STANDARD OPERATING PROCEDURE

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Laboratory of Viral Evolution, Department of Microbiology, Monash University

SOP 3: Fecal sample process

1. INTRODUCTION

This SOP describes the procedure for fecal sample processing from different animals (e.g. Bats, Possums, dogs, cats, ducks, swamphens).

2. SCOPE

This procedure serves as a guide to laboratory personnel engaged in fecal sample processing from different sources.

3. SAFTEY

All lab workers must:

- Wear appropriate personal protective equipment (PPE) (Nitrile gloves, lab coat, closed-toed shoes, and safety glasses when specified by risk assessment) when carrying out the procedure.
- Sample hadling has to carried out in PC2 facilities.
- USE RNAseZap spray to remove RNase contamination from work surface/ equipment/ solutions.
- Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipettes, pipette tips, and tubes.
- Read and understand the recommendations in this SOP to ensure uniformity in practice.
- New staff or students should seek supervision from a senior lab co-worker during first attempt of the procedure.

4. Materials Needed

- Hanks' balanced salt solution (Gibco BRL)
- 0.45-μm filter and a 0.22-μm filter (Millipore)
- Centrifuge that reach to 12000 g at 4°C
- Ultracentrifuge 184,000 × *g*
- Turbo DNase (final concentration, 20 U/ml; Ambion)
- RNase A (final concentration, 0.1 mg/ml; Fermentas)
- Falcon tube

5. Fecal Sample processing

The fecal samples will be transported to the laboratory in dry ice or liquid nitrogen and stored at −80°C until analysis.

1. Resuspend the fecal sample in Hanks' balanced salt solution (Gibco BRL) (1:10, wt/vol) and homogenized thoroughly.
2. Centrifuge the suspension at 5,000 × *g* for 10 min.
3. Transfer the supernatant to fresh tubes and centrifuged at 13,000 × *g* for 15 min.
4. Filter a volume of 150 ml of the supernatant through a 0.45-μm filter and a 0.22-μm filter (Millipore) to remove eukaryotic and bacterial cell-sized particles.
5. Ultracentrifuge the filtrate at 184,000 × *g* for 3 h at 8°C to pellet the viral particles.
6. Resuspend the pellets in 400 μl Hanks' solution and filter with a 0.22-μm filter.
7. Treat the filtrate with Turbo DNase (final concentration, 20 U/ml; Ambion) and RNase A (final concentration, 0.1 mg/ml; Fermentas) at 37°C for 30 min to digest non-particle-protected nucleic acids.
8. Use the treated sample for RNA extraction.