

**Topic:** South African Approval of Colilert

**Title:** Microbiological analysis of water – General Test methods

**Source:** South African Bureau of Standards, Standard Method 221. Edition 4.

**Date:** January 19, 2001

### **Report Highlights:**

- Colilert® and Defined Substrate Technology® is approved for the detection of coliforms and *E. coli* in drinking water, non-potable water and effluent water.
- Colilert is referred to and approved via footnote in the title of the section. Defined Substrate Technology is classified as method 991.15: described in the Official Methods of Analysis of the Association of Official Analytical Chemists, second supplement, 1991, to the fifteenth edition, 1990, published by the Association of Official Analytical Chemists, Inc.

- Fax Cover Sheet -

**DEHTEQ**

**Date:** 08-03-01

**Pages:** 18

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Dear Manja

To give you the information you requested on the SABS form, it is necessary to supply the whole document. Let me know if there are any transmission problems.

Kind regards

  
Bradley Roe

## Microbiological analysis of water — General test methods<sup>1)</sup>

### Notice

This standard was approved in accordance with SABS procedures on 19 January 2001.

NOTE 1 In terms of the Standards Act, 1993 (Act 29 of 1993), no person shall claim or declare that he or any other person complied with an SABS standard unless

- a) such claim or declaration is true and accurate in all material respects, and
- b) the identity of the person on whose authority such claim or declaration is made, is clear.

NOTE 2 It is recommended that authorities who wish to incorporate any part of this standard into any legislation in the manner intended by section 31 of the Act consult the SABS regarding the implications.

This standard will be revised when necessary in order to keep abreast of progress. Comment will be welcome and will be considered when the standard is revised.

### Foreword

This fourth edition cancels and replaces SABS SM 221:1990.

## 1 Scope and applicability

### 1.1 Scope

This standard describes methods for the detection and enumeration, in potable water, non-potable water and effluent water, of total coliform bacteria, faecal coliform bacteria, *E. coli* and heterotrophic bacteria. It also refers to a method for the detection and enumeration of bacteriophages.

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1) An alternative method for the detection of coliforms and *Escherichia coli* in water is method 991.15: *Defined substrate technology method*, described in the Official Methods of Analysis of the Association of Official Analytical Chemists, second supplement, 1991, to the fifteenth edition, 1990, published by the Association of Official Analytical Chemists Inc.  
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## 1.2 Applicability

1.2.1 For potable water or for clear effluent water suitably diluted, use the membrane filter method or the most probable number method.

1.2.2 For turbid effluent water, which may clog the filters even after dilution, use the most probable number method.

1.2.3 The standard plate count may be used for potable or clear effluent or turbid effluent water, but for effluent water where large numbers of bacteria can be expected, provision shall be made for a greater number of dilutions in the preparation of the tenfold dilution series.

1.2.4 The bacteriophage detection method may be used as a rapid general method for assessing the microbiological quality of potable, non-potable and effluent water.

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this standard. All standards are subject to revision and, since any reference to a standard is deemed to be a reference to the latest edition of that standard, parties to agreements based on this standard are encouraged to take steps to ensure the use of the most recent editions of the standards indicated below. Information on currently valid national and international standards can be obtained from the South African Bureau of Standards.

ISO 10705-1, *Water quality – Detection and enumeration of bacteriophages – Part 1: Enumeration of F-specific RNA bacteriophages.*

SABS 241, *Drinking water.*

SABS ISO 835-4, *Laboratory glassware – Graduated pipettes – Part 4: Blow-out pipettes.*

SABS SM 552, *Equipment for microbiological tests.*

SABS SM 553, *Media and reagents for microbiological tests.*

## 3 Definitions

### 3.1

#### ***Escherichia coli***

faecal coliform bacteria that also produce indole from tryptophan within 24 h of aerobic growth at  $45\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$

