Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Detection of Somatic Coliphages in Water Samples – Performance Study of a Sample Concentration Kit and Ready-to-Use Culture Media

Abstract

ISO 10705-2 describes the cultivation-based detection and enumeration of somatic coliphages in water samples.

As a preliminary step that can be used beforehand, ISO 10705-3 specifies the general principles for assessing the performance of methods for the concentration of bacteriophages from water samples.

This report summarizes a performance study of ready-to-use culture media according to ISO 10705-2 and the performance test of a bacteriophage concentration kit according to ISO 10705-3. Surface water samples as well as drinking water samples spiked with bacteriophage phi X174 were included in the study.

Introduction

Coliphages are viruses that infect coliform bacteria. Since their hosts propagate in the gastrointestinal tract of humans and warm-blooded animals, their presence in drinking-water indicates fecal pollution and hence the possible presence of fecal pathogens (e.g., viruses and bacteria).[1–4]

Somatic coliphages are detectable by relatively simple plaque assays. Such an assay is described in ISO 10705-2.[5] In brief, the water sample to be analyzed and a fresh culture of an appropriate host for the virus (e.g., *Escherichia coli* ATCC 13706) are mixed with a liquified soft agar medium. The mixture is subsequently poured on an agar medium pre-cast in Petri dishes. After an overnight incubation the host cells grow into a lawn. The presence of coliphages leads to cell lysis and thus distinct plaques in the lawn of the host cells.

ISO 10705-2 describes three culture media required for the plaque assay:

1. Modified Scholten's broth (for propagation of the *E. coli* host)
2. Modified semisolid Scholten's agar (soft agar used to mix sample and host)
3. Modified Scholten's agar (agar pre-cast in Petri dishes)

We provide these culture media in a ready-to-use format. In this study the performance of these media was determined by two accredited water laboratories in comparison to freshly prepared in house media.

To reduce the sample volume and thus to vastly reduce the number of Petri dishes needed for testing by 95%, ISO 10705-3 specifies the general principles for assessing the performance of methods for the concentration of bacteriophages from water. Annex A gives examples of methods that have been found to be satisfactory, and their fields of application.

One method described is the membrane filtration method. It is based on the adsorption of coliphages to cellulose ester membranes in the presence of a specific binding buffer.[6] The subsequent desorption is performed in a lower volume of a specific elution buffer. Typically, a 100 mL sample is concentrated to a volume of 5 mL. In this study the performance of a commercial concentration kit (VIRAPREP® kit from GL Biocontrol)[7, 8] was determined in comparison to the direct method without sample pre-concentration. In both cases, subsequent virus quantification was performed according to ISO 10705-2.

Material

Inhouse preparation of Scholten's media was performed as described in ISO 10705-2. Ready-to-use Scholten`s media are manufactured by Condalab acc. ISO 10705-2 in an industrial scale and supplied by Merck (see **Table 1**).

All three media (broth, semisolid and solid agar) are supplied in bottles (**Table 1**). Semisolid and solid agar media were liquified at 98–100 °C in a water bath, then cooled to 47.5 ±2.5 °C in a second water bath and subsequently supplemented with 0.6 mL (per 100 mL) of a $CaCl_2$ x $2H_2O$ solution containing 14.6 g/100 mL before use.

For the studies described here Petri dishes with a diameter of 150 mm as well as 90 mm were used. Scholten's agar was filled into the Petri dishes one day before usage (50 mL per 150 mm and 20 mL per 90 mm plate). Plates were prewarmed to room temperature before use.

Escherichia coli ATCC® 13706 or ATCC® 700078 were used as host strain. Pre-cultures were cultivated as described in ISO 10705-2. For spiking of water samples bacteriophage phi X174 (*E. coli* 13706-B1) was used.

The VIRAPREP® kit is manufactured by GL Biocontrol[7, 8] (supplied by Merck: **Cat. No. AR00206**).

The water samples tested in this study are described in **Tables 2** and **3**.

The laboratories that conducted the tests were:

BIOFAQ LABORATOIRES SAS
Marion Mimbielle
491 rue Ch. Nungesser
Mas des Cavaliers 2
F-34130 MAUGUIO

TZW: DVGW-Technologiezentrum Wasser
Abteilung Wassermikrobiologie
Dr. Beate Hambsch
Karlsruher Straße 84
D-76139 Karlsruhe

Table 1: Ready-to-Use Scholten's media according to ISO 10705-2 supplied by Merck

Culture medium	Volume of medium in bottle (mL)	Total capacity of bottle (mL)	Cat. No.
Modified Scholten's broth (MSB)	25	125	**5249**
Modified semisolid Scholten's agar (ssMSA)	100	125	**5248-10X100ML**
Modified Scholten's agar (MSA)	200	250	**5247-10X200ML**

*Condalab media supplied by Merck

Methods

Quantification of coliphages in water samples was performed as described in ISO 10705-2.

