Document Number:	**SASoM/EQUIP/092.v2**
Title:	**Use of the Shandon Cytospin**
Version:	**v2**
Author:	**Peter Mullen**

Effective from:	01/12/2019
Valid to:	31/11/2024

SOP History		
Number	Date	Reason for Change
v1	01/12/2014	Original
V2	01/12/2019	Update

1.0 Purpose –
The purpose of this SOP is to outline the principles of the routine use of the Shandon Cytospin for the preparation and fixation of cells in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to routine use and maintenance of the Shandon Cytospin within the SASoM.

3.0 Responsibilities –
It is the responsibility of all users of the Shandon Cytospin within the SASoM to comply with this SOP.

4.0 Procedure –

The Cytospin is an instrument used for depositing cell suspensions onto glass microscope slides. The instrument functions in the same manner as a centrifuge and therefore should be correctly equally balanced each time it is used.

The rotor is NOT fixed to the spindle at any time and therefore it is ESSENTIAL that it is correctly balanced before use.

The rotor should ALWAYS be removed form the instrument before removing / replacing the rotor.

Operation:

Switch on the instrument by pressing the Power Switch at the lower left hand corner. The green 'Power' light should illuminate.

Press the lid-release button (front left hand corner just in front of the glass cover). Open the glass-covered lid.

Removing the rotor:

The rotor is not fixed to the drive shaft but simply rests on top of it.

Remove the rotor from the centrifuge by lifting vertically upwards and then sitting firmly on the bench. Firmly press the silver button in the centre of the lid until it 'pops'. Lift the lid off the rotor. Never press the release buton with the rotor inside the cytospin as this may damage the shaft.

Load samples according to the descriptions / diagrams below.

Replace the rotor lid by first pulling the silver button out as far as it will go and then placing the lid on top of the rotor. Firmly press the silver button down until it 'pops' into place. Carefully place the rotor inside the instrument, taking care to not disturb the samples. Check that the rotor will freely rotate and then close the glas-top cover of the instrument.

Preparation and Loading of samples:

Remove lid from sealed head to load samples. Cell samples to be put into cytospin should be at a concentration of 0.2 x 10^6 cells/ml. 100µl of cell sample is pipetted into each sample chamber ensuring the pipette tip is inserted into the outlet port and not just the mouth of the funnel.

When transferring the sealed head to the Cytospin 2 keep the head in a horizontal plane and do not swing it around when carrying it, this could result in sample fluid(s) coming into premature contact with the filter card(s)

Programming the instrument:

The instrument can be programmed for speed (rpm x10), time (mins) and acceleration rate (hi/lo). **A typical run would be for 5mins at 800rpm with high accelaration**.

Press the 'set time' button and then select the desired time using the numerical pad. Press Enter to confirm.

Press the 'set speed' button and then select the desired speed (x10) using the numerical pad. A speed of 800rpm will be entered as '80'. Press Enter to confirm.

Select the acceleration rate by pressing 'accel' to toggle between 'hi' and 'lo'.

Press 'Start' to begin the run. Press 'Stop' to terminate a run at any time.

Removal and Fixation of samples:

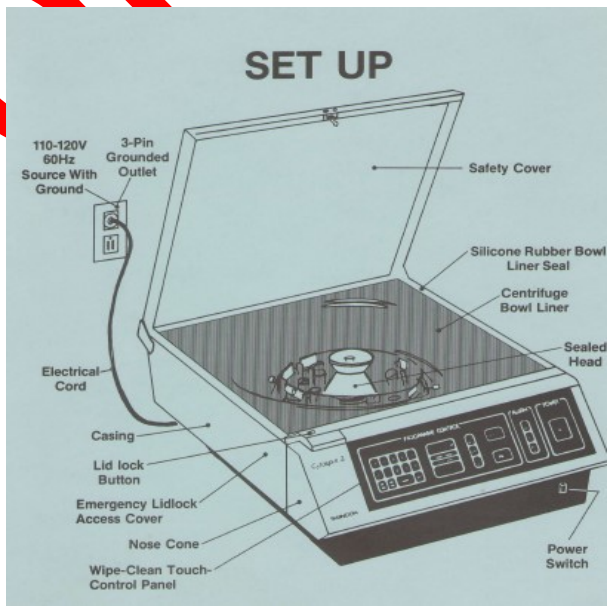
At the end of the run, remove the rotor from the instrument as described above and then remove the rotor lid.

Remove the chamber assemblies one at a time and carefully disassemble, taking care that the blotting paper does not come into contact with the cell preparation.

