



Protocol

Envirochek® HV Sampling Capsule

This bulletin describes the recommended method of use of the Envirochek HV capsule for the concentration and recovery of *Giardia sp.* cysts and *Cryptosporidium sp.* oocysts from water. Detection using Envirochek HV Sampling Capsules is references in USEPA Methods 1622, 1623 & 1623.1 and ISO Standard 15553.

CAUTION: This method does not address all of the safety concerns that are associated with pathogen concentrating and testing. It is the responsibility of the user of this device and method to determine and employ all applicable safety measures to ensure the safety of themselves and others. Check for regulatory limitations that may apply prior to using this device.

1.0 Sample Collection Materials

- 1.1 Envirochek HV sampling capsule, one filter per sample. NOTE: The filters are not reusable.
- 1.2 Hose-type tubing 12.7 mm (0.5 in.) I.D. Tygon♦ formula R-3603 (clear laboratory tubing) or equivalent.
- 1.3 Clamps
- 1.4 Water Meter (flow totalizer)
- 1.5 Flow control valve, 2 L/min (0.5 gal/min)
- 1.6 Pump, electric or gasoline powered
- 1.7 Laboratory shaker (See Figure 2) with arms or equivalent agitation apparatus (Pall Laboratory Part No. 4821A or 4822A)
- 1.8 Ice chest or cooler with cold packs
- 1.9 250 mL conical centrifuge tube
- 1.10 50 mL polypropylene tubes
- 1.11 Elution Buffer
 - 1 g Laureth-12 (Pall Laboratory Part No. 4820)
 - 10 mL 1 M Tris, pH 7.4 (See 1.11.1)
 - 2 mL 0.5 M EDTA, 2 Na, pH 8.0 (See 1.11.2)
 - 150 µL Antifoam A
 - 1 L Deionized (DI) Water

1.11.1 Preparation of 5% w/v Sodium Hexametaphosphate Solution

Dissolve 50 g of sodium hexametaphosphate ($(\text{NaPO}_3)_n$) in 600 mL of reagent water. Dilute to final volume of 1000 mL using reagent water. Store in a plastic or glass container at room temperature for up to 3 months. Discard when expiration date is reached or sooner if microbial growth is apparent.

1.11.2 Preparation of 1 M Tris, pH 7.4

Dissolve 121.1 g Tris in 700 mL of reagent water and adjust pH to 7.4 using 1N HCL or NaOH. Dilute to a final 1000 mL with reagent water and adjust the final pH. Filter-sterilize the sample through a 0.2 μ m membrane (Pall Laboratory Part No. 4632) into a sterile plastic container and store at room temperature.

1.11.3 Preparation of 0.5 M EDTA, 2 Na, pH 8.0

Dissolve 186.1 g ethylenediamine tetraacetic acid, disodium salt dihydrate in 800 mL of reagent water and adjust to pH 8.0 with 1 N HCL or NaOH. Dilute to a final volume of 1000 mL with reagent water and confirm pH.

1.11.4 Preparation of buffer solution

Weigh 1 g Laureth-12 in a glass beaker and add 100 mL of DI water. Heat the beaker to melt the Laureth-12 using a hot plate or microwave and transfer the solution to a 1000 mL volumetric flask. Rinse the beaker several times to ensure the transfer of the detergent to the cylinder. Add 10 mL of Tris solution, pH 7.4; 2 mL of EDTA solution, pH 8.0; and 150 μ L Antifoam A. Bring solution to a final volume of 1000 mL with reagent water. The buffer will have an opaque appearance.

2.0 Sample Collection

- 2.1 Retain the vinyl caps included with the Envirochek HV capsule for sample shipping and the elution process.
- 2.2 The exact installation is dependent on whether the water is from a pressurized water source. If the source is not pressurized, a pump is required. If a pump is required, the system will appear as in Figure 1a. If a pump is not required, the system will appear as in Figure 1b.
- 2.3 Before connecting the sampling system to the pressurized system or sample source, turn the pressurized system or pump on, allowing water to purge residual debris from the water lines for 2-3 minutes, or until the turbidity becomes uniform.
- 2.4 Turn off the pressurized source or pump and connect the sampling system without the Envirochek HV capsule.
- 2.5 Turn on the pressurized source or pump and adjust the flow rate to 2 L/min.
- 2.6 Allow approximately 10 L (2.6 gal) to flush the system.
- 2.7 Install the Envirochek HV capsule in line, securing the inlet and outlet ends with the appropriate fittings/clamps.
- 2.8 With a water-resistant marking pen, record the sampling location, name of the person performing the sampling, date, meter reading and turbidity of the sample on the label provided on the filter housing.
- 2.9 Initiate water flow.
- 2.10 Vent the residual air using the bleed valve by turning it in a counter clockwise direction. When the filter housing is full of water, close the bleed valve.