### 3.2

#### **faecal coliform bacteria**

coliform bacteria capable of acid, aldehyde and gas production within 24 h of aerobic growth at  $45\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$  on a culture medium containing lactose as the carbon source or capable of producing typical blue colonies on mFC medium

### 3.3

#### standard plate count

a method for estimating the total number of viable heterotrophic bacteria in water. Also known as "heterotrophic plate count"

### 3.4

#### total coliform bacteria

bacteria that are gram-negative, oxidase-negative, non-sporing rods capable of growing aerobically on an agar medium containing bile salts, and able to ferment lactose within 48 h at 35 °C to 37 °C with the production of both acid and gas or capable of producing colonies with a typical golden-green metallic sheen on m-Endo medium

## 4 Apparatus

### 4.1 Pipettes

#### 4.1.1 Type and capacity

Blow-out type graduated pipettes of capacity 10 ml, 5 ml and 1 ml and that comply with the relevant requirements of SABS ISO 835-4. (Disposable plastics pipettes pre-sterilized by reliable commercial manufacturers may also be used.)

#### 4.1.2 Sterilization

Protect the mouth end of the pipettes with cotton wool plugs, and place them in suitable (but not copper) containers or wrap them in paper. Sterilize in a hot-air oven at 170 °C ± 2 °C for 1 h, or autoclave at 121 °C ± 2 °C for 20 min.

### 4.2 Sample bottles

#### 4.2.1 Type

Neutral glass sample bottles or equally efficient containers made of material such as polypropylene, of nominal capacity at least 1 l (preferably wide-mouthed), that have ground-glass or screw-capped closures, that are autoclavable and are made of bacteriologically inert materials.

#### 4.2.2 Dechlorination

If the water to be sampled contains (or is likely to contain) traces of chlorine, chloramine or both, add, before sterilization, enough of a solution of sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) to the clean, dry sample bottle to neutralize any trace of these substances. Use twice the quantity of sodium thiosulfate necessary to react with the expected total amount of chlorine in the sample. (The presence of 0,8 ml of a 100 g/l solution of sodium thiosulfate is sufficient to neutralize up to 60 mg of residual chlorine without affecting the microbiological quality).

#### 4.2.3 Sterilization

Sterilize the bottles in an autoclave at 121 °C ± 2 °C for 20 min. Alternatively, the bottles may be sterilized by placing them in a hot-air oven at 170 °C ± 2 °C for 1 h, or by another suitable method.

Use autoclave tape to indicate that autoclaving has been done. Check a batch for sterility by rinsing a bottle with sterile water, plating out with plate count agar (see 5.4), incubating at 37 °C for 48 h and examining for growth.

### 4.3 Membrane filters

#### 4.3.1 Types

Membrane filters (preferably marked with a grid) that have been proved to provide full bacterial retention and satisfactory speed of filtration, to be stable in use, and to be free from chemicals that retard the growth and development of bacteria. Use membrane filters with a maximum pore size not exceeding 0,45 µm.

#### 4.3.2 Sterilization

Individually packed membrane filters pre-sterilized by reliable commercial manufacturers are recommended. If membranes are to be sterilized, moisten the membranes with sterile, distilled water (to prevent curling), interleave with filter paper, and pack them tightly into Petri dishes that are kept closed. Sterilize by immersing in boiling water for 1 h or by autoclaving at 115 °C ± 2 °C for 15 min. (Over-heating the membranes, i.e. heating in excess of 115 °C for longer than 15 min, will result in a serious deterioration in their filtering efficiency.)

### 4.4 Membrane filter holders

#### 4.4.1 Type

A membrane filter holder constructed of a non-corrosive bacteriologically inert material that permits all fluid being filtered to pass through the membrane.

#### 4.4.2 Sterilization

Assemble the filter-holder loosely, ensuring that the porous plate is seated flush with the top surface of its receptacle. Wrap the assembled filter holder in brown paper or other suitable material and sterilize by autoclaving at 121 °C ± 2 °C for 20 min or by disinfecting with 70 % alcohol and rinsing with sterile water. Disinfection of the filter holders between successive samples shall be carried out.

