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The quickest, most accurate and easy-to-use viral indicator tests available for **microbial water quality assessment**

# Enumeration of Somatic Coliphages

## Easy Kit for 1-10 ml

According to the ISO 10705-2 method

### Application

Raw and treated wastewater, surface water, recreational water, shellfish extracts, sediments and sludge extracts after dilution where necessary

A NEW APPROACH FOR WATER TESTING

# User **Guide**

SAVE LIVES · SAVE TIME · SAVE COSTS

70 assays

Cat. No, BP1601

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## 1. GENERAL INTRODUCTION

Historically water quality control has been mainly done through bacterial indicators, but currently, viral indicators have emerged to improve quality controls of water, biosolids and food. Thus, many regulations have been created in different countries consolidating bacteriophages, virus infecting bacteria, as viral indicators.

Bacteriophages provide complementary advantages to bacterial indicators since they are present in a way similar but usually persist longer in the environment and provide information about viral pathogens which are not properly represented by studying only bacterial indicators. Issues such as resuscitation or recovery of injured bacteriophages do not seem to occur. This is an advantage when clear effects of the treatment process need to be evaluated and certified.

Somatic coliphages are bacteriophages of enteric origin that infect *Escherichia coli* through cell surface receptors. The presence of somatic coliphages in a water sample usually indicates pollution by human or animal faeces or by wastewater containing these excreta. They thus provide a relatively rapid and simple method for faecal pollution detection, and their resistance in water and food resemble that of human enteric viruses more closely than faecal bacteria, commonly used as water or food quality indicators.

Somatic coliphages are present in water, wastewater, biosolid and food guidelines and regulations complementing the use of bacterial indicators such as *E. coli* and enterococci.

Somatic coliphages are detected by lysis of suitable bacteria (host bacteria). The standardised method (ISO 10705-2) for quantifying them is based on generating a zone of cell destruction (plaque) in a monolayer of the suitable host bacteria.

## 2. INTENDED USE

This quantitative detection kit is designed for the specific detection and enumeration of somatic coliphages by incubating the sample with the appropriate host strain. The method is applicable to raw and treated wastewater, surface water, recreational water, sediments and sludge extracts after dilution where necessary. The method is also applicable to shellfish extracts.

## 3. KIT PRINCIPLE AND DESCRIPTION

This detection and enumeration kit is based on ISO 10705-2. It contains all the consumables and biological material required to perform the analysis, including freeze-dried specific host-cells for the somatic coliphage group which are ready for use after 120-150 min of incubation.

The sample or the appropriate dilution is mixed with a small volume of semi-solid nutrient medium. The host strain culture is added and plated on a solid nutrient medium. After this, incubation and reading of plates for visible plaques takes place. The results are expressed as the number of plaque-forming units (pfu) per unit of sample volume.

## 4. KIT CONTENTS

### 4.1. Contents

| | | Reagent/Material | Description |
|---|---|---|---|
| 10 x | | *E.coli* strain WG5 known as strain CN | Three mL glass vial with septum containing a tablet of *E.coli* strain WG5 |
| 1 x | | Nalidixic acid | Five mL glass vial with septum containing powder to reconstitute at 25 mg/mL |
| 1 x | | Distilled water | Five mL glass vial with septum containing 3 mL of distilled water for nalidixic acid reconstitution |
| 1 x | | Sodium hydroxide 1.0 M | Two mL tube with bottle screw cap containing 1 mL NaOH solution for nalidixic acid reconstitution |
| 1 x | | Calcium chloride 1.0 M | Two mL tube with reconstitution containing 1.8 mL $CaCl_2$ solution |
| 5 x | | Bacteriophage ϕx174 | Five mL glass vial with septum containing lyophilised positive control |
| 5 x | | Phosphate Buffered Saline (PBS) | Two mL tube with reconstitution containing 2 mL of PBS to reconstitute bacteriophages |
| 10 x | | Semi-solid Modified Scholtens' Agar (ssMSA) | Fifty mL Falcon tube containing 23 mL of ssMSA |
| 5 x | | Blank | Two mL tube with reconstitution containing 2 mL of sterile diluent to be used as a blank |
| 10 x | | Modified Scholtens' Broth (MSB) | Fifty mL Erlenmeyer with bottle cap containing 10 mL of MSB |
| 70 x | | Tube 10 mL | Empty 10 mL sterile tube for mixing ssMSA, inoculum culture and sample |

### 4.2 Additional material not provided in the kit

| | | Reagent/Material | Description |
|---|---|---|---|
| 70 x | | Sterile Petri dishes | 90 mm Petri dishes used to prepare the MSA plates, which are used during the overnight incubation |
| 1 x | | Modified Scholten's Agar power media (BP1638) | Ready to use powder media to prepare MSA plates |

## 5. REAGENTS PREPARATION, STORAGE AND SHELF LIFE

### *E. coli* (WG5) tablets

- Store *E. coli* tablets packed in vials and inside the box at (-20±2)°C.

