|  |
| --- |
| **CYTM Cytology for Malignant Cells in Body Fluids** |
| **Purpose** | This procedure provides instructions for CYTM CYTOLOGY FOR MALIGNANT CELLS IN BODY FLUIDS. The cell counting and concentration of body fluids (CSF, pleural, pericardial, etc.) for morphologic examination remains a first line diagnostic procedure in patients with metastatic tumor, leukemia, lymphoma and primary brain tumors who are suspected of having meningeal spread. |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology testing, the section supervisor, and section pathologist.
 |
| **Materials** | **Equipment** | **Reagents/Solutions** | **Supplies** |
|  | * Sysmex XN 3000 (SP-10 Slide Maker /Stainer) with Cytopro rotor (St.Paul), Wescor Cytopro Cytocentrifuge model 7621 (Mpls.)
* Neubauer or Spencer Brightline counting chamber
* Microscope
 | * Sodium chloride, available in Blood Bank
* 22% Albumin (Ortho). Store at 2-8°C. Check for visible contamination before each use
* 3% acetic acid:
* Add 3.0-mL glacial acetic acid
* To 97.0 mL Type I deionized water
* Store at room temperature, indefinitely
 | * Wescor sample chambers with fast, white Cytopads, caps, pk 48. Part # SS-113.
* Wescor sample chambers with slow, tan Cytopads, caps, pk 48. Part # SS-114.
* Microscope slides, ½ G, uncoated for Cytopro. Part # SS-117.
* Microscope Slides, frosted with rounded / clipped corners 76 x 26mm; 0.9 - 1.2 mm thick Chc# 30455.
* 22 x 30 mm coverslips
* MLA pipettors and tips
* 12 x 75 test tubes
* Plain capillary pipettes

|  |
| --- |
|  |

 |
| **Sample** | 1. Cerebrospinal fluid or other body fluids
* Minimum of 500 µL fluid
 |
| **Quality Control** | Review and validate annually the worksheet calculation Sunquest GUI uses to provide the final result.The pathologist completes a check of the cell count. S/he will bring any discrepancies to the attention of the technologist involved, and to the section supervisor. |
| **Procedure** | Follow the activities in the table below for CYTM CYTOLOGY FOR MALIGNANT CELLS IN BODY FLUIDS. |
|  | Step | **Action** | **Related Document** |
|  | 1 | NOTE: COUNTS MUST BE PERFORMED AS SOON AS POSSIBLE AFTER THE SPECIMEN HAS BEEN RECEIVED. ANY CELLS PRESENT DISINTEGRATE WITHIN A SHORT TIME.Place a portion of the specimen label on the Log sheet.Append the comment code **–DELA** to specimens that are delayed in transport to the lab more than an hour. |  |
|  | 2 | On the Log sheet, circle the appropriate fluid type. |  |
|  | 3 | On the Log sheet, note and record appearance of the fluid:1. Record volume
2. Record color: See [Table C – Sunquest GUI Color and Clarity Result Codes](http://khan.childrensmn.org/Manuals/Lab/SOP/UA/Res/200666.pdf).
3. Record clarity: [See Table D – Clarity Definitions](http://khan.childrensmn.org/Manuals/Lab/SOP/UA/Res/200667.pdf).
 |  |
|  | 4 | Perform cell counts:1. Mix the fluid to resuspend the cells evenly.
2. Clear Fluid
	* Using a capillary pipette, charge both sides of a counting chamber.
	* Allow the chamber to sit ten (10) minutes allowing the cells to settle.
	* Count the WBCs and RBCs in all of the squares, both sides.
3. Cloudy or Bloody Fluid
* Using a pipettor, make a 1:10 dilution in a 12 x 75 test tube.
	+ Use 0.1-mL fluid and 0.9-mL saline.
	+ Dilutions are adjusted depending on the sample volume and turbidity.
* Write dilution used on the Log sheet.
* Mix sample; plate both sides of the chamber.
* Allow the chamber to sit ten (10) minutes for the cells to settle.
* Count the RBCs in the middle square on both sides of the chamber.
* If the RBCs are too numerous (overlapping), the four corners and the middle square of the chamber’s center square are used.
* Count the WBCs in the four corners on both sides of the chamber.
* Bloody fluids may require lysing of the RBCs with 3% glacial acetic acid
* Make a 1:2 dilution (equal parts) of fluid with 3% acetic acid
* If the count is high, make a larger dilution or count fewer squares and adjust the calculation accordingly.
 |  |
|  | 5 | Record raw count on the Manual Log form:1. Input raw count, dilution and area counted.
2. Press ↵ ENTER.
3. SmarTerm calculates and displays the final result (may require scrolling up on the computer screen).
 |  |
|  | 6 | Label two (2) frosted-end slides with patient last name, accession number and “cyto.” |  |
|  | 7 | Cytocentrifugation: Prepare two slides:1. Remove the sealed head from the instrument.
2. Release the button in the center of the sealed head to remove the cover.
3. Place a labeled frosted-slide into the rotor, making sure that the labeled side is facing into the rotor. Slides can be loaded without depressing the release lever.
4. Place the microscope slide and cytofunnel/filter card assemblies into position on the rotor by pressing down on the release lever. Use Tan Cytopads for thin fluids (CSF) and White Cytopads for thick fluids (Body Fluids, Bronchs) Release the lever while gently pressing down on the top of the chamber frame to ensure that the chamber is properly seated.

