore size) with the surface held at 45° angle until dense detritus reaches pitcher lip. (Usually about 9/10 of the pitcher is poured off) If bottom-to-surface depth is 20 to 30 cm, collect duplicate samples by holding a 325 mesh sieve (45 µm pore size) at 90° angle near the bottom. Using hand rake, stir bottom sediments so they roil up in a dense cloud in front of sieve. Let cloud settle about 10 s, then move sieve into cloud about 2.5 to 5 cm above the bottom. Bring sieve out of water while holding it at a 45° angle.

4) Aquatic plants, plant or inorganic debris. Randomly collect one species of live floating or submerged plants from target site and place in 1 L jars filled with collection site water.

Do not fill more than half of jar with plant material. If several plant species are present, take two or more samples. Place plant and inorganic debris (sticks, leaves, pebbles, etc.) in 1 L jar to about half its volume.

b. Sample concentration:

Concentrate detritus on sieve into approximately one quarter of sieve surface by washing tap water across tilted sieve face (45° angle) from top to bottom. Place tilted sieve on lip of a clean, empty 250 ml beaker and wash detritus into beaker by turning sieve upside down and flushing tap water from another beaker or wash bottle through the back side of the sieve thereby flushing material into the jar.

c. Sample transport and storage:

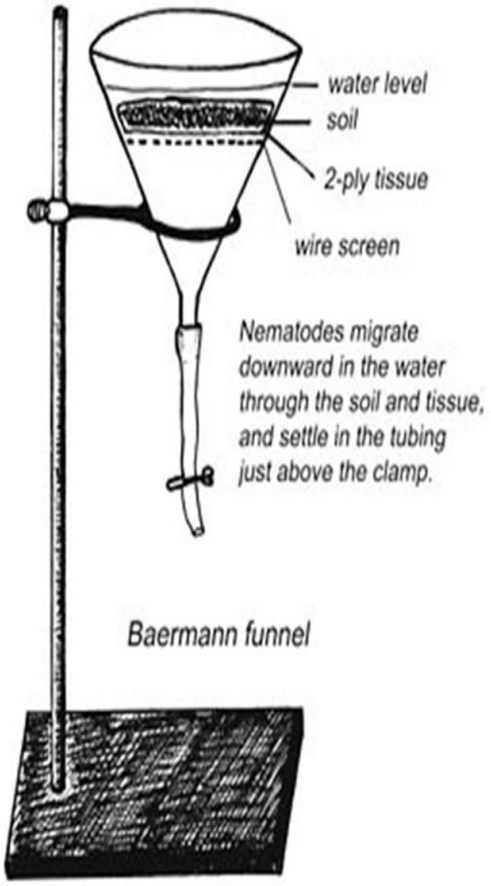
Regardless of collection mode, keep sample jars cool. On very hot days, use ice to cool them. Taxonomic determination is most accurate when nematodes are live and healthy. Because nematode mortality, deterioration, and obfuscation of diagnostic characters begins at time of collection, process samples for diagnosis within 24 h and complete diagnostic processing within 48 h.

Cold storage retards, but does not halt, deterioration and rot. Plan survey so samples can be processed on the same day they are collected. In an emergency, preserve entire sample indefinitely in 4% formalin (never use alcohol). Add equal volume of 8% formalin solution to sample. If sample jar is more than half full, decant excess water after at least 40 min of settling. Preserved specimens will shrink to some degree, and body pores and lumens may become obscure.

4. Sample Processing

For a current review of sample-processing techniques, see Hodda and Eyualem-Abebe.

disposable pipet



Baermann funnel

a. Specialized apparatus:

1) Custom pipet, for clean-water samples. Take a 29 cm long disposable pipet and place a piece of 12 cm long rubber tube snugly over about 3 cm of the conical pickup end. Add a wire buret clamp on the rubber tube. (Clean by removing clamp and flushing with a syringe).

2) Baermann funnels, for samples containing debris. Use a glass funnel with a 15.5-cm top opening and 1.5 cm tube. Fit a rubber tube to the exit tube and close with a buret wire clamp.

Place an 8 to 10 cm diamter, coarse screen (3 mm pores) wire disk in the funnel opening. Add tap water until it lies just above the wire disk. Insert a facial tissue over the disk.

4. Procedure:

1) Clear or relatively clear water. Shake to obtain homogenous mix, then pour slowly onto the surface of a 7.6 cm, 325 mesh sieve (45 µm pore size).Concentrate detritus on sieve into approximately one-quarter of sieve surface by washing tap water across tilted sieve face (45° angle) from top to bottom. Place tilted sieve on lip of a clean, empty 250 ml beaker and wash detritus into beaker by turning sieve upside down and flushing tap water from another beaker or wash bottle through the back side of the sieve thereby flushing material into the jar. Pour concentrated residues into 50-mL conical bottom centrifuge tube or tubes. Let nematodes settle for 40 min. insert a custom pipet, with rubber tube closed by finger pressure, to tube bottom. Depress rubber tube to take up the ball of nematodes on bottom of cone. Discharge about 0.05 ml (small drop) of pipet contents onto a microscope slide.

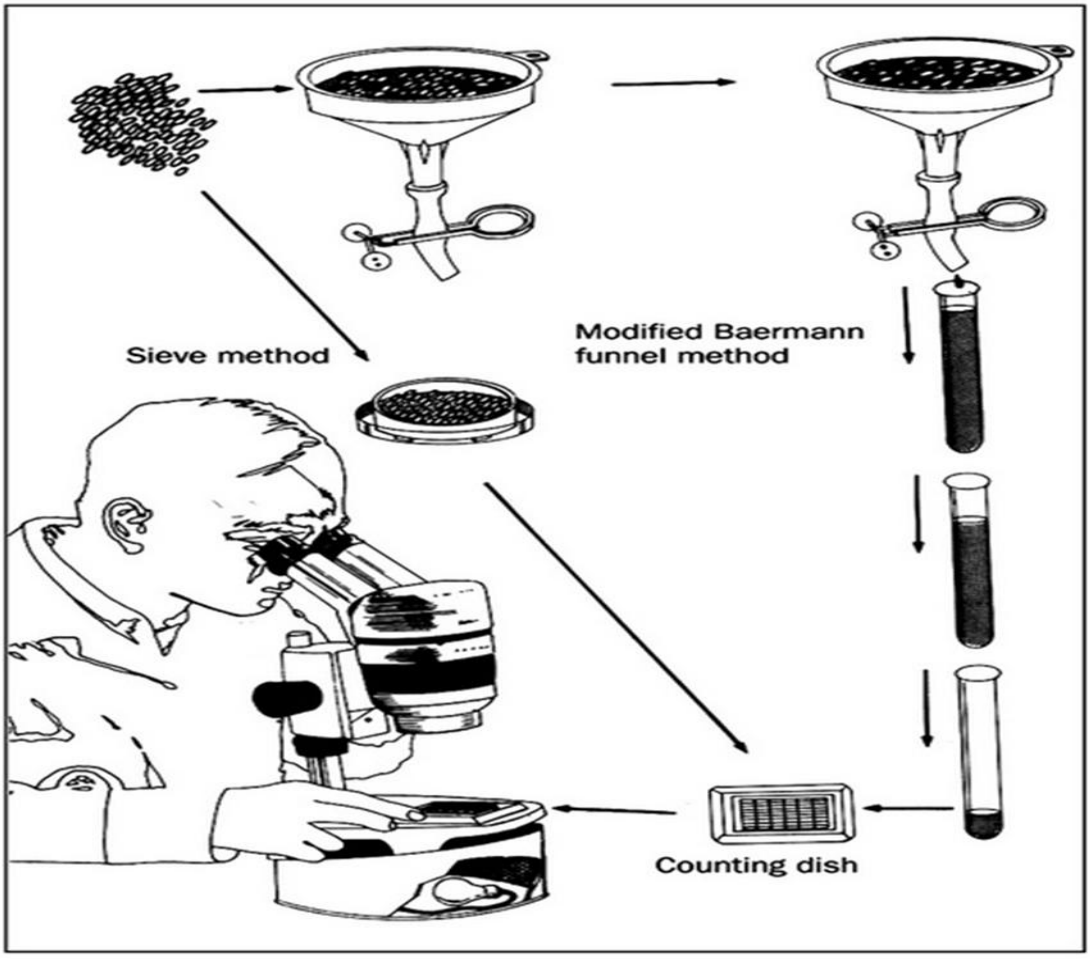
Cover drop with a 22 mm cover slip. Use a compound microscope to diagnose nematodes.

