

	STANDARD OPERATING PROCEDURE	SOP VITAL 002
		Version: 2
	Sampling and virus concentration from harvesters' hands	Date: 28/10/2008
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EU FP VII PROJECT “VITAL”
STANDARD OPERATING PROCEDURE

SOP VITAL 002

**Sampling and virus concentration
from harvesters' hands**

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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 obtain a wash-off from the harvesters' hands to be used for analysis for viruses.

PRINCIPLE

Worker's hands are sampled using a sterile gauze swab moistened in buffer. Sampling is carried out on the right hand (or left hand if left-handed) at a pre-determined time of day ie. immediately before lunch or afternoon coffee break. The workers selected for sampling are not allowed to wash hands before sampling. After sampling, the gauze swab is placed in a sterile plastic bag containing enough buffer to protect the swab against drying out, kept refrigerated and sent to the lab for analysis.

EQUIPMENT

- Micropipettes of a range of sizes, 1000µl, 200µl and 20µl
- Micropipette tips of a range of sizes, 1000µl, 200µl and 20µl
- Latex or Vinyl Gloves
- Centrifuge and microcentrifuge tubes/bottles of a range of sizes, 1.5 ml, 15 ml, 50 ml. 1.5-ml tubes with screw caps are necessary. The exact selection of tubes required will depend on the centrifuges and rotors available in each laboratory.
- Centrifuge
- Refrigerator
- Freezer
- Sterile plastic bags containing sterile gauze swab in 20 ml of sterile gentamycin-containing PBS solution. This "sampling set" can be prepared in advance from non-sterile items and autoclaved prior to use.
- 10×10 cm sterile gauze swab (supplied, for example, by Smith & Nephew A/S" (www.smith-nephew.com)).

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

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PROCEDURE

1. Sampling:

1. Use sterile plastic bags containing a sterile gauze swab in 20 ml of gentamycin-containing PBS solution.
2. Prior to each sampling, wear a new set of gloves.
3. Open the sterile plastic bag. Remove excess buffer by squeezing the gauze swab while keeping it in the bag.
4. With a firm pressure rub the back of the right hand back and forth five times. Using the same technique, sample the palm of the hand. Finally rub gently the spaces between the fingers and all the fingertips.
5. Using sterile tweezers, remove the swab from the bag.
6. Using firm pressure rub the back of the harvester's hand back and forth five times. Using the same technique, sample the palm of the hand. Finally rub gently the spaces between the fingers and all the fingertips (try to swab under fingernails also).
7. After sampling place swab back into the sterile plastic bag.
8. Label the plastic bag for traceability, including the following details:
 - Analyst
 - Date of sampling
 - Location
 - Reference number (for traceability, use the same number for the rest of the analysis process)
9. Maintain the sample at $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$ (max. 24 h).

2. Virus Concentration

1. Squeeze the gauze swab in the bag to release the contents of the swab.
2. Discard swab
3. Pour the contents from the plastic bag into a clean 50 ml centrifuge tube.
4. Add $10\mu\text{l}$ of the sample process control virus to the tube.
5. Vortex at full speed for 20 seconds.
6. Centrifuge at $3,000 \times g$ for 5 minutes.
7. Transfer the supernatant into a clean 50 ml centrifuge tube, using a graduated pipette. Record the volume.
8. 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 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.