Iron Chloride Precipitation of Viruses from Seawater

Seth John, Bonnie Poulos, Christine Schirmer

Abstract

This protocol describes a technique to recover viruses from natural waters using iron- based flocculation and large-pore-size filtration, followed by resuspension of virus- containing precipitates in a pH 6.5 buffer. This Fe-based virus flocculation, filtration and resuspension method (FFR) is efficient (> 90% recovery), reliable, inexpensive and adaptable to many aspects of marine viral ecology and genomics research. Recovered viruses are amenable to gene sequencing, and a variable proportion of phages, depending upon the phage, retain their infectivity when recovered if using oxalic acid in the resuspension buffer. Particles lose infectivity if resuspended with ascorbic acid in the buffer.

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Guidelines

PCR inhibition: Note, that due to the likely issue with the EDTA in the resuspension buffer interacting with the MgCl in a PCR reaction, direct PCR is ineffective. To circumvent this, extract DNA using Wizard columns from Promega (see Tucson Marine Phage Lab's DNA Extraction of Viruses Using Wizard Columns protocol).

Materials:
- Seawater
- Tris-base (FW=121.14)
- Na2-EDTA dihydrate (FW=372.24)
- MgCl2 hexahydrate (FW=203.3)
- Ascorbic acid (FW=176.12) and/or Oxalic acid (FW=126.07)
- NaOH, 5N
- HCl
- $FeCl_3 \cdot 6H_2O$ (FW=270.3)
- MilliQ water
- pH paper
- Whatman glass fiber filter GF/A (cat. 1820-150; 1.6µm retention; 150mm diameter)
- Millipore Express PLUS filter (cat. GPWP14250; 142mm, 0.22µm)
- Pall Supor-800 filter (cat. 60114; 142mm, 0.8um)
- GE Polycarbonate Membrane filter (cat. K10CP14220; 142mm, 1.0µm), Available through Midland Scientific Inc. (cat. MAINE 1216611)
- pH meter
- 142mm filtering towers (Need 2 for efficiency)
- Peristaltic pump with a pressure gauge

Notes:
Alternative Pre-filtering Method: An alternative method for preparing seawater is to pre-filter the seawater through a Whatman GF/D glass fiber filter (cat. 1823-150; 2.7µm retention; 142mm diameter) to remove large particles. The filtrate is filtered again using a Millipore Steripak GP10 or GP20 (cat. SPGPM10RJ or SPGPM20RJ; 0.22µm). The GP 10 works for <10L volumes and GP20 works for <20L volumes. Larger capsule filters will easily filter 100-200 liters: Pall Acropak 200, 500, 1000 and 1500. The cellular fraction is retained on the filter while the virus fraction is in the filtrate.

Filtration should be done at a maximum pressure of 15 psi. A peristaltic pump applies the pressure and a stainless steel Millipore filter rig (PN YY3014236) holds filters. For less expense, use an acrylic or polycarbonate 142 mm filter holder (Geotech Environmental Equipment, acrylic or PC) and pressurize carboy with an air pump. Most lab vacuum pumps have an air-pressure outlet on the other side that can easily be adjusted to provide 15 psi pressure. Nalgene sells caps for their 20L carboys with fittings in the top that can be used for pressurization.

$FeCl_3$ Formulation: The solution of ferric chloride hexahydrate is calculated based on the amount of iron, not on the amount of the salt. The stock solution has 10g Fe per liter. For precipitation, the final optimal concentration of Fe in seawater (SW) is 1mg Fe per liter SW which is equal to 2.9mg $FeCl_3$ per liter SW or to 4.83mg $FeCl_3 \cdot 6H_2O$ per liter SW.

Equipment Care: Carboys and collection bottles should be washed with 1N HCl and rinsed with MilliQ water before use. Checking tubing before use and replace if worn. Always rinse stainless steel filter rigs with MilliQ water after use and air dry to prevent corrosion.

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Materials

- Millipore Express PLUS filter 0.22 µm, 142 mm GPWP14250 by Contributed by users
- Pall Supor-800 filter 60114 by Contributed by users
- GE Polycarbonate Membrane filter MAINE 1216611 by Contributed by users

Protocol

Virus Precipitation

Step 1.
Assemble two 142mm filtering towers and attach the filter apparatus to a peristaltic pump with a pressure gauge (maximum pressure = 15 psi)

NOTES

Lauren Chittick 29 Jun 2015
See Guidelines note for an alternative pre-filtration method.

ANNOTATIONS
Lauren Chittick 17 Feb 2016
For smaller volume testing, we used 1.0-1.5L filtrate per 47mm tower.

Virus Precipitation

Step 2.
Pre-filter 20L of seawater using a 150mm Whatman GF/A filter.

NOTES
Lauren Chittick 30 Jun 2015
Wear gloves and use forceps for handling all filters.

Virus Precipitation

Step 3.
Filter again to remove bacteria using a 0.22um, 142mm Millipore Express Plus filter.

REAGENTS
Millipore Express PLUS filter 0.22 µm, 142 mm GPWP14250 by Contributed by users

NOTES
Lauren Chittick 23 Jun 2015
See note in guidelines for alternative pre-filtration method.
Lauren Chittick 30 Jun 2015
Wear gloves and use forceps for handling all filters.

Virus Precipitation

Step 4.
Treat the virus fraction (0.22µm filtrate) with $FeCl_3$ to precipitate the viruses by adding 1ml of 10g/L Fe Stock Solution to each 20L of filtrate. Shake vigorously for 1 minute.

AMOUNT
1 ml Additional info:
DURATION
00:01:00

PROTOCOL

. 10g/L Fe Stock Solution

CONTACT: Bonnie Poulos

NOTES
Lauren Chittick 13 Jul 2015
Solution is acidic and should be handled with care.

Discard solution if a cloudy precipitate forms. Do not dilute solution as iron hydroxide precipitate will form quickly.

For use on board ship, it is best to pre-weigh iron chloride into 50ml centrifuge tubes and add water as needed during the cruise: pre-weigh 0.966g $FeCl_3 \cdot 6H_2O$ into several 50ml tubes. Add 20ml MQ-H_2O when ready to precipitate viruses in seawater.
Step 4.1.

Measure out 4.83g $FeCl_3 \cdot 6H_2O$.

AMOUNT
5 g Additional info:

REAGENTS
Iron(III) chloride hexahydrate 236489 by Contributed by users

Step 4.2.
Dissolve $FeCl_3 \cdot 6H_2O$ in 100ml MilliQ water.

AMOUNT
100 ml Additional info:

Step 4.3.
Store at room temperature or 4°C.

Virus Precipitation

Step 5.
Add an additional 1ml of 10g/L Fe stock solution to each 20L of filtrate (for a total of 2ml Fe Stock Solution per 20L filtrate). Shake vigorously for 1 minute.

AMOUNT
1 ml Additional info:

DURATION
00:01:00

PROTOCOL

. 10g/L Fe Stock Solution

CONTACT: Bonnie Poulos

NOTES
Lauren Chittick 30 Jun 2015
For use on board ship, it is best to pre-weigh iron chloride into 50ml centrifuge tubes and add water as needed during the cruise: pre-weigh 0.966g FeCl3-6H2O into several 50ml tubes. Add 20ml MQ-H2O when ready to precipitate viruses in seawater.
Lauren Chittick 30 Jun 2015
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Lauren Chittick 30 Jun 2015
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Step 5.1.
Measure out 4.83g $FeCl_3 \cdot 6H_2O$.

AMOUNT
5 g Additional info:

REAGENTS
Iron(III) chloride hexahydrate 236489 by Contributed by users

Step 5.2.
Dissolve $FeCl_3 \cdot 6H_2O$ in 100ml MilliQ water.

AMOUNT
100 ml Additional info:

Step 5.3.

Store at room temperature or 4°C.

Virus Precipitation

Step 6.

Let the $FeCl_3$ treated filtrate sit for 1 hour at room temperature.

DURATION

01:00:00

Virus Precipitation

Step 7.

Filter the $FeCl_3$ treated filtrate using a 1.0µm, 142mm, polycarbonate (PC) membrane filter on top of a 0.8µm, 142mm, Supor support filter and attaching the filter apparatus to a peristaltic pump with a pressure gauge (maximum pressure = 15 psi).

REAGENTS

- GE Polycarbonate Membrane filter MAINE 1216611 by Contributed by users

NOTES

Lauren Chittick 30 Jun 2015

The FeCl3 precipitate will be captured on the PC filter; the Supor is just for support in the 142mm stainless steel filtration apparatus. Although the 20L can be collected on a single 142mm PC membrane, it is faster to change the PC membrane one or two times during the filtration; normally 3 PC membranes and 1 Supor membrane are used per 20L seawater.

Virus Precipitation

Step 8.

Place all of the polycarbonate filters from the 20L into a 50ml centrifuge tube being careful not to scrape off any of the $FeCl_3$ on the edge of the tube (having precipitate facing out aids in dissolving the precipitate).

Virus Precipitation

Step 9.

Discard the Supor support filter.

NOTES

Lauren Chittick 30 Jun 2015

The filters can be stored in the 50ml tubes at 4°C until ready to resuspend the precipitated viruses. Be sure the caps are on securely so that the filters do not dry out.

Resuspension

Step 10.

Prepare fresh 0.1M EDTA-0.2M $MgCl_2$-0.2M Ascorbate Buffer, pH6.5.

PROTOCOL

. 0.1M EDTA-0.2M MgCl2-0.2M Ascorbate Buffer

CONTACT: Bonnie Poulos

ANNOTATIONS

Lauren Chittick 30 Jun 2015

Fresh buffer with no precipitate and a pH of 6.0 are critical!

Lauren Chittick 20 Jun 2016

For an easier formulation, see the protocol for making 1M Citrate and Magnesium Resuspension

Buffer.

1x Buffer

Step 10.1.
Dissolve 1.51g Tris-base in 80ml Milli Q water.

1x Buffer

Step 10.2.
Dissolve 3.72g Na2-EDTA dihydrate into solution.

NOTES
Bonnie Poulos 15 Jun 2015
pH will be ~10.0

1x Buffer

Step 10.3.
Once EDTA is in solution, dissolve 4.07g MgCl2.

NOTES
Bonnie Poulos 15 Jun 2015
pH will drop to ~8.0

1x Buffer

Step 10.4.
Add 3ml of NaOH.

NOTES
Bonnie Poulos 15 Jun 2015
This will drop the pH to ~4.5 and the solution will become cloudy which indicates that the EDTA is coming out of solution.

1x Buffer

Step 10.5.
Dissolve the reductant (3.52g of ascorbic acid or 2.52g of oxalic acid).

NOTES
Bonnie Poulos 15 Jun 2015
The pH will increase to ~8.3 and the solution will clear up.

ANNOTATIONS
Uri Neri 10 Apr 2018

Dear Bonnie,

We're trying to create the resuspension buffer for the VLPs-Iron precipitates resuspension step, with the reductant agent being oxalic acid (anhydrous, FW=90.04). net weight for the acid concentration was calculated to adjust for the anhydrosity and is ~1.808 [g]. Other than this minor change, I've made no modifications to the protocol, yet I am unable to create the buffer without the solution becoming 'murky' after the addition of 3ml NaOH(5N), and after some time without stirring, visible precipitates are formed (and the solution becomes clear).

We've followed the protocol to the letter, and continuously measured pH.

Your thoughts on the matter would be greatly appreciated.

With best regards,

Uri Neri

1x Buffer

Step 10.6.

Once the reductant is in solution, add the last 1ml of NaOH.

1x Buffer

Step 10.7.

Check the pH using pH paper (the buffer should be at pH 6.0 - 6.5)

NOTES

Bonnie Poulos 23 Jun 2015

The solution may need some minor adjusting with NaOH or HCl to achieve a pH of 6.0.

Bonnie Poulos 23 Jun 2015

pH 6.0 is ideal for good recovery of viruses.

1x Buffer

Step 10.8.

Check the volume and add MilliQ water for a total volume of 100ml.

1x Buffer

Step 10.9.

Store the buffer in the dark (bottle wrapped in foil) and visually inspect prior to use. It should be clear without precipitates.

NOTES

Bonnie Poulos 15 Jun 2015

At this point, 10-15ml of buffer can be sacrificed for a final pH check using a pH meter.

Bonnie Poulos 23 Jun 2015

The buffer will start to change color after about 24 hours. It is okay to use if slightly discolored, but do not use after about 36 hours (eventually the buffer will turn almost orange!).

Resuspension

Step 11.

Add 1ml buffer for each 1mg Fe, which is 20ml of 1x buffer for the 20L seawater precipitate (10ml of a 2X concentrated buffer). Shake the tube vigorously.

Resuspension

Step 12.

Place tubes on a rotator and rotate at 4°C overnight.

DURATION

18:00:00

NOTES

Lauren Chittick 30 Jun 2015

You will see the solution change colors a number of times – that is okay. However, if precipitates form, there is a possibility that viruses are trapped or adhered to the particles. Centrifuge gently to pellet the precipitate, and try using additional buffer to get the pellet back into solution. Although it may not go back into solution, the pellets will have been washed and may recover some viruses.

13. Leave stored at 4°C until ready to analyze or further purify the viral fraction.

Resuspension

Step 13.

Leave stored at 4°C until ready to analyze or further purify the viral fraction.

Resuspension

Step 14.

To collect the resuspended precipitate, open the tube and collect liquid into a fresh tube.

Resuspension

Step 15.

Using a sterile pipet, applicator stick, or bleached and rinsed forceps pull an edge of the filter onto the tube edge so it is caught in the lid when secured.

Resuspension

Step 16.

Centrifuge at low speed (less than 1000 rpm) for 3-5 minutes to recover more buffer.

DURATION

00:05:00

Resuspension

Step 17.

If the filter has precipitate on it, add more buffer, incubate again for a couple hours, and collect liquid as stated above.

ANNOTATIONS

Lauren Chittick 17 Feb 2016

Citrate buffers were a little more "stubborn" so I briefly vortexed and rinsed with the collected buffer (instead of adding new buffer).

Warnings

Do NOT let the filters dry out before the precipitated viruses are resuspended! This will cause a loss in yield. - It is important that the filters remain moist until resuspension. The excess liquid on top of the filter can provide enough humidity if the tubes are well sealed with a tight-fitting cap and parafilm. One or two ml of sterile water or 0.2um filtered SW from the same source can be added if necessary to keep the filters moist. - Keep filters at 4°C until processed, and processing is best if done soon after collection (although we have successfully resuspended particles after several years if stored properly).