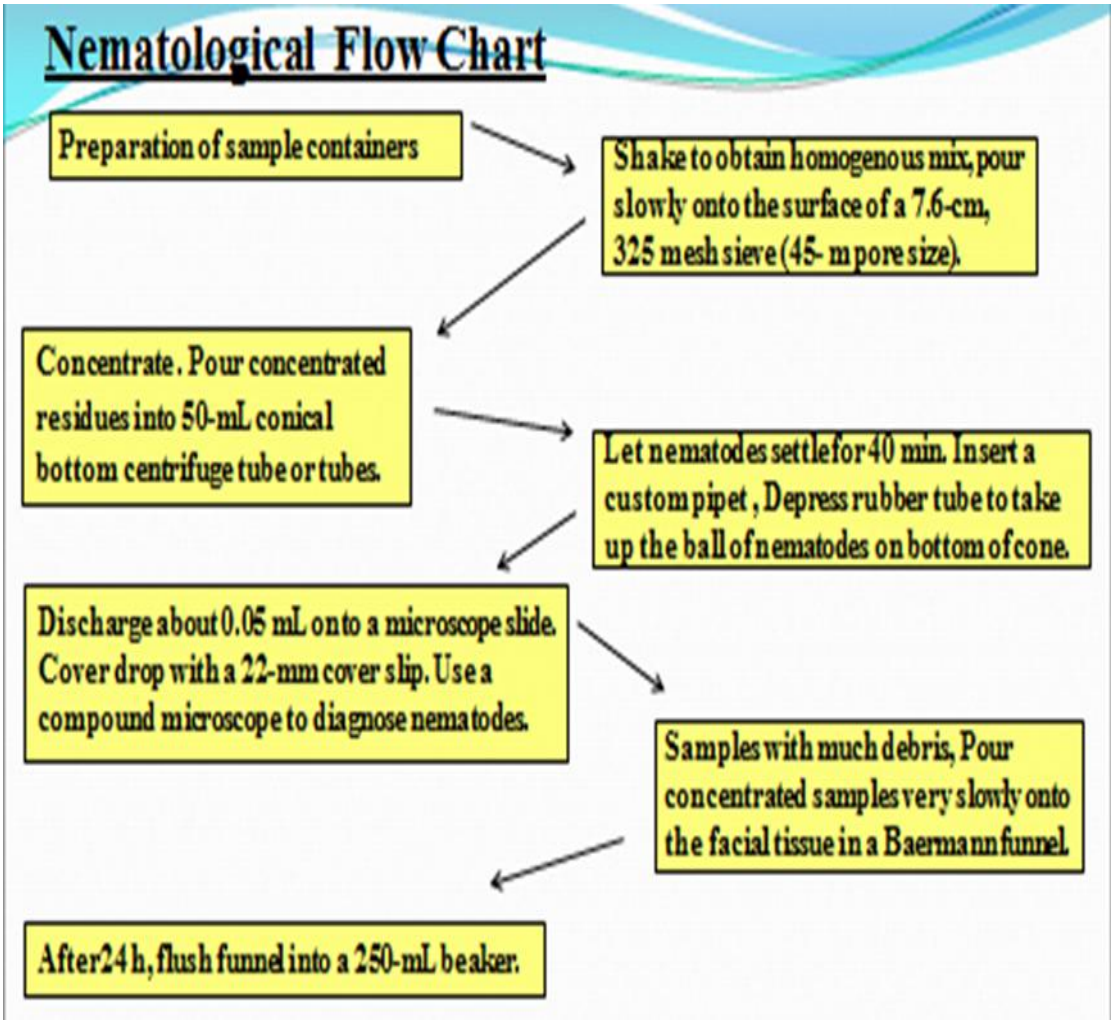
2) Samples with much debris pour concentrated samples very slowly onto the facial tissue in a Baermann funnel.

After 24 h, flush funnel into a 250 ml beaker. (Process as above)

3) Samples containing live plants, plant debris, or inorganic material process samples immediately on return to laboratory. Shake vigorously and pour contents into beaker.

Concentrate detritus on sieve into approximately one quarter of sieve surface by washing tap water across tilted sieve face (45° angle) from top to bottom. Place tilted sieve on lip of a clean, empty 250 ml beaker and wash detritus into beaker by turning sieve upside down and flushing tap water from another beaker or wash bottle through the back side of the sieve thereby flushing material into the jar and, depending on sample clarity, proceed according to ¶above. If the laboratory is equipped to process samples with an excess of debris using the centrifugal flotation technique, 2 preferably use this technique.


Sieve method
Modified Baermann funnel method
Counting dish


Nematological Flow Chart
Preparation of sample containers
Shake to obtain homogenous mix, pour slowly onto the surface of a 7.6-cm, 325 mesh sieve (45- m pore size).
Concentrate. Pour concentrated residues into 50-mL conical bottom centrifuge tube or tubes.
Let nematodes settle for 40 min. Insert a custom pipet, Depress rubber tube to take up the ball of nematodes on bottom of cone.
Discharge about 0.05 mL onto a microscope slide. Cover drop with a 22-mm cover slip. Use a compound microscope to diagnose nematodes.
Samples with much debris, Pour concentrated samples very slowly onto the facial tissue in a Baermann funnel.
After 24 h, flush funnel into a 250-mL beaker.

5-Plate count
Introduction

This method is taken from Standard Methods for the Examination of water and wastewater edition 19:1995" Heterotrophic Plate Count 9215. And Bacteriological Method in Water Quality Control Programs EPA 1980.

The standard plate count attempts to provide a standardized means of determining the density of aerobic and facultative anaerobic heterotrophic bacteria in water.

Bacteria occur singly or in pairs; chains, cluster or packet, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in water sample.

However, the heterotrophic plate count is a good measure of water treatment plant efficiency, after growth in transmission lines, and general bacterial composition of source water.

Three different methods and three different media are used:

1- Pour plate method.
2- Membrane filters method.
3- Spread plate method.

2 Scope

This procedure describes how to measure Heterotrophic Plate Count in chlorinated effluent

Personal Responsible

Title	Chemist
Responsibilities	Responsible to measure Heterotrophic Plate Count in chlorinated effluent.

4 Reagents

Hazards

Laboratory coats must be worn at all times.

Eye protection and a fume cupboard must be used when handling samples.

Water

Distillation should be carried out behind a safety screen.

Plate count agar (treptone glucose yeast agar):

Dissolve 23.5 g of treptone glucose yeast agar in 1 liter, pH should be 7.0 after autoclaving at 121 °C for 15 minutes, use for pour and spread plat method.

M-HPC agar:

Dissolve 70 g of M-HPC agar in 1 liter; pH should be 7.1 after autoclaving at 121 °C for 15 minutes, use for membrane filter method.

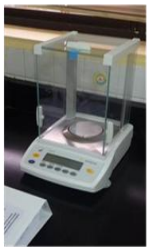
R2A agar:

Dissolve 18.2 g of R2A agar in 1 liter, pH should be 7.2 after autoclaving at 121 °C for 15 minutes, use for pour and spread plat method.

-Autoclave

-Refrigerator.

- pH meter.



-Balances.

-Colony Counter.

-Incubator.

-Membrane filtration equipment.

-Microscope

Apparatus

1 Hot- Air sterilizing oven
2 Autoclave
3 pH meter
4 Balances
5 Pipettes and graduated cylinders
6 Pipette containers
7 Refrigerator
8 Dilution bottles and tubes
9 Incubator
10 Petri dishes
11 Membrane filtration equipment
12 Sample bottles
13 Microscope
14 Colony Counter

Procedure

1 Pour Plate method for heterotrophic bacteria:

1.1 Preparation of sample containers

Wash hands thoroughly with soap and water. Car must be taken to prevent contamination when conducting bacterial tests. All material used for containing or transferring samples must be sterile either pre-sterilized plastic bags, or glass or plastic bottles may be used to collect samples.

1.2 Glass or plastic bottles (at least 125 ml) may be used instead of plastic bags, these containers should be prepared by wash it in hot water and detergent,throughly rise with hot tap water ,followed by a distilled water rinse to make sure that all detergent is removed If dechlorinating agent is need (for chlorinated potable water)add one decholrinating reagent powder pillow to each 125 ml sample container .Add two powder pillows to a 250 ml sample container Steam sterilize glass and autoclaveable plastic containers at 121 °C for 15 minutes, glass sample containers may be sterilized by hot air at 170 °C for one hour. Store sterile containers tightly capped in a clean environment until needed.

1.2 Samples collections and preservation

2.1 Collect at least 100 ml of sample in sterile glass or plastic sample bottle or in a pre-sterilized plastic bag.

2.2 Sample containers should not be filled completely.

2.3 Maintain at least 2-5 cm of air space to allow adequate space for mixing the sample prior to analysis.

2.4 Analyze as soon as possible after collection. The maximum time between collection and examination of samples should be 8 hours.

2.5 If time between collection and analysis will exceed 8hours, maintain the sample at or below 4 °C but don’t freeze.

2.6 Maximum time between collection and analysis should not exceed 24 h.

1.3 Sample dilution

3.1 The pour plate method requires use of 1 ml, 0.1 ml, 0.01 ml, or 0.001 ml of sample .because the two smaller volumes, 0.01 and 0.001 ml are difficult to measure and to work with, sample dilution must be prepared.

