TRAINING UPDATE

Lab Location:	GEC, SGAH & WAH	**Date Distributed:**	4/23/2014
Department:	Core	**Due Date:**	5/30/2014

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Cell Count and Differential, CSF GEC.H06, SGAH.H08, WAH.H09 v3

Body and Synovial Fluid Analysis GEC.H07, SGAH.H09, WAH.H10 v2

Description of change(s):

Techs have already been trained on the new dilution process. This update and quiz are intended to reinforce your knowledge of the change

The revised SOPs will be implemented on May 1, 2014

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

Technical SOP

Title	**Cell Count and Differential, CSF**	
Prepared by	Cynthia Reidenauer	Date: 3/21/2011
Owner	Robert SanLuis	Date: 11/26/2013

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

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Cell Count and Differential, CSF	Manual/Microscopic	CCTD
CSF Cell Ct diff ER ONLY (WAH only)		CCNDE
CSF Cell Ct tube number 1 ER ONLY, additional test (SGAH)		CRB1

Synonyms/Abbreviations
CSF Count, Cerebrospinal Fluid Cell Count, Spinal Fluid Count

Department
Hematology

2. ANALYTICAL PRINCIPLE

Gross examination of the specimen is performed to determine the appearance. A microscopic examination is performed for the Total Nucleated Cell count (TNC) and Red Blood Cell count (RBC). Smears for cell identification are prepared using cyto-centrifuge or conventional centrifuge. Nucleated cell identification/ differential counts are done on Wright's Stained smears prepared using a cyto-centrifuge or smeared sediment from clinical centrifugation.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Not Applicable
Specimen Collection and/or Timing	Not Applicable
Special Collection Procedures **All SGAH and GEC patients;** **WAH other than ED patients** **SGAH other than ED patients**	Specimens are usually collected in three sterile tubes labeled 1, 2, and 3 in the order in which they are withdrawn. Tube 1 is used for chemical and serological tests, tube 2 is used for Microbiology, tube 3 is used for the cell count because it is the least likely to contain cells introduced by the spinal tap procedure. A minimum of 0.5 mL is required for the cell count.
Special Collection Procedures **WAH ED patients only**	Specimens are usually collected in four sterile tubes labeled 1, 2, 3 and 4 in the order in which they are withdrawn. Tube 1 is used for Cell count, tube 2 is used for chemical and serological tests, tube 3 is used for Microbiology, tube 4 is used for the cell count and diff because it is the least likely to contain cells introduced by the spinal tap procedure. A minimum of 0.5 mL is required for the cell count.
Special Collection Procedures **SGAH ED patients only**	Perform testing as described for non-ED patients and add cell count on tube 1. A minimum of 0.5 mL is required for the cell count.
Other	Not Applicable

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	CSF – tube #3 or #4 (see Section 3.1) *If only one tube is received, perform all testing on that tube.* **Note: If only 3 tubes are received for a WAH ED or SGAH ED patient, follow the testing pattern for non-**

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Criteria		
-Other Acceptable	**ED patients but also do a cell count on tube #1 before it is spun for chemistry / serological testing.** None	
Collection Container	Sterile Plastic Conical Tube	
Volume - Optimum **- Minimum**	2.0 mL 0.5 mL	
Transport Container and Temperature	Sterile Plastic Conical Tube at room temperature	
Stability & Storage Requirements	Room Temperature:	Rapid deterioration and cell lysis occurs on prolonged standing in CSFs, the sample should be processed STAT and the count should be performed as soon as it is received. Stable for 24 hrs.
	Refrigerated:	Same as above.
	Frozen:	Unacceptable
Timing Considerations	Not Applicable	
Unacceptable Specimens & Actions to Take	**Clotted specimens** - perform counts and append the comment: "*Specimen contains clots, counts may not be accurate."* **Specimens received after 24 hours -** perform the counts and append the comment: *"Counts may not be accurate due to the age of the specimen."* Due to nature of specimen, do not reject, unless frozen. If the specimen is received frozen, cancel the test with the comment: *"Specimen unsuitable for assay; received frozen."* Notify a caregiver and document in the LIS.	
Compromising Physical Characteristics	None defined	
Other Considerations	None defined	

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Rinse	Wescor, SS-035A
Thiazin	Wescor, SS035/049B
Eosin	Wescor, SS-035C
Methanol	Wescor, SS-MEOH
Aerofix	Wescor, SS-148

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0.9% Saline	Thermo 0.9% Saline cat # 23535435
22% Albumin (Obtain from Blood Bank)	Immucor CE 0088
Diff Quick Stain Pak (GEC only)	Siemens
0.005% Methylene Blue Diluting Fluid	Chantilly reagent room

4.2 Reagent Preparation and Storage

NOTE: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

Reagent	**Wescor Aerospray Rinse**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**Wescor Aerospray Thiazin**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**Wescor Aerospray Eosin**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**Wescor Aerospray Aerofix**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Add 10 ml to Methanol and mix well prior to use.

Reagent	**0.9% Saline** (Obtain fresh daily from Blood Bank)
Container	Plastic Bottle
Storage	Room temperature

Stability	24 hours, working supply in hematology. Open expiration on container in Blood Bank is 30 days.
Preparation	Ready to use

Reagent	**22% Bovine Albumin**
Container	Glass Bottle 10 ml
Storage	1°-10° C for long term storage
Stability	Stable until expiration date on the bottle. If turbid, discard.
Preparation	Ready to use

Reagent	**Diff Quick Stain Pack (GEC)**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**0.005% Methylene Blue Diluting Fluid. Obtain when needed from the reagent room in Chantilly.**
Container	Brown Glass Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date. Aliquot small amount to use when needed. Stability of aliquot is 24 hours.
Preparation	Ready to use

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Control	Supplier & Catalog Number
Cell-Chex 2ml each of L1-UC, L1-CC and L2	Streck Laboratories, Inc. Cat # 212431

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	Cell-Chex Level L1-UC, L1-CC and L2
Preparation	None. It is not necessary to warm the controls to room temperature before using.
Storage/Stability	• Store upright at 2-8ºC • Closed-vial stability 180 days. • Open-vial stability 30 days

