bluephage®

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

Enumeration of Somatic Coliphages

Easy Kit for 100 ml

Based on ISO 10705-2 and US-EPA 1602, 1642 and 1643 methods and using the host strain *E. coli* WG5

Application

Drinking water, reclaimed water and other samples with expected low counts of somatic coliphages

A NEW APPROACH FOR WATER TESTING

User **Guide**

SAVE LIVES · SAVE TIME · SAVE COSTS

10 assays

Cat. No. BP1604

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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 control 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 included 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 methods (ISO 10705-2, US-EPA 1602, 1642 and 1643) for detecting 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 a 100 mL water sample with the appropriate host strain. This method is applicable to samples with expected low counts of bacteriophages (e.g. drinking water and reclaimed water).

3. KIT PRINCIPLE AND DESCRIPTION

This detection and enumeration kit is based on ISO 10705-2 and US-EPA 1602, 1642 and 1643 methods. 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 100 mL water sample is mixed with a fresh culture of the host strain. This mixture is added to a melted solid nutrient medium and distributed by plating on five 140 mm diameter Petri dishes. After this, incubation and reading of plates for visible plaques takes place. The results are added up and expressed as the number of plaque-forming units (pfu) per 100 mL.

4. KIT CONTENTS

4.1. Contents

	Reagent/Material	Description
10 x 	*E.coli* strain WG5 known as strain CN	Screw-capped plastic vial containing the *E.coli* WG5 host strain and PBS for resuspension
10 x 	Modified Scholtens' Broth (MSB) with calcium	Sixty mL glass bottle containing 20 mL of MSB supplemented with calcium chloride.
5 x 	Bacteriophage ϕx174	Screw-capped plastic vial containing lyophilised positive control and 1.5 mL of PBS for resuspension
10 x 	2X Solid Modified Scholtens' Agar (MSA) with nalidixic acid	Two hundred and fifty mL glass bottle with microwave safety cap containing 100 mL of 2X MSA supplemented with nalidixic acid

4.2. Additional material not provided in the kit

	Reagent/Material	Description
50 x 	Sterile Petri dishes	140 mm Petri dishes ready to use

5. REAGENTS PREPARATION, STORAGE AND SHELF LIFE

***E. coli* (WG5) vials**

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

Reference bacteriophage for positive control

- Store the bacteriophage ϕX174 supplied lyophilised in vials at (-20±2)°C.

2X Modified Scholtens' Agar (MSA) with nalidixic acid

- 2X MSA is supplied in sterile 250 mL bottles. It contains nalidixic acid at 0.31 mg/ml to prevent growing of bacteria other than *E. coli* WG5.
- Store at room temperature or refrigerated.

Modified Scholtens' Broth (MSB) with calcium

- MSB is supplied in sterile glass bottles. It contains calcium chloride to favor coliphage adsorption to the bacterial host strain.
- Store at room temperature or refrigerated.

6. REAGENTS, MATERIALS AND EQUIPMENT NOT INCLUDED

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

Reagents & Materials

- 50 units of 140 mm sterile Petri dishes.
- Micropipette tips, aerosol resistant or positive displacement.

Equipment

- Incubator, thermostatically controlled at (36±2)°C.
- Water bath, thermostatically controlled at (49±1)°C.
- Microwave oven or autoclave.
- Incubator with shaking, thermostatically controlled at (36±2)°C (optional).
- Micropipettes (200-1000 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 10 assays with one 100 mL water sample in each one.

9. GENERAL RECOMMENDATIONS

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

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

10. PROCEDURE

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

2. Prewarm one *E.coli* WG5 vial at room temperature. Remove the seal from the cap and gently rotate it clockwise to release the host strain. Shake vigorously to resuspend the host strain and let it stand for 10 minutes upside-down at room temperature.

3. Open the *E.coli* WG5 vial and check that all the contents in the cap have been resuspended. Pour the host strain into the MSB bottle and 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 2X MSA bottle (one bottle per assay). Remove the red seal and heat the contents in the microwave oven during 3 min at 800 W or autoclave it at 121 ºC for 15 min (standard cycle). It is important to check that the agar is totally melted before its use. Replace the red seal in the cap to prevent contamination.

5. Place the melted 2X MSA bottle in a water bath at (49±1)°C and let it reach this temperature. The use of this temperature for the maintenance of the melted MSA is very important to prevent the formation of condensation drops later.

