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| Cytocentrifuge Slide Preparation |
| **Purpose** | This procedure provides instructions for CYTOCENTRIFUGE SLIDE PREPARATIONTo prepare a uniform deposit of cells from CSF and other body fluids suitable for staining and analyzing, the CytoFuge 2 deposits cells in a monolayer onto standard microscope slides while the suspension fluid spreads along the slide and is absorbed by the filter card. |
| **Policy Statements** | This procedure applies to Microbiologists/virologists who perform culture set-up |
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| **Materials** | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram Stain reagents
 | * Sterile disposable pipettes
* Glass Slides
* Filter concentrators (product #FF01)
* Reusable clips (product #FFCL, 4/bag)
 | * StatSpin CytoFuge 2

The CytoFuge 2 is not provided with an on/off switch. The centrifuge is normally left plugged in and “on”. The cover is usually left down but not latched. | * Refer to the Sunquest specimen label for media information.
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| Specimen | 1. Acceptable specimens: CSF, Ventricular or Peritoneal Dialysate fluid.
2. SDES codes/Specimen type
* CSF – Cerebrospinal fluid
* LCSF – lumbar puncture CSF
* RST – Reservoir tap CSF
* SHF – VP shunt fluid
* SUB – Subdural fluid
* VEN – Ventriculostomy
* VF – Ventricular fluid
* PD – Peritoneal Dialysate
1. Specimen Collection and Transport
* Refer to *Microbiology Lab Test Directory*
1. Specimen assessment
2. Refer to the policy *Specimen Rejection Criteria*
3. Gross blood and clotted specimens are not suitable for cytocentrifugation
4. Special instructions
5. Handle CSF as a STAT specimen
6. Report the CSF Gram stain within 60 min of receipt.
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| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. [*Biohazard Containment*](file:///G%3A%5CLAB%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20201%20%20%20Biohazard%20Containment%20R.doc)
2. [*Safety in the Microbiology/Virology Laboratory*](file:///G%3A%5CLAB%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20202%20Safety%20in%20the%20Microbiology%20Lab%20Policy%20R.docx)
* [*Biohazardous Spills*](file:///G%3A%5CLAB%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20204%20Biohazardous%20Spills%20R.docx)
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| **Quality Control** | 1. Each run
	1. Check inside of rotor for broken or other debris, and remove any from the base.
	2. Check that each load is properly balanced.
	3. Clean up any spills immediately (See Maintenance section below)
2. If there is an instrument malfunction, document failure, calls Biomed, and file online St. Croix System report. Notify Microbiology Supervisor.
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| **Procedure** | Starting Sample Concentration1. The approximate cell concentration of the specimen should be established prior to slide preparation on the CytoFuge2, by examination of the specimen and evaluating the turbidity.
* Clear: Crystal clear fluid
* Slightly cloudy: Turbidity clearly present; print easily read through tube
* Cloudy: Print not easily read through tube
* Very cloudy: Print cannot be seen through tube
1. Samples containing higher than optimal cell concentration will result in slides with cells too closely packed or overlapping.
2. 500-1500 cells per µl are optimal.
3. If the cell count is greater than 1500 cells per µl, the specimen should not have a cytospin slide. **Make a thin direct smear instead.**
4. In general, clear, and slightly cloudy fluids do not need dilution.
5. For cloudy, and very cloudy specimens, make a direct thin smear
6. Do not use the BD Normal Saline (0.5 ml tubes) or THIO to make the dilution of the specimen; they periodically contain non-viable gram staining organisms, leading to misinterpretation of the gram.
7. CytoFuge 2 Speed and Time Settings

**Note: Set CytoFuge 2 at 2200 for 8 min**1. For Microbiology, the recommended speed range is from 1600-3200 rpm with a time range of 4 to 10 minutes.
2. As a general guideline, for small particles, (e.g. bacteria), increase speed and time.

