Document Number:	**SASoM/EQUIP/040.v1**
Title:	**Use of the Coulter Counter Model Z1**
Version:	**v1**
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SOP History		
Number	Date	Reason for Change
v1	26/09/2013	Original

1.0 Purpose –
This SOP describes the current procedure for performing cell counts using the Coulter Counter 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 electronic cell counting using the Coulter Counter for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –
All staff involved in cell counting 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 –

NB: Before using the machine, it must be calibrated if data on the cell type you are using is not already available (see below for details of calibration procedure).

1. Dispense 20ml Isoton into the counting vials using the automatic pipette.

2. Use a vial with Isoton only to determine the background count.

3. Add an appropriate volume of cell suspension depending on the cellularity of the sample. You want counts that are not too low i.e. less than 1000 or too high i.e. more than 60,000. Note the volume added to later calculate cellularity from the dilution. Cap the vial and gently invert to mix.

4. Press SET-UP and field S2 will appear. Use up and down arrow keys to move up and down. At aperture check that set to 100 microns and metered volume is 0.5 ml. This is the usual setting but need to check if someone had changed these.

5. Press SET-UP again field S3 appears press SET-UP again field S4 appears press SET-UP again and field S1 appears.

6. In field S1 select units. Can either be fl or microns, use fl. Can toggle between these using the < or > key. Scroll down to Set size. The value used will depend on the cell type you are counting and determined from the calibration curve e.g. for human epithelial cells and tumours is currently 350fl.

7. Pull down the vial support (there is a spring loaded clamp under the platform) and place the counting vial (containing the sample) under the aperture probe. Carefully slide the platform into the counting position again depressing the spring loaded clamp and holding the platform to control the movement.

8. Correcting for coincidence. If the count rate is high then there is a finite probability that more than one cell will pass through the aperture at the same time. This will register as one large cell. Thus the count will be underestimated and a coincidence correction is required. This is calculated using the following formula :

True count (C) + (S - B) + ((S - B)/1000)2 x 2.5

where S = sample count and B = background count (Isoton only)

On this machine the correction is automatically calculated for you by activating the appropriate display mode.

9. Press the START/STOP button to count.

10. Take 2 readings and average these. Subtract the background and calculate the cellularity of the cell suspension. Remember the count on the display is the cell number in 0.5ml. Thus the number in the vial is x2 x20 (x40). This is the number of cells in the volume you added to the vial eg. In 100 µl.

11. There is a mirror system and light which allows the aperture to be projected onto a screen. Adjust the position of the vial and focus the aperture so that this can be viewed while counting. If the aperture becomes blocked then lower the vial and gently brush the debris away using the paint brush provided. The aperture can also be cleaned by using the unblock button on the keyboard.

Calibration of the Coulter Counter.

Before the Coulter can be used for cell counting, it is necessary to calibrate the instrument for the particular cell population you wish to count. Different cells have different volumes and thus appropriate settings have to be selected. The size setting acts as a gating system for the pulses. As the size setting is increased, pulses with heights above this level will be counted. As all cell populations have a range of cell sizes, they will generate a spectrum of voltage pulses.

Plot a volume threshold curve for the cell line you are using.

1. Dilute the cell suspension in a 100 ml. beaker.

2. Press SET-UP and field S2 will appear. Use up and down arrow keys to move up and down. At aperture check that set to 100 microns and metered volume is 0.5 ml.

3. Press SET-UP again field S3 appears press SET-UP again field S4 appears press SET-UP again and field S1 appears.

4. In field S1 select units. Can either be fl or microns, use fl. Can toggle between these using the < or > key. Scroll down to Set size. This value will be varied to count particles above a set volume size.

5. Press START / STOP key to count. Field A2 appears and counting commences.
When completed A4 field gives count and volume setting.

6. Press SET-UP key to return to S1 field and alter set size then recount.
As the volume setting is increased, the count will initially fall rapidly as pulses derived from electronic noise and small debris are gated out. The count should then plateau as all pulses generated from the cells are larger then the volume set. Further increase in the volume results in some of the smaller cells being gated out.

The optimal setting for counting is the value in the middle of the plateau.

Points to note when using the Coulter Counter.

- **Do not touch the electrodes while counting or you may receive a small electric shock.**
- **Move the spring loaded sample platform up and down carefully.**
- **Make sure the liquid waste reservoir does not overfill or liquid will enter the pump and damage it. Empty when approaching the marked line.**

- **Make sure the clean reservoir is filled with Isoton above the indicator mark. This is used to flush the system and the unblock cycle.**
- **When counting is complete, leave a reservoir vial with clean Isoton in it under the glass aperture and flush through several times to clean.**

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 using the Coulter Counter before starting any cell counting .

8.0 Related documents –
8.1 Risk assessments – need to be made in relation to the samples you use.
RA/COSHH/002 (Disposal of Waste material)
RA/COSHH/004 (Cell Culture)

9.0 Approval and sign off –

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Signature: Date:

Management Approval:

Name: Mary Wilson

Position: Laboratory Manager

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