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Full SOP title	Reception, aliquoting and storage of Phocine Herpes Virus 1 (PhHV) stock solution
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1. Introduction

Molecular biological methods have become increasingly relevant to the diagnosis and control of parasitosis. Since various factors may affect the sensitivity of PCR assays, including DNA yield and purity, an optimal extraction method is essential. Losses of a parasite's DNA during extraction may significantly impair its detection by PCR and lead to false-negative results. PCR inhibitors are common in stool samples and can lead to false negative results as well. When performing DNA extraction, it is therefore of the utmost importance to use an internal control to exclude possible losses of genetic material during the purification process and inhibition of the PCR amplification. The internal control used in the context of the ALIVE trial is the Phocine Herpes Virus 1 (PhHV), obtained from European Virus Archive Global (EVAg), Erasmus MC Rotterdam. The PhHV is an exogenous DNA spiked into the sample during DNA extraction co-purified with the sample DNA and subsequently detected by real-time PCR. The PhHV is spiked at a known concentration and therefore should become positive at a known Ct-value when DNA is extracted properly and inhibition factors do not interfere with the PCR. The amplification is considered to be hampered by inhibitory factors if the Ct-value in the PhHV specific PCR is 3.3 cycles higher than expected. If the Ct-value of the PhHV PCR is not greater than 3.3 cycles of the average of uninhibited samples, the loss of genetic material and the presence of inhibitors can be excluded and the result of the specimen can be considered a valid result.

2. Scope and application

This Standard Operating Procedure (SOP) describes the steps required for (i) ordering, (ii) receiving, (iii) determining the concentration of the working solution to be added to each sample, and (iv) aliquoting of the stock solution of PhHV when used as DNA extraction internal control in the context of the ALIVE trial.

3. Responsibilities

Investigator/field workers: must know the contents of this SOP, follow it strictly and instruct the participants on safety and procedures for the sample collection process.

Laboratory technicians: must know the contents of this SOP and perform the laboratory activities under the responsibility and guidance of the Head of Site Laboratory.

4. Safety

5. Always wear gloves on the hands during manipulation of biologic materials;

Universal precautions should be always followed, such as:

- ✓ Clean the work surface after the work is finished with 1% bleach and 70% alcohol.

6. Materials

- Virus culture stock of Phocine Herpes Virus 1 (PhHV), (EVAg cat.N. 011V-00884) storage at -80°C

All materials required to perform DNA extraction and real-time PCR are listed in the corresponding SOPs (see below).

Other SOPs

- SOP_007 (ST_EXTR: Stool DNA extraction for multiplex qPCR and resistance studies).

- SOP_008 (ST_PCR: Multiplex real-time PCR for the detection of STH)

7. Procedure

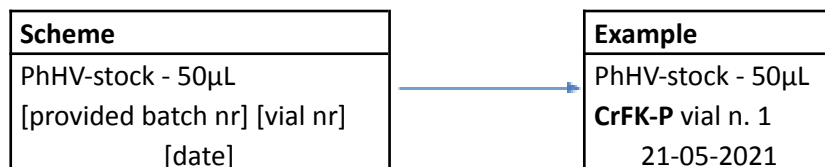
Ordering

- The virus culture stock of PhHV, can be ordered directly from the EVAg website (<https://www.european-virus-archive.com/>)
- Create an EVAG user account and log in (see Appendix A)
- Find the product of interest in the Portal/catalogue (EVAg cat.N. 011V-00884), and add it to the enquiry cart
- Validate the cart and complete the online enquiry form.
- Investigators outside the Netherlands can order the PhHV stock free of charge. Contact the supplier for confirmation (<https://www.european-virus-archive.com/contact-us>) (see Appendix B)

Reception

The virus culture stock of PhHV, is shipped in dry ice in a quantity of 1 mL. After arrival, check the parcel for temperature compliance and proceed by aliquoting and storing the stock solution before proceeding with the quantification:

- Thaw completely the stock solution
- Thoroughly mix the stock solution by pipetting up and down several times (very important)
- Divide the stock solution in aliquots of 50 μ L (20 in total)
- Label the aliquots with the EVAG stock batch (written on the vial received from EVAg), the volume of stock in the vial (50 μ L), the progressive number (from 1 to 20) and the date of aliquoting (see example below)



- Store the aliquots at -80°C and register storage location in freezer
- Always work from highest number to lowest number, so start with vial n. 20 for the verification