6.3 Frequency

- **Cell Count and Cytocentrifuge QC** is performed every 8 hours of patient testing for manual body fluid counting and per technologist.
  QC menu each level of controls is as follows:
  L1-UC perform cell count and crystal exam
  L1-CC perform a cytospin differential and a crystal exam
  L2 perform cell count only
  Note: crystal exam only pertains to body fluid
- **Automated or Manual stain methods** is performed once per day. A smear must be reviewed on a daily basis to verify that the staining is adequate for differential of the various cells. The result of this review is documented in the manual Hematology QC book.
- Diluting fluid must be checked daily for contamination and documented. Refer to section 8.2

6.4 Tolerance Limits

a) **Cell count by Manual Hemacytometer:**
QC values for Manual Hemacytometer are lot specific so check package insert for lot number and expiration date. The lot number and ranges for each lot in use will be available on the Cell Chex Log.
- If both QC values are within 2 SD, patient results may be released.
- If a control value is >2SD, repeat the control before running patient samples. If the repeat control is within 2SD, patient samples may be run.
- If the repeat of the control value is still >2SD, further investigation is required before running patient samples.

b) **Differential %:**
QC values for Differential % are lot specific so check package insert. The lot number and ranges for each lot in use will be available on the Cell Chex Differential Log.

c) **Corrective Action**:
- All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed.

- Corrective action documentation must include the following: The QC rule(s) (or specific QC criteria) violated, the root cause of the problem, steps taken to correct the problem, how patient samples were handled, and the date and initials of the person recording the information.

d) **Review of QC**
- Refer to SOP Laboratory Quality Control Program for more details.
- Upon weekly and monthly review of QC, if the SD's or CV's are greater than the above maximums, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Review Patient Data

Since only a few patient samples may be tested in one day, daily review for trends may not be applicable.

6.6 Documentation

QC results are recorded on the Cell Chex QC log sheets.

6.7 Quality Assurance Program

The laboratory participates in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Not applicable

7.2 Equipment

Equipment	Supplier
Microscope	Not specified
Wescor Aerospray Cytocentrifuge	Wescor, Inc
CytoTek centrifuge (GEC only)	Shandon

7.3 Supplies

Supply	Supplier & Catalog Number
Disposable Pipettes	Fisher Brand or equivalent
Hemacytometer (disposable) C-CHIP	InCyto co, Ltd DHC-N01-5 neubauer improved
MLA pipette and tips	Not specified
Disposable tubes	Not specified
Cover glass	Fisher Scientific, Cat. #12-544-10 or equivalent

Supply	Supplier & Catalog Number
Microscope Slides	Fisher Scientific, Cat. #12-550-13 or equivalent
Petri Dish	Fisher Scientific, Cat. #08-757-12 or equivalent
Applicator Sticks	Bulk Pack, Multiple Vendors
Cytopro	Wescor, Cat. #SS-113

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

PROMPT EXAMINATION AFTER RECEIPT OF CSF IN THE LABORATORY IS ESSENTIAL FOR ACCURATE RESULTS. Cellular disintegration may occur if there is a delay in testing. If delay is unavoidable, refrigerate until testing can commence.

8.1 Color and Appearance

Step	Examination for Appearance and color
1.	Examine the CSF for appearance and color.
2.	If bloody, centrifuge the tube used for chemistry testing for 5 min. at 3400 rpm to remove the cellular elements and report the color of the supernatant.
3.	Compare the supernatant to distilled water. Use the term xanthochromia for any orange, pink or yellow color.

8.2 Concentration

Step	Specimen Preparation
1.	Place a drop of Methylene Blue diluting fluid on a slide and coverslip. Examine under 100X for contamination with artifacts, crystals or bacteria, replace fluid if necessary. Record the examination on the Cell Count Worksheet. If the diluting fluid is acceptable to, proceed to specimen dilution.
2.	Inspect specimen to determine the appropriate dilution. a. All specimens will be diluted with 0.005% Methylene Blue Diluting fluid. b. The minimum dilution is 1:2. This will ensure distinction between RBC and TNC. Red Cells will not pick up the methylene blue stain and will appear agranular. Methylene Blue allows the visual distinction of nucleated cells by staining the granules a faint blue.
3.	Mix specimen well and make the appropriate dilution. Refer to dilution tables below.

Step	1:2 Dilution
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.

Step	1:2 Dilution
2.	Mix specimen well. Using a 100µL pipette, add 100µL of body fluid to 100µl of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes. **Dilution Factor is 2**
3.	Charge the two chambers of the hemacytometer by touching the tip of the pipette to the coverslip edge where it meets the chamber floor. The chamber will fill by capillary action if the hemacytometer and coverslip are clean.
4.	If the hemacytometer is overcharged, it must be discarded and a fresh one used.
5.	Place the charged hemacytometer in a humidified Petri dish for 10 minutes to allow the cells to settle.
6.	Place the hemacytometer on the microscope and examine. The area to be counted is adjusted according to the sample. • If less than 20 cells are present in one square, count all the squares. • If greater than 20 cells are present in one square, count the four corner squares only. • If greater than 200 cells are present in one square count 5 of the 25 squares in the middle square. ALWAYS USE THE AVERAGE COUNT FROM BOTH SIDES OF THE CHAMBER IN THE FORMULA. Count the total number of rbcs and nucleated cells present on both sides. The sides should agree within 20%.
7.	Calculate the total number of RBCs and nucleated cells. Follow instructions on the Cell Count Worksheet to calculate results.
8.	All calculations must be recorded on worksheet.

Step	Diluted Specimen 1:10
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 100µL pipette, add 100µL of body fluid to 900µl of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes. **Dilution Factor is 10**
3.	Charge a counting chamber (one pipette per side), using proper technique.
4.	Place in a Petri dish for about 10 minutes to let the cells settle.
5.	For counting guidelines, follow steps 6 through 8 for 1:2 Dilution

Step	Diluted Specimen 1:20
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 50µL pipette, add 50µL of body fluid to 950µl of Methylene Blue diluting fluid. Mix dilution well. Let sit 10-15 minutes. **Dilution Factor is 20**
3.	Charge a counting chamber (one pipette per side), using proper technique.
4.	Place in a Petri dish for about 10 minutes to let the cells settle.
5.	For counting guidelines, follow steps 6 through 8 for 1:2 Dilution

Step	Diluted Specimen 1:50
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 20µL pipette, add 20µL of body fluid to 980µl of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes. **Dilution Factor is 50**
3.	Charge a counting chamber (one pipette per side), using proper technique.
4.	Place in a Petri dish for about 10 minutes to let the cells settle.
5.	For counting guidelines, follow steps 6 through 8 for 1:2 Dilution

Step	Diluted Specimen 1:100
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 10µL pipette, add 10µL of body fluid to 990µl of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes. **Dilution Factor is 100**
3.	Charge a counting chamber (one pipette per side), using proper technique.
4.	Place in a Petri dish for about 10 minutes to let the cells settle.
5.	For counting guidelines, follow steps 6 through 8 for 1:2 Dilution

8.3 Differential Count

- Perform a differential cell count on a cytocentrifuged specimen using Wright's stain or equivalent. The leukocytes are classified and reported as a percentage. Examine smear for the presence of immature or abnormal cells.
- If abnormal or immature cells are noted, refer to a Pathologist for review.

