Document Number: **SASoM/METHOD/044.v4**

Title: **Exosome Quantification Using NanoSight Tracking Analysis**

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 Quantification Using NanoSight Tracking Analysis** 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 Quantification Using NanoSight Tracking Analysis** 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 –

1. Clean the NanoSight sample chamber prior to use:
    a. Disassemble the chamber and clean the mounting plate/viewing port with lens cleaning tissue.
    b. Reassemble the chamber, tightening the screws of the viewing port to a finger-tight seal.
    c. Use a 1 ml syringe to fill the chamber serum free RPMI medium slowly, to avoid the formation of air bubbles in the chamber. (MilliQ water or PBS can be used, although serum free RPMI medium has the lowest background and may be best for cleaning prior to use with tissue culture samples.)
    d. Flush the chamber three times (3 ml) with solution.

2. Ensure the chamber is clean:
    a. Ensure the chamber is filled with solution slowly to expel 1-2 drops of solution at the far end port.
    b. Place the chamber on the microscope stage and attach the appropriate power source (638 nm laser for dark field microscopy, 488 nm laser for fluorescence work – ensure the appropriate camera is used).
    c. Turn on the laser and open the NanoSight NTA 2.3 software on the desktop.
    d. (If viewing sample on the desktop screen, confirm the microscope is set to view samples on this by ensuring the toggle is at the 'in' position – toggle found on the left binocular head, at the base of the eyepiece. If choosing to view the sample through the eyepiece, pull the toggle out. Videos can only be captured with the toggle in, viewing the sample on the desktop screen.)
    e. Orientate the chamber to view the sample between the laser thumb-print and point of exit (vertical line on screen), then focus.
    f. The solution should be devoid of exosome-sized particles.
    g. Remove the solution by withdrawing on the syringe.

3. Prepare sample (exosome samples are often highly concentrated, needing dilution):
    a. Blood serum samples often need around 1 in 50 dilution, urine samples around 1 in 5.

4. Load the sample:
    a. Use a 1 ml syringe to fill the chamber with sample, avoiding air bubbles as before.
    b. Return chamber to microscope as before.
    c. Ensure attachment of the appropriate power source.
    d. Attach the temperature probe to the chamber (the rate of movement of exosomes by Brownian motion must be standardised for temperature).

5. Analyse the sample:

a. N.B. The most accurate scans are achieved when >100 exosomes are tracked per video, and the concentration of exosomes is <10 x 10^8/ml.
b. Ensure focus of the miscroscope (note that focus varies when viewing through the eyepiece and on the screen).
c. Record a video by selecting 'capture' on the software.
d. Choose a camera level that is appropriate to your practice. (Either select the optimal level for each individual sample and accept this is not a standard level, or standardise by using the same level for every sample (e.g. 15) and accept that this may not be optimal for all of them.)
e. Record a 30-60 second video (depending on concentration and homogeneity of the sample) and save the file on the computer hard drive in named folder by date.
f. Remember to enter the temperature at the end of video capture.
g. Record three videos for each sample.
h. Clean the chamber between samples.
i. Process each file by choosing 'open file' and selecting the video the process. (Choosing a maximal screen gain, setting 10, will optimise the ability to see exosomes captured in the video and will not affect analysis of the sample. Either select the optimal detection threshold for each individual sample and accept this is not a standard level, or standardise by using the same level for every sample (e.g. 6) and accept that this may not be optimal for all of them.) Preferably, choose one detection threshold for samples with a single experimental set.
j. (This generates a PDF of the sample video, providing information on the number of tracks, concentration of exosomes and mode/mean/median size of the exosomes.)

6. Save the data:
   a. The data files will be saved to the computer hard drive.
   b. To avoiding slowing the computer transfer these files to the attached external Toshiba hard drive. (For ultimate security of precious data, also make a copy to a personal portable hard drive of all the data.)
   c. The report PDFs can be used for further work (i.e. copy to own computer).

7. Clean the chamber:
   a. Flush the chamber three times (3 ml) with solution.
   b. Disassemble the chamber and clean the mounting plant/viewing port with lens cleaning tissue.
   c. Reassemble the chamber, tightening the screws of the viewing port on to a finger-tight seal.

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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