Figure 1a – Assembly of Envirochek HV sampling system with pump.

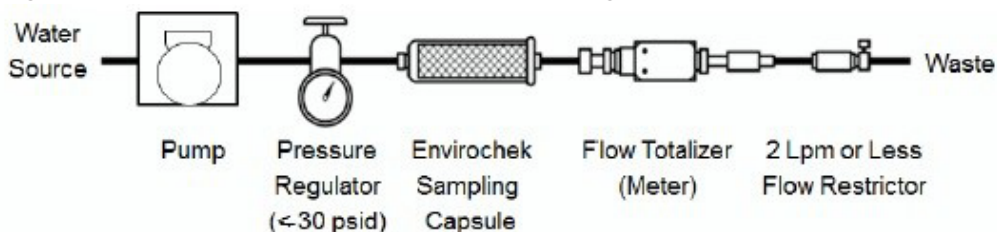
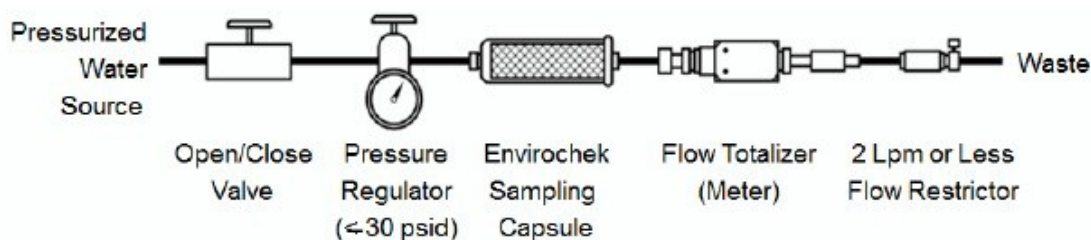


Figure 1b – Assembly of Envirochek HV sampling system without pump.



- 2.11 After you have collected your sample through the Envirochek HV capsule, shut off your water source. Allow the pressure to decay until flow stops.
 - 2.12 Record the stop time and final meter reading in the appropriate spaces on the label on the filter.
 - 2.13 Disconnect the inlet end of the Envirochek HV capsule, making sure not to spill any of the water remaining in the capsule. This water is part of your sample.
 - 2.14 Seal the inlet of the capsule with the vinyl end cap that was saved from step 2.1.
 - 2.15 Loosen the outlet end and allow water to drain as much as possible. Water drainage from the capsule through the outlet is acceptable as the sample has passed through the membrane.
 - 2.16 Seal the outlet of the capsule with the vinyl end cap that was saved from step 2.1.
 - 2.17 The Envirochek HV capsule should be placed on cold packs for storage or transport to the testing facility.
- NOTE:** Sample should be stored at 1°C - 10 °C from time of filtration. Sample elution must be initiated within 96 hours of sample collection.

3.0 Elution Method

- 3.1 Assemble the laboratory shaker with the arms at full extension and the clamps aligned in a vertical position so that the capsules will be aligned horizontally as demonstrated in Figure 2.

Figure 2 – Proper assembly of laboratory shaker



- 3.2 Prepare sufficient elution buffer so all the samples to be eluted that day can be eluted with the same stock elution buffer solution. (See 1.11.)
- 3.3 Designate a 250 mL conical centrifuge tube for each sample and label with the sample number.
- 3.4 If the upper chamber of the capsule has water remaining in it, carefully decant this water into an appropriately labeled conical centrifuge tube to be used in section 4.0.
- 3.5 Hold the capsule in a vertical position with the inlet end up by using a ring stand or other means. Remove the end cap.

- 3.6** Add elution buffer to the inlet end of the capsule and allow the elution solution level to stabilize. Sufficient elution buffer should be added to the capsule to ensure the covering of the pleated white filter module by 12.7 mm (0.5 in.) (See Diagram 1.) Replace the vinyl end cap to the inlet end of the capsule.

Diagram 1 – Elution buffer level.



- 3.7** Securely clamp the capsule in one of the clamps on the shaker such that the bleed valve is facing toward you. Make sure the bleed valve is in the 12 o'clock position (See Figure 3.)