6. Prewarm 100 mL of water sample in a bottle at (36±2)°C. The *E.coli* WG5 culture stored in the refrigerator must also be tempered at (36±2)°C.

7. Aseptically add the 20 mL of the *E.coli* WG5 culture to the flask containing the 100 mL water sample and keep it at (36±2)°C for 5-10 minutes. Do not exceed this time. Increased contact time may result in replication of phages such that the initial concentration of phages is overestimated. After this, proceed with the next step immediately.

8. Aseptically pour the water sample with the *E.coli* WG5 culture into the melted 2X MSA bottle and mix carefully avoiding the formation of air bubbles with rotation movements (never shaking). It is very important that in this step the mix and the agar are well temperate.

9. Pour the contents onto five 140-mm diameter Petri dishes with an even distribution (approx. 40 - 45 mL per dish).

10. Allow to solidify on a horizontal surface at room temperature. It is highly recommended not to stack the Petri dishes to avoid formation of water drops due to condensation.

11. Incubate the plates upside-up at (36±2)°C for (18±2)h. It is also recommended to do not stack the Petri dishes to avoid the formation of water drops by condensation. If stacking is required for space reasons, stack the smallest possible number of Petri dishes.

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).

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.

Prewarm the lyophilised positive control at room temperature. Remove the seal from the cap and gently rotate it clockwise to release the reference bacteriophage. Shake vigorously to resuspend the lyophilised bacteriophage and let it stand for 10 minutes upside-down at room temperature.

Add 1 mL of the reconstituted coliphage solution to PBS or sterile diluent (e.g. sterile water, Ringer, etc.) up to a final volume of 100 mL. Then proceed as described above for the analysis of samples.

The final concentration of the suspension is marked on the Certificate of Analysis included in the kit.

Discard the reference control samples if the mean number of pfu/mL is lower or higher than the values indicated.

12. INTERPRETATION OF RESULTS

Count plaques in each plate. Note that plaques may have different sizes and appearances. Avoid misunderstandings due to bubble formation or condensed water drops. Bubbles have much sharper borders than plaques. Water drops produced by condensation have an irregular shape. Add up the number of plaques counted in all five plates, and express the result as the number of plaque-forming units (pfu) of somatic coliphages in 100 mL.

APPENDIX

A1. TROUBLESHOOTING

Problem	Possible cause	Suggestions
There are agar clumps on the plates that impede plaque counting.	The MSA has partially solidified before use or it has not been adequately melted.	When melting the MSA, check that all the agar has been melted. Before using the melted MSA, check that it does not contain agar clumps. Prewarm the water sample with the host-bacteria culture before use.
Difficulties in distinguishing between bubbles and plaques.	Presence of bubbles in the plates.	Avoid bubble formation when plating the mixture of sample, host bacteria and MSA. In order to distinguish between bubbles and plaques, bear in mind that bubbles have much sharper borders than plaques. Water drops produced by condensation have irregular shapes.
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 host strain. 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 or when lower or higher PFU values are obtained than those indicated on the Certificate of Analysis.	1. Something has gone wrong in the analysis. 2. The stock of bacteriophage is degraded.	The results obtained should be discarded and the samples retested. 1. Strictly follow the instructions provided in this manual. 2. Strictly follow the rehydration and storage protocols for bacteriophage stock contained in this manual. Discard any rest of reconstituted positive control and not reuse it. 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. 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.
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US-EPA Office of Water. Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure; EPA 821-R-01-030; US-EPA Office of Water: Washington, DC, USA, 2001.

US-EPA Office of Water. Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure; EPA 821-R-01-029; US-EPA Office of Water: Washington, DC, USA, 2001.

US-EPA Office of Water. Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure; EPA 820-R-18-001; US-EPA Office of Water: Washington, DC, USA, 2018.

US-EPA Office of Water. Method 1643: Male-specific (F+) and Somatic Coliphage in Secondary (No Disinfection Wastewater by the Single Agar Layer (SAL) Procedure; EPA 820-R-18-003; US-EPA Office of Water: Washington, DC, USA, 2018.

A3. RELATED PRODUCTS

- Enumeration of Somatic Coliphages Easy Kit for 1 mL (Cat. No. BP1601).
- 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 Broth (MSB) 0,5kg. Usable with the ISO 10705-2 method (Cat. No. BP1637).

A4. 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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