Verification

- Thaw one stock vial aliquot and prepare a 100-fold dilution of the PhHV in Phosphate Buffered Saline (PBS) by adding 450 μ L of PBS to the 50 μ L of stock solution followed by transferring 100 μ L of the 1:10 dilution to a new vial and adding 900 μ L of PBS
- Spike the AL solution (included in the Qiagen DNA extraction kit) by adding 6 μ L of the PhHV 1:100 dilution to 4 mL of AL buffer and mix properly (do not vortex)
- Perform DNA extraction on 10 PBS samples (200 μ L) according to procedure described in SOP_007: Stool DNA extraction for multiplex qPCR and resistance studies, starting from the 10 minutes incubation at 100°C (step 1, paragraph 6.5). NB. Use AL buffer spiked with the 1:100 PhHV

- Perform real-time PCR on all samples according to procedure described in SOP_008: ST_PCR: Multiplex real-time PCR for the detection of STH. Use only PhHV primers and probes. Replace the primers and probes not included in the reaction with molecular biology grade water
- Put results (Ct-values) of the PhHV PCR in a table
- If the Ct-values of the ten replicates fall within the desired range (Ct-value 28-30) with a difference in Ct-values not higher than 1.5, the PhHV is validated, proceed with aliquoting and storing the PhHV 1:100 dilution as described below
- If the average Ct-values of the ten replicates is above 30, a lower dilution (1:10) of the stock should go through the verification process

Aliquoting and storage of working solution

- Divide the whole volume of the diluted PhHV into 50 μ L aliquots in 1.5 mL screw cap micro tubes, these are the working solutions
- Label the aliquots with the Stock ID (here CrFK-P), Stock number (the progressive number of the stored stock, here **20**), the dilution (here **1/100**) and the date of preparation

PhHV-CrFK-P_20
1/100 - 50 μ L/33 mL
21-05-2021

- Store at -80°C until use (do not thaw and refreeze)
- Register storage location in the freezer and data of preparation of the PhHV working solution, including the expected Ct-value when performed the DNA extraction according to the in-use protocols (SOP_007 and SOP_008)

8. General remarks

- ✓ All local laws and regulations must be followed during the procedures described in this SOP. In case of conflict, the Principal Investigator should be informed, for further escalation.
- ✓ Use all the validated working solution before utilising a new stock aliquot
- ✓ Always verify the PhHV stock aliquot before making a new working solution, because long-term storage might result in deterioration.
- ✓ Storage of the working solution at a temperature of -20°C is accepted for periods not longer than 1 year.

Waste management

Dispose of potentially contaminated material without contaminating the local environment.

Precautions

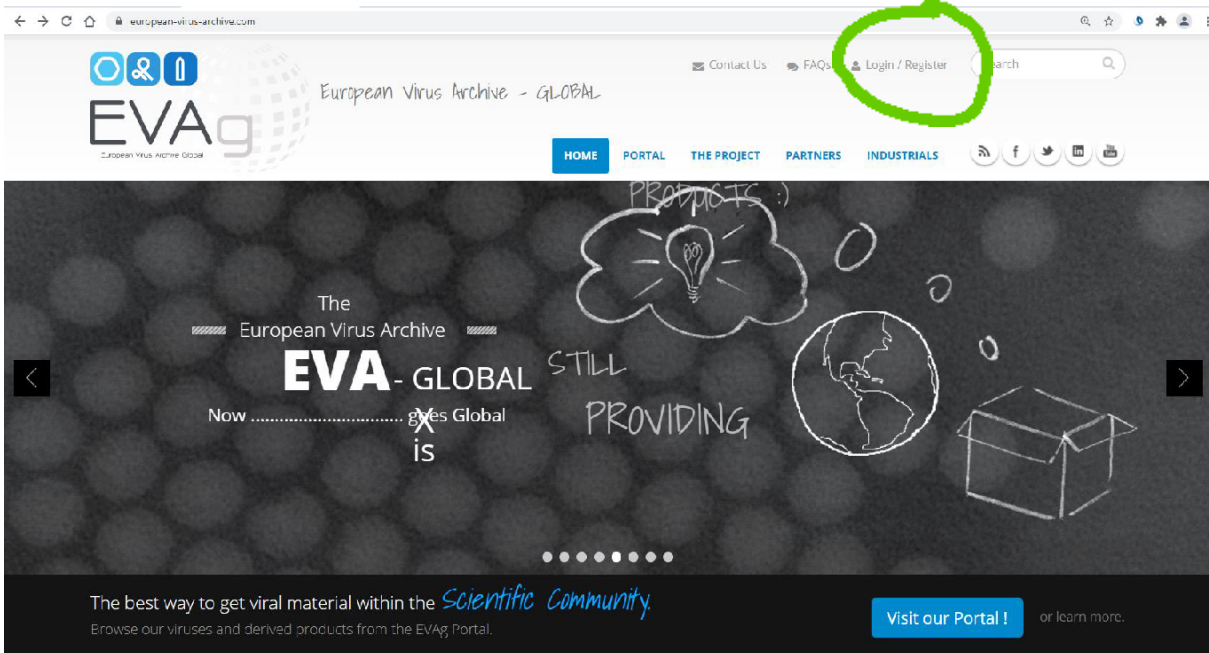
Ensure that local SOPs on screening and sample delivery are available to staff.