### 4.5 Forceps

#### 4.5.1 Type

Round-tipped forceps that have smooth inner sides to their jaws.

#### 4.5.2 Sterilization

Sterilize by dipping in methylated spirits or technical methanol and then igniting the adherent liquid, or by another suitable method.

NOTE Ethanol and methanol are volatile and hygroscopic liquids and should not be used if allowed to stand in an open container for more than 24 h. It is recommended that these liquids be used from freshly opened containers only.

## 4.6 Petri dishes

### 4.6.1 Type

Petri dishes of glass or plastics, or other suitable material, and of such dimensions that the membrane filters can lie flat on the surface of the culture medium in them and can be removed without difficulty.

### 4.6.2 Sterilization

Place the Petri dishes in a Petri dish container (not a copper container) or wrap in paper. Where possible, sterilize in a hot-air oven at  $170\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  for 1 h, or autoclave at  $121\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  for 20 min.

Disposable plastics Petri dishes pre-sterilized by reliable commercial manufacturers are recommended. (Plastics Petri dishes are destroyed by heat and can only be used once.)

## 4.7 Incubators

4.7.1 Water-baths equipped with mechanical stirrers, and maintained at  $35\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$  or  $45\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$  (as relevant).

4.7.2 Good quality incubators circulating warm air at  $35\text{ }^{\circ}\text{C} \pm 1,0\text{ }^{\circ}\text{C}$  or  $37\text{ }^{\circ}\text{C} \pm 1,0\text{ }^{\circ}\text{C}$  (as relevant).

## 4.8 Culture tubes

Rimless test-tubes of nominal dimensions 18 mm × 160 mm and 20 mm × 150 mm or bottles (see SABS SM 552) of nominal capacity 30 ml.

## 4.9 Fermentation (Durham) tubes

Tubes of sizes suitable for use in the culture tubes or bottles.

## 4.10 pH meter

A pH meter accurate to 0,1 pH unit at 25 °C.

## 4.11 Other equipment

See SABS SM 552.

## 5 Culture media<sup>2)</sup> and reagents

### 5.1 Purity of ingredients

Ensure that the purity of the ingredients of media and reagents complies with the relevant requirements given in SABS SM 553. Commercially available culture media of good quality should preferably be used. All culture media shall be constituted as prescribed.

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2) Other media giving a comparable result may be used.

## 5.2 m-Endo agar LES

### 5.2.1 Ingredients

Agar .....	15 g
Lactose .....	9,4 g
Tryptose .....	7,5 g
Castlone .....	3,7 g
Sodium chloride .....	3,7 g
Thiopeptone .....	3,7 g
Dipotassium phosphate .....	3,3 g
Sodium sulfite .....	1,6 g
Yeast extract .....	1,2 g
Monopotassium phosphate .....	1 g
Basic fuchsin .....	0,8 g
Sodium desoxycholate .....	0,1 g
Sodium lauryl sulfate .....	0,05 g
Water (distilled) .....	1 000 ml

### 5.2.2 Preparation

Suspend the ingredients in the water. Add 20 ml of ethanol (a volume fraction of 95 %) and allow to stand for 10 min. Dissolve the ingredients completely by heating to boiling. Cool to 45 °C to 50 °C and dispense into Petri dishes, ensuring that the depth of the agar in each plate is at least 3 mm.

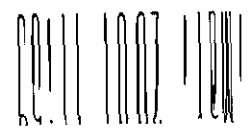
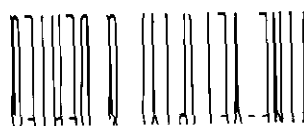
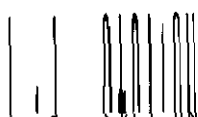
NOTE 1 **WARNING:** Basic fuchsin is a potential carcinogen and care should be taken to avoid inhalation of the powdered dye and contamination of the skin.

