TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	8/22/2014
Department:	Core	**Due Date:**	9/30/2014
		Implementation:	**10/1/2014**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Cytospin CSF / Body Fluid Slide Preparation GEC.H03 v2

Cyto-Tek Maintenance Log AG.F194.1

Description of change(s):

SOP

Specimen Prep:

Add Note for placing slides.

Update Dilution chart to change drops to 2-3, address performing dilution of small quantity sample

LOG:

Change frequency of some maintenance to 'as needed (with patient testing)'

This revised SOP and log will be implemented on October 1, 2014

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training

Non-Technical SOP

Title	**Cytospin CSF / Body Fluid Slide Preparation**	
Prepared by	Leslie Barrett	Date: 12/22/2009
Owner	~~Cynthia Reidenauer,~~ Robert SanLuis	Date: 8/11/2014

Laboratory Approval		
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Issue Date:	Local Effective Date:	

Review:		
Print Name	**Signature**	**Date**

Form revised 3/31/00

TABLE OF CONTENTS

1. PURPOSE

Cytocentrifugation forcefully sediments cells from suspension onto a vertical micro slide as the suspension medium is absorbed by a blotter. This technique allows preparation of Body fluid/CSF slides, which are superior to slides made the conventional way even with low cell counts.

2. SCOPE

This procedure applies to all personnel at the Germantown Emergency Center.

3. RESPONSIBILITY

The Technical Supervisor of the Core Laboratory is responsible for content and review of this procedure.

4. DEFINITIONS

None

5. PROCEDURE

A. Material Required:

Cytocentrifuge
Slide/Sample chamber holder
Disposable sample chamber (1mL), caps and white filter cards
Microscope slides, frosted end
Plastic transfer pipettes
0.9% NaCl
30% Albumin

B. Specimen Preparation: (Prepare duplicate)

1. Label (2) slides with patient last name first name, accession number, date, and source. If not enough sample, use blank cyto well for balance.

2. Prepare sample chambers:
   a. Place a slide into the Cyto-Tek specimen chamber holder.
      Note: The slides must be flush with the chamber holders. Failure to do so could result in glass slides breaking during centrifugation.

   b. Place Cyto-Tek specimen chamber into the holder and snap it in place.
      Note: Refer to the Cyto-Tek operator guide for step by step instructions (with pictures) for assembling the specimen chamber.

3. Place sample chamber into rotor with the funnel-facing center. Ensure that centrifuge is balanced.

4. To ensure that a monolayer of cells is produced the fluid should have less than 300 WBC/µL and less than 5,000 RBC/µL when ~~five~~ two to three drops of fluid are placed in chambers. To arrive at these counts, refer to dilution chart below.

   Dilution Chart *

WBC Count	Dilution factor	Drops fluid	Drops 0.9% NaCl	Drops in chamber
0-300	None	5	0	2-3
301-600	1:2	5	5	2-3
601-900	1:3	5	10	2-3
901-1200	1:4	3	9	2-3
1201-2000	1:6	3	15	2-3
2001-3000	1:10	1	9	2-3

   * If the specimen does not have the sufficient quantity to follow the dilution chart referenced above, perform an appropriate dilution using the required materials based on limited volume to obtain a monolayer of cells.

5. If WBC count is greater than 3000/µL, dilute to 3,000 and then use above chart.

6. If RBC count is greater than 5,000/µL in diluted sample, then continue to dilute until less than 5,000/µL.

7. For viscous fluids make 1:1 dilution with saline and skip step #8.

8. Place one drop 30% Albumin into chamber first then add ~~5~~ 2-3 drops of fluid or diluted fluid.

9. Press caps onto chamber slowly so as not to push prematurely onto slide.

C. Cyto-Tek Centrifuge Procedure:

1. Turn on machine. Switch is located on front lower left side of machine.

2. Open lid to machine by depressing button on top left side.

3. Place rotor with top in place into machine.

4. Close lid and listen for audible click indicating lid is locked.

5. Program Cytospin as follows:

   a. Press: **Set time**, then **5**, then **Enter**
   b. Press: **mode** key and use arrow key to set speed to 20
   c. Press: **Set Speed**, then **20**, then **Enter**
   d. Press: **mode** key again and use arrow key to set time 5
   e. Press **Start**
   f. When Cytospin stops an alarm will be heard

6. Disassembly/Staining:

   a. Remove rotor from machine
   b. Remove sample carriage
   c. Open sample carriage, then pull sample chamber away from microslide. DO NOT brush the two against one another as this will smudge the cell preparation.
   d. Air dry slide before staining. Stain with Wright's stain. Refer to manual Wright Stain procedure (GEC.H05 Diff-Quik Stain Kit) to stain slide.

D. Differential

1. Perform differential under oil immersion. If possible, count 100 cells; if not, count and report percentage of each cell type.

   Note: If the patient is a known tumor patient or the physician suspects tumor cells in the fluid, always examine the slide under low power then proceed to oil immersion to perform the differential.

2. Technical Note:
   Recovery –

Numbers of WBC's counted on chamber	Number of cells counted on cyto slide
0	0 – 40
1 – 5	20 – 100
6 – 10	60 – 150
11 – 20	150 – 250
20	250

Form revised 3/31/00

3. If the slide and chamber counts do not agree according to the chart:

   a. Too many cells on the cytocentrifuge slide – recount the white cell count.
   b. No cells or not enough cells on the cytocentrifuge slide as compared to the chamber count – prepare a new slide making sure that the specimen is well mixed.

4. After the fluid is counted it is saved in the refrigerator for 30 days, after which it is discarded.

6. RELATED DOCUMENTS

Diff-Quik Stain Kit, Hematology procedure
Shakura Cytospin User's Manual
Cyto-Tek Maintenance Log (AG.F194)

7. REFERENCES

- Shakura Cytospin User's Manual.
- Nicky Sherwood and Joanne Combleet Stanford University Medical Center

8. REVISION HISTORY

Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes GEC H022.001		
000	6/16/12	Update owner Section 5: Add sample chamber size; update slide labeling and Cytospin programming, add manual staining Section 6: Add documents Section 9: Add Maintenance Log	D. Patel	R. SanLuis
001	8/11/14	Update owner Section 5: Specimen Prep: Add Note for placing slides. Update Dilution chart to change drops to 2-3, address performing dilution of small quantity sample Section 6: replaced Shandon with Sakura, moved log from section 9 Section 7: replaced Shandon with Sakura Footer: version # leading zero's dropped due to new EDCS in use as of 10/7/13.	H. Genser L. Barrett	R. SanLuis

9. ADDENDA AND APPENDICES

None ~~Cyto-Tek Maintenance Log (see Attachment tab of Infocard)~~

Form revised 3/31/00

Cyto-Tek Centrifuge Maintenance Log

Make / Model #: _____ Serial number:_____

Month: _____ Year: _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Daily																															
As needed / Periodically																															

Instructions:

Daily

1. Wipe the external surfaces with a clean cloth moistened with neutral detergent, pat dry.

As needed (with patient testing)

1. Wipe the rotor bowl with a disinfecting solution, then wipe with a clean cloth moistened with neutral detergent.
2. Disconnect the drain hose at the rear of the instrument, and place a drain pan under the drain port of the instrument. Thoroughly rinse the rotor bowl with clear water. Completely dry the rotor bowl with clean, lint-free cloths.

Periodically

1. Soak the rotor in 5% sodium hypochlorite solution, then soak in a neutral detergent solution. Rinse with clear water, then thoroughly dry.

Weekly review:	Weekly review:	Weekly review:
Weekly review:	Weekly review:	Monthly review: