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Concentration method using Hemoflow ultrafiltration

The protocol

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Colophon

Title
Concentration method using Hemoflow ultrafiltration
(The protocol)

Author(s)
Leo Heijnen, Anke Brouwer and Harm Veenendaal

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Contents

1 General

1.1 Subject

This document describes the procedure to concentrate water samples for microbiological analysis with the use of cross-flow-ultra-filtration (Hemoflow-filter).

1.2 Range of application

Application of this procedure is possible on different types of water (e.g. surface water, groundwater, tapwater).

1.3 Definitions

The Crossflow filter (HF 80S) is a F-Series High-Flux Polysulfone Hemodialysisfilter manufactured by Fresenius.
The filter consists of the following materials:

- Membrane: Fresenius Polysulfone
- Housing: polycarbonate
- Fixation material: polyurethane
- O-ring: silicone.

1.4 Principle

Particles (e.g. particles from organic or anorganic material, sand, protozoa, bacteria, viruses) are concentrated from large volumes of water by cross-flow (ultra) filtration.
Concentrated samples are preserved and transported on ice (0-4^{o}C) to consolidate the composition of the micro-organisms.

1.5 Safety

Microbiological safety precautions have to be taken to avoid personal contact with concentrated water samples. The possible presence of pathogenic micro-organisms, in combination with the high concentration factor makes these concentrated water samples potentially bio-hazardous.

1.6 Determination of the recovery

The water-composition could influence the recovery by the hemoflow concentration method and has to be determined for every sample-type. The recovery is determined by spiking experiments. Known concentrations of target organisms are spiked in water and the concentration of these organisms is determined before and after the hemoflow concentration procedure.
Pre-stained *Cryptosporidium* and *Giardia* (oo)cysts (Colorseed™ C&G, BTF) are used routinely to determine the recovery of these organisms easily.

2 Equipment and tools

2.1 Sterilisation oven
To sterilize glass bottles and other glassware at a temperature of 170 ± 10ºC.

2.2 Autoclave
To sterilize the tubes (20 min. at a temperature of 121ºC ± 3ºC).

2.3 Cooling box with melting ice or ice packs
To transport samples to the laboratory at 5 ± 3ºC.

2.4 Microbiological sample flasks

2.5 Crossflow filter (Hemoflow) (Hemoflow F-series high flux)
Provided by Fresenius Medical Care, part number 5007181

2.6 Tubes for Hemoflow system
Masterflex (Cole-Parmer) tube type 96400– 73.
Silicone tube with a diameter of ± 10 mm.

2.7 Masterflex peristalitic pump
Pump model 77410-10, with Easyload pump head 77601-10, or comparable.

2.8 Water tank
The water tank contains two outlets which are used to connect the tank to the system and one inlet which is used to fill the tank. A float-system is connected to the inlet to make it possible to block the inlet in cases where the tank is filled with water.

2.9 Thermometer
To determine the temperature of the sample at an accuracy of ± 0.5ºC.

2.10 Balance
To determine the mass of concentrated samples at an accuracy of ± 1g.

2.11 Toolbox
Containing tube-connectors, tube clamps and appropriate gear to build-up the Hemoflow system.

3 Procedure

3.1 Preparations

- Clean and sterilize the following equipment parts:
  - Tubes: rinse with clean tap-water and autoclave
  - Glassware: use the standard cleaning laboratory procedures for glassware and sterilize with heat (as described in ISO 8199)
  - A 1L sterile glass flask is used to collect the concentrated sample. Determine and note down the weight of this flask (to determine the weight (volume) of the sample after the Hemoflow concentration procedure).

