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Effective Date: 11/15/18
Date Reviewed/ Date Revised: 11/15/18

Cytospin Preps

Cytospin Centrifuge

I. PRINCIPLE

Cytospin slide preparation system uses centrifugal force to deposit a monolayer of cells onto defined area of a slide for microscopic analysis. When dealing with a smaller amount of specimen, particularly with body fluids, cytospin technique can increase the number of cells for identification and leukocyte morphology than in traditional air dried slide.

II. CLINICAL SIGNIFICANCE

The cytoplasm of the nucleated cells tends to shrink in the low protein concentration of most body fluids. With the cytospin technique the integrity of the cell membrane remains intact and the cells appear much the same morphologically as on a blood smear.

III. SPECIMEN

A. Fresh liquid specimens from any body site may be used. One to five drops of sample are usually adequate.
B. Cerebral spinal fluid collected with no anticoagulant.
C. Synovial and Serous fluid collected into EDTA tube.
D. Miscellaneous body fluids
E. All body fluids should be examined within one hour of collection due to cell degeneration.
F. Highly viscous synovial fluid should be treated with hyaluronidase. Add approximately 5 mg (about the size of a split pea) to 0.5 – 1.0 ml aliquot of fluid and mix gently for several minutes.

IV. REAGENTS

A. 22% Albumin
B. Hyaluronidase
C. Isotonic Diluent:
    1. Nerl Saline from Blood Bank
    2. Cellpack DCL

V. REAGENT/EQUIPMENT

A. Cytospin centrifuge
B. Stainless steel cytoclips
C. Cytospin slides
D. Cytofunnel sample chambers with filer card
E. Disposable pipettes and test tubes

VI. QUALITY CONTROL

A. Quality of the slides produced should meet most of these standards:
   1. Proper staining of cells
   2. Monolayer of cells with minimal overlap.
   3. Sufficient concentration to avoid extensive searching for cells.
   4. Clear morphology with no evidence of stretching or tearing of the periphery.
   5. Flattened nuclei with distinct chromatin.

B. Poor quantity or quality findings may necessitate repeating the preparations if there is sufficient sample. Be sure to use the albumin drop.

VII. PROCEDURE

A. Label the frosted end of the slide with the patients name, accession #, body fluid type and dilution (if any).

B. Prepare the specimen in a disposable aliquot tube. Aliquots or dilutions must be labeled with a small LIS label.
   1. Determine the red and white cell counts according to established methods.
      a. Use the WBC to determine whether dilution is needed.

C. Using the table below, prepare sample dilution based on the WBC count.

WBC/μL	Dilution	Drops of Specimen	Drops of Isotonic Solution
< 100	none	5	0
100 - 300	1/2	6	6
301 - 700	1/4	3	9
701 - 1500	1/10	1	9
1501 - 3000	1/20	1	19

D. Assemble the cytospin sample chamber (cytoclip).
   1. Label one single-ring cytospin slide with the patient's full name and accession number, using a pencil on the frosted area.
   2. Fit the slide into the cytoclips with right-side orientation.
   3. Place a disposable cytofunnel over the glass slide, making sure the hole in the funnel chamber lines up with the ring on the slide.
   4. Lock the cytoclip springs into place.
   5. Prepare a balance clip, unless one is already available.

E. Add prepared dilution to the chambers' specimen funnels.
   1. Add one drop of 22% Albumin prior to adding sample
   2. Add one to five drops of specimen or specimen-dilution to the bottom of the chambers.
      a. Be sure there are no fibrin strands or clots in it.
      b. Don't run specimen down the sides.

c. If the cell count is very low (≤ 100/cmm), use more drops of straight specimen in the chamber. Do not put in more than 0.5 mL (10 drops) liquid totally.
d. It is not necessary to use liquid in an empty balance chamber.

3. Cap the cytospin funnel with the plastic cap provided

F. Install the chambers in the head.
1. Turn on the power to the instrument. Switch is on the bottom left back.
2. Touch OPEN LID button to release the lid. Pull up the center button on the lid until it clicks, then lift off the lid.
3. Load a prepared cytoclip chamber into the head with another opposite it as a balance.

G. Reinstall the head into the cytospin.
1. Lock the lid down and "click-tight" on the head after all chambers are loaded.
2. Close the lid.

H. Program and start the cytospin.
1. Select Program #3 and press START.
2. The program (#3) is for hematology body fluids and is a gentler spin than histology uses routinely.
3. The speed is 600 rpm, time is 10 minutes, and the acceleration is SLOW.

I. Remove slides and air dry.
1. When alarm sounds, open the cover and remove the lid. Lift out chambers.
2. Examine the chambers to check whether the entire specimen has been spun out. Do not let residual specimen flow onto the slide as the slide is removed from the clip because cells could wash off.
3. Allow hematology slides to air dry.
a. Do not spray with fixative.
b. Stand on edge to dry.
4. Use the slide stainer in Hematology to stain the slides.
5. Check slide quality under a microscope and
6. Dispose of funnel chamber.
7. Disinfect and clean the hemacytometer.
a. Cover hemacytometer and coverslip with alcohol.
b. Soak two to five minutes.
c. Rinse with tap water and alcohol and dry.
d. Air dry or polish with lint-free material.
e. Before reusing, use alcohol and lens paper to polish surfaces

VIII. **REFERENCES**

A. Walters, Jeri, "Hemological Analysis of Body Fluids", pg 18 Ap 29, '96. Advance for Medical Laboratories Professions.

B. Walters, Jeri, Procedures for "Synovial Analysis, Body Fluids Exams" and "Cerebral Spinal Fluid Exam" at ACL-Esoteric Central Laboratory, Wallis, WI 1997.

POLICY CREATION :		***Date***
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11-19-18	Kathryn O. Kramer MD	
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REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date

Reviewed by:

Lead	**Date**	**Coordinator/ Manager**	**Date**	**Medical Director**	**Date**
	11-28-18				