NOTE 2 m-Endo agar LES is sensitive to strong artificial light or direct sunlight. It should therefore be stored in the dark and exposed to light for the minimum time when used.

## 5.3 mFC agar

### 5.3.1 Ingredients

Agar .....	13 g
Lactose .....	12,5 g





Tryptose .....	10 g
Proteose peptone No. 3 .....	5 g
Sodium chloride .....	5 g
Yeast extract .....	3 g
Bile salts No. 3 .....	1,5 g
Aniline blue (water blue) .....	0,1 g
Water (distilled) .....	1 000 ml

### 5.3.2 Preparation

Suspend the ingredients in the water and dissolve completely by heating to boiling. Cool to 45 °C to 50 °C and dispense into Petri dishes, ensuring that the depth of the agar in each plate is at least 3 mm.

## 5.4 Plate count agar

### 5.4.1 Ingredients

Agar .....	14,5 g
Tryptone .....	5 g
Yeast extract .....	2,5 g
Dextrose .....	1 g
Water (distilled) .....	1 000 ml

### 5.4.2 Preparation

Dissolve the tryptone, yeast extract and dextrose in the water and so adjust the pH value that it will be  $7,2 \pm 0,1$  after sterilization. Add the agar, and steam until all the ingredients are dissolved. Dispense 15 ml volumes into tubes or bottles. Sterilize by autoclaving at  $121 \text{ °C} \pm 2 \text{ °C}$  for 15 min.

## 5.5 Tryptone water

### 5.5.1 Ingredients

Tryptone .....	10 g
Sodium chloride .....	5 g
Water (distilled) .....	1 000 ml

### 5.5.2 Preparation

Dissolve the ingredients in the water and so adjust the pH value that it will be  $7,4 \pm 0,1$  after sterilization. Dispense 10 ml volumes into tubes or bottles. Sterilize by autoclaving at  $121 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$  for 15 min.

## 5.6 Kovacs reagent

### 5.6.1 Ingredients

Paradimethylaminobenzaldehyde .....	5 g
Amyl alcohol (pyridine free) .....	75 ml
Hydrochloric acid (concentrated) .....	25 ml

### 5.6.2 Preparation

Dissolve the aldehyde in the alcohol by heating gently in a water-bath maintained at  $50 \text{ }^\circ\text{C}$  to  $55 \text{ }^\circ\text{C}$ .

Cool and carefully add the acid and mix. Allow to stand for 24 h prior to use. Protect from light and store at  $4 \text{ }^\circ\text{C}$ . The reagent shall be light yellow to light brown in colour.

NOTE - Only the best quality ingredients should be used, since poor quality ingredients can influence the test results.

## 5.7 Peptone water

### 5.7.1 Ingredients

Peptone .....	1 g
Sodium chloride .....	0,5 g
Water (distilled) .....	1 000 ml

### 5.7.2 Preparation

Dissolve the ingredients in the water. Adjust the pH value to  $7,0 \pm 0,1$  and dispense 9 ml volumes into screw-cap bottles of nominal capacity 30 ml. Sterilize by autoclaving at  $121 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$  for 15 min.

## 5.8 Lauryl tryptose broth (single strength)

### 5.8.1 Ingredients

Tryptose .....	20 g
Lactose .....	5 g
Sodium chloride .....	5 g

Dipotassium phosphate .....	2,75 g
Monopotassium phosphate .....	2,75 g
Sodium lauryl sulfate (specially pure) .....	0,1 g

### 5.8.2 Preparation

Dissolve the tryptose, sodium chloride, lactose and phosphates in distilled water by warming, and dilute to 1 l. Add the sodium lauryl sulfate and mix gently to avoid froth formation. Adjust the pH value to  $6,8 \pm 0,2$  and dispense 10 ml volumes into tubes or bottles each containing an inverted fermentation (Durham) tube. Sterilize by autoclaving at  $121 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$  for 15 min.