From each water sample 100 mL were analyzed for a test.

- Where the phage concentration kit was used, the 100 mL of sample were concentrated to 5 mL before analysis, otherwise unconcentrated sample was processed directly.
- For each 150 mm agar plate, 5 mL of either concentrated or unconcentrated sample was mixed with a suspension of the host cells and subsequently with melted semisolid Scholten's agar and spread onto the plate.
- For each 90 mm plate, 1 mL of either concentrated or unconcentrated sample was mixed with a suspension of the host cells and subsequently with melted Scholten's semisolid agar and spread onto the plate.
- Phage concentration using the VIRAPREP® kit from GL Biocontrol was performed as outlined in the VIRAPREP® protocol (VIRAPREP® illustrated protocol).[8]

The phage quantification tests were performed by two independent laboratories. Samples selected by the two laboratories included drinking water, surface water and, only at BIOFAQ, wastewater (**Table 2** and **3**).

Water samples were spiked with coliphage phi X174 as indicated in **Tables 2** and **3**.

Table 2: Water samples and testing scheme performed at TZW

Water sample	Source (City) - Germany	Spiking (PFUs)	Testing
Drinking water 1	Hirschberg	54	1. Direct with inhouse media 2. Direct with Media from Merck* 3. VIRAPREP® Kit + Media from Merck*
Drinking water 2	Cölbe	54	1. Direct with inhouse media 2. Direct with Media from Merck* 3. VIRAPREP® Kit + Media from Merck*
Drinking water 3	Karlsruhe	54	1. Direct with inhouse media 2. Direct with Media from Merck* 3. VIRAPREP® Kit + Media from Merck*
Surface water 1	Rhine (Karlsruhe)	no	1. Direct with inhouse media 2. Direct with Media from Merck*
Surface water 2	Neckar (Ladenburg)	no	1. Direct with inhouse media 2. Direct with Media from Merck*

PFU (plaque forming units) per 100 mL sample

*provided by Condalab

Table 3: Water samples and testing scheme performed by BIOFAQ

Water sample	Source (City) - France	Spiking	Testing
Drinking water 1	Mauguio	10 PFU/ 100 mL	1. Direct with inhouse media 2. Direct with Media from Merck* 3. VIRAPREP® Kit + inhouse media 4. VIRAPREP® Kit + Media from Merck*
Drinking water 2	Mauguio	50 PFU/ 100 mL	1. Direct with inhouse media 2. Direct with Media from Merck* 3. VIRAPREP® Kit + inhouse media 4. VIRAPREP® Kit + Media from Merck*
Drinking water 3	Mauguio	100 PFU/ 100 mL	1. Direct with inhouse media 2. Direct with Media from Merck* 3. VIRAPREP® Kit + inhouse media 4. VIRAPREP® Kit + Media from Merck*
Surface water 1	Seine (Choisy le Roi)	no	1. Direct with inhouse media 2. Direct with Media from Merck* 3. VIRAPREP® Kit + inhouse media 4. VIRAPREP® Kit + Media from Merck*
Surface water 2	Marne (Neuilly-sur-Marne)	no	1. Direct with inhouse media 2. Direct with Media from Merck* 3. VIRAPREP® Kit + inhouse media
Waste water	-	no	1. Direct with inhouse media 2. Direct with Media from Merck*

The same drinking water sample was used but spiked with different numbers of coliphage phi X174. PFU = plaque forming units
*provided by Condalab

Results

The results of the study performed at TZW are summarized in **Table 4**. Recovery of coliphages for one spiked drinking water sample was identical for the Merck ready-to-use and the inhouse media. For two spiked drinking water samples and the two surface water samples, the PFU counts were higher on Merck RTU (ready-to-use) media. Detection after sample concentration with the VIRAPREP® kit was performed only for the spiked drinking water samples and in combination with Merck RTU media. Two samples could be evaluated. Compared to the direct method, recovery rates after *coli*phage concentration were 47% and 72%, respectively.