8.4 Cytospin

Step	Cytospin		
1.	Assemble sample chamber and glass microscope slide in the Wescor Aerospray cytocentrifuge carousel. At GEC, follow Cytopsin procedure.		
2.	**IF**	**THEN**	
	Nucleated cell count is <300	Place 3-5 drops of fluid plus 1 drop of albumin into a disposable cytofunnel and place into the Cytospin centrifuge. The albumin is used to make the cells adhere to the slide better before the staining procedure.	
	Nucleated cell count is >300	**Cells/ µL**	**Dilution**
		301-700	1:2 (5 drops CSF + 5 drops saline)
		701-1500	1:5 (2 drops CSF + 10 drops saline)
		1501-3000	1:10 (2 drops CSF + 20 drops saline)
		>3000	1:20 (2 drops CSF + 40 drops saline)
		Mix dilution well and place 3-5 drops into the Cytospin funnel. Add 1 drop of albumin.	
3.	**Centrifuge Sample**: See procedure Aerospray Hematology Slide Stainer Cytocentrifuge		

Step	Cytospin
	(SGAH/WAH) or Cytospin CSF/Body Fluid Slide Preparation (GEC) as appropriate.
4.	Stain slide using the Aerospray stainer or Diff Quick Stain Pack as appropriate

9. CALCULATIONS

Formula for Hemacytometer

$$\frac{\text{Cells Counted}}{\text{\# of squares counted}} \times 10 \times \text{dilution}$$

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required.

10.2 Rounding

Results for cell counts are rounded to whole numbers.

10.3 Units of Measure

Parameter	Units
RBC	Cells/µL
TNC	Cells/µL
Differential Counts	%

10.4 Clinical Reportable Range

Not applicable.

10.5 Repeat Criteria and Resulting

Any duplicate counts not agreeing within 20% must be repeated.

All CSF counts must be reviewed by a second technologist prior to resulting. Calculations must be rechecked and proper placement and documentation of cell counts on the worksheet must be verified. In addition, once typed into the computer a second technologist must verify the proper placement of the counts **PRIOR TO ACCEPTING THE RESULTS.**

Second tech review for Germantown Emergency Center ONLY:
Due to the fact that there is only one person working per shift, if a CSF cell count is performed then it will be the first duty of the next shift tech to review the cell count worksheet and compare it to the results entered into the computer. The reviewing tech will initial that the second tech review was performed.

All fluids needing a pathology review are to be taken to the pathologist on call for Hematology. All slides are to be accompanied by an IRA report from the LIS and the pathologist slide review form.

11. EXPECTED VALUES

11.1 Reference Ranges

Parameter/Units of Measurement	Both Male and Female	
	< 60 Days	≥ 60 days to Adult
Color	Colorless	
Appearance	Clear	
RBC cells/µL	<10	<10
TNC cells/µL	<20	<6
Lymphocyte %	<70	<70
Monocyte %	<30	<30
Eosinophil %	<10	<10

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

CSF Appearance		
Appearance	**Cause**	**Most Significance**
Crystal Clear		Normal
Hazy, turbid, cloudy, smoky, milky	WBC's; RBC's Microorganisms Protein	Meningitis, Hemorrhage, Traumatic tap Meningitis Disorders that affect blood-brain barrier, Productions of IgG within CNS
Oily	Radiographic Contrast Material	
Bloody Xanthochromic	RBC's Hemoglobin Bilirubin Merthiolate Carotene Protein	Hemorrhage Old Hemorrhage Lysed cells from traumatic tap RBC Breakdown Elevated serum bilirubin Contamination Increased serum levels See above

CSF Appearance		
Appearance	**Cause**	**Most Significance**
Clotted	Protein Clotting Factors	See above Introduced by traumatic tap
Pellicle Formation	Protein Clotting Factors	Tubercular meningitis

The CSF is the third major fluid of the body. It provides a physiologic system to supply nutrients to the nervous system, remove metabolic wastes and produce a mechanical barrier to cushion the brain and spinal cord against trauma. Identification of cell types present in the CSF has become a valuable diagnostic aid most frequently associated with meningitis. High WBC counts with neutrophilic majority are associated with bacterial meningitis while lymphocyte/monocyte predominance indicates viral, tubercular, etc., origin. The differential can impart diagnostic information based on abnormal cell types found indicating metastatic carcinoma, central nervous system involvement of leukemia or parasitic infections. Refer to the table below for a more complete list.

Predominant Cells Seen in CSF		
Type of Cell	**Major Clinical Significance**	**Microscopic Findings**
Lymphocyte	Normal Viral, tubercular and fungal meningitis Multiple Sclerosis	All stages of development may be found.
Neutrophil	Bacterial meningitis Early cases of viral, tubercular, or fungal meningitis Cerebral hemorrhage	Granules may be less prominent than in blood. Cells disintegrate rapidly.
Monocyte	Chronic bacterial meningitis Viral, tubercular, and fungal meningitis Multiple Sclerosis	Found mixed with lymphocytes and neutrophils.
Eosinophil	Parasitic infections Allergic reactions Intracranial shunts (hydrocephalus)	Same appearance as seen in blood.
Macrophages	Viral and tubercular meningitis RBC's in spinal fluid	May contain phagocytized RBCs appearing as empty vacuoles or ghost cells and hemosiderin granules.
Pia arachnoid mesothelial (PAM) cells	Normal, mixed reactions, including neutrophils, lymph-ocytes, monocytes and plasma cells	Resemble young monocytes with a round, not indented, nucleus.
Blast forms	Acute leukemia	Lymphocytes or myeloblasts.
Plasma cells	Multiple Sclerosis Lymphocyte reactions	Transitional and classic forms seen.

