OSU LTB Laboratories Procedure Preparation of cytological slides from mononuclear cell fraction of bone marrow aspirate or peripheral blood[1]			Effective: 11/13/2014
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1.0 PRINCIPLE

Cytological "Cytospin" preparations have been described in the literature for their application in the diagnosis of various disease conditions. Cytospins may be prepared from a variety of samples such as sputum[1]and cerebrospinal fluid[2]. The Cytospin centrifuge is a special purpose instrument designed to deposit cells evenly onto a glass slide. The instrument when used correctly produces a monolayer cell deposition in a defined area of the slide using centrifugal force. Cytological specimens may also be deposited onto slides by techniques such as direct smear, touch preps or filter techniques. Cytospin preparation consistently produces uniform preparations of cells that are easily stained and evaluated. The primary requirements are that the specimen is a cell suspension - preferably of single cells - and that the cells are fresh and intact enough to be evaluable microscopically.

2.0 SPECIMENS

Mononuclear cell fractions are prepared by Ficoll separation and cells are resuspended in sterile, isotonic buffer solution such as Dulbecco's PBS (Ca/Mg free). Cells are obtained from bone marrow or peripheral blood collected with an anticoagulant such as heparin or EDTA.

3.0 MEDIA AND SUPPLIES

Specimen cells are held in sample holders, each consisting of:

- Stainless steel Cytoclip™ slide clips
- Fisher Superfrost™ Plus charged microscope slides (Fisher# 12-550-15)
- Thermo-Electron filter cards
- Disposable chamber (part no. 5991040) or double chamber (part no. 5991039) Cytofunnel ™ sample chamber.

Also required:

Dulbecco's PBS, supplemented with 2%FBS (Invitrogen – 19140-144/16140-071)

100% Methyl Alcohol – (Sigma-Aldrich – M1775)

70% Isopropyl Alcohol for cleaning slides

Coplin jar for fixing slides

Wexcide solution and 70% Isopropanol for surface decontamination

Sterile pipets

Pipet-aid

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Micropipetter (1000µl)
Sterile 1000 µL pipet tips

4.0 EQUIPMENT

The Shandon Cytospin 3 Cell Preparation System

5.0 QUALITY CONTROL AND SAFETY

All samples should be handled using universal precautions for biohazards. D-PBS supplemented with FBS can be a source of bacterial contamination of slides. Care must be taken to insure that D-PBS/FBS solution is always handled with aseptic technique. Fresh reagents should be used and/or prepared each time cytospin slides are prepared. Slides should be cleaned with 70% isopropanol and air-dried prior to use. Filter cards should be stored in a dry place at room temperature. Filters funnels should be cleaned immediately after use with a disinfectant cleaner and rinsed and dried thoroughly before the next use. Methanol is flammable and must be stored appropriately. It is recommended that specimen collection be carried out in accordance with NCCLS document M29T2. No known test sample can offer complete assurance that human blood samples will not transmit infection. Therefore, all derivatives are potentially infectious. Always spray alcohol on the caps before opening solutions. The alcohol can be dried off using gauze. If you have been out of the hood for a while and are wearing the same pair of gloves, use a new pair of gloves. Remember not to touch the sides (inside or outside) of any bottles with your pipette – if you do, dispose of the pipette and start again.

6.0 PROCEDURE

6.0.1. Samples are prepared according to mononuclear cell separation protocol[2].

6.0.2. Once a cell count is obtained a volume of sample containing 1000-5000 cells will be removed from the total cell suspension of the original sample.

6.0.3. Cells are placed in D-PBS/2%FBS, 100-500µl. In other words, use 100µl for every 10^3 cells. Aliquot cells into a sterile conical tube labeled with accession number or other sample ID.

6.0.4. For each sample, two cytological slides are made. Clean each slide briefly with 70% isopropyl alcohol and label with sample ID.

6.0.5. Match each slide face-up with a Thermo-Electron filter card and a plastic funnel (open end facing the labeled end of the slide).

6.0.6. Slide the assembly into slide clip and shut the mechanism. The clip handle should fall on the same end as the funnel opening.

6.0.7. Assemble Cytospin sample chamber and insert into the sealed Cytospin head. Gently invert the sample and transfer no more than 0.5 ml of sample to each sample chamber, slowly allowing ample opportunity for air to be displaced by the sample. It is critical that the sample does not contact the slide or filter before the Cytospin is started.

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6.0.7. Centrifuge sample for 4 minutes at 1000 rpm, to allow for complete fluid absorption. (Pre-set Program 7)

6.0.8. Place assemblies flat on absorbent toweling; release clips. Allow slides to dry 2-24 hours on toweling or in slide box.

6.1 SPECIMEN FIXATION

6.1.0. Slides should be fixed with cold (4°C) methanol immediately after drying. One slide is placed in a labeled storage box, while the other is be placed in a separate box for Wright/Giemsa staining.

6.1.1. Slide clips and cytofunnels should be washed in warm water/Alconox® solution, rinsed thoroughly and allowed to air dry for next use. Do not autoclave cytofunnels.

7.0 LIMITATIONS

Samples, which have undergone shipping stress such as exposure to abnormally high or low temperatures, may yield poor quality cells with low overall viability.

8.0 REFERENCES

1. Users guide for Cytospin 3 – Thermo-Shandon, Pittsburgh, PA. 1997.
2. Popov T, Gottschalk R, Kolendowicz R, Dolovich J, Powers P, Hargreave FE. The evaluation of a cell dispersion method of sputum. *Clin Exp Allergy* 1994; 24:778–783.
3. Yamashita J, Oda Y, Takeuchi J, Nakao S, Iwaki K, Handa H. [Cerebrospinal fluid (CSF) cytology using "cytospin" in patients with brain tumors. No Shinkei Geka. 1979 Aug; 7(8): 751-8.