	STANDARD OPERATING PROCEDURE	SOP VITAL 001
		Version: 2
	Sampling and virus concentration from faeces	Date: 15/07/2008
		Page 1 of 4


EU FP VII PROJECT “VITAL”

STANDARD OPERATING PROCEDURE

SOP VITAL 001

**Sampling and virus concentration
from faeces**

<p>CREATED:</p> <p>David Rodriguez-Lazaro: 15/07/08</p>	<p>REVISED:</p> <p>CSL: 28/10/2008</p>	<p>APPROVED:</p> <p>Wim Van der Poel: 07/11/08</p>
--	---	---

	STANDARD OPERATING PROCEDURE	SOP VITAL 001
		Version: 2
	Sampling and virus concentration from faeces	Date: 15/07/2008
		Page 2 of 4

— WARNING —

Adenoviruses, noroviruses, and hepatitis A and E viruses are viral pathogens. All samples and controls shall be handled by trained staff in a laboratory with appropriate equipment. Staff must be fully vaccinated against Hepatitis A and poliovirus. Persons using this SOP must be familiar with normal virology laboratory practice. This SOP does not presume to address fully all of the safety issues associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

AIM

To concentrate virus from 250 mg of faecal contents into a final volume of 140 µl of PBS buffer.

PRINCIPLE

Two hundred fifty mg of soft faecal contents is suspended in buffer, homogenised by vortexing, clarified by centrifugation and supernatant retained for examination.

EQUIPMENT

- Micropipettes of a range of sizes, 1000µl, 200µl and 20µl
- Micropipette tips of a range of sizes, 1000µl, 200µl and 20µl
- Gloves
- Vortex mixer
- Centrifuge and microcentrifuge tubes.
- Centrifuge
- Refrigerator
- Freezer

REAGENTS (SEE APPENDIX)


When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

- Phosphate Buffered Saline (PBS)
- Gentamycin stock (10 mg/ml)
- Deionised water

PROCEDURE


1. Sampling:

1. Place aseptically a representative sample of faeces (at least 1 g) into a sterile plastic bag.
2. Label the plastic bag for traceability, including, at least, the following details:
 - Analyst
 - Date of sampling
 - Location
 - Reference number (for traceability, use the same number for the rest of the analysis process)
3. Maintain the sample at 4°C ± 3°C (max. 24 h) or freeze at -20°C ± 4°C until it is processed.
NOTE: It is better to store the original faecal samples at -20°C rather than the subsequent 10% suspension.

	STANDARD OPERATING PROCEDURE	SOP VITAL 001
		Version: 2
	Sampling and virus concentration from faeces	Date: 15/07/2008
		Page 3 of 4

2. Virus concentration

1. Weigh 250 mg of faecal contents and transfer the sample to a centrifuge tube.
2. Add 2.25 ml of gentamycin-containing PBS solution, and 10 μ l of the positive process control virus to the sample.
3. Mix using a vortex mixer at full speed for 1 - 1.5 minutes.
4. Centrifuge at 3000 \times g for 15 min, and transfer the supernatant into a clean microcentrifuge tube.
5. Store at -20 °C.

	STANDARD OPERATING PROCEDURE	SOP VITAL 001
		Version: 2
	Sampling and virus concentration from faeces	Date: 15/07/2008
		Page 4 of 4

APPENDIX

Phosphate Buffered Saline (PBS)

- 10 Dulbecco's PBS tablets in 1 litre deionised water
or 2 Invitrogen or 2 Gibco PBS tablets in 1 litre deionised water

Using a magnetic stirrer, dissolve the PBS tablets in the deionised water. Once dissolved, aseptically dispense 10ml volumes into sterile universals.

If commercial PBS tablets are unavailable, please make in-house as set out below

In-house preparation:

Add 8g NaCl, 0.2g KCl, 1.15g Na₂HPO₄, 0.2g KH₂PO₄ and 1000ml molecular grade water to a bottle. Mix by stirring until the solids are dissolved. Adjust the pH to 7.3.

Sterilise all solutions according to local procedures e.g autoclave at 121°C for 15 minutes. Check the sterility, of each batch made by plating out 100µl onto Nutrient Agar plates and record on the QC sheet. Label with the volume, batch number and the expiry date.

	Storage	Expiry
Supplier's Stock	Room temperature	Supplier's use-by date
In-house Stock	Room temperature	4 months
In-use	4°C (± 3°C)	4 months
In-media	4°C (± 3°C)	1 week

Gentamycin-containing Phosphate Buffered Saline (PBS)

Add 500 µl gentamycin stock solution (10 mg/ml) to 100 ml PBS for having a final concentration of 50 µg/ml of gentamycin.