Predominant Cells Seen in CSF		
Type of Cell	**Major Clinical Significance**	**Microscopic Findings**
Ependymal Cells Choroidal Cells	Normal trauma Diagnostic procedures	Seen in clusters with distinct nuclei and distinct cell walls.
Malignant Cells	Metastatic carcinoma	Seen in clusters with fusing of cell borders and nuclei.

General Patterns of Laboratory Findings on CSF in Disease					
	Normal	**Pyogenic Infections**	**Tuberculosis Meningitis**	**Virus Infection**	**Syphilitic Infection**
Appearance	Clear, Colorless	Turbid to purulent	Clear to slightly cloudy	Clear to milky	Clear
Coagulability	Does not coagulate	Apt to clot	Fibrin web or pellicle may form	Rarely clots	Small clots in acute syphilitic meningitis and paresis
Cell Count/µL	Adults: 0-10 Children under 5 Years: 0-20	100 - Several thousand	20 - 1000	10 - Several hundred	10 - 100
Predominating Cell Type	Mononuclears	Polynuclears	70-90% mononuclears	Mononuclears	Mononuclears
Total Protein	15-45 mg/dL	50-1500 mg/dL	Normal-400 mg/dL	60-300 mg/dL	Normal-100 mg/dL
Glucose	(True Glucose) 50-80 mg/dL	Markedly decreased	Decreased, usually falls progressively	Normal	Normal or slightly decreased
Chloride (as NaCl)	118-132 mEq/L (adult)	Decreased	Decreased greatly	Normal	Normal
Colloidal (curve)	Negative	Meningitic	Meningitic	Variable	Tabetic, paretic, etc. depending on type or location of the infection
Pressure	70-150 mm H_2O (adult)	Usually increased	Usually increased	Normal to slightly increased	Normal except in acute meningitis.

13. PROCEDURE NOTES

- **FDA Status:** Laboratory Developed Test (LDT) without message
- **Validated test modifications:** not applicable

- Perform cell counts as soon as possible since cells deteriorate with time.
- If there is a clot, perform count on available liquid and make notation in the report. Counts on partially clotted samples may be affected depending whether or not cells are trapped in the clot.
- Low power scanning should be performed on smear to evaluate cell distribution and evaluate for presence of malignant cells.

Quest Diagnostics Nichols Institute
Site: GEC, SGAH & WAH
Title: **Cell Count and Differential, CSF**

14. LIMITATIONS OF METHOD

Not applicable

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Hematology Slide Stainer Cytocentrifuge, Wescor Aerospray® Model 7151, SGAH / WAH Hematology SOP
3. Cytospin CSF/Body Fluid Slide Preparation, GEC Hematology SOP
4. Diff Quick Stain Kit, Hematology SOP
5. Cell Count Worksheet, AG.F12
6. Cell Chex Control Log, AG.F87

17. REFERENCES

1) Body Fluids, Third Edition, Kjeldsberg, C.R., and Knight, J.A., American Society of Clinical Pathologists Press, Chicago, 1993.
2) Clinical Hematology and Fundamentals of Hemostasis, Second Edition, Harmening, Denise M., F.A. Davis Company, Philadelphia, 1992.
3) Urinalysis and Body Fluids, Edition 2, Strasinger, S.K., F.A. Davis Company, 1989
4) Defining CSF WBC Count Reference Values in Neonates and Young Infants, Kestenbaum Ebberson et al Pediatrics 2010;125;257-264
5) CSF Analysis, D. Seehusen et al American Family Physician September 15,2003; Vol. 68; Number 6, 1103-1108

Form revised 7/01/01

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP SGAH-WAH H019.000		
000	06/06/12		Update owner	L Barrett	J Buss, RSL
000	06/06/12	6.1, 6.7	Add diluting fluid check to match Cell Count Worksheet	J Buss	J Buss, RSanLuis
001	11/26/13		Update owner	L Barrett	R SanLuis
001	11/26/13	4	Add Methylene Blue diluting fluid and stain components	L Barrett	R SanLuis
001	11/26/13	6	Update QC material, frequency clarified	L Barrett	R SanLuis
001	11/26/13	7, 8	Remove use of alcohol swabs, filter paper and non disposable hemacytometer,	L Barrett	R SanLuis
001	11/26/13	8.2	Add Methylene Blue as diluting fluid, add process to make each dilution	L Barrett	R SanLuis
001	11/26/13	10.5	Add second review process for GEC	L Barrett	R SanLuis
001	11/26/13	13	Add handling for clots	L Barrett	R SanLuis
001	11/26/13	15	Update to standard wording	L Barrett	R SanLuis
001	11/26/13	16	Add forms, update SOP titles	L Barrett	R SanLuis
001	11/26/13	19	Remove forms	L Barrett	R SanLuis
001	11/26/13	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
2	3/12/14	8.3	Correct 1:1 dilution to 1:2. Add dilution factors	C Reidenauer	R SanLuis
2	3/12/14	8.4	Change Isoton to saline	C Reidenauer	R SanLuis

19. ADDENDA

None

Technical SOP

Title	**Body and Synovial Fluid Analysis**	
Prepared by	Cynthia Reidenauer / Cathy Keifer	Date: 11/22/2011
Owner	Robert SanLuis	Date: 11/26/2013

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

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

Quest Diagnostics Nichols Institute
Site: GEC, SGAH & WAH
Title: **Body and Synovial Fluid Analysis**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Cell Count and Diff, Pleural Fluid Cell Count and Diff, Peritoneal Fluid Cell Count and Diff, Pericardial Cell Count and Diff, Fluid, Other	Hemacytometer, Microscope	FCCD for all Body fluids EXCEPT Synovial (see below)
Cell Count and Diff, Synovial fluid to include Crystal exam		SFCC

Note: For CSF, refer to procedure 'Cell Count and Differential, CSF' (GEC.H06, SGAH.H08, WAH.H09)

Synonyms/Abbreviations
Body fluid cell count/Body Fluid Exam Synovial Fluid cell count/Synovial Fluid Exam

Department
Hematology

Form revised 7/01/01

2. ANALYTICAL PRINCIPLE

The total nucleated cell count in body fluids is performed manually using a hemacytometer. A differential cell count is performed via cytospin. The color, appearance and volume of the fluid are also reported.