3.2 Sample dilutions are prepared by pipe 1 ml of undiluted sample into 99 ml of buffered dilution water .diluting the sample allow 1ml diluted sample to be used instead of 0.01 ml of undiluted sample, and 0.1 ml of diluted sample instead of 0.001 ml of undiluted sample .

1.4 Plating procedure:

4.1 Melt the sterile solid agar medium by placing a tube of plate count agar in a beaker of boiling water. Keep the melted media in a water bath between 44 and 46 °C until used. Pipette the appropriate amount of undiluted or diluted sample (1 ml or 0.1 ml) into the sterile Petri dish .prepare at least two plates for each different volume of undiluted or diluted sample used.

4.2 Pour at least 10 to 12 ml of liquefied medium (1/2 of the contents plate count agar tube) into the dish by gently lifting the cover just high enough to pour.

4.3 Mix the melted medium thoroughly with the sample in the Petri dish by rotating the first in one direction and then in the opposite direction, or by rotating and tilting.

4.4 Place the plates n a level surface and let them solidify this generally take 10 minutes.Invert the plates, place them in a plastic bag , and seal the bag . Place the bag in an incubator which has been pre warmed to 35 °C.

4.5 Incubate the plates for 48 ± 3 h ate 35+ or -0.5 °C.

4.6 Using a Quebec colony counter, count all colonies on the plates promptly after incubation.

1.5 Counting, computing and recording results

5.1 Count all colonies on selected plates promptly after incubation if counting must be delayed temporarily, store plate at 5 to 10 °C for no more thane 24 h, but avoid this as routine practice.

5.2 Quebec colony counters feature a built in grid to simplify counting, the easiest way to count colonies is to follow aback and forth pattern, moving down the grid.

5.3 Report all counts as colony forming units /ml include in the report of the method used, the incubation temperature and time, and medium.

5.4 Generally, results are obtained by averaging the number of colonies on all plates from the same undiluted or diluted sample volume, and multiplying by a dilution factor. In this case, results should be rounded to two significant digits, to avoid creating false precision.

For three digit results raise the middle digit if the last digit is 5 or greater. Raise the middle digit if the last digit is 4 or smaller. The last digit will be zero.

1.6 Average number of colonies / plate

The average number of colonies per plate is derived by dividing the total number of colonies on all plates which were inoculated with the same sample volume or dilution volume, and dividing that sum by the number of plates used.

For example:

$$\frac{89 \text{ colonies} + 103 \text{ colonies}}{2 \text{ plate}} = 96 \text{ colonies/plate}$$

1.7 Colony forming units

This the unit used for reporting bacterial density .to derive the number of colony forming units ,multiply the average number of colonies / plate by dilution factor of the incubated sample .

1.8 Dilution factor

The dilution factor is the reciprocal of the volume of original, undiluted sample plated and is used to standardize the results according to this sample volume.

1.9 Representative colony distribution

When counting colonies in a specified number of squares, try to count those squares that appear to have an average number of colonies .in other words try to avoid counting squares that have many less or many more colonies than most of the other squares on the plate .

1.10 No colonies

If plates from all dilution of any sample have no colonies, report the count as less than on (<1) times the dilution factor for the smallest volume of original sample used.

1.11 Less than 30 colonies / plate.

Ordinary, no more than 1.0 ml of sample is plated. Therefore, when the total number of colonies developing from 1.0 ml is less than 30, record the number of colonies as "Colony forming units", CFU

1.12 30 to 300 Colonies / plate

Compute bacterial count per ml by multiplying the average number of colonies /plate by the dilution factor. Report count as "Colony forming units" CFU per ml.

1.13 More than 300 colonies /plate

If there is no plate with 30 to 300 colonies and on or more plates have more than 300 colonies use the plate having a count closest to 300 colonies compute the count by multiplying the average number of colonies / plate by the dilution factor and report as "estimated colony forming units" per ml.

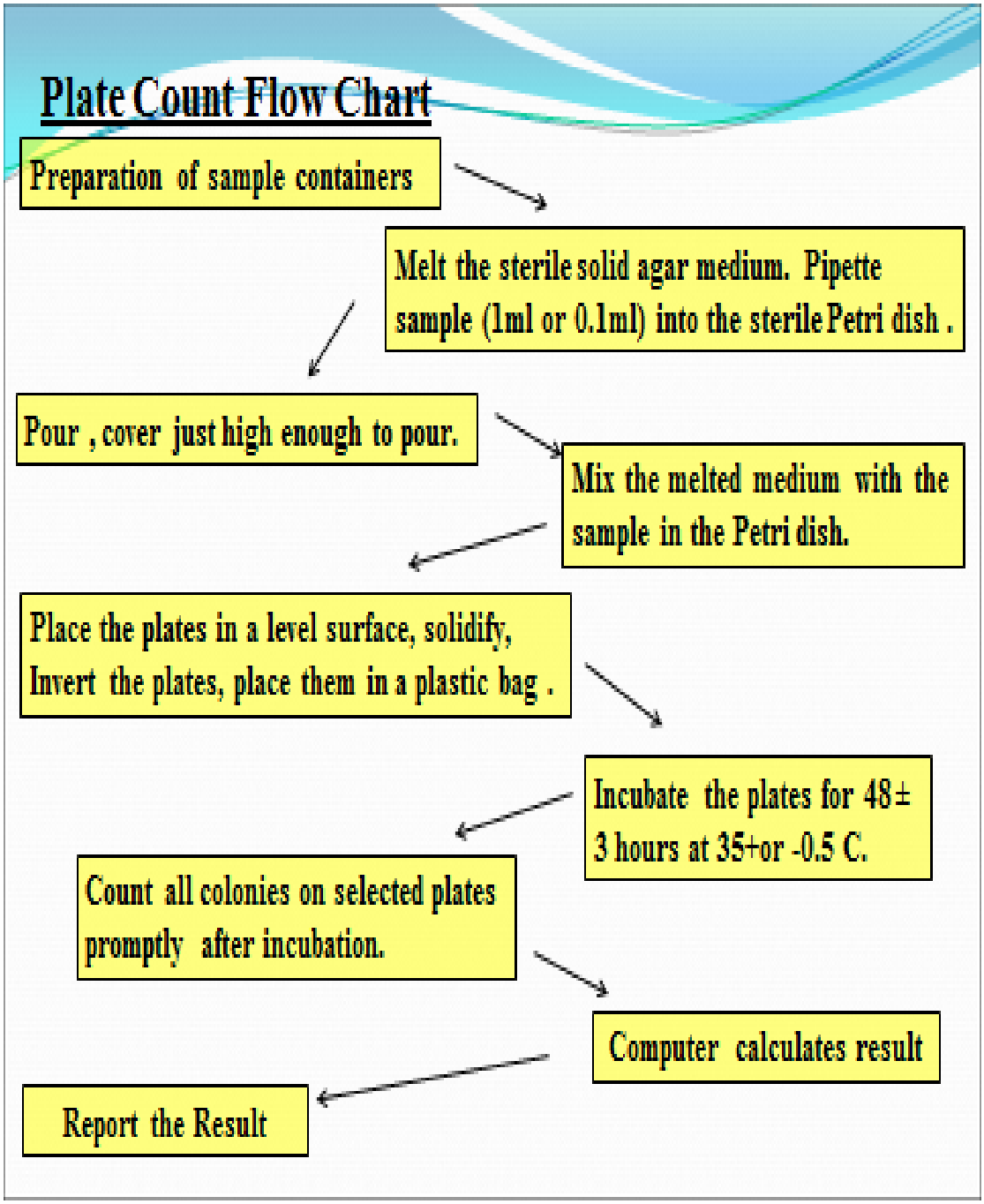
1.14 For more than 300 colonies /plate

If there are far more than 300 colonies / plate do not report the result as "too numerous to count" (TNTC) .Instead follow these guide lines for reporting.

Reporting results

Save the spread sheet and print out the Heterotrophic Plate Count in chlorinated effluent report sheet pass to the senior scientist or laboratory manger to sign off.

When the Heterotrophic Plate Count in chlorinated effluent report sheet has been signal off the daily sludge report can be prepared.



6-Microscopic examination
Introduction

This procedure is taken from the following references:

Practical animal biology vol. 11 Published by: Dar El Maaref.

Manual of instruction for Wastewater Treatment Plant Operators vol. 11 New York State 1988.

Aerobic biological Wastewater Treatment Facilities Process Control Manual 1977.

Microscopic examination of the MLSS can be a significant aid in the evaluation of the activated sludge process. The presence of various microorganisms within the sludge floc can rapidly indicate good or poor treatment. The most important of these microorganisms are the heterotrophic and autotrophic bacteria, which are responsible for purifying the wastewater. In addition, protozoa play an important role in clarifying the wastewater and act as indicators of the degree of treatment.

Protozoa, which mean “first animals”, are single celled microscopic animals. They are a diverse assemblage grouped together for convenience. Protozoa are very small, usually measuring 5 to 1000 µm in size, and visible only with the aid of a microscope.