### Nalidixic acid

- Store at (5±3)°C until the date marked on the vial.
- Dissolve nalidixic acid powder using 0.6 mL of NaOH solution, add 2.4 mL of distilled water and mix well to obtain a 25 mg/mL solution.
- Reconstituted solution can be stored at (5±3)°C in the dark for no longer than 8 h or at (-20±2)°C for up to 6 months, so once reconstituted it is recommended to prepare aliquots of the volume to be used.

### Calcium chloride

- Store in the dark at (5±3)°C.

### Reference bacteriophage for positive control

- Store the bacteriophage ϕX174 supplied lyophilised in glass vials at (-20±2)°C.
- Reconstitute with 0.5 mL of PBS and after gently shaking add 1 mL more. Allow 10 min to complete reconstitution. The final concentration of the suspension will be 20-90 pfu/mL, depending on the batch, and will be marked on the vial.

### Semi-solid Modified Scholtens' Agar (ssMSA)

- ssMSA is supplied in sterile 50 mL tubes.
- Store in the dark at (5±3)°C.

### Modified Scholtens' Broth (MSB)

- MSB is supplied in sterile 50 mL Erlenmeyers.
- Store in the dark at (5±3)°C.

## 6. REAGENTS, MATERIALS AND EQUIPMENT NOT INCLUDED

Apart from the kit, the following reagents and equipment are needed to perform the analysis:

### Reagents & Materials

- Modified Scholtens' Agar (MSA) powder media (Cat. No. BP1638).
- 70 units of 90 mm sterile Petri dishes
- Micropipette tips, aerosol resistant or positive-displacement.

### Equipment

- Incubator, thermostatically controlled at (36±2)°C.
- Water bath, thermostatically controlled at (55±1)°C.
- Boiling water bath.
- Incubator with shaking, thermostatically controlled at (36±2)°C (optional).
- Micropipettes (20-200 µL, 200-1,000 µL, 1,000-5,000 µL).
- Refrigerator, temperature set at (5±3)°C.
- Freezer, thermostatically controlled at (-20±2)°C.

## 7. ASSAY TIME TO RESULTS

One day, including incubation for (18±2)h.

## 8. NUMBER OF TESTS

This kit is designed to perform 70 assays, distributed as convenient by each user based on the number of dilutions and replicates per sample (e.g. ten samples can be analysed by duplicate and up to three different dilutions, including five positive controls and five negative controls).

## 9. GENERAL RECOMMENDATIONS

It is important to maintain aseptic conditions during the procedure to avoid cross-contaminations between samples and contamination from external sources.

In the case of samples with an expected high bacterial load, it is recommended to filter the sample beforehand through a 0.22 µm pore size, low-protein-binding (PES) membrane.

Since bacteriophages tend to generate aggregates, it is recommended to shake the samples gently before their analysis and always conduct positive controls.

## 10. PROCEDURE

### 10.1. FOR 1 ML SAMPLES

1. Prewarm one MSB Erlenmeyer per sample at (36±2)°C.

2. Aseptically add the *E. coli* WG5 strain tablet to the Erlenmeyer. Allow 10 min for rehydration.

3. Incubate at (36±2)°C for 120-150 min in a shaking rotator at 100-150 rpm. After the incubation time, quickly cool the culture by placing it in a refrigerator or in melting ice. Use the inoculum culture within the same working day.

4. Melt the ssMSA tubes (one tube per 7 assays). Slightly loosen the cap of the tubes and put them in a boiling water bath. It is important to check that the agar is totally melted before its

use. There are alternative methods to melt ssMSA tubes as the use of a microwave. However, such uses are always under client's responsibility.

5. Place in a water bath at (55±1)°C and let them reach this temperature.

6. Aseptically add 0.14 mL of the calcium chloride solution, prewarmed at room temperature.

7. Aseptically add 0.23 mL of the nalidixic acid solution, previously rehydrated, as described above.

8. Distribute 2.5 mL aliquots into red screw-capped tubes and place in a water bath at (55±1)°C.

9. Add 1 ml of the original sample (diluted or concentrated) prewarmed at room temperature to each red screw-capped tube. Examine each aliquot at least in duplicate.

10. Once the inoculum culture is ready, add 1 mL to each red screw-capped culture tube and mix carefully avoiding the formation of air bubbles.