In Synovial Fluids only, crystals are first observed microscopically with polarizing lenses, and if present, are identified.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Not applicable
Specimen Collection and/or Timing	None defined
Special Collection Procedures	Fluid is collected in sterile vacuum bottle or other collection container and then aliquoted as needed into lavender top (EDTA), green top (Heparin), screw cap plastic vial.
Other	Not applicable

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	*Site specified on collection* 3 mL fluid in EDTA for Count, Diff 1 mL fluid (unpreserved) for Crystal 3 mL fluid in Heparin or Plastic Vial
Collection Container - Alternate	Lavender Top Tube Heparin (Green Top Tube) or Plastic Vial
Volume - Optimum **- Minimum**	3.0 mL 1.0 mL *If less than 1.0 mL is received, call the physician and ask the priority of tests needed. Note: In the case of a small volume synovial fluid the crystal exam may be the top priority*
Transport Container and Temperature	Collection container at room temperature
Stability & Storage Requirements	**Room Temperature**: 48 hours
	Refrigerated: 48 hours
	Frozen: Unacceptable
Timing Considerations	Not applicable

Criteria		
Unacceptable Specimens & Actions to Take	Since body fluid specimens are more difficult to obtain and are frequently "one time only" samples, the criteria for an acceptable sample should not be as rigid as those for peripheral blood. Samples with small clots should not be rejected, but the presence of clot should be noted.	
	Condition	**Then**
	Small clots noted	Perform test, append results with free text comment: *Cell count may be inaccurate due to presence of clots in sample*
	Solid clot	In the presence of a solid clot a cell count cannot be performed. A slide can be made on the surrounding fluid and an examination should be made for cellular content. (ie many rbc, few wbc noted)
	Age of specimen	Since each specimen deteriorates at unpredictable ranges, aged specimens are to be tested and evaluated for significant deterioration of TNC. Append results with free text comment, "Count performed on specimen #-days old, appearance of cells may be affected." Only results deemed valid will be reported. Unacceptable results cannot be reported and the test should be cancelled. Result the test as: *Unsuitable for analysis due to the age of the specimen. Test has been cancelled.* Perform CRW to credit the test. Notify a caregiver
Compromising Physical Characteristics	None defined	
Other Considerations	None defined	

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Rinse	Wescor, SS-035A
Thiazin	Wescor, SS035/049B
Eosin	Wescor, SS-035C
Methanol	Wescor, SS-MEOH

Aerofix	Wescor, SS-148
0.9% Saline	Thermo 0.9% Saline cat # 23535435
22% Albumin (Obtain from Blood Bank)	Immucor CE 0088
Diff Quick Stain Pak (GEC only)	Siemens
0.005% Methylene Blue Diluting Fluid	Chantilly reagent room

4.2 Reagent Preparation and Storage

NOTE: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

Reagent	**Wescor Aerospray Rinse**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**Wescor Aerospray Thiazin**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**Wescor Aerospray Eosin**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**Wescor Aerospray Aerofix**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Add 10 ml to Methanol and mix well prior to use.

Reagent	**0.9% Saline** (Obtain fresh daily from Blood Bank)
Container	Plastic Bottle
Storage	Room temperature
Stability	24 hours, working supply in hematology. Open expiration on container in Blood Bank is 30 days.
Preparation	Ready to use

Reagent	**22% Bovine Albumin**
Container	Glass Bottle 10 ml
Storage	1°-10° C for long term storage
Stability	Stable until expiration date on the bottle. If turbid, discard.
Preparation	Ready to use

Reagent	**Diff Quick Stain Pack (GEC)**
Container	Plastic Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**0.005% Methylene Blue Diluting Fluid. Obtain when needed from the reagent room in Chantilly.**
Container	Brown Glass Bottle
Storage	Room temperature
Stability	Manufacturer's expiration date. Aliquot small amount to use when needed. Stability of aliquot is 24 hours.
Preparation	Ready to use

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

Control	Supplier & Catalog Number
Cell-Chex - 2mL each of Level L1-UC, L1-CC and L2	Streck Laboratories, Inc. Cat. #212431

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4)

expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	Cell-Chex Level L1-UC, L1-CC and L2
Preparation	None. It is not necessary to warm the controls to room temperature before using**.**
Storage/Stability	• Store upright at 2-8ºC • Closed-vial stability 180 days. • Open-vial stability 30 days

6.3 Frequency

- **Cell Count and Cytocentrifuge QC** is performed every 8 hours of patient testing for manual body fluid counting and per technologist.
  QC menu each level of controls is as follows:
  L1-UC perform cell count and crystal exam
  L1-CC perform a cytospin differential and a crystal exam
  L2 perform cell count only
- **Automated or Manual stain methods** is performed once per day. A smear must be reviewed on a daily basis to verify that the staining is adequate for differential of the various cells. The result of this review is documented in the manual Hematology QC book.
- Diluting fluid must be checked daily for contamination and documented. Refer to section 8.3

6.4 Tolerance Limits

a) **Cell count by Manual Hemacytometer:**
QC values for Manual Hemacytometer are lot specific so check package insert for lot number and expiration date. The lot number and ranges for each lot in use will be available on the Cell Chex Log.
- If both QC values are within 2 SD, patient results may be released.
- If a control value is >2SD, repeat the control before running patient samples. If the repeat control is within 2SD, patient samples may be run.
- If the repeat of the control value is still >2SD, further investigation is required before running patient samples.

b) **Differential %:**
QC values for Differential % are lot specific so check package insert. The lot number and ranges for each lot in use will be available on the Cell Chex Differential Log.

c) **Crystal exam**
Note the absence or presence of crystals and using the polarizer attachment identify the type of crystal present; Monosodium Urate (uric acid) or Calcium Phosphate.
The lot number and ranges for each lot in use will be available on the Cell Chex Log.

d) **Corrective Action**:
- All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed.
- Corrective action documentation must include the following: The QC rule(s) (or specific QC criteria) violated, the root cause of the problem, steps taken to correct the problem, how patient samples were handled, and the date and initials of the person recording the information.

e) **Review of QC**
- Refer to SOP Laboratory Quality Control Program for more details.
- Upon weekly and monthly review of QC, if the SD's or CV's are greater than the above maximums, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Review Patient Data

Since only a few patient samples may be tested in one day, daily review for trends may not be applicable.