Aerobic wastewater treatment plants have been colonized successfully by protazoa, and their presence was noted almost as soon as the process was developed. In recent years the significance of these organisms has begun to emerge as the types, and sometimes species, of protozoa have been related to effluent quality and plant performance.

The majority of protozoa inhabiting activated sludge units are associated with mixed liquors flocs.

The protozoan populations may by analyzed quantitative or qualitatively by extracting samples and examining them directly. Various methods for estimating the populations have been described.

The use of counting chambers such as hemocytometers or slide with cover slip, and lowpower stereo microscopes to count the numbers of protozoa in a small drop of known volume are common methods.

Generally two types of methods are used to sample for protozoa in wastewater treatment process: direct sampling and use of artificial solid support media.

Artificial support media

For the isolation of every species

Polyurethane foam has been used successfully for sampling Protozoa in activated sludge units.

Small “1 cm^3” units of foam are left immersed in an aeration tank for several days, and these are squeezed to release the protozoa.

Direct Sampling

This procedure describes the direct sampling technique.

Scope

This procedure describes how to Measurement of Activated Sludge Protozoa

Personal Responsible

Title	Biologist
Responsibilities	Responsible to Measurement of Activated Sludge Protozoa.

Reagents
Hazards

1 Laboratory coats must be worn at all times.

2 Eye protection and a fume cupboard must be used when handling concentrated acids.

3 The biological room of the lab should be clean and dry.

4 All chemical containers must be clearly labeled.

5 After samples and reagents have been used; any residual material adhering to the outside of the bottle should be wiped or rinsed off to prevent contact with the hands during future handlings.

6 The habit of placing discarded pipettes on tabletops, laboratory carts, or in sinks without adequate decontamination presents an unnecessary health risk to the laboratory personnel.

7 Disinfectant solutions in the discard container should be replaced each morning to ensure maximum disinfection.

8 Using disposable laboratory items will eliminate many washing problems.

9 When hands are contaminated, they should be thoroughly washed in warm water using mild soap or detergent.

Water

Distillation should be carried out behind a safety screen.

1% Nickel sulphate

Methylene blue stains Solution:

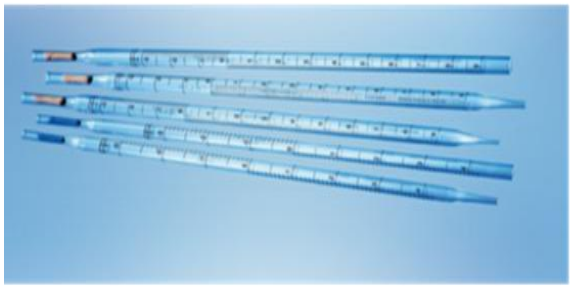
Dissolve 0.01 g of Methylene blue in 100 ml of absolute alcohol.

Lugol’s iodine stains solution:

Dissolve 10 g of Potassium iodide 5 g of Iodine crystals in 100 ml distilled water.

Mix reagent and filter into brown bottle.

Stopper tightly and store away from light.



-Pipette

-Beakers.

-Microscope

-Slides & Slide cover .

Apparatus

1 Binocular microscope 10x and 40x objective

2 Hemocytometers, Counting chambers

3 Beakers 250 ml

4 Pipette 1 ml

5 Pasteur pipette

6 Slides

7 Slide covers 22 x 22 mm cover slips

Procedure

Preparation of microscope slide

1 Take a clean cover slip and slide.

2 Use a 1 ml pipette to pick up sludge. Put finger on top of pipette until the immersed end of a wide tip pipette reaches the bottom of the activated sludge sample. Release your finger to allow sludge into the pipette.

3 Replace your finger on top of pipette and remove the pipette from the sample beaker. A long tipped eyedropper may also be used.

4 Allow one drop of sludge from the pipette to drop in the middle of the clear area of the glass slide by lifting your finger from the top of the pipette momentarily, and then replacing your finger.

5 Pick up the cover slip by two corners. Do not touch the central area.

6 Pull cover slip along glass slide towards drop of sludge.

7 As soon as cover slip touches the drop of sludge, allow cover slip to fall onto glass slide.

8 Pick up glass slide. Place on microscope stage.

9 Move stage up to within approximately 1/8 inch of objective.

10 Look at glass slide through the eyepiece of the microscope.

11 Use the coarse adjustment on the microscope to bring the sludge into the field of focus.

12 Use fine adjustment to refine focus to suit your eyes.

13 Identify organisms in the sludge using the examination procedure

Procedures for examination

When performing a microscopic examination of Protazoa in activated sludge, a sheet of paper should be kept handy to sketch the types of protozoa observed. In the event that unknown varieties of microorganisms are observed these may be determined later by referring to reference books. The objective of the examination is to determine relative predominance of microorganisms.

Examination procedures:

1 Record the date, time, temperature, and location of sample on the worksheet.

A minimum of three slides per sample should be examined.

2 Scan each slide and count the number of microorganisms in each group.

Provide a mark for each microorganism on the worksheet.

Note:

To slow down rapidly moving protozoa you can use one drop of 1% nickel sulphate, on the slide.

Film Stain bacteria

Methylene blue or lugol's iodine stain can be used to aid the examination of microorganisms.

Procedures

1 Add one drop of the stain to a drop of sludge on the slide and cover with cover slip.

2 Examine the slide under the microscope

3 The easiest way to scan microorganism is to follow a back and forth pattern, moving down the slide.

4 Provide a mark for each type of microorganisms on the worksheet (See appendix 2).

5 Types of microorganism, which is normally found in activated sludge. (See appendix 1 for pictorial representation of microorganisms)

Floc Structure

Daily microscopic examination of floc by the laboratory technician can alert the operator to changes in the activated sludge treatment process. Floc types include:

a. Compact, well-formed floc which settles easily, leaving a clear supernatant.

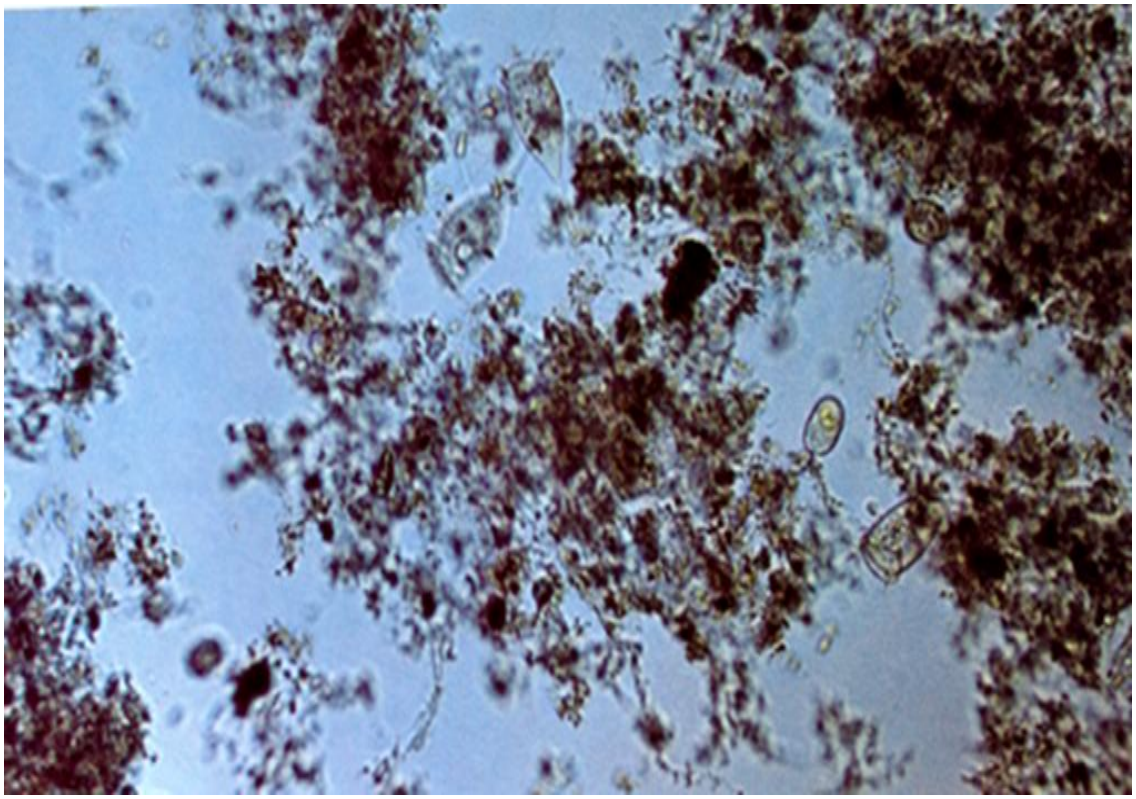
b. Pin floc tiny very compact floc, often with few bacteria that may or may not settle well, but leaves floc suspended in the supernatant.

c. Straggler floc loose floc structure, which is characterized by large, amorphous floc which may or may not have filamentous bacteria. Generally settles slowly.

d. Bulking floc usually characterized by filamentous bacteria. Filamentous bacteria extend between floc particles, connecting them together, and thus preventing them from compacting and settling well.

e. Dispersed floc loose small floc structure, very little organization. Poor settling usually has a very turbid supernatant.