Table 4: Summary of the results of the study conducted at TZW

Water sample	TZW inhouse media (PFU)	Merck RTU media (PFU)	Sample concentration + Merck media (PFU)
Drinking water 1	26	45	21
Drinking water 2	35	46	33
Drinking water 3	33	33	n.a.
Surface water 1	365	657	n.a.
Surface water 2	674	941	n.a.

n.a. = not analyzed. PFU (plaque forming unit) values per 100 mL sample.

The results of the study performed at BIOFAQ are summarized in **Table 5**. Recovery of coliphages on BIOFAQ inhouse media and Merck supplied media was comparable, both when using the direct method and after sample concentration with the VIRAPREP® kit. Detection after sample concentration with the VIRAPREP® kit was performed for the spiked drinking water and the two surface water samples, each in combination with both media, inhouse and Merck supplied RTU. For the drinking water samples the recovery rates after concentration were between 75% and 92% of the counts of the samples analyzed directly. For the two surface water samples the values obtained with the direct method were too low for an accurate quantification. Here, only the samples after concentration could be evaluated.

Table 5: Summary of the results of the study conducted at BIOFAQ

Water sample	Direct analysis - BIOFAQ inhouse media (PFU)	Direct analysis - Merck Media (PFU)	Sample concentration + BIOFAQ inhouse media (PFU)	Sample concentration + Merck media (PFU)
Drinking water 1	16	13	12	12
Drinking water 2	50	67	42	59
Drinking water 3	125	120	97	98
Surface water 1	estimated at 300*	estimated at 500*	580	640
Surface water 2	<100*	<100*	120	120
Waste water (pfu/1 mL)	9500	14000	n.a.	n.a.

n.a. = not analyzed. PFU (plaque forming unit) values per 100 mL sample, if not otherwise indicated. *At detection limit

Discussion

Quantification of somatic coliphages in water samples according to ISO 10705-2 requires three different culture media. Merck offer these media in a ready-to-use format (**Table 1**). In this study the test results of two independent laboratories suggest that the Merck provided RTU media are well suited for the detection of somatic coliphages. In both laboratories the Merck media performed equally well or even slightly better when compared to freshly prepared inhouse media.

For the analysis of a 100 mL water sample according to the method described in ISO 10705-2, 20 Petri dishes with a diameter of 15 cm or 100 Petri dishes with a diameter of 9 cm are required. To reduce the number of plates, Annex A of ISO 10705-3 gives examples of methods that have been found to be satisfactory for the concentration of bacteriophages. The VIRAPREP® commercial concentration kit follows a method described in ISO 10705-3 and allows the concentration of a 100 mL sample to 5 mL. Hence, the number of plates required in the subsequent analysis according to ISO 10705-2 is reduced by 95% to one 15 cm plate or five 9 cm plates. The analysis at BIOFAQ shows that phage recovery after concentration is ≥75% of the corresponding direct analysis counts. In the study of TZW two values could be evaluated, of which one was significantly below 75%. This could be an outlier.

Conclusion

The ready-to-use culture media MSB, MSA, and ssMSA provided by Merck are suitable for detecting somatic coliphages in accordance with ISO 17025-2. Additionally, the VIRAPREP® kit supplied by Merck is suitable for concentrating coliphages from diluted water samples in accordance with ISO 17025-3, offering high accuracy and yield.

Literature

1. Singh S., Pitchers R. and Hassard F. (2022) Coliphages as viral indicators of sanitary significance for drinking water. Front. Microbiol. 13: 941532.
2. World Health Organization (2017) Guidelines for drinking water quality. 4. Edition
3. Lamy M-C., Sanseverino I., Niegowskaand M., Lettieri T. (2020) JRC Technical Reports – Microbiological parameters under the drinking water directive
4. Directive (EU) 2020/2184 of the European Parliament and of the Council – Quality of water intended for human consumption
5. ISO 10705-2 (2000): Water quality – Detection and enumeration of coliphages – Part 2: Enumeration of somatic coliphages
6. ISO 10705-3 (2003): Water quality – Detection and enumeration of coliphages – Part 3: Validation of methods for concentration of bacteriophages from water
7. GL Biocontrol; 9 Avenue de l'europe, 34830 Clapiers, France – VIRAPREP® Product data sheet
8. GL Biocontrol; 9 Avenue de l'europe, 34830 Clapiers, France – VIRAPREP® Illustrated protocol
9. ATCC® American Type Culture Collection (ATCC) is a registered trademark of a nonprofit company

To place an order or receive technical assistance:
SigmaAldrich.com/order

Technical Service:
SigmaAldrich.com/techservice

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

SigmaAldrich.com

MK_AN13963EN Ver. 1.0 58179 09/2024