### 5.9 Lauryl tryptose broth (double strength)

Proceed as in 5.8, but double the quantities of the ingredients.

### 5.10 Brilliant green bile broth

#### 5.10.1 Ingredients

Ox bile (Dehydrated, purified) .....	20 g
Lactose .....	10 g
Peptone .....	10 g
Brilliant green solution (0,1 g of brilliant green per 100 ml of water) .....	13,3 ml

#### 5.10.2 Preparation

Dissolve the peptone in 500 ml of distilled water. Add the 20 g of dehydrated ox bile dissolved in 200 ml of distilled water. Ensure that the pH value of the solution is in the range 7,0 to 7,5. Make up to approximately 975 ml with distilled water, add the lactose, and adjust the pH value to 7,4. Add the brilliant green solution and make up to 1 l with distilled water. Dispense 10 ml volumes into tubes or bottles each containing an inverted fermentation (Durham) tube. Sterilize by autoclaving at  $121 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$  for 15 min.

## 6 Microbiological examination of water using the membrane filter method

### 6.1 Examination for total coliform bacteria

#### 6.1.1 Assembly of membrane filter holder

Ensure that the water to be tested is in a sterile sample bottle as specified in 4.2.

Immediately before use, attach the sterile filter holder (see 4.4) to a filter flask. Dismantle the holder and, using sterile forceps (see 4.5), place a sterile membrane filter (see 4.3) over the porous plate, grid-side uppermost. Re-assemble the holder.

### 6.1.2 Agar medium

Ensure that the surface of the m-Endo agar LES (see 5.2) in each Petri dish is free from excess moisture.

### 6.1.3 Procedure

**6.1.3.1** Thoroughly mix the water sample by rapidly inverting and righting the sample container approximately ten times (by rapid movement of the wrist). Aseptically transfer 100 ml of the sample to the assembled membrane filter holder (see 6.1.1) and filter by applying suction to the filter flask.

**6.1.3.2** After the 100 ml water sample has been filtered, rinse the funnel three times with 20 ml to 30 ml volumes of sterile water. Carefully dismantle the holder, and, using sterile forceps, aseptically transfer the membrane, grid-side uppermost, to a plate of m-Endo agar LES (see 6.1.2). Ensure that no air-bubble is trapped between the membrane and the surface of the agar and that good wetting contact is maintained.

**6.1.3.3** Invert and incubate the m-Endo plates at  $35\text{ }^{\circ}\text{C} \pm 1,0\text{ }^{\circ}\text{C}$  for 18 h to 24 h in a dark container. Examine the plates and count the number of coliform colonies, i.e. colonies that have a pink to dark-red colour with a golden-green metallic sheen. If more than one filter was used to filter the 100 ml water sample, count all the coliform suspect colonies on all the filters. The sheen area might vary in size from small pinhead to complete coverage of the colony surface. Colonies that lack sheen are considered to be non-coliform.

**6.1.3.4** If it is suspected that the water sample contains more than 30 coliform bacteria per 100 ml, suitable dilutions may be prepared and filtered.

**6.1.3.5** Calculate the number of coliform bacteria ( $TN_c$ ) per 100 ml of water sample as follows:

$$TN_c = \frac{Cc \times DF \times 100}{M_s}$$

where

$Cc$  is the total number of colonies counted;

$DF$  is the dilution factor;

$M_s$  is the millilitres of sample filtered.

Carry out the test at least in duplicate and preferably in triplicate.

## 6.2 Examination for faecal coliform bacteria

**6.2.1** Proceed as in 6.1 but use a plate of mFC agar (see 5.3), and incubate the mFC agar plates at  $45\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$  for 18 h to 24 h.

**6.2.2** Examine the plates and count the number of colonies that exhibit a blue centre with a translucent periphery. These colonies are faecal coliforms. Non-faecal coliform colonies are grey to cream coloured.



7.2.4 Transfer 1 ml of each of the subsequent dilutions (see 7.1) into each of three tubes or bottles containing single-strength lauryl tryptose broth (see 5.8). Use a fresh sterile pipette for each dilution.