11. Pour the contents onto a layer of complete MSA on a 90 mm plate pre prepared before starting the enumeration and prewarmed at room temperature. Distribute evenly and allow to solidify on a horizontal, cool surface.

12. Incubate the plates upside-down at (36±2)°C for (18±2)h. Do not stack more than six plates.

### 10.2. FOR 10 ML SAMPLES

For samples with low coliphage counts, 10 mL can be analyzed directly (ISO 10705-2, Section 11.4). Proceed as for 1 ml samples with the following modifications at steps 8-11:

Mix 5 mL of the sample with 10 mL of supplemented ssMSA and 1 mL of inoculum culture and pour onto a layer of complete MSA on a 140 mm plate. Repeat for the remaining 5 mL.

## 11. QUALITY CONTROL

### Negative Control

It is always recommended in each series of samples to examine a procedural blank using sterile diluent as the sample (ISO 10705-2). Materials for up to five negative samples are supplied in this kit.

### Positive Control

It is always recommended in each series of samples to examine a reference control of ϕX174. Materials for up to five positive controls are supplied in this kit.

Reconstitute the standard provided with the kit following the instructions described above and use the suspensions as the sample. Plate the reconstituted positive control following the procedure described above. The number of plaques obtained should be in the range of concentration indicated on the label of the positive control vial.

Discard the reference control samples if the mean number of pfu/mL is lower or higher than the Control Limits indicated on the label and the Certificate of Analysis.

## 12. INTERPRETATION OF RESULTS

Count plaques in selected plates with well-separated plaques, and preferably more than 30 and fewer than 200 whenever present. If only counts below 30 per plate are found, select plates inoculated with the largest volume of sample. Note that plaques may have different sizes and appearances. Using the number of plaques counted, calculate the number X of plaque-forming units (pfu) of somatic coliphages in 1 mL of the sample as follows:

$$X = \frac{N}{(n_1 V_1 F_1) + (n_2 V_2 F_2)}$$

Where

$X$ number of pfu/mL

$N$ total number of plaques counted

$n_1, n_2$ number of replicates counted

$V_1, V_2$ test volume (mL)

$F_1, F_2$ dilution factor (F=1 for an undiluted sample; F=0.1 for a ten-fold dilution)

If only one dilution/concentrate is counted, simplify the formula to:

$$X = \frac{N}{nVF}$$

Refer to ISO 8199 for further details.

