Document Number:	**SASoM/METHOD/043.v4**
Title:	**Exosome Isolation Using Centrifugation**
Version:	**v4**
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SOP History		
Number	Date	Reason for Change
v1	02/08/2013	Original
v2	10/08/2015	Update
v3	10/08/2017	Update and revised author
V4	10/08/2019	Update

1.0 Purpose –
This SOP describes the current procedure for **Exosome Isolation Using Centrifugation** for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in **Exosome Isolation Using Centrifugation** work.

3.0 Responsibilities –
All staff involved in cell culture are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

By 'samples', we mean either (i) cell suspension, (ii) urine, (iii) serum or (iv) cell culture supernatants.

N.B. It is important to grow cells in exosome-free medium. Therefore, any serum added to cell culture medium should be depleted of exosomes by ultracentrifugation at 100,000 x g overnight, at 4°C prior to use (turn off the deceleration brake: after centrifugation the exosomes will be left undisturbed by a gradual slowing of the spin cycle).

N.B. It is also advisable to cut down the concentration of serum to the minimum required for cell type, as BSA will often still aggregate on spin down in ultracentrifuge, may distort gels in 70kDa region, and add false signal to protein estimations.

1. Transfer (45 ml) sample to a conical tube.

2. Centrifuge at 150 x g (Heraeus Instruments Megafuge 1.0R; 1000-1500 RPM) for 10 minutes at room temperature to pellet any cells.

3. Transfer (30 ml of) the supernatant to ultracentrifuge tubes. (The cell pellet, containing whole cells, can be discarded unless needed for further work).

4. Centrifuge the supernatant at 10,000 x g (Beckman Coulter Optima L-100 XP Ultracentrifuge; SW 32 Ti rotor; 7,500 RPM) for 30 minutes at 4°C to further remove cells, cell debris and larger microvesicles.

5. (Some protocols recommend filtering the supernatant through a 0.2µm filter to remove particles larger than 200 nm in diameter. However this can lead to filtration of aggregates and the membrane can become clogged filtering single exosomes.)

6. (The supernatant after step 4 can be used to quantify exosome content of the sample using NTA.)

7. Transfer the supernatant to new centrifuge tubes. The pellet, containing cell fragments, can again be discarded.

8. Centrifuge the supernatant at 100,000 x g (Beckman Coulter Optima L-100 XP Ultracentrifuge; SW 32 Ti rotor; 24,000 RPM) for 120 minutes at 4°C to pellet the exosomes.

9. Discard the supernatant.

10. The exosomes can be resuspended from the pellet using a small volume of appropriate buffer (this depends on the downstream experiments planned):
    a. Lysis buffer can be used for protein and RNA isolation.

b. PBS for electron microscopy and flow cytometry.
c. Exosome-depleted or serum free RPMI medium (or resuspension in a small amount of supernatant) may be preferred for functional studies.

11. The exosomes are stable when stored at -20^{o}C or lower.

N.B. If the sample needs to be further purified the exosome pellet can be floated on a sucrose gradient: the exosomes will be primarily found in the fraction representing a density of 1.13-1.19 g/ml[2].

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training -
All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1 Risk assessments –COSHH/004 and RA/BIOL/004

8.2 SOP SASoM/METHOD/042
Exosome Isolation Using Antibody Coated Beads

8.3 SOP SASoM/METHOD/043
Exosome Isolation Using Centrifugation

8.4 SOP SASoM/METHOD/044
Exosome Quantification Using NanoSight Tracking Analysis

8.4 SOP SASoM/METHOD/044
Exosome Red Fluorescent Labelling

9.0 Approval and sign off –

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