3.2 Sample collection

3.2.1 Connecting the hemoflow system

A picture (A) and a schematic overview (B) of the connected system are shown in figure 1

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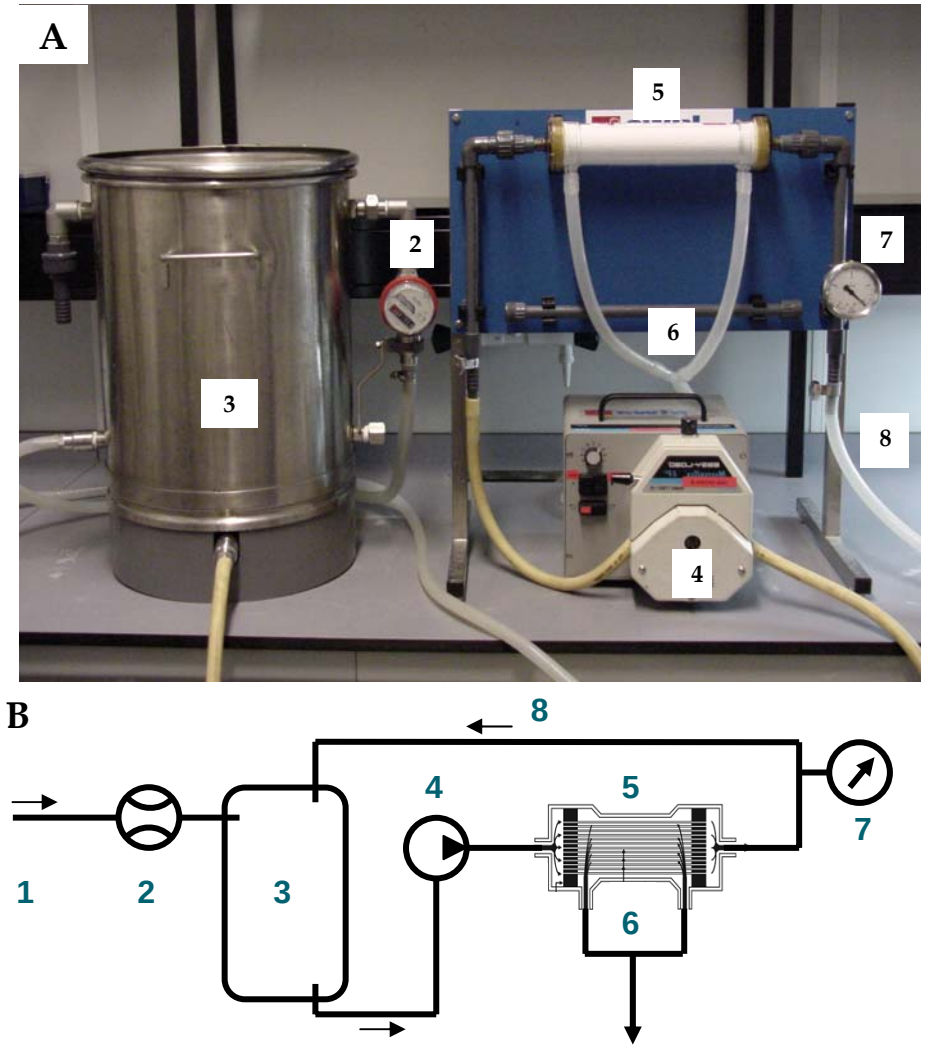

A picture (A) and a schematic overview (B) of the Hemoflow-system
1, sample-collection point; 2, water meter; 3, water tank with float; 4, pump; 5, Hemoflow-filter; 6, filtrate; 7, pressure meter; 8, returning tube; 9, tube clamp; a, b, c en d connection points for tubes

- Connect a tube to the sample collection point (1), this sample collection point (1) can be a tap.
- Connect the other end of the tube to the water meter (2) and connect the water meter with the water tank (3).
- Connect a sterile tube (Masterflex 96400 -73), using a clamp to the connection point (a).
- Lead the tube from left to right through the pump (4).
- Connect the tube to the pump head using a clamp.
- Connect the other end of the tube to the Hemoflow-filter (5) at point b.
- Connect tubes to the two side-outlets of the Hemoflow-filter. These tubes will transport the (clean) filtrate to a waste (6). Optionally, these tubes can be combined with a T-connector to a single waste tube.
- Connect a sterile silicone tube to the pressure-meter (7), the pressure meter is connected to the other end of the Hemoflow-filter (5).
- Connect a tube clamp (9) to this tube (8).