7.2.5 Incubate the inoculated tubes or bottles at  $37\text{ }^{\circ}\text{C} \pm 1,0\text{ }^{\circ}\text{C}$  for 48 h.

7.2.6 Examine the cultures after incubation and regard as positive reactions those that show turbidity due to bacterial growth and gas formation. Gas formation is indicated by an amount of gas at least sufficient to fill the concavity of the top of the fermentation (Durham) tube.

7.2.7 For each dilution, count and record the number of tubes or bottles showing a positive reaction.

### 7.3 Confirmatory tests

#### 7.3.1 Coliform bacteria

To confirm the presence of coliform bacteria, subculture from each tube or bottle of lauryl tryptose broth giving a positive result, into a tube or bottle of brilliant green bile broth (see 5.10) and incubate at  $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ . Examine for gas formation within 48 h (see 7.2.6). The formation of gas confirms the presence of coliform bacteria.

#### 7.3.2 Faecal coliform bacteria

To confirm the presence of faecal coliform bacteria, proceed as in 7.3.1, but incubate in a water-bath, maintained at  $45\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$ , for 24 h. Examine for gas formation (see 7.2.6). The formation of gas confirms the presence of faecal coliform bacteria.

#### 7.3.3 *Escherichia coli*

To confirm the presence of *E. coli*, proceed as in 7.3.2 but also subculture into a bottle of tryptone water (see 5.5). Incubate in a water-bath maintained at  $45\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$  for 24 h. After incubation, test for the formation of indole by adding 0,3 ml to 0,5 ml of Kovacs reagent (see 5.6) to each bottle of inoculated tryptone water, shake gently and allow to stand for 10 min. The development of a red colour denotes the presence of indole. Bottles showing gas formation and the presence of indole confirm the presence of *E. coli*.

NOTE The detection of *E. coli* is regarded as satisfactory evidence of faecal pollution. However, further tests for the confirmation of *E. coli* may be carried out if considered necessary.

#### 7.3.4 Enumeration

For each dilution, count and record the number of tubes or bottles showing a positive reaction.

### 7.4 Expression of results

#### 7.4.1 Calculation

From the number of tubes or bottles of lauryl tryptose broth giving positive reactions, calculate, by reference to table 1, the most probable number (MPN) of coliform bacteria, faecal coliform bacteria and *E. coli*.

#### 7.4.2 Selection of dilutions

For each sample examined, select three consecutive dilutions in accordance with one of the following rules, as appropriate:

- a) Select the highest dilution (i.e. that having the lowest concentration or the smallest amount of sample) yielding positive results together with the two preceding sets of dilutions (see 7.4.4, examples A and B).
- b) If fewer than three sets of dilutions give positive results, start with the set containing the lowest dilution (i.e. that having the highest concentration or the largest amount of sample) (see 7.4.4, example C).
- c) If only one set of dilutions gives a positive result, use this dilution and the one higher and lower (see 7.4.4, example D), except when the set of dilutions giving a positive result is found at the level of the first dilution prepared. In this case it is necessary to select the first three dilutions for the calculation of the MPN.

#### 7.4.3 Determination of the MPN Index

Determine the MPN Index by the number of positive bottles in each of the three consecutive dilutions selected (in accordance with the rules in 7.4.2) from table 1.

Example: Should the number of positive bottles for the three consecutive dilutions selected read 3, 2, 1 (see 7.4.4, example B), then by consulting table 1, find in the first three columns the appropriate sequence of numbers, i.e. 3, 2, 1 and in the fourth column, read the MPN Index, which, in this case, is 15.

#### 7.4.4 Calculation of MPN

Calculate the number of coliform bacteria, faecal coliform bacteria and *E. coli* per 100 ml of water sample by multiplying the MPN index (see 7.4.3) by the reciprocal of the lowest dilution selected (i.e. that having the highest sample concentration) times 100. When the lowest dilution selected corresponds to the tubes or bottles inoculated with 10 ml, first divide the MPN index by 10. The result may be expressed as a number between 1,0 and 9,9 multiplied by  $10^x$  where x is the appropriate power of 10.