6.6 Documentation

QC results are recorded on the Cell Chex QC log sheets.

6.7 Quality Assurance Program

The laboratory participates in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Not applicable

7.2 Equipment

Equipment	Supplier
Wescor Aerospray Cytocentrifuge	Wescor, Inc
Microscope	Not specified
CytoTek centrifuge (GEC only)	Shandon

7.3 Supplies

Supply	Supplier & Catalog Number
Disposable Pipettes	Fisher Brand or equivalent
Hemacytometer (disposable) C-CHIP	InCyto co, Ltd DHC-N01-5 neubauer improved
MLA pipette and tips	Not specified
Disposable tubes	Not specified
Cover glass	Fisher Scientific, Cat.#12-542-1B or equivalent
Microscope Slides	Fisher Scientific, Cat.#12-550-15 or equivalent
Petri Dish	Fisher Scientific, Cat.#08-757-12 or equivalent
Applicator Sticks	Bulk Pack, Multiple Vendors
Cytopro	Wescor, Cat. #SS-113

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1 Color: Determine the color of the body fluid and report as:

IF	THEN	IF	THEN
Amber	AMB	Gray White	GRAY
Blue	BLUE	Light Yellow	LYEL
Brown	BRWN	Orange	ORNG
Colorless	COLR	Pale Yellow	YEL
Dark Yellow	DYEL	Red	RED
Green	GRN	Straw	STRW
		Yellow	YEL

8.2 Appearance: Determine the appearance of the body fluid and report as:

IF	THEN	IF	THEN
Bloody	BLDY	Clotted	CLTD
Bloody, cloudy	BLDY-CLDY	Hazy	HAZY
Clear	CLER	Turbid	TUR
Cloudy	CLDY	Slightly Cloudy	SLCL

8.3 Concentration

Step	Specimen Preparation
1.	Place a drop of 0.005% Methylene Blue diluting fluid on a slide and coverslip. Examine under 100X for contamination with artifacts, crystals or bacteria,

Step	Specimen Preparation
	replace fluid if necessary. Record the examination on the Cell Count Worksheet. If the diluting fluid is acceptable to, proceed to specimen dilution.
2.	Inspect specimen to determine the appropriate dilution. a. All specimens will be diluted with 0.005% Methylene Blue Diluting fluid. b. The minimum dilution is 1:2. This will ensure distinction between RBC and TNC. Red Cells will not pick up the methylene blue stain and will appear agranular. Methylene Blue allows the visual distinction of nucleated cells by staining the granules a faint blue.
3.	Mix specimen well and make the appropriate dilution. Refer to dilution tables below.

Step	1:2 Dilution
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 100µL pipette, add 100µL of body fluid to 100µl of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes **Dilution Factor is 2**
3.	Charge the two chambers of the hemacytometer by touching the tip of the pipette to the coverslip edge where it meets the chamber floor. The chamber will fill by capillary action if the hemacytometer and coverslip are clean.
4.	If the hemacytometer is overcharged, it must be discarded and a fresh one used.
5.	Place the charged hemacytometer in a humidified Petri dish for 10 minutes to allow the cells to settle.
6.	Place the hemacytometer on the microscope and examine. The area to be counted is adjusted according to the sample. • If less than 20 cells are present in one square, count all the squares. • If greater than 20 cells are present in one square, count the four corner squares only. • If greater than 200 cells are present in one square count 5 of the 25 squares in the middle square. ALWAYS USE THE AVERAGE COUNT FROM BOTH SIDES OF THE CHAMBER IN THE FORMULA. Count the total number of rbcs and nucleated cells present on both sides. The sides should agree within 20%.
7.	Calculate the total number of RBCs and nucleated cells. Follow instructions on the Cell Count Worksheet to calculate results.
8.	All calculations must be recorded on worksheet.

Step	Diluted Specimen 1:10
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 100µL pipette, add 100µL of body fluid to 900µl of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes.

	Dilution Factor is 10
3.	Charge a counting chamber (one pipette per side), using proper technique.
4.	Place in a Petri dish for about 10 minutes to let the cells settle.
5.	For counting guidelines, follow steps 6 through 8 for 1:2 Dilution

Step	Diluted Specimen 1:20
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 50µL pipette, add 50µL of body fluid to 950µl of Methylene Blue diluting fluid. Mix dilution well. Let sit 10-15 minutes. **Dilution Factor is 20**
3.	Charge a counting chamber (one pipette per side), using proper technique.
4.	Place in a Petri dish for about 10 minutes to let the cells settle.
5.	For counting guidelines, follow steps 6 through 8 for 1:2 Dilution

Step	Diluted Specimen 1:50
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 20µL pipette, add 20µL of body fluid to 980µl of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes. **Dilution Factor is 50**
3.	Charge a counting chamber (one pipette per side), using proper technique.
4.	Place in a Petri dish for about 10 minutes to let the cells settle.
5.	For counting guidelines, follow steps 6 through 8 for 1:2 Dilution

Step	Diluted Specimen 1:100
1.	Perform the diluting fluid check as described above. If the diluting fluid is acceptable to use, proceed to dilution of the specimen.
2.	Mix specimen well. Using a 10µL pipette, add 10µL of body fluid to 990µl of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes. **Dilution Factor is 100**
3.	Charge a counting chamber (one pipette per side), using proper technique.
4.	Place in a Petri dish for about 10 minutes to let the cells settle.
5.	For counting guidelines, follow steps 6 through 8 for 1:2 Dilution

8.4 Cytospin

Step	Cytospin	
1.	Assemble sample chamber and glass microscope slide in the Wescor Aerospray cytocentrifuge carousel. At GEC, follow Cytospin procedure.	
2.	**IF**	**THEN**
	Nucleated cell count is <300	Place 3-5 drops of fluid plus 1 drop of albumin into a disposable cytofunnel and place into the Cytospin centrifuge. The albumin is used to make the cells adhere to the slide better before the staining procedure. Synovial fluids do not require albumin added.