- Connect the end of this tube (8) to the lower connector (d) on the water tank with float (3). This connection closes the circuit and makes it possible to circulate the water between the tank and the hemoflow filter.

3.2.2 *Concentrating a water sample*

- **First concentration step**
  - Read the water meter and write down the readout.
  - Fill the water tank partly with water (approximately ¼ part).
  - Switch on the pump (velocity between 4 (40) and 5 (50)). Water should flow from left to right through the hemoflow-filter (as shown in the schematic overview of the system), change the sense of rotation of the pump in case water flows in the opposite direction. Water flows from the water tank, through the pump, through the hemoflow-filter and then back to the water tank now.
  - The water pressure in the hemoflow-filter can be adjusted by tightening or loosening the tube clamp (9). Tighten the tube clamp to obtain a pressure of 0.2 bar, as show on the pressure meter (7).
  - Catch up the filtrate (6) during a time period of one minute and measure the filtrate volume to determine the flow-rate. The flow-rate should be adjusted to ± 900 ml/minute by changing the pump speed without letting the pressure exceed 0.6 bar.
  - Take care that enough water is floating back in the tank (± 4 liter/ minute), to be sure that the micro-organisms in the water-concentrate are circulating and that they are not captured on the filter.
  - Fill the water tank with water by opening the tap at the sample collection point (in case of a tap water sample), or by switching on the pump between the water meter and the sample collection point (in case of an environmental water sample). Arrange a rising water level in the tank by loosening the tube clamp (9).
  - The float in the water tank will close the inlet and stop the supply of water in case of a full water tank.
  - Monitor the volume that passed the water meter and close the tap at the sample collection point (1) as soon as the desired volume has been reached.

- **Second concentration step (after reaching the desired volume)**
  - Take of the lid from the water tank (3).
  - Fill a bottle with 500 ml of filtrate (6).
  - Concentrate the sample further until the tank is almost empty.
  - Rinse the wall of the tank the 500 ml filtrate (6).
  - Concentrate the sample further until the tank is empty. Only the hemoflow filter and the tubes contain water-concentrate at this stage.
  - Switch off the pump (4).
  - Disconnect the returning tube between the hemoflow filter and the tank (8) at the connection with the tank.
  - Bring the end of this tube (8) in a sterile bottle.
  - Switch on the pump (4).
  - The water concentrate will be collected in the bottle.

- Switch off the pump (4) directly after emptying the tube (8).
- Remove the tube clamp (9) from the returning tube (8).
- Leave the returning tube in the bottle.
- Rinse the walls of the water tank with 400 ml of sample water.
- Switch on the pump. The filter and tubes will be rinsed with the water from the tank. This water will be collected in the bottle and pooled with the concentrated sample.
- Switch off the pump as soon as rinse water has passed through the tubes and the filter.
- Transport the concentrated sample on melting ice or ice packs to the laboratory.
- Dismantle the complete system and clean the different parts.

3.2.3 Cleaning of the system

- Connect a tube (or synthetic pipe) to the system at the position of the hemoflow filter.
- Disconnect the returning tube (8) at point d
- Fill the water tank with hot (60°C to 80°C) water and pump this water through the system.
- Use at least 100 liter hot water to rinse the water tank and the system.
- Air-dry the tank and the rest of the system.
- Tubes are sterilized in the autoclave (20 min. 120°C)

Remark: it is not clear if this cleaning procedure suffices in all circumstances (e.g. after processing samples with high organism concentrations). The use of disinfecting agents (like chlorine) might be needed in certain cases.

3.2.4 Determination of the volume of the concentrated sample

The volume of the concentrated water sample is determined by weighing. The mass difference between the pre-weighed empty sample-bottle and the sample-bottle containing the concentrated water-sample is used to determine the volume of the concentrated water-sample. One gram is used as the equivalent of 1 ml of concentrated water-sample.