Confidence limits are given in table 1.

Example: With reference to example A in the text, the MPN index of 15 (see 7.4.3) is divided by 10, i.e. the volume of the inoculum in the lowest dilution selected. The quotient thus obtained, i.e. 1,5, is multiplied by 1,0, i.e. the reciprocal of the lowest dilution ( $10^0$ ) selected. The product 1,5 is then multiplied by 100, the specified reference volume, to obtain the number of bacteria per 100 ml of water sample.

NOTE "+" implies a positive result, "-" a negative result.

Example A:

Test sample (dilution 10 <sup>0</sup> )	(10 ml)	: 3	tubes + ;	0 tubes -
Test sample (dilution 10 <sup>0</sup> )	(1 ml)	: 2	tubes + ;	1 tube -
Dilution 10 <sup>-1</sup>	(1 ml)	: 1	tube + ;	2 tubes -
Dilution 10 <sup>-2</sup>	(1 ml)	: 0	tubes + ;	3 tubes -
Dilution 10 <sup>-3</sup>	(1 ml)	: 0	tubes + ;	3 tubes -

From table 1, the MPN index is 15 and the calculation gives an MPN of  $\frac{15}{10} \times 1 \times 100$ , i.e.  
1,5 × 10<sup>2</sup> bacteria per 100 ml of water sample.

Example B:

Test sample (dilution 10 <sup>0</sup> )	(10 ml)	: 3	tubes + ;	0 tubes -
Test sample (dilution 10 <sup>0</sup> )	(1 ml)	: 3	tubes + ;	0 tubes -
Dilution 10 <sup>-1</sup>	(1 ml)	: 3	tubes + ;	0 tubes -
Dilution 10 <sup>-2</sup>	(1 ml)	: 2	tubes + ;	1 tube -
Dilution 10 <sup>-3</sup>	(1 ml)	: 1	tube + ;	2 tubes -
Dilution 10 <sup>-4</sup>	(1 ml)	: 0	tubes + ;	3 tubes -

From table 1, the MPN index is 15 and the calculation gives an MPN of  $\frac{15}{10} \times 10 \times 100$ , i.e.  
1,5 × 10<sup>3</sup> bacteria per 100 ml of water sample.



Example C:

Test sample (dilution 10 <sup>0</sup> )	(10 ml)	: 2	tubes + ;	1 tube -
Test sample (dilution 10 <sup>0</sup> )	(1 ml)	: 1	tube + ;	2 tubes -
Dilution 10 <sup>-1</sup>	(1 ml)	: 0	tubes + ;	3 tubes -
Dilution 10 <sup>-2</sup>	(1 ml)	: 0	tubes + ;	3 tubes -
Dilution 10 <sup>-3</sup>	(1 ml)	: 0	tubes + ;	3 tubes -

From table 1, the MPN index is 1,5 and the calculation gives an MPN of  $\frac{1,5}{10} \times 1 \times 100$ , i.e.  
1,5 × 10<sup>1</sup> bacteria per 100 ml of water sample.

Example D:

Test sample (dilution 10 <sup>0</sup> )	(10 ml)	: 0	tubes + ;	3 tubes -
Test sample (dilution 10 <sup>0</sup> )	(1 ml)	: 1	tube + ;	2 tubes -
Dilution 10 <sup>-1</sup>	(1 ml)	: 0	tubes + ;	3 tubes -
Dilution 10 <sup>-2</sup>	(1 ml)	: 0	tubes + ;	3 tubes -
Dilution 10 <sup>-3</sup>	(1 ml)	: 0	tubes + ;	3 tubes -

From table 1, the MPN index is 0,30 and the calculation gives an MPN of  $\frac{0,3}{10} \times 1 \times 100$ , i.e.  
3 × 10<sup>0</sup> bacteria per 100 ml of water sample.