Assembly of the Filter Concentrator1. Remove a Filter Concentrator.
2. Unhinge the backing plate while holding the funnel side downward.
3. Place your pre-labeled slide atop the filter card (labeled side toward filter).
4. Carefully close the backing plate over the slide.
5. Slide the Clip onto the Filter Concentrator so the flat side of the clip settles into the “footprint” on the backing plate. See diagram below.

1. Adding Specimen to the Filter Concentrator-**THIS STEP MUST BE PERFORMED IN THE HOOD**

**Note: Use 2 to 3 drops per slide**1. Use a sterile disposable plastic transfer pipette to add specimen to the bottom of the funnel of the Filter Concentrator.
2. Avoid getting droplets onto the walls of the funnel.
3. Use care not to get the specimen on the filter paper or the slide during the loading process.
4. Do not overfill the device.
5. The volume ranges from 50 µl (minimum) to 500 µl (maximum).
6. The optimum volume is 100-400 µl.
7. This approximates to 2-8 drops from the transfer pipette.
8. If necessary, 2 slides can be prepared, using different amounts of drops per slide, so that the “better” slide can be chosen for gram stain exam.
9. Loading Filter Concentrators in the Rotor
10. The rotor must be balanced.
11. Install the fully assembled Filter Concentrators (with slides inside) opposite each other.
12. If only one slide is made, use the “blank” for a balance.
13. Maintain the “rest” angle while loading.
14. Make sure the Concentrators are fully seated and will be able to pivot during spinning.
15. Operation of the CytoFuge
16. Screw on rotor lid, close and latch the cover; press the “Start” button.
17. When the timed cycle is complete, the rotor will stop, three beeps will be heard.
18. The interlock mechanism will release and the cover latch can be squeezed to open the cover.
19. Unscrew the rotor lid, remove concentrators, disassemble them, and recover the slides.
20. Heat fix and Gram stain as usual.
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| **Maintenance** | 1. Clean monthly: Document on Maintenance Schedule, date and initials.
2. Clean outside surfaces and switch overlay panel with a water-dampened cloth and mild detergent.
3. Clean the inner surface or bowl, a powder-coated steel surface, with a mild detergent and disinfected if necessary by wiping with a cloth dampened with 70% alcohol or 10% bleach.
4. IMPORTANT!!! DO NOT SPRAY the bowl or outer surfaces with detergent or bleach.
5. In case of instrument malfunction, document failure and notify Micro Supervisor and call BioMed for repair, file on-line St Croix report.
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| **Method Performance Specifications** | 1. Slide breakage can result from spinning an improperly assembled or installed concentrator.
2. Addition of excess amounts of liquid (overfilling) to filter concentrators will result in fluid being “spun out” during centrifugation.
3. Never operate the CytoFuge 2 without the rotor cover in place.
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| **References** | 1. Clinical and Laboratory Analysis of Cytospin-Prepared Gram Stains for Recovery and Diagnosis of Bacteria from Sterile Body Fluids. Chapin-Robertson, Kimberle, et al. JCM February 1992. Vol. 30 No. 2
2. CytoFuge 2 Operators Manual. StatSpin. An IRIS Company. Norwood, Massachusetts 1998
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | * 1. Direct observation
	2. Complete written exam
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Becky Carlson | 01/15/1999 | Initial Version |
| 1.1 | Eddy Morrow/Becky Carlson | 02/12/2005 |  |
| 1.2 | Pat Ackerman | 08/15/2007 | Placed centrifuge on a monthly cleaning schedule. Revised Maintenance section. |
|  | 1.3 | Becky Carlson | 05/05/2013 | Removed specimen dilution instructions due to non-viable bacteria in sterile saline. |  |  |
| 1.4 | Tina Gronquist | 07/28/2014 | Updated into online format |
| 2 | Becky Carlson | 4/4/2015 | Re-numbered from MC 804 |
| 2 | Susan DeMeyere | 8/8/2017 | Update logo |
| **Archived by:** |  | **Archived Date:** |  |