

	STANDARD OPERATING PROCEDURE	SOP VITAL 003
		Version: 2
	Sampling and virus concentration from animal-derived fertilizer	Date: 28/10/2008
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EU FP VII PROJECT “VITAL”

STANDARD OPERATING PROCEDURE

SOP VITAL 003

**Sampling and virus concentration
from animal-derived fertilizer**

<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: XXXXXX</p>
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— 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 create a 10% suspension from 250 mg of animal-derived fertilizer contents to be used for nucleic acids extraction.

EQUIPMENT

- Micropipettes of a range of sizes, 1000 µl, 200 µl and 20 µl
- Micropipette tips of a range of sizes, 1000 µl, 200 µl and 20 µl
- Gloves
- Vortex mixer
- Centrifuge and microcentrifuge tubes of 1.5 ml
- Centrifuge
- Refrigerator
- Freezer

REAGENTS (SEE APPENDIX)

When working with chemicals, always wear suitable Personal Protective Equipment including lab coat, disposable gloves, and protective goggles.

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

PROCEDURE

1. Sampling:

1. Place animal-derived fertilizer (at least 1 g) into a sterile plastic bag.
2. Label the plastic bag for traceability, to include 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 (max. 24 h) or freeze at -20°C until it is processed.
NOTE: It is better to store the original faecal samples, rather than the subsequent 10% suspension, at -20° C

2. Virus concentration

1. Weigh 250 mg of animal-derived fertilizer and transfer the sample to a centrifuge tube.
2. Add 2.25 ml of gentamycin-containing PBS solution and 10 µl 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 × g for 15 min, and transfer the supernatant into a clean microcentrifuge tube.
5. Store at -20°C.

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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 8 g NaCl, 0.2 g KCl, 1.15 g Na₂HPO₄, 0.2 g KH₂PO₄ and 1000 ml 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.

Supplier's Stock	Storage	Expiry
In-house Stock	Room temperature	Supplier's use-by date
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.