Common Microorganisms in activated sludge sample

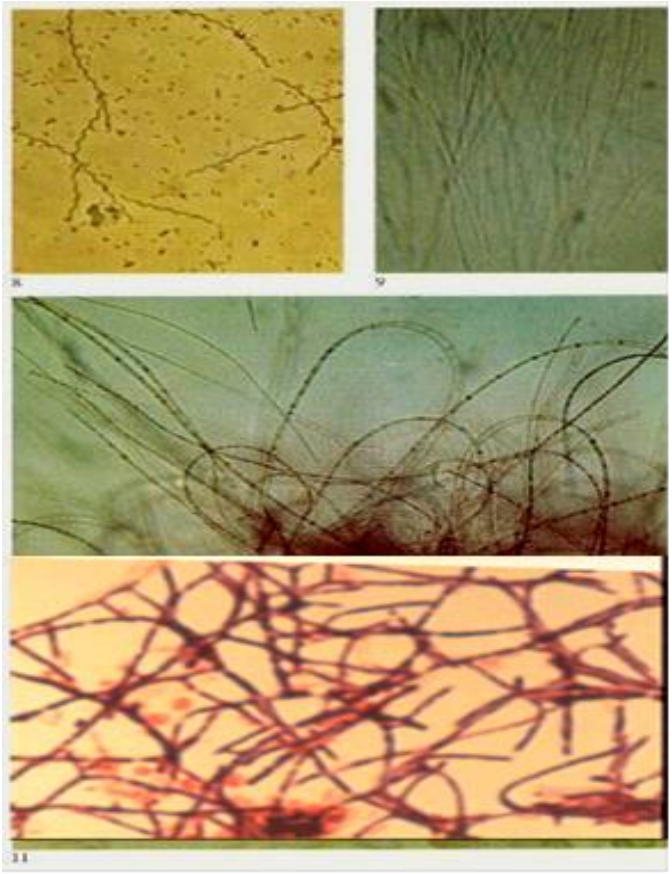


Bacteria

Bacteria are the major work force in waste treatment. There have many different strange names but most fit in the category called heterotrophs. Under the microscope, bacteria will appear as very small black dots some will be stationary others will be motile.

The presence of lots of bacteria indicates very early treatment and high Food/Microorganism ratio (BOD). Bacteria can barely be seen under the 400x magnification (40x objectives).

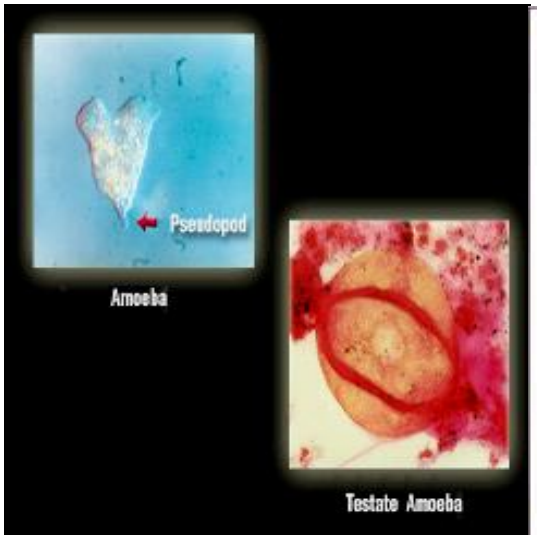
There is a second type of bacteria called filamentous bacteria. They provide similar waste stabilization as normal bacteria except they create problems because they don't settle well. Filamentous bacteria begin to predominate when the environment is not “happy” such as low pH or low dissolved oxygen.

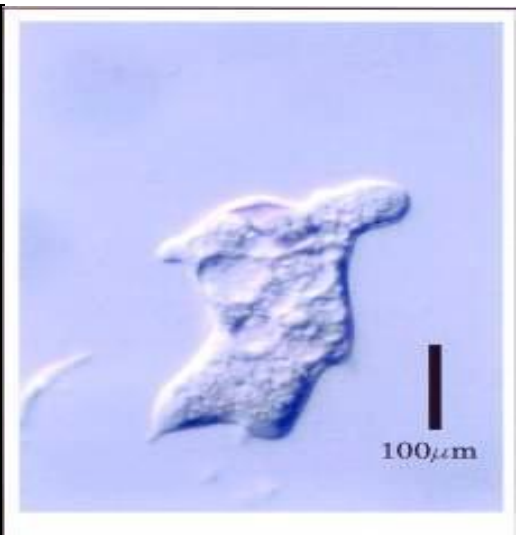


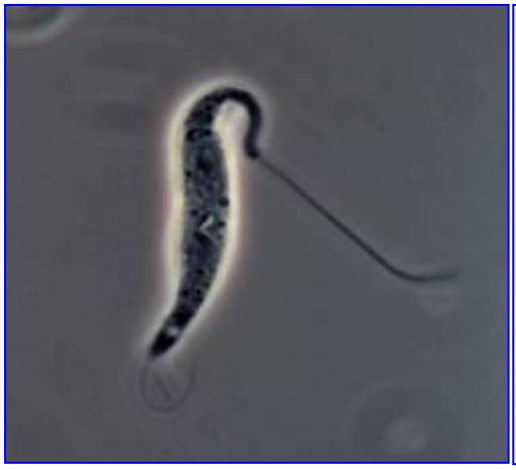
Protozoans

Protozoans are one-celled, animal like organisms, which include amoeba, flagellates, ciliates, and sporozoans. Protozoans can be free living or parasitic. Common parasitic protozoans found in water cause amoebic dysentery Giardiasis, and Cryptosporidasis.

Amoeba







Flagellates

Euglena is a genus of microscopic, one celled organisms in the Protista kingdom.There are about 150 species of euglenas.They live in fresh water, and are especially common in warm seasons when they may form a green scum on the surfaces of small ponds or drainage ditches.

Peranema are small flagellates and ranges in size from 20-70 µm in diameters.They are very active predators and scavengers.

This flagellate is common in waters rich in organic nutrients, especially in water in which decay is taking place.

Ciliates

• Ciliates are protozoans having hair-like structures (cilia) covering all or part of their cell membrane. The beating cilia either move the cell or cause currents in the water which aid in food gathering. They use bacteria or particulate organic matter as food. The movement of the cilia forces the particles into the cell's gullet.

• Ciliates can be divided into free-swimming, grazers, and stalked ciliates.

• Free-swimming ciliates are present in high numbers when the bacteria population is large.

• Two types of free swimmers include those that cruise between floc eating whatever particulate matter is available; and grazers, those that usually stay within or on the floc and feed on the floc as cows would munch on grass in a field.

Free-swimming Ciliates

The presence of free-swimmers indicates the activated sludge system is approaching optimum treatment, although many grazers seem to indicate a better treatment than the presence of the cruisers.

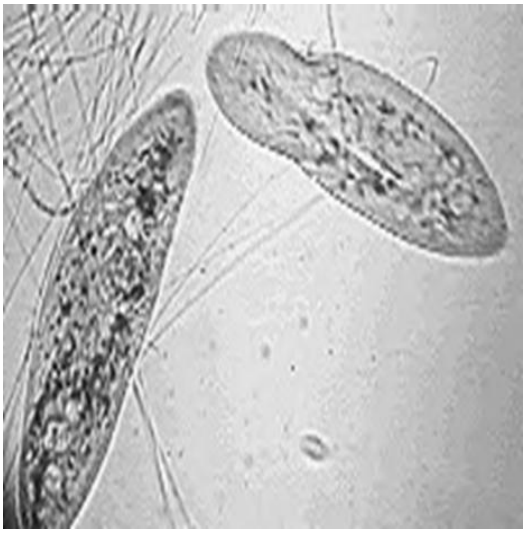


Figure: Colonized Stalked Ciliates

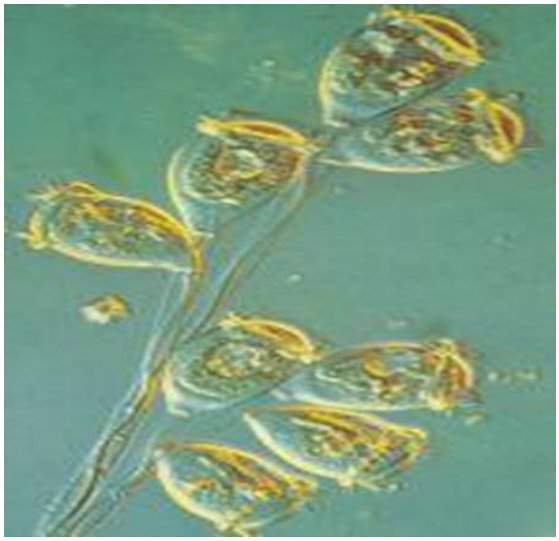


Figure: Grazing ciliate

Stalked Ciliates

• Stalked ciliates use a stalk to hold onto the floc.