Table 1 — MPN index and confidence limits

1	2	3	4	5	6
Number of positive tubes for the three dilutions selected <sup>1)</sup>			MPN Index	95 % Confidence limits	
First	Second	Third		Lower	Upper
0	0	0	< 0,30	0,00	0,94
0	0	1	0,30	0,01	0,95
0	1	0	0,30	0,01	1,00
0	1	1	0,61	0,12	1,70
0	2	0	0,62	0,12	1,70
0	3	0	0,94	0,35	3,50
1	0	0	0,36	0,02	1,70
1	0	1	0,72	0,12	1,70
1	0	2	1,1	0,4	3,5
1	1	0	0,74	0,13	2,00
1	1	1	1,1	0,4	3,5
1	2	0	1,1	0,4	3,5
1	2	1	1,5	0,5	3,8
1	3	0	1,6	0,5	3,8
2	0	0	0,82	0,15	3,50
2	0	1	1,4	0,4	3,5
2	0	2	2,0	0,5	3,8
2	1	0	1,5	0,4	3,8
2	1	1	2,0	0,5	3,8
2	1	2	2,7	0,9	9,4
2	2	0	2,1	0,5	4,0
2	2	1	2,8	0,9	9,4
2	2	2	3,5	0,9	9,4
2	3	0	2,9	0,9	9,4
2	3	1	3,6	0,9	9,4
3	0	0	2,3	0,5	9,4
3	0	1	3,8	0,9	10,4
3	0	2	6,4	1,8	18,1
3	1	0	4,3	0,9	18,1
3	1	1	7,5	1,7	19,9
3	1	2	12	3	36
3	1	3	18	3	38
3	2	0	9,3	1,8	36,0
3	2	1	15	3	38
3	2	2	21	3	40
3	2	3	29	9	99
3	3	0	24	4	99
3	3	1	45	9	198
3	3	2	110	20	400
3	3	3	> 110		

<sup>1)</sup> See de MAN, J.C. MPN tables corrected *Eur. J. Appl. Microbiol. Biotechnol.* 17, 1983, 301 - 305.

## 8 Standard plate count to estimate the total number of viable heterotrophic bacteria in water samples

**8.1** Thoroughly mix the water sample by rapidly inverting and righting the sample container approximately ten times (by rapid movement of the wrist). Aseptically transfer 1 ml of the sample, or 1 ml of appropriate ten-fold dilutions of the sample in a diluent, to each of two or three Petri dishes (see 4.6) of diameter approximately 85 mm to 90 mm, to enable the test to be carried out in duplicate and preferably in triplicate.

**8.2** Within 20 min. of transferring the samples to the Petri dishes, add to each of the Petri dishes 15 ml of plate count agar (see 5.4) previously melted and cooled to 45 °C to 50 °C.

**8.3** Keeping the Petri dishes flat on the bench, immediately mix the contents of each dish by a combination of rapid but gentle to-and-fro and rotary movements for a period of 10 s. Allow the Petri dishes to stand until the agar has solidified.

**8.4** Invert the Petri dishes and incubate at 35 °C ± 1 °C for 48 h.

**8.5** Count the colonies on those plates that contain between 30 and 300 colonies. From the counts so obtained, calculate the average total plate count (TPC<sub>a</sub>) per millilitre of sample as follows:

$$TPC_a = \frac{TNC}{P_N} \times DF$$

where

*TNC* is the total number of colonies counted;

*P<sub>N</sub>* is the number of plates counted;

*DF* is the dilution factor.

Count colonies on plates from the same dilution only and use these in the calculation.

## 9 Microbiological examination of water, using the coliphage detection method

Use ISO 10705-1.

## Bibliography

DE MAN, J.C. MPN tables corrected. *European Journal of Applied Microbiology and Biotechnology*, 1983, vol. 17, p. 301-305.