## 13. APPENDIX

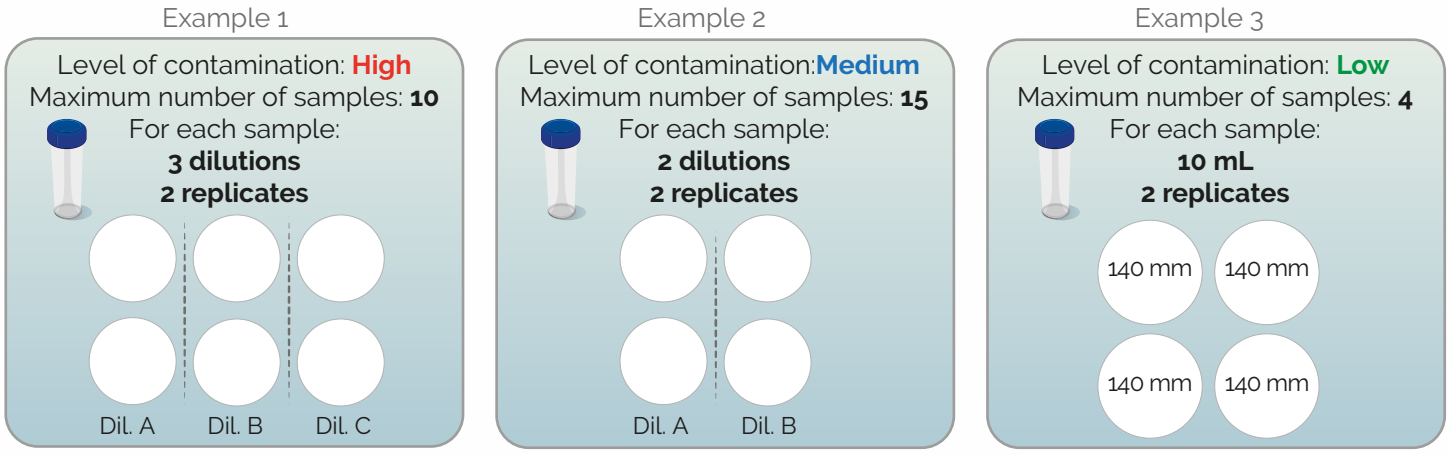
### A1. TROUBLESHOOTING

| Problem | Possible cause | Suggestions |
|---|---|---|
| There are agar clumps on the plates that impede plaque counting. | The ssMSA has partially solidified before use or it has not been adequately melted. | When melting the ssMSA, check that all the agar has been melted. Before adding the ssMSA to the tubes, check that it does not contain agar clumps. Prewarm the $CaCl_2$ and nalidixic acid before using them. Prewarm the inoculum culture before use. Prewarm samples at room temperature before use. Prewarm the MSA plates to room temperature before adding the mixture of sample, ssMSA and host bacteria. |
| Difficulties in distinguishing between bubbles and plaques. | Presence of bubbles in the ssMSA layer. | Avoid bubble formation when plating the mixture of sample, ssMSA and host bacteria. In order to distinguish between bubbles and plaques, bear in mind that bubbles have much sharper borders than plaques. |
| The bacterial lawn is not thick enough for the correct visualisation of plaques. | The inoculum culture grown has not been completed. | Make sure that the MSB is prewarmed to (36±2)°C before adding the *E. coli* WG5 strain tablet. This will facilitate bacterial growth. In addition the incubation time can also be increased slightly (15 - 30 minutes) but never to more than 180 minutes. |
| Negative control is positive. | There is cross-contamination with a positive sample. | The results obtained should be discarded and the samples retested, strictly following the instructions provided in this manual. Use new aliquots of the reagents and change the consumables batch. |
| Positive control is negative. | 1. Something has gone wrong in the analysis.<br>2. The stock of bacteriophage is degraded. | The results obtained should be discarded and the samples retested.<br>1. Strictly follow the instructions provided in this manual.<br>2. Strictly follow the rehydration and storage protocols for bacteriophage stock contained in this manual. Discard the current tube stored refrigerated and rehydrate a new one. |
| A shaking rotator is not available | | It is recommended to shack frequently by hand (around every 30 minutes) incubating at (36±2)°C for 120-150 min. |

### A2. USAGE EXAMPLES

The 70 assays supplied in this kit may be performed in a single working session or in different combinations up to a maximum of 10. The following figure shows some examples depending on the level of contamination of the sample. *E.coli* concentrations are used as a reference for the level of contamination: High (e.g., wastewater), $10^5$-$10^6$ CFU/100mL; Medium (e.g., surface water), $10^3$-$10^4$ CFU/100mL; Low (e.g., bathing water), $10^1$-$10^2$ CFU/100mL.

Remember to add positive and negative controls for each working session. The maximum number of samples that can be analysed also depends on the number of positive and negative controls that the user decides to perform in their trials.




## A3. REFERENCES

Coliphages 2018. http://www.coliphages.com

ISO 10705–2:2000. Water quality - Detection and enumeration of bacteriophages - Part 2: Enumeration of somatic coliphages. International Organisation for Standardisation, Geneva, Switzerland.

Jofre, J., F. Lucena, A. R. Blanch, and M. Muniesa. 2016. Coliphages as Model Organisms in the Characterization and Management of Water Resources. Water 8:199.

Jebri, S., Muniesa, M. and Jofre, J. 2017. General and host-associated bacteriophage indicators of fecal pollution. In: J. B. Rose and B. Jiménez-Cisneros, (eds) Global Water Pathogens Project. Part 2 Indicators and Microbial Source Tracking Markers. A. Farnleitner and A.R. Blanch (eds). UNESCO Global Water Pathogen Project.
http://www.waterpathogens.org/book/coliphage

## A4. RELATED PRODUCTS

- Enumeration of Somatic Coliphages. Easy kit for 100 ml (Cat. No. BP1604).
- Biological Material for the Enumeration of Somatic Coliphages. Usable with the ISO 10705-2 method (Cat. No.BP1603).
- Positive control for the Enumeration of Somatic Coliphages Usable with the ISO 10705-2, US-EPA 1601, 1602, 1642 and 1643 methods (Cat. No.BP1626).
- Bacterial Host Strain for the Enumeration of Somatic Coliphages Usable with the ISO 10705-2 method (Cat. No.BP1628).
- Modified Scholten's Agar (MSA) 0,5kg. Usable with the ISO 10705-2 method (Cat. No. BP1638).
- Modified Scholten's Broth (MSB) 0,5kg. Usable with the ISO 10705-2 method (Cat. No. Bp1637).

## A5. LEGAL NOTICE

**Product warranty**

This product has been designed for enumeration of somatic coliphages, and its performance is guaranteed in the manner described in this brochure. The purchaser must determine the suitability of the product for its particular use. Bluephage rejects any implicit warranty for any other use or adaptation to particular purposes. No other licence is granted expressly, impliedly, or by estoppel.

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