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| **Cytocentrifugation of Body Fluids** | | | | | | | | | |
| **Purpose** | This procedure provides instructions for CYTOCENTRIFUGATION OF BODY FLUIDS. | | | | | | | | |
| **Principle** | The Aerospray model 7152 with Cytopro rotor ( St.Paul) and the Cytopro Cytocentrifuge model 7621(Mpls.) are designed for rapid and standardized deposition of body fluids directly onto microscope slides. Under the influence of centrifugal force, the sample is driven through the outlet port; the cells are deposited onto the microscope slide, while the suspension fluid spreads along the slide and is absorbed by the filter card. | | | | | | | | |
| **Policy Statements** | * This procedure applies to all laboratory staff making slides from body fluids, section supervisor, and pathologist. | | | | | | | | |
| **Materials** | **Equipment** | | | | | **Supplies** | | | |
|  | * Sysmex XN 3000 (SP-10 Slide Maker /Stainer) * Wescor Aerospray model 7152 with Cytopro rotor (St.Paul), Wescor Cytopro Cytocentrifuge model 7621(Mpls.) | | | | | * Wescor sample chambers with fast, white Cytopads, caps, pk48. Part # SS-113. * Wescor sample chambers with slow, tan Cytopads, caps, pk48. Part # SS-114. * Microscope Slides, 1/2 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. * Gamma Biologicals 22% albumin; product # 7-024; pH 7.2; store at 2 - 8°C. * 12 x 75 glass or plastic test tubes. * Marker – Moist Mark Plus – Cancer Diagnostics, chc# 22062., or lead pencil. | | | |
| **Sample** | 1. Cerebrospinal fluid or other body fluids. 2. Minimum volume: 0.5 to 1.0 mL  * Because of the difficulty obtaining specimens, make every effort to use the submitted sample, whatever the volume.  1. Criteria for rejection: 2. Massively clotted specimens  * Process fluids with small clots.  1. Fluids < 0.2 mL 2. Process ASAP – cells begin disintegrating in fluids that sit more than an hour. | | | | | | | | |
| **Procedure** | Follow the activities in the table below for CYTOCENTRIFUGATION OF BODY FLUIDS. | | | | | | | | |
|  | **Step** | **Action** | | | | | | | **Related Document** |
|  | 1 | Keep the cytocentrifuge power switch ON. | | | | | | |  |
|  | 2 | Remove the sealed head from the instrument. | | | | | | |  |
|  | 3 | Release the button in the center of the sealed head and remove the cover. | | | | | | |  |
|  | 4 | On the frosted-end slide(s), write last name, test code and accession number. Make sure that the labeled side is facing into the rotor. Slides can be loaded without depressing release levers. | | | | | | |  |
|  | 5 | 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. | | | | | | |  |
|  | 8 | Distribute cytofunnels by balancing them evenly in the sealed head. | | | | | | |  |
|  | 9 | Pipet one drop of 22% albumin into one or more cytofunnels. | | | | | | |  |
|  | 10 | Pipet 300 μL of sample into the cytofunnel(s). | | | | | | |  |
|  | 11 | Place plastic caps on the cytofunnel. | | | | | | |  |
|  | 13 | Replace the lid on the sealed head of the rotor by lifting the center button, center cover on head, press down on the center button to lock and seal the head onto the rotor. | | | | | | |  |
|  | 14 | Return the rotor to the instrument by placing it on the tapered boss. | | | | | | |  |
|  | 15 | Check that the settings are correct on the instrument panel, reset if incorrect.  In St.Paul select the “Cyto” icon on the stainer and then check settings.    Settings should be as follows:  • Speed required: 600rpm  • Time required: 10 minutes  • Acceleration rate: high | | | | | | |  |
|  | 16 | Press [START] to begin the run. | | | | | | |  |
|  | 17 | The instrument plays a tune when centrifugation is complete. | | | | | | |  |
|  | 18 | To remove the specimen:   1. Remove sealed head from instrument. 2. Pull up on button of sealed head to remove cover. 3. Press down on the release lever on the bowl. 4. Remove and discard cytofunnel. 5. Remove slide, air dry. | | | | | | |  |
| **Procedure Notes** | 1. Dilute specimens with a WBC > 250 cells/mm3 to provide a monolayer of cells for easier cell identification. To determine the dilution required, divide the total WBC by 250. Example:   Total chamber WBC = 687  687/250 = 2.748 (almost a 1:3 dilution)  to 100 μl sample add 200 μl saline (round up to facilitate diluting)   1. Keep the sealed head level when it contains specimens. 2. Deposit the 22% albumin and specimen directly into the base of the chamber, do not touch the side of the chamber. 3. 22% albumin minimizes the problem of broken cells and causes cells to round up. If cells are still difficult to identify make new slides using albumin diluted 1:10 with saline. 4. Remake slides that contain numerous overlapping cells or unidentifiable cells (small, pyknotic cells). 5. Clean the instrument thoroughly with 10% bleach monthly or immediately after a spill. 6. Periodically lubricate the lid latch mechanism by turning the lid upside down and applying a small amount of oil directly into the lid locking pin receptacle. Work the locking pin back and forth a number of times to allow the oil to penetrate the mechanism. Wipe off any excess at the mouth of the lid locking pinhole. | | | | | | | | |
| **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. Cytopro Methods Manual 57-0185-01B ELITech Biomedical Systems, 2012 4. Cytopro Cytocentrifuge Rotor Applications Manual 57-0021701A ELITech Biomedical Systems, 2010. | | | | | | | | |
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| **Historical Record** | **Version** | | **Written/Revised by:** | | **Effective Date:** | | **Summary of Revisions** | | |
| 1 | | Margaret Starcevic | | 01/1996 | | Initial version | | |
| 2 | | MIN: Laura Rachford  STP Chuck Stevenson | | 07/1997 | | Update | | |
| 3 | | Laura Rachford | | 08/2002 | | MIN obtained new instrument; rewritten as system procedure | | |
| 4 | | Al Quigley | | 06/01/11 | | Revised, reformatted, and renamed | | |
| 5 | | Al Quigley | | 02/03/14 | | Cytopro Application | | |
|  | 6 | | Al Quigley | | 05/25/17 | | Routine settings 600rpm/10min/high accelaeration.  XN 3000 (SP-10 Slide Maker/Stainer) application. | | |