Immerse the slides in ice cold methanol for 2 minutes and allow to air dry for 5 minutes. Slides can be stored at -20 in a suitable holder to be stained at a later date.

Ensure any disposable cytofunnels or filter cards are deposited in biohazard waste bin.

Wipe away any liquid spillage from Cytospin head with 70% Ethanol.



The Cytospin 2 is an instrument with an operating speed of 200 - 2000 r.p.m. for depositing cells onto microscope slides using centrifugal force. Three parameters - Speed, Time and Acceleration Rate are programmable. The parameters, once selected, are held in a volatile memory.

The Cytospin 2 is supplied with a 12 position sealed head as standard equipment. This head rests on a tapered drive boss and lifts out for routine use.

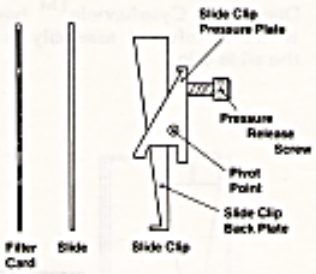
Samples are centrifuged in reusable plastic sample chambers OR disposable Cytofunnels. Each Sample Chamber Assembly - comprising Sample Chamber, Filter Card and Glass Microscope Slide - is held in position using a special stainless steel slide clip. Up to 12 Sample Chamber Assemblies fit into the sealed head. During centrifugation (at 200 rpm) the Sample Chamber Assemblies tilt from an angled loading position into an upright operating position.

1. Never open the sealed head when it is in the instrument. Remove the sealed head from Cytospin2.

   Release the button in the center of the sealed head. Remove the lid and see how the two seals fit into place. The top seal is just under the release button, and the lid seal is the large seal running around the outside of the bowl.

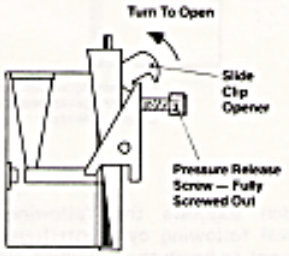
   Replace the lid and lock by pushing down on the button in the center of the lid. The sealed head should always be opened and closed while it is outside the instrument.

2. Place a clean microscope slide and filter card against each sample chamber and place them in a slide clip. Use the slide clip opener to release the tension on the spring. The pressure release screw may be used, but when using the clip opener, make sure the pressure release screw is turned out as far as it will go. Assemble all 12 slide clips with sample chambers, slides and filter cards.

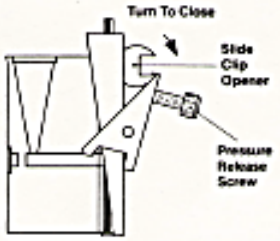


3. How to use the slide clip opener:

   a) Ensure that the pressure release screw is fully screwed out. This is important.
   b) Hold slide clip opener in one hand, slide clip in the other hand.
   c) Insert the slide clip opener on top of clip (Fig. 1). Turn flat side of clip opener to flat side of slide clip.
   d) Slide clip will remain in place allowing you to remove or insert the sample chamber. Slide, sample chamber and filter card should be inserted together.
   e) To close the slide clip, turn and release the slide clip opener (Fig. 2).



TO OPEN SLIDE CLIP — FIG. 1

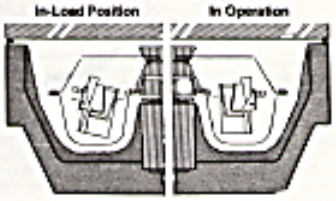


TO CLOSE SLIDE CLIP — FIG. 2

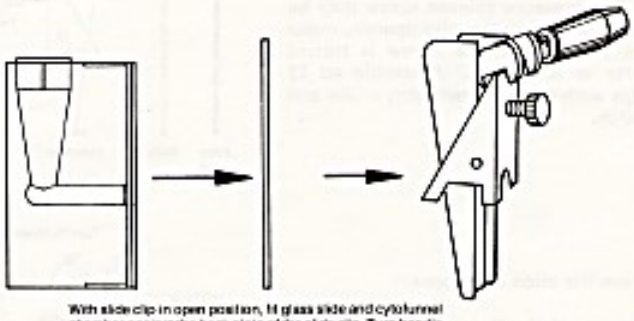
4. Place all 12 chambers and clip assemblies in the slots provided in the bowl. Make sure that each is free to move forward to an upright position and tilt when released. The clip assembly should tilt back toward the center of the head easily of its own accord.

   The reason the chamber tilts is so that when the sample is placed into the chamber, it does not run out to the front of the chamber and start to be absorbed by the filter card.