• Because they do not have to propel themselves through the water, they do not expend as much energy

• As the free swimmers and therefore live in water with lower organic loadings. Since they are attached to the floc, they cannot move to more oxygenated areas, so the dissolved oxygen must be fairly high for them to survive.

• The rhythmic beating of the cilia at the anterior end (head) produces currents, which drive bacteria and organic particles into their gullet.

• Some stalked ciliates have a contractile protein in their stalk that routinely contracts into a tightly wound coil, then springs out. This "spring action" stirs the water and helps the stalked ciliates gather food.

• Stalked ciliates may occur individually or form colonies. They begin to predominate when free swimming ciliates are unable to compete for food because the bacteria population is small.

• When stalked ciliates are present, floc formation is usually good and the effluent is clear. Ciliates are easily visible under 100x and 200x

Metazoans

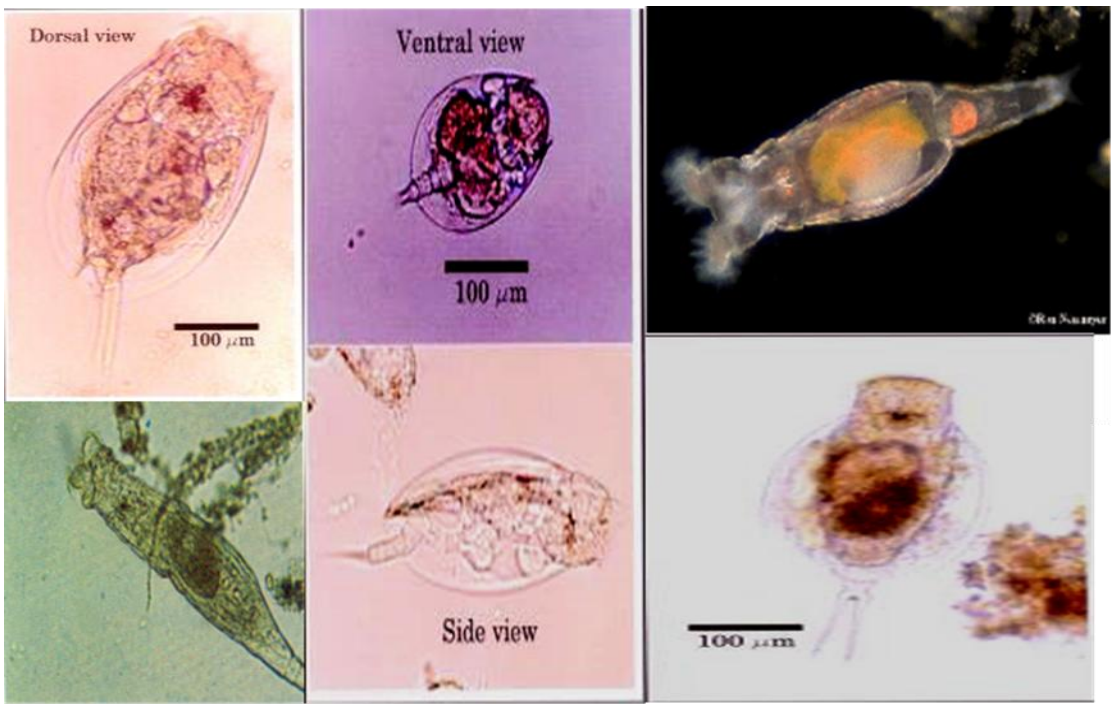
Metazoans are the largest organisms activated sludge system. They include rotifers, nematodes and bristle worms.

Rotifers

• Rotifers are found mainly in older activated sludge. At the head end, most rotifers have a conspicuous corona with cilia used for both locomotion and food gathering. Just below the corona is the mouth, which is connected to a grinding structure and called the mastax. The mastax is usually easily seen in rotifers and can be used to differentiate rotifers from other microorganisms.

• The foot, at the posterior end has spurs and retractile toes, which allow the rotifer to attach to floc.

• Rotifers have a high DO requirement and will die when the DO is too low. The presence of stalked ciliates and rotifers indicates a good activated sludge floc and produce a clear effluent.



Nematodes

• Nematodes commonly found in wastewater are non-segmented roundworms. They have a long, slender body with one end usually sharply pointed while the other end tapers to a blunt tip (little fire hoses).

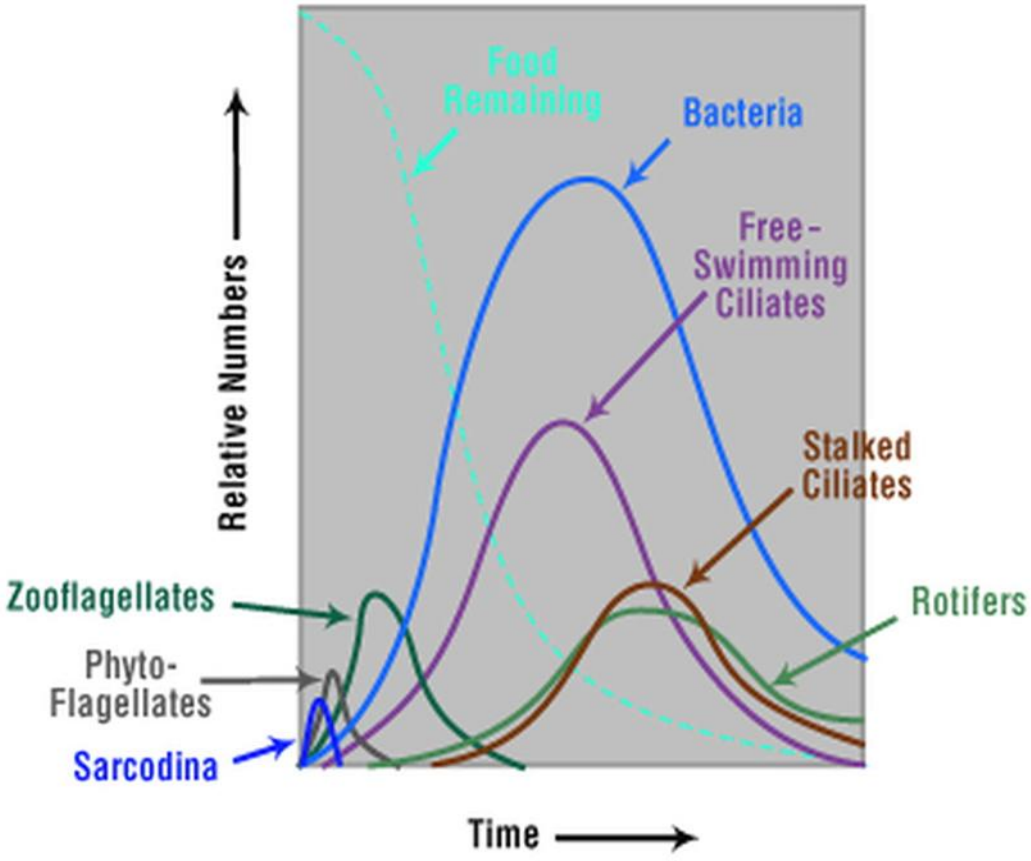
• They feed on bacteria, protozoan, ciliates, rotifers, and floc.

• The presence of nematodes indicates an older sludge. Sludge worms are easily seen under the 4x objective.