Step	Cytospin		
	Nucleated cell count is >300	**Cells/ µL**	**Dilution**
		301-700	1:2 (5 drops fluid+ 5 drops saline)
		701-1500	1:5 (2 drops fluid + 10 drops saline)
		1501-3000	1:10 (2 drops fluid + 20 drops saline)
		>3000	1:20 (2 drops fluid + 40 drops saline)
		Mix dilution well and place 3-5 drops into the Cytospin funnel. Add 1 drop of albumin.	
3.	**Centrifuge Sample**: See procedure Aerospray Hematology Slide Stainer Cytocentrifuge (SGAH/WAH) or Cytospin CSF/Body Fluid Slide Preparation (GEC) as appropriate.		
4.	Stain slide using the Aerospray stainer or Diff Quick Stain Pack as appropriate		

8.5 Differential Count

IF	THEN
Cell count is <10	Do not perform differential. Result with **NOTP-;**due to an insufficient number of cells in the sample.
Cell count is >10	Perform a 5 part differential of 100 cells on a cytocentrifuged specimen using Wescor slide stainer, or a manual stain (GEC). The nucleated cells are classified and reported as a percentage. Examine smear for the presence of immature or abnormal cells, crystals and bacteria. Refer to a Pathologist if abnormal or immature cells are noted.

8.6 Crystal Examination

Step	Crystal Examination
1.	Place a drop of fluid on a clean glass slide and cover slip. Examine the preparation using polarized light to detect monosodium urate or calcium pyrophosphate dihydrate or cholesterol crystals.
3.	Using 40X lens, scan for presence of refractile material, crystals normally are either needle shaped or rod shaped and may be intra or extracellular (exceptions being cholesterol plates; irregular shaped steroid crystals and contaminants).
5.	Having located a crystal, carefully rotate the full wave plate to the right so that it now overlaps onto the illuminator. Moving the orientation handle while observing the crystal will result in a color change of the crystal. To properly identify crystals it is necessary to find at least one crystal oriented in North-South (vertical) and one in East-West (horizontal) position.
6.	This setting is such that if the long axis of a crystal lined up horizontally to the front is blue in this position it is positively birefringent. If the crystal is yellow in this position it is negatively birefringent. When the red compensator is rotated 90 degrees to the right side, the positively birefringent

Step	Crystal Examination
	crystal turns yellow and the negatively birefringent crystal turns blue.
7.	Monosodium uric acid crystals are oriented parallel to the slow north-south axis and will be yellow in color. The east west will be blue. If the polarizer orientation handle is moved to the extreme right, the north-south crystals will be blue and the east-west crystals will be yellow.
8.	Calcium pyrophosphate crystals (pseudogout) are parallel with the north-south axis will be blue. The east-west ones will be yellow. Moving the orientation handle to the extreme right will switch the colors.
9.	Cholesterol crystals are rhombic or rectangular notched plates. They may polarize into many colors.

9. CALCULATIONS

Refer to cell count worksheet. The master cell count formula is:

$$\frac{\text{Cells Counted}}{\text{\# of squares counted}} \times 10 \times \text{dilution}$$

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

All results are rounded to whole numbers.

10.3 Units of Measure

Parameter	Units
Red Blood Cell Count	Cells/µl
Total Nucleated Cell Count (TNC)	Cells/µl
Differential Counts	%

10.4 Clinical Reportable Range

None defined

10.5 Repeat Criteria and Resulting

Any duplicate counts not agreeing within 20%.

All Body fluid and Synovial fluid counts must be reviewed by a second technologist prior to resulting. Calculations must be rechecked and proper placement and documentation of cell counts on the worksheet must be verified. In addition, once typed into the computer a second technologist must verify the proper placement of the counts **PRIOR TO ACCEPTING THE RESULTS.**

Second tech review for Germantown Emergency Center ONLY:
Due to the fact that there is only one person working per shift, if a Body fluid or a Synovial fluid is performed then it will be the first duty of the next shift tech to review the cell count worksheet and compare it to the results entered into the computer. The reviewing tech will initial that the second tech review was performed.

All fluids needing a pathology review are to be taken to the pathologist on call for Hematology. Unless it has a cytology order, all slides must be accompanied by a Pathologist slide review request.

10.6 Crystal Resulting

Report the presence or absence of crystals seen under high power using the following guidelines. Also note if crystals are intra- or extra-cellular or both.

a. None seen
b. Crystals seen. Report the type of crystal seen to include Monosodium Urate, Calcium Pyrophosphate or Cholesterol.

11. EXPECTED VALUES

11.1 Reference Ranges

Parameter/Units of Measurement	Reference Range
Color	Pleural Fluid – Pale Yellow Peritoneal Fluid – Pale Yellow/Straw Pericardial Fluid – Pale Yellow/Straw Synovial Fluid-Pale Yellow/Straw
Appearance	Clear
Red Blood Cells/µl	Not Established
Total Nucleated Cells/µl	Not Established
Neutrophils/ %	Not Established
Lymphocytes/ %	Not Established
Monocyte/Macrophage/ %	Not Established
Eosinophils/ %	Not Established
Mesothelial/ %	Not Established
Crystal	None Seen

11.2 Critical Values

None established

11.3 Priority 3 Limits

None established

12. CLINICAL SIGNIFICANCE

12.1 Pleural and Ascitic Fluid

These fluids are classed as either transudates or exudates. The class indication is of great diagnostic importance.

- Transudates are due to alterations in the formation or reabsorption and are mechanical rather than pathologic in nature.
- Exudates are caused by an increase in the formation and decrease in reabsorption of the fluid (pleural or ascetic). Inflammation of the pleural or peritoneal lining or other diseases causes the formation of this fluid.

To differentiate fluids into transudates and exudates:

Parameter	Transudates	Exudates
Specific Gravity	<1.016	>1.016
Protein	<3.0 g/dl	>3.0 g/dl
LDH	<200 IU	>200 IU
Total Nucleated Cell Count	<1000/nm3 (Predominant cell type mononuclear)	>1000/nm3
Cultures	Negative	Positive or Negative

Some causes of ascetic fluid effusions are:

- **Transudates**: Congestive heart failure, cirrhosis, hypoproteinemia, and diffuse hepatic metastases.
- **Exudates**: Infections (either primary or secondary peritonitis), malignant disorders, trauma, and pancreatitis.
- **Chylous**: Trauma, carcinoma, lymphoma, and tuberculosis.

12.2 Peritoneal Dialysate

- Is used frequently for home renal dialysis patients. Samples of this fluid may be sent to the lab to check for leukocytosis due to bacteria infection. A large proportion of these patients develop peritonitis in the first year of treatment.
- A WBC count of more than 100/mm^3 with >50% neutrophils is the criteria used to establish an infection. The Wright stained smear will frequently show both intracellular and/or extracellular bacteria.