1. Distribute cytofunnels by balancing them evenly in the sealed head.
2. Pipet one (1) drop 22% albumin in each cytofunnel; add 300-μL sample.
3. Place plastic caps on each assembly.
4. Place the sealed head on the cytocentrifuge’s center cone.
5. Check instrument settings, adjust if necessary:
* Speed: 600 rpm
* Time: 10 minutes
* Acceleration: high
1. Press START.
2. To remove completed slides preps:
* Open lid
* Remove sealed head
* Release and lift off cover
* Press down on release lever on bowl
* Remove and discard cytofunnel
* Remove slide, air dry
 |  |
|  | 8 | Print an Interim report for the accession number in Sunquest (function IRA). Place a fluorescent label, provided by Histology on the Interim report. Fill in the requested information: 1. Hematology tech initials.
2. Quality of specimen (Adequate vs. Inadequate).
3. Enter dilution made to obtain a monolayer of cells or indicate with N/A if no dilution was needed.(see Procedure Notes #2 below).
4. Check the QNS 2nd slide box when the amount of fluid submitted is < 500-μL.
 |  |
|  | 9 | Take slides and completed form to Histology.After hours and on weekends, fix slides for 5 minutes in methanol and air dry. |  |
|  | 10 | Additional Notes:1. If in the presence of excess albumin the cells round up and become difficult to identify, it may be necessary to make new slides using albumin diluted 1:10 with saline.
2. Count all squares when the first large square has 5 cells or less.
3. Count an equal number of squares on each side of the hemacytometer.
4. The number of cells counted on each side of the hemacytometer should agree ± 10%.
5. CSF or Body Fluids that have been collected from shunts or drainage reservoirs can contain a marked amount of cellular debris and/or breakdown products, making an accurate cell count impossible. Result these samples in SmarTerm function MEM by appending the code ECD (Unable to perform cell count due to extreme cellular degeneration) to CSFC (CWBC,CRBC); BFC (FWBC,FRBC); CYTM (CYWB,CYRB); BRON (BWBC).

Order differentials for CSF (CSFS); BFC (FLDI), prepare slides, and perform according to procedure.1. If the specimen is unacceptable, answer the CYWB and CYRB using code **SMCL** (unable to do count, small clots present), and notify the unit.
 |  |
| **Calculations** | 1. Basic cell count calculation used if Sunquest GUI is down:

# cells counted x dilution x depth = cells/μL area counted* *Example of clear fluid count (no dilution):*

# cells counted x 1 x 10 = cells/μL 18* *Example of bloody fluid count diluted 1:10*

# RBCs counted x 10 x 10 = RBCs/μL 2/5Count the number of RBCs in five squares of the center sq. mm on both sides of the chamber (counted 1/5 of the center square on two sides = 2/5).* *Example of cloudy fluid count diluted 1:10*

# cells counted x 10 x 10 = cells/μL 2One RBC square (entire middle square) per side counted.1. Cytocentrifugation calculation used to create a monolayer of cells:

# cells = # cellsundiluted diluted# cells = # cells1. x
* *Example:* WBC = 687

200 = 678 then x = 687 ÷ 200 and x = 3.4 total volume 1 x so to 0.1-μL sample add 0.2-μL saline |
| **Interpretation/****Results/Alert Values** | A pathologist interprets the slides. |
| **Reference Intervals** | Reference Range: No malignant cells present. |
| **Result Reporting** | Enter the fluid count results in Sunquest GUI as below:Function: MEMWorksheet: CYTest-1: <CR>CAP Method: (A)cceptWorkload Data: <CR>ACCN NO: Enter specimen ID #CYAP: Enter code for appearance (color/clarity separated by a hyphen)CYVO: Enter volume of fluid (x.xx)CYWB: Enter number (no commas) Enter dilution Enter area countedCYRB: Enter number (no commas) Enter dilution Enter area countedCYTY: Enter fluid type if not answered at time of orderAccept: (Y)es |
| **References** | 1. Cytospin® 3 Cell Preparation System Operator Guide, Shandon Scientific Limited, 1994.
2. Cytospin® 4 Cell Preparation System Operator Guide, English Issue 1.1, Thermo Shandon, 2002.
3. Harmening, DM, Clinical Hematology and Fundamentals of Hemostasis, 3rd edition, F.A. Davis Company, Philadelphia, PA, 1997, pp.593 - 598.
4. Henry, J, Todd and Sanford Clinical Diagnosis And Management By Laboratory Methods, W.B. Sanders Co., 1979, pp. 640-641.
5. Kjeldsberg, C., et al., Body Fluids, 3rd edition, American Society of Clinical Pathologists Press, Chicago, 1993, pp. 65-157.
6. Mayo Clinic Workshop, Morphology of Body Fluid, June 1988.
7. Cytopro Methods Manual 57-0185-01B ELITech Biomedical Systems, 2012.
8. Cytopro Cytocentrifuge Rotor Applications Manual 57-0021701A ELITech Biomedical Systems.
 |
| **Appendices** | Hematology Manual Result formCytology Report form |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Julie Schulte (MIN)Margaret Stacevich (STP) | 11/199011/1993 | Initial Version for each site |
| 2 | Deb Oman (MIN)Joan Jones (STP) | 09/199408/1995 | Updated for STP conversion to Sunquest |
| 3 | Laura Rachford | 07/2001 | Procedural change |
| 4 | Laura Rachford | 10/2002 | Added procedure notes 1 – 3 |
| 5 | Laura Rachford | 04/2004 |  |
|  | 6 | Al Quigley | 05/2010 | Added ECS coded comment for samples containing degenerated cells |
|  | 7 | Al Quigley | 06/01/11 | Revised, reformatted, and renamed |
|  | 8 | Al Quigley | 02/03/14 | Cytopro Application |
|  | 9 | Al Quigley | 05/25/17 | Added 600rpm/10min/high acceleration as routine setting on Cytopro. Sysmex XN 3000 application. |