EU FP VII PROJECT "VITAL"

STANDARD OPERATING PROCEDURE

SOP VITAL 013

Nucleic acids extraction from waters, or harvesters' hands wash-off

CREATED:	REVISED:	APPROVED:
David Rodriguez-Lazaro: 19/04/2010	FERA: 19/04/2010	Wim Van der Poel: 19/04/2010

— 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 extract the nucleic acids from the concentrates of waters (irrigation water, processing water, slaughter house effluents etc; see SOP VITAL 004.) or harvesters' hands wash-off. (see SOP VITAL 002).

PRINCIPLE

This protocol is based on the NucliSENS® miniMAG® (bioMérieux) using Boom technology and magnetic silica.

EQUIPMENT

- Micropipettes of a range of sizes, 1000µl, 200µl and 20µl
- Sterile, RNase-free micropipette tips of a range of sizes, 1000µl, 200µl and 20µl
- Disposable gloves
- 1.5-ml microcentrifuge tubes.
- Microcentrifuge (with rotor for 1.5 ml and 15 ml tubes) (20,000 × *g*)
- Freezer (-70ºC)
- Magnetight™ Separation Stand (for one 15-ml tube, one 50-ml tube and four 1.5-ml tubes), (Cat. No. 69964-3), Novagen, or any alternative magnetic support (miniMAG®).
- thermoshaker (60 ºC and 1,400 rpm)
- Vortex mixer
- Fine –tipped plastic pastettes (e.g.Fisher PMK-500-010R)

REAGENTS

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

- NucliSENS® easyMAG Lysis Buffer, 4x1000ml (Cat. No 280134), bioMérieux, (or 500 ml Cat. No 284135).
- NucliSENS® Magnetic Extraction Reagents including silica magnetic particles, WB1, WB2, WB3 and elution buffer. 48 tests (Cat. No. 200 293), bioMérieux

PROCEDURE

1. Transfer 5 ml of the hand washings (see SOP2) or water concentrate (see SOP VITAL 004) into a clean centrifuge tube.
2. Add 10 ml of NucliSENS® lysis buffer to the tube, and mix by vortexing briefly. Incubate for 10 min at room temperature.
3. Centrifuge for 2 min at 1,500 × *g* to ensure that entire sample is brought down into the tube.
4. Add 50µl of well-mixed magnetic silica solution to the tube and mix by vortexing briefly. Incubate for 10 min at room temperature.
5. Centrifuge for 2 min at 1,500 × *g* then carefully discard supernatant*.
6. Add 400µl wash buffer 1 and resuspend the pellet by pipetting/vortexing.
7. Transfer the suspension to a 1.5 ml screw-cap tube. It is very important to avid creating foam at this stage. Very gentle pipetting should be done when using wash-buffer 1, because of the GuSCN incorporated into it. This will avoid any loss of nucleic acids.
8. Wash for 30 sec using the automated wash steps of the miniMAG® extraction systems or by vortexing. After washing allow silica to settle using magnet of the miniMAG® extraction systems or magnetic rack. Discard the supernatant carefully using a plastic pastette*.
9. Separate tubes from magnet, then add 400 µl wash buffer 1. Resuspend pellet, wash for 30 sec, allow silica to settle using magnet then discard supernatant.
10. Separate tubes from magnet, then add 500 µl wash buffer 2. Resuspend pellet, wash for 30 sec, allow silica to settle using magnet then discard supernatant.
11. After this first washing, transfer the total sample to a clean 1.5 ml tube to eliminate GTC residues.
12. Repeat step 10.
13. Separate tubes from magnet, then add 500 µl wash buffer 3. Wash for 15 sec, allow silica to settle using magnet then discard supernatant.
14. Add 50 µl (pig chain) or 150 µl (vegateble chain) elution buffer, and transfer the tubes to thermoshaker or equivalent.
15. Incubate for 5 min at 60ºC with shaking at 1,400 rpm.

16. Place tubes in magnetic rack and allow silica to settle, then transfer eluate to a clean tube.
17. Repeat steps 14-18 with another volume of 50 µl (pig chain) 150 µl (vegateble chain) elution buffer (total volume of 100 µl for the pig chain or 300 µl for the vegetable chain)
18. Retain at 4ºC for a maximum of 24 hrs or -80ºC for up to one week.

    NOTE: Any Washbuffer 3 and elution buffer remaining must be discarded after use.

19. Before use, centrifuge for 2 min at 1,500 × *g* to ensure all traces of silica are pelleted to the bottom of the tube. If any silica is present in the PCR, it will cause inhibition in the samples.

**NOTE: NucliSens Lysis buffer contains guanidinium. Therefore, either dispose the filtrate in a high pH environment (e.g. into a 500 ml waste flask that contains 10 g of NaOH), or refer to local special waste procedures*