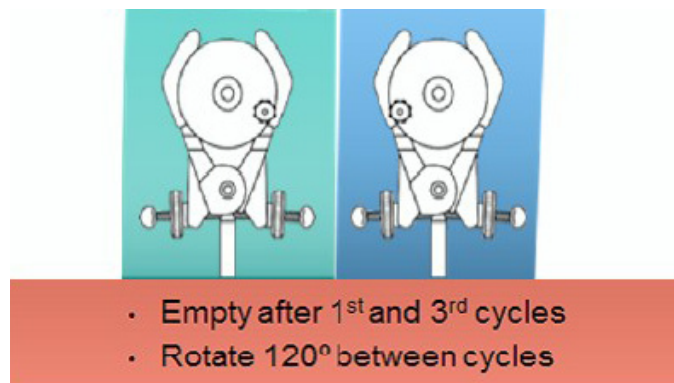
Figure 3 – Proper orientation of Envirochek capsule during the first cycle of the elution procedure



- 3.8** Turn on the shaker and set the speed at approximately 600 rpm and agitate for 5 minutes.
- 3.9** Remove the capsules from the shaker. Carefully remove the inlet end cap and decant the contents of the capsule into a 250 mL conical centrifuge tube.
- 3.10** Add elution buffer to the inlet end of the capsule and allow the elution solution level to stabilize. Sufficient elution buffer should be added to the capsule to ensure the covering of the pleated white filter module by 12.7 mm (0.5 in.) (See Diagram 1.) Replace the vinyl end cap to the inlet end of the capsule.
- 3.11** Again secure the capsule in one of the clamps on the shaker such that the bleed valve is facing toward you. This time make sure the bleed valve is at the 4 o'clock position which is roughly 120 degrees from the first position.
- 3.12** Turn on the shaker and set the speed at approximately 600 rpm and agitate for 5 minutes.
- 3.13** After this shake is complete, remove the capsule from the clamp (do not decant contents of the capsule) and rotate the capsule so that the bleed valve is at the 8 o'clock position, roughly 120 degrees from the second position (See Figure 4).

- 3.14 As before, remove the capsules from the shaker, carefully remove the inlet end cap, and decant the contents of the capsule into the same 250 mL conical centrifuge tube used for that sample.

Figure 4 – Proper alignment of Envirochek HV capsule during the second cycle of the elution process



4.0 Concentration: Adjustment of pellet volume

- 4.1 Centrifuge the sample at a minimum of 1500 X G for 15 minutes. Allow the centrifuge to coast to a stop. **NOTE:** DO NOT USE THE BRAKE.
- 4.2 Record the volume of the pellet with date and the time that the concentration was completed. Use a Pasteur pipette to carefully remove the supernatant to a volume low enough to the pellet which does not disturb the pellet. It is common to leave a bit more in the vial to ensure that any cysts and oocysts which are not in the pellet are still recovered.

4.2.1 If the pellet volume (volume of solid) is 0.5 mL or less, using a pipette, carefully aspirate the supernatant to 5mL above the pellet. **NOTE:** Care must be taken to avoid aspirating oocysts and cysts during this step. To resuspend the pellet, vortex the tube for 10-15 seconds. Record the resuspended pellet volume, and proceed to step 5.0.

4.2.2 If the pellet volume is greater than 0.5 mL, the concentrate must be separated into multiple subsamples. **NOTE:** No more than 0.5 mL of pellet must be processed at a time. Use the following formula to determine the total volume required in the centrifuge tube before separating the concentrate into two or more subsamples, rounding the result up to the nearest multiple of 5.

Aspirate the supernatant from the centrifuge tube leaving 5 mL of fluid for every 0.5 mL of pellet. Record the volume. Proceed to step 5.0.

$$\text{Total Volume (mL) Required} = \frac{\text{Pellet Volume}}{0.5 \text{ mL}} \times 5 \text{ mL}$$

Example:

If packed pellet volume is 1.2mL, the total volume required is 15 mL.

5.0 Detection

- 5.1 The sample is now ready for the detection stage of the process. Refer to the current approved detection method for your region.

Reference EPA Method 1623.1: *Cryptosporidium* in Water by Filtration/IMS/FA; USEPA 1622; USEPA 1623; ISO 15553

WARNING: Employment of the products in applications not specified, or failure to follow all instructions contained in this technical bulletin, may result in improper functioning of the product, personal injury, or damage to property or the product. See Statement of Warranty in our most recent catalog.



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