Technical Assistance
HYDROLOGY PROJECT

World Bank & Government of The Netherlands funded

Training module # WQ - 23

How to measure coliforms

New Delhi, June 1999

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DHV Consultants BV & DELFT HYDRAULICS

with
HALCROW, TAHAL, CES, ORG & JPS

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1. Module context

This module describes a laboratory exercise on measurement of coliform bacteria. Modules in which prior training is required to complete this module successfully and other related modules in this category are listed below.

While designing a training course, the relationship between this module and the others, would be maintained by keeping them close together in the syllabus and place them in a logical sequence. The actual selection of the topics and the depth of training would, of course, depend on the training needs of the participants, i.e. their knowledge level and skills performance upon the start of the course.

No.	Module title	Code	Objectives
1	Basic water quality concepts[a]	**WQ - 01**	• Become familiar with common water quality parameters • Appreciate important water quality issues
2	Basic chemistry concepts[a]	**WQ - 02**	• Convert units from one to another • Understand the basic concepts of quantitative chemistry • Report analytical results with the correct number of significant digits
3	How to prepare standard solutions	**WQ - 04**	• Recognise different types of glassware • Use an analytical balance and maintain it • Prepare standard solutions
4	Introduction to microbiology[a]	**WQ - 20**	• classify different types of microorganisms • identify certain water borne diseases
5	Microbiological laboratory techniques[a]	**WQ - 21**	• Explain methods of bacteria identification • Discuss methods of bacteria enumeration • Follow methods of good laboratory practice
6	Coliforms as indicators of faecal pollution[a]	**WQ - 22**	• Identify the main water quality problems caused by microorganisms • Explain why coliform bacteria are good indicators • Explain the principles of the coliform analysis method

a- prerequisite

2. Module profile

Title	:	How to measure coliforms
Target group	:	HIS function(s): Q2, Q3, Q5, Q6
Duration	:	1 session of 160 min and 2 sessions of 45 min each
Objectives	:	After the training the participants will be able to • Measure total and faecal coliforms in water samples
Key concepts	:	• Culture media preparation • Serial dilution • Inoculation • Reading MPN table
Training methods	:	Lecture, laboratory exercises
Training tools required	:	Board, flipchart, OHS, laboratory
Handouts	:	As provided in this module
Further reading and references	:	• Standard Methods: for the Examination of Water and Wastewater, APHA, AWWA, WEF/1995. APHA Publication

3. Session plan

No	Activities	Time	Tools
1	***Preparations*** • Collect/prepare the following samples – Sample A: Surface water – Sample B: Contaminated tap water (1 mL sewage/ 10 Ltap water) – Sample C:Boiled sample B • Estimate the requirement of dilution and culture tubes,and pipettes for the presumptive test • Prepare dilution water, culture medium accordingly and dispense in tubes. Culture tubes should contain an inverted durham tube. Put caps or cotton plugs. • Sterilise glassware, culture and dilution tubes		
2	***Introduction***: • Ask participants to name a few water borne diseases • Recapitulate use of coliforms as indicators of faecal pollution	10 min	
3	***Bacteriological testing*** • Discuss importance of advance preparations • How to correctly estimate requirements • What steps are required in preparations and testing • Take the group around the laboratory and show the incubators and the sterilisers	30 min	OHS
4	***Exercise*** *Session I* • Briefly describe the exercise and ask the participants to read the main text and SAP for total coliforms • Demonstrate serial dilution and inoculation of tubes using aseptic technique. Ask participants to practice under supervision. • Divide the participants in groups of 3. Ask them to prepare dilutions and inoculate lauryl tryptose tubes for presumptive test. • The inoculated tubes should be immediately put in the incubator. • Simultaneously the groups should prepare BGBL and EC broths and dispense them in tubes, sterilise and store for use in subsequent sessions	20 min 20 min 40 min 40 min	handout

No	Activities	Time	Tools
4	***Exercise (contd.)*** *Session II* • Ask participants to record the positive tubes after incubation for 24 h. Explain that in order to complete the exercise, the negative tubes will not be incubated for additional 24 h as required in the standard procedure • The participants will start the confirmed test for total coliforms and test for faecal coliforms by transferring inocula from the positive tubes to the respective culture media • Ascertain that the incubators are set at required temperature	45 min	
5	***Exercise (contd.)*** *Session III* Ask participants to record the number of positive tubes and determine MPN of total coliforms and faecal coliforms	15 min	
6	***Conclusion*** Ask participants to write report and discuss results	30 min	

4. Overhead/flipchart master

OHS format guidelines

Type of text	Style	Setting
Headings:	OHS-Title	Arial 30-36, with bottom border line (not: underline)
Text:	OHS-lev1 OHS-lev2	Arial 24-26, maximum two levels
Case:		Sentence case. Avoid full text in UPPERCASE.
Italics:		Use occasionally and in a consistent way
Listings:	OHS-lev1 OHS-lev1-Numbered	Big bullets. Numbers for definite series of steps. Avoid roman numbers and letters.
Colours:		None, as these get lost in photocopying and some colours do not reproduce at all.
Formulas/ Equations	OHS-Equation	Use of a table will ease horizontal alignment over more lines (columns) Use equation editor for advanced formatting only

How to measure coliforms

1. Preparations
2. Sample inoculation
3. Total coliform bacteria
4. Faecal coliform bacteria
5. Reading MPN

Preparations

- Test once started cannot be delayed
- Preparations according to number & source of samples
  - *culture medium and culture tubes*
  - *dilution water and dilution tubes*
  - *pipettes, transfer loop*
  - *sterilisation, autoclave, hot air oven, flame*
  - *incubators at required temperature*