   DO NOT PUT FLUID IN THE CHAMBER YET.

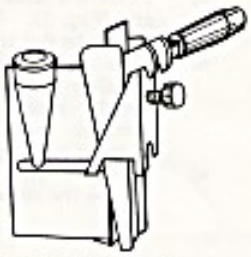


5. Disposable Cytofunnels™ have permanently attached filter cards. In use, the entire Cytofunnel assembly is coupled with a clean microscope slide and placed in the slide clip.

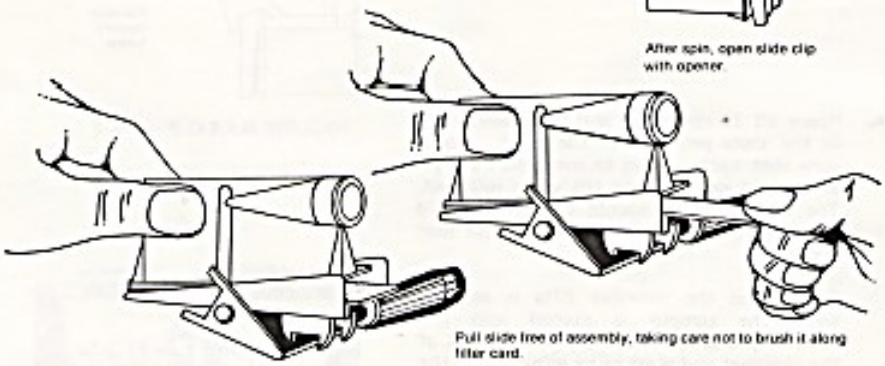


With slide clip in open position, fit glass slide and cytofunnel chamber against the back plate of the slide clip. Turn handle to close slide clip.

Shandon suggests the following method for slide removal following cytocentrifugation. Care must be taken not to brush the specimen along the filter card.



After spin, open slide clip with opener.

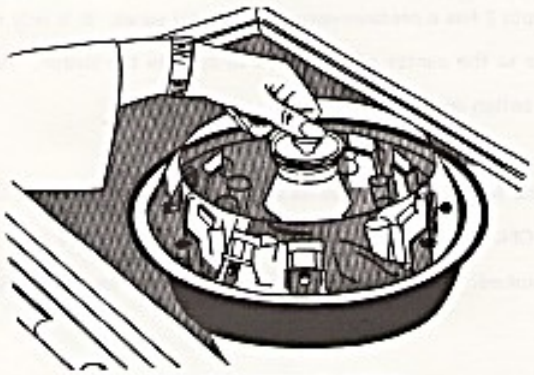


Pull slide free of assembly, taking care not to brush it along filter card.

Remove cytofunnel from clip and discard.

Grasp cytofunnel with other hand and turn to horizontal position — suspending the entire slide clip assembly by the used sample chamber. This will separate the cytofunnel from glass slide.

6. With all 12 clip assemblies in place, replace the lid on the sealed head by lifting the center button; now press the center button to lock and seal the head.

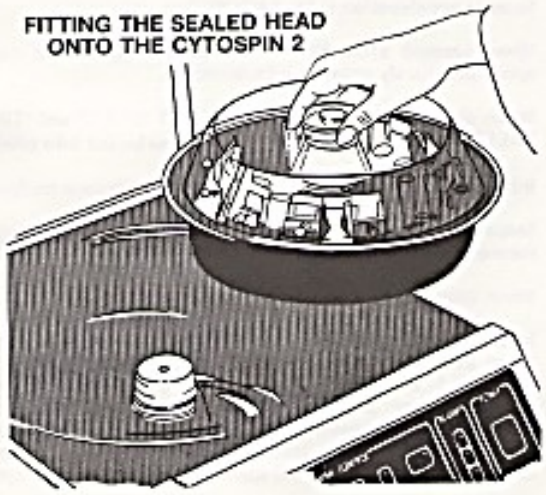


CLOSING THE SEALED HEAD

7. Return the sealed head to the instrument and place it on the center cone. Note there is some silicone grease on the cone. Do not wipe this grease off.