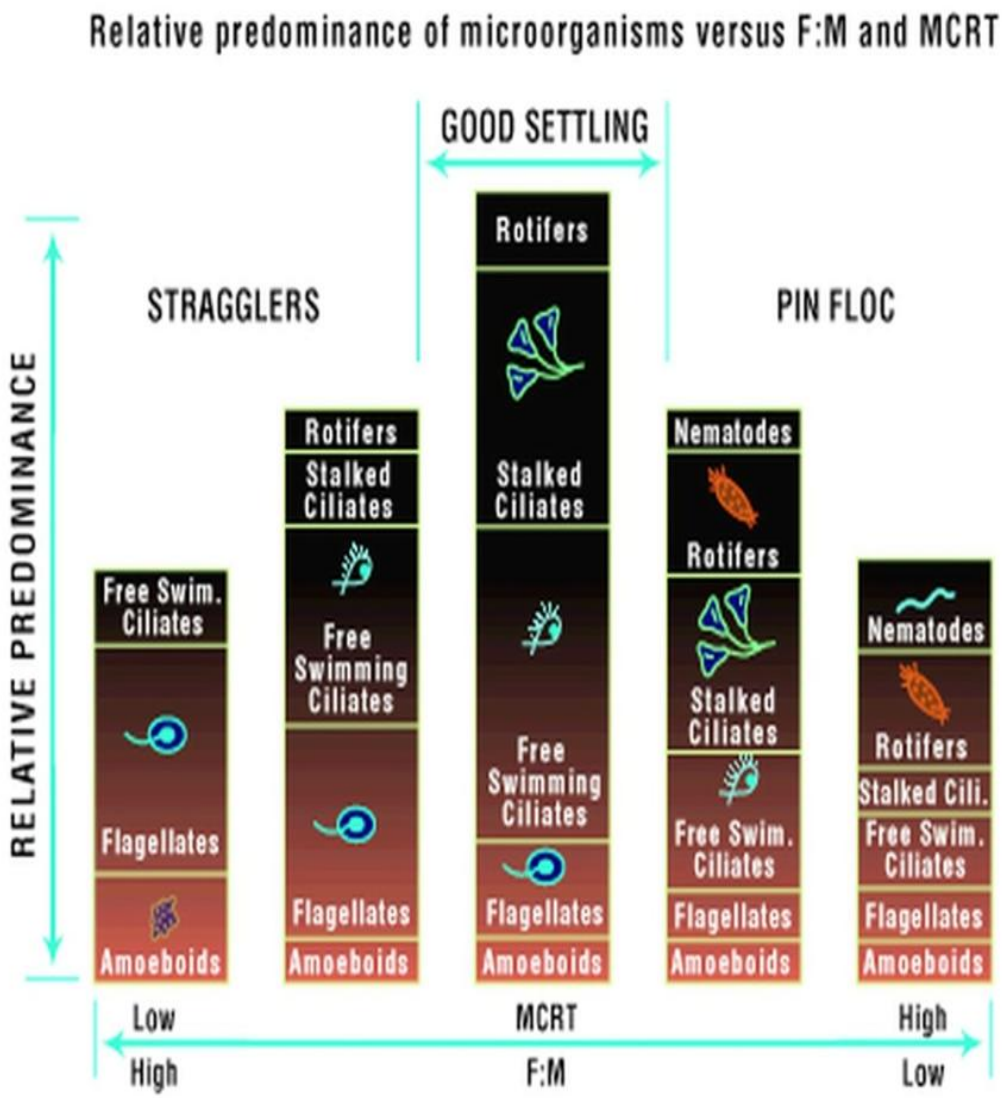
Bristle worms

Aeolosoma is the segmented worm commonly found in very old activated sludge. It has bristles, which extend from its sides and burnt orange dots on its surface. Bristle worms consume organic sludge

Once again, the microscope can be a valuable tool to help the lab technician identify types of microorganisms and relate them to the effluent quality (BOD and TSS).



Microorganism's growth against the aeration time



Qualitative Assessment of Protozoa

When the data from the worksheet is entered into the computer spreadsheet, the computer will provide a qualitative assessment of the activated sludge, as follows:

1 Based on the five following categories

Absent

Frequent

Regular

Occasional

Absent

Make a qualitative assessment for each type of protozoa and mark the worksheet against one category.

Vitality

Based on the five following categories:

- Very vigorous
- Vigorous
- Ok
- Stressed
- Very stressed

Make a qualitative assessment for:

i. Stalked ciliated protozoa.
j. Other protozoa and make a mark on the worksheet against one category for the two types of protozoa.

Flock strength

Based on the following assessment of floc size assign, a category of floc strength.

Strong	more than 500 µm
Dense	400 – 500 µm
Moderate	250 – 400 µm
Weak	150 – 250 µm
Very weak	50 – 150 µm

Filamentous

The worksheet contains an assessment for filamentous bacteria; these are not covered in this procedure.

After an assessment for each of the above categories has been entered.

The computer will provide on eof the following assessments:

Frequency and vitality

- Excellent
- Good
- Ok
- Bad
- Very bad

Floc strength

- Strong
- Dense
- Moderate
- Weak
- Very weak

Filamentous bacteria

- Within floc only
- Present
- Absent
- Protruding
- Dominating

An overall assessment will be made by the computer spreadsheet based on the three categories above as follows:

- Excellent
- Good
- Ok
- Bad
- Very bad

Chlorophyll Estimation
Introduction

Photosynthetic pigment concentrations are used extensively to estimate phytoplankton biomass.1, 2. All green plants contain chlorophyll a, which constitutes about 1 to 2% of the dry weight of planktonic algae. Other pigments in phytoplankton include chlorophylls b and c, xanthophylls, phycobilins, and carotenes. Important chlorophyll degradation products found in the aquatic environment are chlorophyllides, pheophorbides, and pheophytins. The presence or absence of various photosynthetic pigments is used, among other features, to identify major algal groups.

Three methods to determine chlorophyll a in phytoplankton are the spectrophotometric, fluorometric and high performance liquid chromatographic (HPLC) techniques. Fluorometry is more sensitive than spectrophotometry, requires fewer samples, and can be used for in-vivo measurements.These optical methods can significantly under- or overestimate chlorophyll a concentrations; part because the absorption and fluorescence bands of co-occurring accessory pigments and chlorophyll degradation products overlap.

Pheophorbide a and pheophytin a, two common degradation products of chlorophyll a, can interfere with the determination of chlorophyll a because they absorb light and fluoresce in the same region of the spectrum as chlorophyll a does. If these pheopigments are present, significant errors in chlorophyll a values will result. Pheopigments can be measured either via spectrophotometry or fluorometry, but in marine and freshwater environments, the fluorometric method is unreliable when chlorophyll b co-occurs

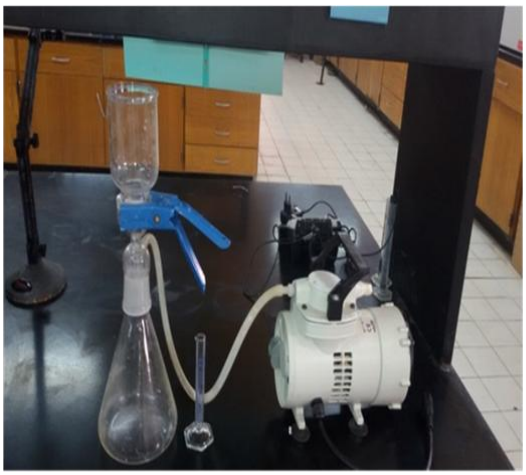
Sample collection:

Once sampling locations, depths, and frequency have been determined, prepare for field sampling. Use opaque sample containers because even brief light exposure during storage will alter chlorophyll values. Sample-storage bottles should be made of polyethylene or glass to avoid metallic ion contamination, which can lead to significant errors when making algal assays or productivity measurements. Similarly, in multi-analyte sampling Programs, store algal pigments in bottles without acid residues. For example, do not use bottles containing acidic preservatives for nutrients or Lugol's solution when microscopically enumerating phytoplankton: acidic preservatives preclude analyses for chlorophyll and other pigments.

Glass fiber filters are preferred for removing algae from water. The glass fibers help break the cells during grinding, larger volumes of water can be filtered, and no precipitate forms after acidification. Filters taken from water with pH above 6 may be placed in airtight plastic bags and stored frozen for 28 days. Process samples from naturally acidic water with pH less than 6 promptly after filtration to prevent possible chlorophyll degradation from residual acidic water on the filter.

Chlorophyll can be extracted from cells, typically collected on a filter, with several solvents (e.g., acetone, ethanol, and methanol. The procedure described here uses acetone. Conduct this procedure with chlorophyll extracts in subdued light to avoid degradation. Use opaque containers or wrap with aluminum foil. The pigments are extracted from plankton concentrate with aqueous acetone and the extract's absorbance (optical density) is determined via a spectrophotometer. The ease with which chlorophylls are removed from cells varies considerably with different algae. For completely extract pigments consistently, disrupt the cells mechanically with a tissue grinder.

Spectrophotometer



Filtration Unit

- Tissue grinder.

Centrifuge

Equipments (Apparatus)

- Tissue grinder.

- Clinical or bench top centrifuge (5000 to 7500 rpm).
- Centrifuge tubes, 15 ml graduated, and screw cap.
- Filtration equipment, filters, glass fiber, or membrane (0.45 µm porosity, 47 mm diameter); vacuum pump; solvent resistant filter assembly
- Saturated magnesium carbonate solution:

Add 1.0 g finely powdered $MgCO_3$ to 100 ml distilled water.

- Aqueous acetone solution: Mix 90 parts acetone (Reagent grade BP 56 °C) with 10 parts saturated magnesium carbonate solution.

Extraction method:

- Concentrate sample by centrifuging or filtering as soon as possible after collection. If processing must be delayed, hold samples on ice or at 4 °C and protect from exposure to light. Use opaque bottles because even brief exposure to light during storage will alter chlorophyll values. Rinse sample storage container with about 20 ml organic-free lab water (which is also passed through the same sample filter to make sure all cells are collected).

- Use glassware and cuvettes that are clean and acid free. Add approximately 2 ml of $MgCO_3$ solution to sample just before filtering process is completed. $MgCO_3$ solution acts as a pH buffer to keep chlorophyll from degrading.

- Place sample in a tissue grinder, cover with 2 to 3 ml 90% aqueous acetone solution, and macerate at 500 rpm for 1 min. Use TFE/glass grinder for a glass-fiber filter and glass/glass grinder for a membrane filter

- Transfer sample to a screw-cap centrifuge tube, rinse grinder with a few milliliters 90% aqueous acetone, and add the rinse to the extraction slurry. Adjust total volume to 10 ml with 90% aqueous acetone.

- Use solvent sparingly and avoid excessive dilution of pigments. Steep samples at least 2 h at 4 °C in the dark. Glass fiber filters of 25- and 47-mm diameters have dry displacement volumes of 0.03 and 0.10 ml, respectively, and introduce error of about 0.3 and 1.0% if a 10 ml extraction volume is used.

- Clarify by filtering through a solvent-resistant disposable filter or by centrifuging in closed tubes for 20 min at 500 g or 3000 rpm.

- Decant clarified extract into a clean, calibrated, 15 ml, screw cap centrifuge tube and measure total volume.

Spectrophotometric Determination of Chlorophyll:

- Use spectrophotometer, with a narrow bandwidth (path) (0.5 to 2.0 nm) because the chlorophyll absorption peak is relatively narrow.

At a spectral bandwidth of 20 nm, the chlorophyll a concentration may be underestimated by as much as 40%.

- Cuvettes, with 1-, 4-, and 10-cm path lengths.
- Pipets, 0.1- and 5.0-ml.
- Hydrochloric acid, HCl, 0.1N**.**

Procedure:

- Spectrophotometric procedure:

- Transfer extract to 1 cm cuvette and measure absorbance at 750, 664, 647, and 630 nm.

- Choose a cell path length or dilution to give absorbance 664 between 0.1 and 1.0.

- Use the absorbance readings at 664, 647, and 630 nm to determine chlorophyll a, b and c, respectively.

The absorbance reading at 750 nm is a correction for turbidity.

- Subtract this reading from each of the pigment absorbance values of the other wavelengths before using them in the equations below.

Because the extract's absorbance at 750 nm is sensitive to changes in the acetone-to-water proportions, adhere closely to the 90 parts acetone: 10 parts water (v/v) formula for pigment extraction.

Turbidity can be removed easily via filtration through a disposable, solvent resistant filter attached to a syringe or by centrifuging for 20 min at 500 g.

Calculation:

Calculate the concentrations of chlorophyll a, b, and c in the extract by inserting the corrected optical densities in following equations:

a) C_a = 11.85 (absorbance 664) - 1.54(absorbance 647) - 0.08 (absorbance 630)

b) C_b = 21.03 (absorbance 647) - 5.43 (absorbance 664) - 2.66 (absorbance 630)

c) C_c = 24.52 (absorbance 630) - 6.70 (absorbance 647) - 1.67 (absorbance 664)

Where:

C_a, C_b and C_c= concentration of chlorophyll a, b, and c, respectively, mg/l and absorbance 664,647 and 630

After determining the concentration of pigment in the extract, calculate the amount of pigment per unit volume as follows:

Chlorophyll a, mg/m^3 = (c_a × extract volume, L / volume of sample, m^3)

Unit:

The result is recorded as mg/l.

Reference:

(Standard methods for the examination of water and wastewater ^{TM},22 nd edition.10200H)

- Test results shall be adjusted to exclude algae using the results of Chlorophyll A testing.
- Influent and effluent sample test results shall be recorded daily.
- Chlorophyll A tests shall be conducted and recorded twice per week, initially.
- Frequency of testing for Chlorophyll A may be reduced after three months if it can be demonstrated that satisfactory results can be achieved by testing on a less frequent basis

- Chlorophyll A testing shall be performed twice per week on a portion of the sample taken from the effluent pumping station and the facultative lagoon effluent.

a. The concentration of Chlorophyll A in mg/L shall be divided by 0.015 to determine the concentration of TSS in mg/L attributed to algae (TSS_A).

b. Multiply the concentration of TSS_A by 0.8 to determine the increase in BOD caused by algae content (BOD_A).

c. Subtract the TSS_A from the TSS found in the sample (TSS_S) to determine the TSS corrected for algae content (TSS_C).

d. Subtract the $B0D_A$ from the sample BOD (BOD_s) to determine the BOD corrected for algae content ($B0D_c$).

So:

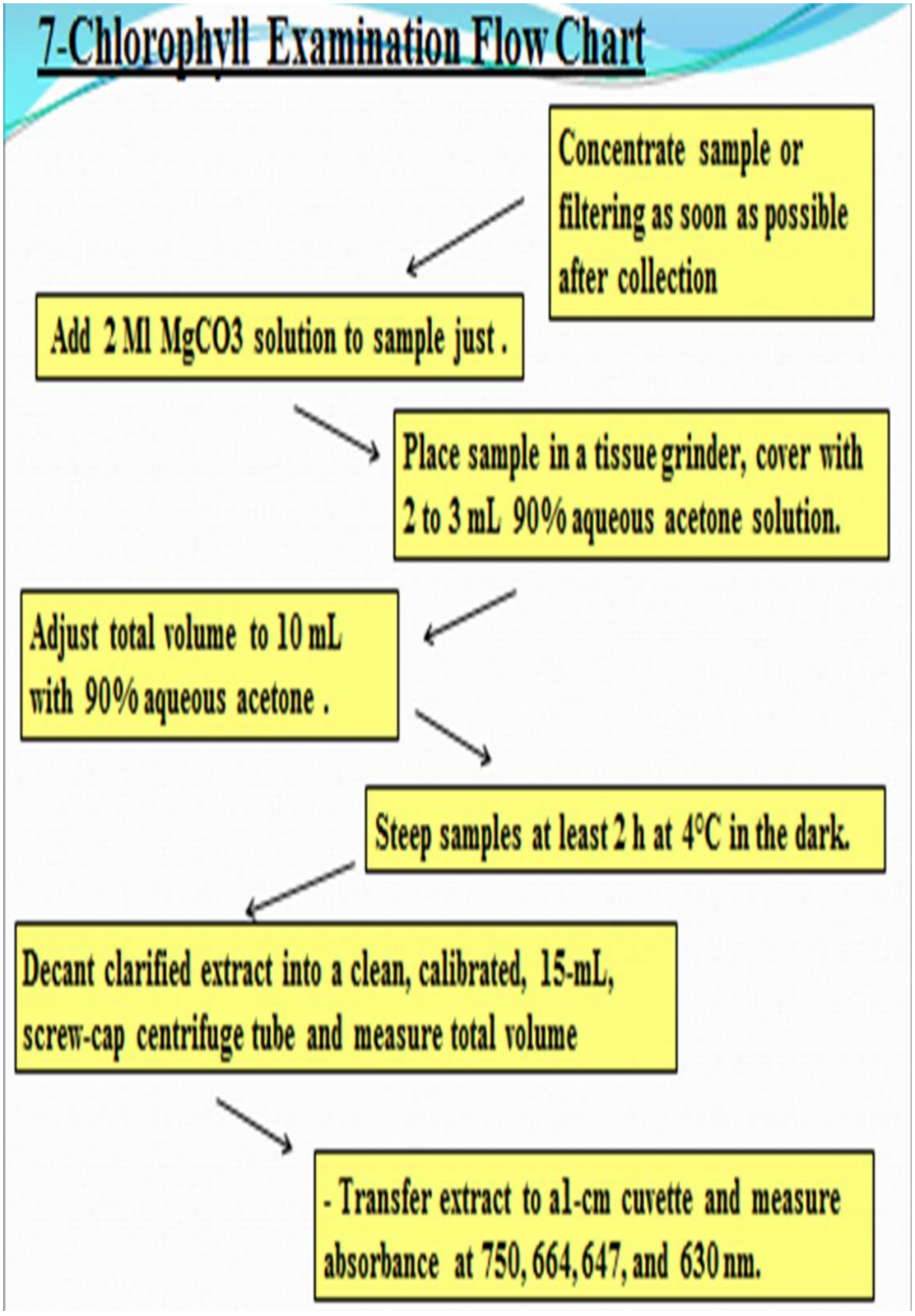
TSS_A (mg/l) = C_a (mg/l) / 0.015

BOD_{5A} (mg/l) = TSS_A(mg/l) × 0.8

And then

TSS_C (mg/l) = TSS_S (mg/l) – TSS_A (mg/l)

BOD_{5C} (mg/l) = BOD_{5S} (mg/l) – BOD_{5A} (mg/l)


7-Chlorophyll Examination Flow Chart
Concentrate sample or filtering as soon as possible after collection
Add 2 Ml MgCO3 solution to sample just .
Place sample in a tissue grinder, cover with 2 to 3 mL 90% aqueous acetone solution.
Adjust total volume to 10 mL with 90% aqueous acetone .
Steep samples at least 2 h at 4°C in the dark.
Decant clarified extract into a clean, calibrated, 15-mL, screw-cap centrifuge tube and measure total volume
- Transfer extract to a1-cm cuvette and measure absorbance at 750, 664, 647, and 630 nm.

المراجع

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