12.3 Synovial Fluid: Categorization of Arthritides or Joint Diseases

Except for the identification of crystals and culture for microorganisms, synovial fluid examination usually does not elicit a specific diagnosis. However, examination of the following characteristics is often valuable in categorizing a joint disease and in facilitating the establishment of a diagnosis: volume, clarity, color, viscosity, mucin clot formation, spontaneous glucose, crystals, and microbiologic culture.

By evaluating these characteristics of the fluid, joint disorders can be separated into five disease groups:

Disease Groups	Joint Disorders
Group I Non - inflammatory	Degenerative joint disease, Trauma, Osteochondritis dissecans, Osteochondromatosis, Neuropathic osteoarthropathy, Pigmented villonodular synovitis
Group II Inflammatory	Rheumatoid arthritis, Reiter's syndrome, Alkylosing spondylitis, Rheumatic fever, System lupus erythematosus, Scleroderma, Arthritis with Chronic ulcerative colitis or Regional enteritis
Group III Infections	Bacterial, Fungal
Group IV Crystal - induced	Gout, Pseudogout
Group V Hemorrhage	Hemorrhagic diatheses including – Hemophilia, Trauma, Neuropathic osteoarthropathy

Synovial Fluid Test Results According to Group of Arthritides

Test	Normal	Group I Noninflammatory	Group II Inflammatory	Group III Infectious	Group IV Crystal Induced	Group V Hemorrhagic
Clarity	Clear	Clear or Cloudy	Cloudy	Very Cloudy	Cloudy	Very Cloudy
Color	Yellow	Yellow	Yellow	Gray-white	Opalescent or colorless	Bloody
Leukocyte Count, per nm3	<200	200-3,000	3,000 - >100,000	10,000 - >100,000	1,000 - 100,000	>5,000
% PMN (Segs)	<25	<30	>50	>80	>70	>25
Crystals	No	No	No	No	Yes	No

12.4

Crystals are seldom seen except in arthritides Group IV. Urate crystals are seen in gout; calcium pyrophosphate crystals are seen in pseudogout; and corticosteroid crystals may be present following therapeutic intra-articular injection of steroid. The presence of cholesterol crystals has been described in osteoarthritis, rheumatoid arthritis, and

familial hypercholesterolemia. Oxalate crystals will be seen if the synovial fluid was collected in tubes containing oxalate anticoagulant.

12.5 Corticosteroid crystals are usually needle-shaped. They can be present in leukocytes, and have varying birefringence patterns depending on the particular steroid preparation used for therapeutic injection. Consequently, for correct interpretation of needle-shaped crystals, one must know whether a prior therapeutic injection has been given. Cholesterol crystals appear as notched plates, are not present in leukocytes, and are strongly birefringent.

12.6 Additional Microscopic Findings:
The microscopic examination of synovial fluid may show red cells, leukocytes, and crystal-bearing leukocytes, as previously described. The presence of synoviocytes (synovial lining cells) in the fluid is associated with pigmented villonodular synovitis, rheumatic fever and osteoarthritis. Synovial cells are round and much larger than leukocytes. Cartilage cells, when present in the synovial fluid, are associated with traumatic arthritis, osteoarthritis, and pseudogout. Cartilage cells are much larger than leukocytes and irregular in outline. RA cells, also called ragocytes, are segmented neutrophils containing round inclusions in their cytoplasm. These inclusions contain immunoglobulin and complement. As the name implies, RA cells occur in rheumatoid arthritis, but are not specific for the diagnosis. Wright-stained smears from patients with systemic lupus erythematosus (SLE) may show typical LE cells in the synovial fluid.

13. PROCEDURE NOTES

- **FDA Status:** Laboratory Developed Test (LDT) without message
- **Validated Test Modifications:** None

- Perform cell counts as soon as possible since cells deteriorate with time.
- If there is a clot, perform count on available liquid and make notation in the report. Counts on partially clotted samples may be affected depending whether or not cells are trapped in the clot.
- Low power scanning should be performed on smear to evaluate cell distribution and evaluate for presence of malignant cells.
- If crystal examination is ordered, perform this test first to help estimate the dilution needed for the cell count.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range

None defined

14.2 Precision

Not applicable

14.3 Interfering Substances

- Contamination with birefringent talcum powder may interfere with crystal analysis.
- Use of powdered EDTA or oxalate as an anticoagulant may interfere with crystal analysis.

14.4 Clinical Sensitivity/Specificity/Predictive Values

None defined

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Hematology Slide Stainer Cytocentrifuge, Wescor Aerospray® Model 7151, SGAH / WAH Hematology SOP
3. Cytospin CSF/Body Fluid Slide Preparation, GEC Hematology SOP
4. Diff Quick Stain Kit, Hematology SOP
5. Cell Count Worksheet, AG.F12
6. Cell Chex Control and Cell Chex Differential Control Log, AG.F87

17. REFERENCES

Body Fluid Analysis procedure, Hematology BPT, QDHE749 v1.2

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	11/26/13		Update owner	L Barrett	R SanLuis
000	11/26/13	4	Add Methylene Blue diluting fluid	C Reidenauer	R SanLuis
000	11/26/13	6.3	Re-format to clarify process	L Barrett	R SanLuis
000	11/26/13	7.2	Remove model number of stainers	L Barrett	R SanLuis
000	11/26/13	7, 8	Remove use of non disposable hemacytometer.	C Reidenauer	R SanLuis
000	11/26/13	8.3	Add Methylene Blue as diluting fluid to all dilution steps	C Reidenauer	R SanLuis
000	11/26/13	8.5	Add process for count <10	L Barrett	R SanLuis
000	11/26/13	8.6	Add cholesterol crystal to step 1	L Barrett	R SanLuis
000	11/26/13	10.6	Add specific crystals to be reported	C Reidenauer	R SanLuis
000	11/26/13	16	Add forms, update SOP titles	L Barrett	R SanLuis
000	11/26/13	19	Remove forms	L Barrett	R SanLuis
000	11/26/13	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
1	3/12/14	8.3	Correct 1:1 dilution to 1:2. Add dilution factors	C Reidenauer	R SanLuis

19. ADDENDA

None