Reapply grease monthly

FITTING THE SEALED HEAD ONTO THE CYTOSPIN 2

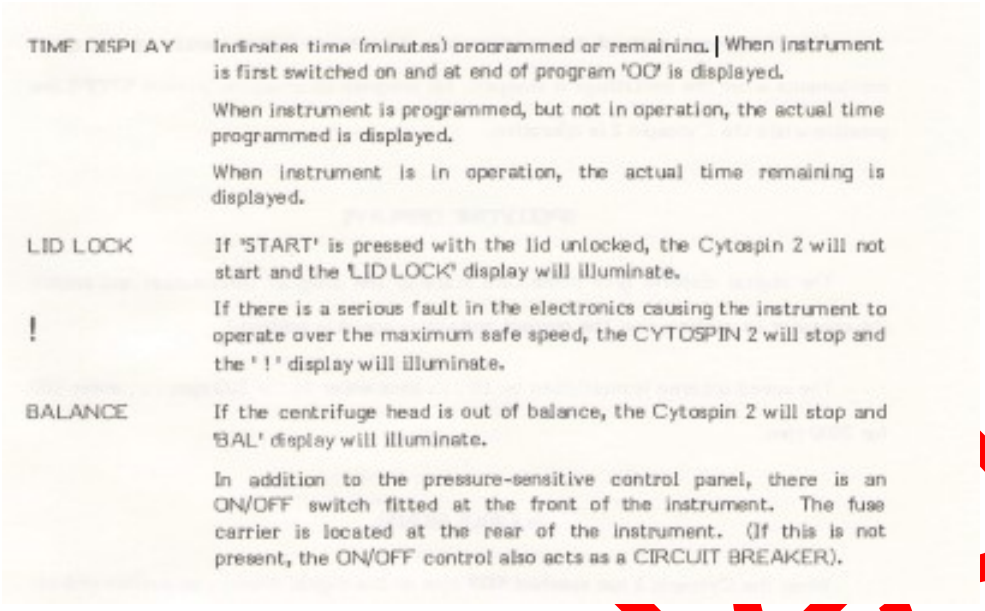


CONTROL PANEL DETAIL

The Cytospin 2 has a pressure-sensitive control panel. It is only necessary to apply a slight pressure to the center of each area to operate the switch. An audible signal is heard when the switch operates.

Pressing the following areas has no effect: HI . . . LO . . . BAL (balance) . . . ! . . . LID LOCK . . . ON. These areas are informational only; they are NOT SWITCHES and do not need to be pushed. Full details of the function of each area are given below.

CONTROL	FUNCTION
SET TIME	Selects time (minutes) parameter in program memory. Minimum: 1 Minute; Maximum: 99 Minutes.
SET SPEED	Selects speed parameter in program memory. Minimum: 200 rpm Maximum: 2000 rpm
ACCELERATION	Selects acceleration rate. ('ACCEL')
CANCEL	When pressed after 'SET TIME' or 'SET SPEED' it cancels time or speed previously entered into memory.
ENTER	When pressed after 'SET TIME' or 'SET SPEED' and 'TIME' or 'SPEED VALUE' it transfers the time or speed selected into program memory.
START	Initiates program entered in memory and displays on front panel.
STOP	Stops centrifuge immediately when pressed. The program stored in memory is not affected by using this control.
ON	When illuminated it indicates main power is switched on.
SPEED DISPLAY	Indicates speed programmed or achieved as a 3-digit display. When instrument is first switched on and also when 'SET SPEED' and 'CANCEL' are operated '000' is displayed. When instrument accelerates or decelerates, the display will change as the speed increases or decreases. When the programmed speed is achieved, the speed display remains stable. When the instrument is stopped, the display indicates the actual speed entered into the program memory.



TIME DISPLAY	Indicates time (minutes) programmed or remaining. When instrument is first switched on and at end of program '00' is displayed.
	When instrument is programmed, but not in operation, the actual time programmed is displayed.
	When instrument is in operation, the actual time remaining is displayed.
LID LOCK	If 'START' is pressed with the lid unlocked, the Cytospin 2 will not start and the 'LID LOCK' display will illuminate.
!	If there is a serious fault in the electronics causing the instrument to operate over the maximum safe speed, the CYTOSPIN 2 will stop and the '!' display will illuminate.
BALANCE	If the centrifuge head is out of balance, the Cytospin 2 will stop and 'BAL' display will illuminate.
	In addition to the pressure-sensitive control panel, there is an ON/OFF switch fitted at the front of the instrument. The fuse carrier is located at the rear of the instrument. (If this is not present, the ON/OFF control also acts as a CIRCUIT BREAKER).

5.0 Personal protection –
Howie coat and disposable gloves must be worn at all times.

6.0 Training –
All users have to be trained before using the Instrument by a designated person.

7.0 Related documents –

7.1 Equipment Manuals - Shandon –cytospin-2-operator guide

8.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE