**Preparation of cells for staining and subsequent imaging using Cytospin**

1. Count the number of viable cells using a hemocytometer.
2. Centrifuge cells at 300RPM for 6 min.
3. Remove media and resuspend cells in 2% Paraformaldehyde, vortex, incubate at 4C for 15-20 min
4. Centrifuge cells at 300RPM for 5 min. Remove liquid
5. Resuspend the cells in PBS at a final concentration of 1 to 5 x105 cells/ml in a volume of at least 100ul but no more than 200 ul

Note: If the cell suspension is too dilute, there will not be enough cells immobilized on the slide after the cytospin centrifugation. If the cell suspension is too concentrated, the cells will tend to clump together and be poorly dispersed following the cytospin.

**Cytospin Located in CBI**



1. Label the glass microscope slides.
2. Assemble the glass slides, filter cards (A), and sample chambers (B) in the cytospin centrifuge, ensuring that the centrifuge is balanced. The glass slide should be placed frosted label side facing the filter towards the sample chamber. The sample chamber can divide the volume between two regions of the filter so account for 2xsample within the maximum volume of 200 ul/sample chamber.
3. Donnenberg protocol use of 1 x105 cells/sample in ~100 ul to 200 ul of volume/sample
4. Pipette 100–200 ul of cell suspension into each of the sample chambers.
5. Centrifuge at 300 RPM for 6 min.
6. Remove the slides, filter cards, and sample chambers from the centrifuge. Disassemble carefully, so as not to damage the cells on the slide. Discard filter cards and sample chambers.
7. Mark the position of the cells with a permanent marker on the underside of the slide (the side without cells).
8. Examine the cells under a microscope to ensure that they are not damaged. There should be an adequate number of cells on the slide and the cells should be evenly dispersed.
9. Store at slides in a slide box at 4C until ready for Immunofluorescent staining. Donnenberg lab immediately stains with Giemsa-Wright

Note: The optimal speed of centrifugation may vary between cell lines. Cells may be damaged or flattened if the centrifugation speed is too high, and may not adhere properly to the slide if the speed is too low

Reference:

Shandon Filter Cards ref 5991023 from Thermo Scientific 200/box

Corning Cell-Tak Cell and Tissue Adhesive Fisher Scientific cat no. CD-40240 $302.00/1mg

<http://www.ihcworld.com/_protocols/histology/cytospin.htm>