Sample inoculation

- Drinking water: no dilution, 10 mL in 10 tubes
- Surface waters: 10,1,0.1 mL in 5 tubes each
- Polluted waters: 1,0.1,0.01 mL in 5 tubes each
- Grossly polluted water: smaller inocula
- Good practice to have more than 3 inocula

Total coliform bacteria

- Presumptive test
  - *lauryl tryptose broth, enrichment medium for lactose fermenters*
  - *incubate at 35 ± 0.5 °C*
  - *check for gas & turbidity, 24±2 h, 48±3 h*
- Confirmed test
  - *positive presumptive tubes*
  - *brilliant green lactose bile broth, selective medium for coliforms*
  - *incubate at 35 ± 0.5 °C*
  - *check for gas & turbidity, 24±2 h, 48±3 h*

Faecal coliform bacteria

- Positive presumptive tubes
- EC medium
- Incubate at 44.5 $\pm$ 0.2 ^{o}C
- Elevated temperature is selective for faecal coliforms
- Check for gas & turbidity, 24$\pm$2 h, 48$\pm$3 h

MPN /100 mL

- Bacteria are randomly distributed in the sample
- Multiple tubes inoculated with various sample sizes
- Smaller the sample, greater the chance of negative reaction
- The density is statistically estimated

5. Evaluation sheets

6. Handout

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Add copy of Main text in chapter 8, for all participants.

7. Additional handout

These handouts are distributed during delivery and contain test questions, answers to questions, special worksheets, optional information, and other matters you would not like to be seen in the regular handouts.

It is a good practice to pre-punch these additional handouts, so the participants can easily insert them in the main handout folder.

8. Main text

Contents

How to measure coliforms

1. Background

Coliform bacteria ferment lactose with production of gas. this property is utilised to determine its presence or absence in analysis of water for its bacteriological quality.

In the presumptive test, the sample is inoculated in lauryl tryptose broth. The broth is an enrichment medium for the coliform group. It contains lactose as the main carbon source and sodium chloride and sodium lauryl sulphate which act as inhibitors for non-coliform organisms. The medium allows growth of environmentally stressed coliforms also.

The confirmed test is used to substantiate or deny the presence of coliforms in a positive presumptive test. A small inoculum from a positive lactose broth tube is transferred to a tube containing brilliant green lactose bile broth. The green dye and bile salts in this broth inhibit non- coliform growth. The presence of coliform is confirmed by growth and gas production within 48 hour at 35 ^{o}C.

Some times ‘completed test' may be performed to determine the faecal origin of the coliforms. These tests involve subculturing of the positive tubes on various solid and liquid media and testing for further bio-chemical reactions.

Elevated temperature test for the separation of organisms of coliform group into those of faecal and non-faecal origin may also be performed. In this test, transfers from all positive presumptive tubes are made to culture tubes of EC medium, which contains bile salts and sodium chloride as selective agents along with nutrients. The inoculated tubes are incubated at 44.5 $\pm$ 0.2 ^{o}C. The elevated temperature of incubation further differentiates in favour of organisms of faecal origin. Gas production within 24 hour is considered a positive reaction indicating coliforms of faecal origin.

The multiple tube fermentation technique is used to enumerate the organisms in a water sample. The method is applicable to many different water samples including those obtained from potable, fresh, brackish and salt waters. The test can also be used for the estimation of coliform bacteria in muds, sediments and sludges.

Multiple tubes of culture medium are inoculated with various volumes (dilutions) of a water sample. Because of random nature of distribution of the organisms in the sample, there is always a chance that an inoculum may not contain any organism. Smaller the volume of the inoculum higher is the chance that the result would be negative. The combined results of all the tubes are statistically interpreted to arrive at the most probable number (MPN) density. A detailed discussion of the steps in the enumeration is discussed in the module on ‘Coliforms as indicators of faecal pollution’.

2. Aim

a. To determine the MPN/100mL of total and faecal coliform bacteria in various samples of water
b. To demonstrate the effect of sterilisation on MPN of total coliforms.

3. Method

a. Familiarise yourself with the operation of hot air oven and autoclave steriliser.
b. Estimate the number of culture tubes and dilution tubes required for different samples for total coliform determination. An indication of the required dilution may be obtained from the information given in the SAP for total coliform analysis.
c. Calculate the volume of lauryl tryptose and brilliant green bile broths required based on the number of dilutions for each sample, prepare the media, distribute in culture tubes and sterilise as described in the SAP.
d. Proceed with the presumptive and confirmatory tests for total coliforms as described in the SAP.
e. Prepare EC medium and simultaneously with the confirmatory phase, determine faecal coliforms according to the SAP for faecal coliform determination.
f. Use aseptic technique while transferring samples and cultures.

4. Observations & calculations

a. Record number of positive tubes for various inocula sizes for the three tests for each sample.
b. Read MPN/100 mL values for the appropriate set of positive tubes for total coliform (brilliant green bile broth) and faecal coliforms (EC medium) from the tables given in SAP for total coliform determination.

Sample	**Lauryl tryptose broth**				**Brilliant green bile broth**				**EC medium**			
	Inoculum size, mL				Inoculum size, mL				Inoculum size, mL			
A												
B												
C												

Sample	Total coliforms	Faecal coliforms
A		
B		
C		

5. Report

When writing your report the following aspects should be addressed:

Need for presemptive test, enrichment culture, brilliant green bile broth and EC broth as selective media, ratio of total to faecal coliforms.