9. References

1. <https://www.european-virus-archive.com/virus/phocid-herpes-virus-type-1>

Appendix A

Create an EVAG user account



European Virus Archive - GLOBAL

HOME PORTAL THE PROJECT PARTNERS INDUSTRIALS

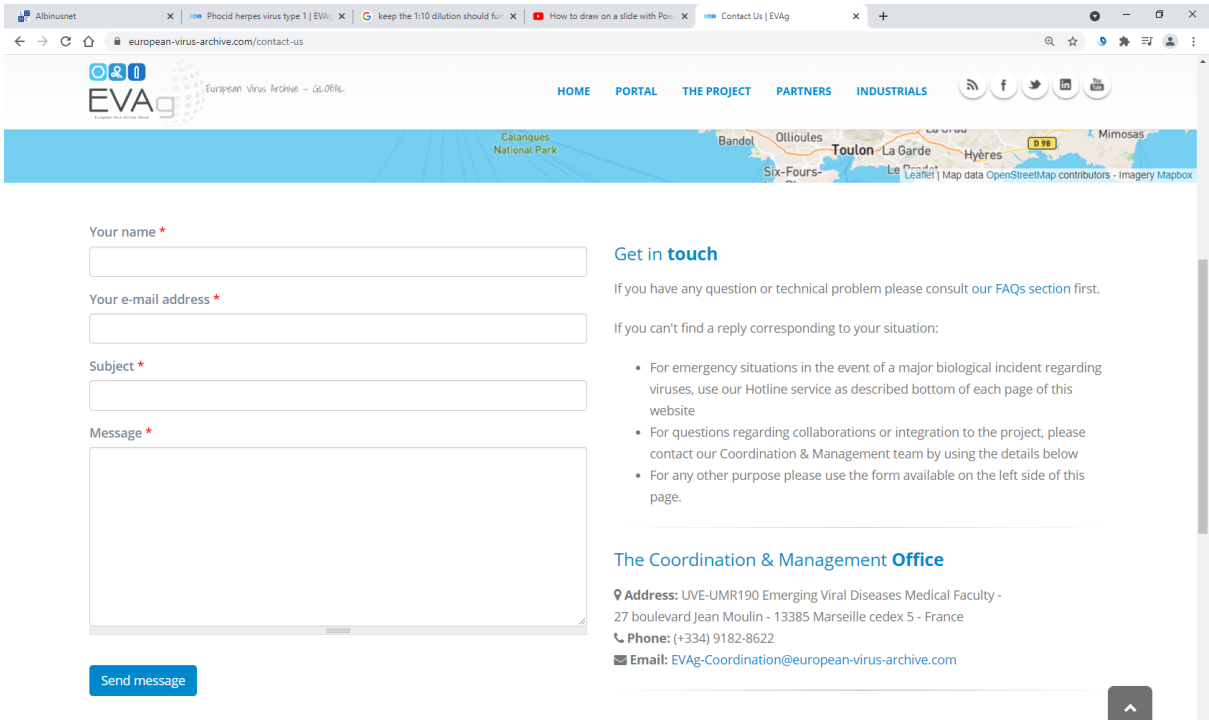
The European Virus Archive
EVA-GLOBAL
 Now gives Global
 is

STILL PROVIDING

The best way to get viral material within the *Scientific Community*.
 Browse our viruses and derived products from the EVAg Portal.

Visit our Portal! or learn more.

Appendix B



Albinusnet | Phocid herpes virus type 1 | EVA | keep the 1:10 dilution should fu | How to draw on a slide with Poi | Contact Us | EVAg

European Virus Archive - GLOBAL

HOME PORTAL THE PROJECT PARTNERS INDUSTRIALS

Calanques National Park

Bandol Ollioules Toulon La Garde Hyères Mimosas
 Six-Fours- Le Cap d'Antibes
 Leaflet | Map data OpenStreetMap contributors - Imagery Mapbox

Your name *

Your e-mail address *

Subject *

Message *

Send message

Get in touch

If you have any question or technical problem please consult [our FAQs section](#) first.

If you can't find a reply corresponding to your situation:

- For emergency situations in the event of a major biological incident regarding viruses, use our Hotline service as described bottom of each page of this website
- For questions regarding collaborations or integration to the project, please contact our Coordination & Management team by using the details below
- For any other purpose please use the form available on the left side of this page.

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