Quest Diagnostics

TRAINING UPDATE

Lab Location:	GEC, SGAH & WAH	**Date Distributed:**	7/2/12
Department:	Hema	**Due Date:**	7/31/12

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Cell Count and Differential, CSF GEC.H06, SGAH.H08, WAH.H09 v001

Description of change(s):

Section	Reason
6.1, 6.7	Add diluting fluid check to match Cell Count Worksheet

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

Approved draft for training all sites (version 001)

Technical SOP

Title	**Cell Count and Differential, CSF**		
Prepared by	Cynthia Reidenauer	Date:	3/21/2011
Owner	Jean Buss, Robert SanLuis	Date:	6/6/2012

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

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

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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

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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ED patients**	Specimens are usually collected in three sterile tubes labeled 1, 2, and 3 in the order in which they are withdrawn.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-**

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
Bovine Albumin, 22%	Immucor, Cat. # 122-2
Wescor Aerospray Stain pack (or equivalent for other stainer)	Wescor
Diff Quick Stain Pak (GEC only)	Siemans
Isotonic Saline	Any brand
CSF diluting fluid	ENG Scientifics Cat # 5000.

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	Bovine Albumin, 22%
Container	Glass bottle
Storage	2-8°C
Stability	Manufacturers expiration date
Preparation	No preparation needed

Reagent	CSF diluting fluid
Container	Glass or Plastic
Storage	Room temperature
Stability	Manufacturers expiration date
Preparation	Ready to use.

Reagent	Wescor Aerospray Stain
Container	Plastic
Storage	Room temperature
Stability	Until Expiration Date Listed
Preparation	Ready To Use

Reagent	Isotonic Saline
Container	Plastic bottle
Storage	Room temperature
Stability	Manufacturer’s expiration date
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

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Control	Supplier & Catalog Number
Cell-Chex - 2x2mL each of Level 1&2	Streck Laboratories, Inc. Cat. #212419

The diluting fluid is checked for non-specimen background particulates every eight hours on days that CSF analysis is performed and changed when indicated. Checking can be done by examining samples of these fluids under the microscope. Each lot must be examined visually for uniformity of filling and clarity. See section 6.7

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 1&2
Preparation	None. It is not necessary to warm the controls to room temperature before using**.**
Storage/Stability	• Store upright at 2-8ºC • Open-vial stability 30 days

6.3 Frequency

QC should be tested every eight hours on days that CSF analysis is performed, and per technologist.

Batch Size or Max Time Limit	Minimum Number of QC Samples	QC % of Batch Size
Batch size = variable	Two levels of QC must be tested every 8 hours for cell count	N/A
Batch size = variable	Two levels of QC must be included with each batch of patient samples. If different technologists perform testing in an 8 hour period, each technologist must perform two levels of QC.	N/A
Wescor Slide-Stainer Cytocentrifuge or other stain method (Diff Quick for GEC)	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.	N/A

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6.4 Tolerance Limits

a) **Cell count by Manual Hemacytometer:**
QC values for Manual Hemacytometer are lot specific so check package insert.

- 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.) **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.

c) **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

Document the QC values in the LIS

6.7 Quality Assurance Program

- Place a drop of diluting fluid on a slide with cover slip. Observe 10 fields under high power on the microscope for the presence of debris or bacteria. Document completion on the "Diluting Fluid Check" section of the Cell Count Worksheet.
- The laboratory participates in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Not applicable

7.2 Equipment

Equipment	Supplier
Microscope	Any light microscope
Wescor Aerospray Slide Stainer Cytocentrifuge SGAH and WAH	Wescor
CytoTek centrifuge (GEC only)	Shandon

7.3 Supplies

Supply	Supplier & Catalog Number
Microhematocrit Tubes	Fisher Scientific, Cat. #21-176-5
Bright line Hemacytometer	Any brand
MLA pipette and tips	Any brand
Disposable tubes	Any brand
Cover glass	Fisher Scientific, Cat. #12-544-10
Microscope Slides	Fisher Scientific, Cat. #12-550-13
Petri Dish	Fisher Scientific, Cat. #08-757-12
Alcohol Swabs	Fisher Scientific, Cat. #06-669-62
Filter Paper	Fisher Scientific, Cat. #11-996
Applicator Sticks	Fisher Scientific, Cat. #01-340
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.

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8.2 Concentration

Step	Specimen Preparation
1.	Inspect specimen to determine the appropriate dilution.
2.	Bloody or cloudy CSF may require dilution with normal saline prior to counting. Since counts vary widely in CSF from zero to thousands, there is no standard dilution. Each case must be evaluated by the technologist. Examining the undiluted sample in a hemacytometer will help in this evaluation.
3.	Examine the CSF in the hemacytometer to determine whether a dilution is needed. Overlapping cells or cells packed in a single layer indicate the original fluid should be diluted with isotonic saline and the hemacytometer chamber recharged with this dilution.
4.	If it is difficult to differentiate between RBCs and TNCs, use the CSF diluting fluid
5.	Once the CSF is diluted, examine the diluted specimen for the presence of interfering substances such as bacteria, stain precipitate or foreign objects. If present use fresh diluting fluid and redilute. Document this check of the diluting fluid on the cell count worksheet.
6.	If the CSF is clear, it may be counted undiluted.

8.3 Differential Count

Step	Count
1.	Clean a hemacytometer with an alcohol swab and then wipe dry. Place coverslip on hemacytometer
2.	Fill a micro hematocrit tube or disposable pipette with well-mixed body fluid.
3.	Charge the two chambers of the hemacytometer by touching the tip of the capillary 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, clean and recharge the hemacytometer.
5.	Place the charged hemacytometer in a humidified Petri dish for 10 minutes to allow the cells to settle.
6.	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.
7.	Place the hemacytometer on the microscope and count the number of nucleated cells present on both sides. The sides should agree within 20%. If not, the cells are not evenly distributed and the chamber must be recharged and counted again. Average both sides and use this number in the calculation
8.	Calculate the total number of nucleated cells. See Section 9 for calculation formula.
9.	All calculations should be recorded on worksheet.

IF	THEN
Nucleated cell count is >0	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.
Abnormal or immature cells are noted	Refer to a Pathologist for review.

8.4 Cytospin

<table>
<tr><th>Step</th><th colspan="3">Cytospin</th></tr>
<tr><td>1.</td><td colspan="3">Assemble sample chamber and glass microscope slide in the Wescor Aerospray cytocentrifuge carousel. At GEC, follow Cytopsin procedure.</td></tr>
<tr><td rowspan="7">2.</td><td>IF</td><td colspan="2">THEN</td></tr>
<tr><td>Nucleated cell count is <300</td><td colspan="2">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.</td></tr>
<tr><td rowspan="5">Nucleated cell count is >300</td><td>Cells/ µL</td><td>Dilution</td></tr>
<tr><td>301-700</td><td>1:2 (5 drops CSF + 5 drops Isoton)</td></tr>
<tr><td>701-1500</td><td>1:5 (2 drops CSF + 10 drops Isoton)</td></tr>
<tr><td>1501-3000</td><td>1:10 (2 drops CSF + 20 drops Isoton)</td></tr>
<tr><td>>3000</td><td>1:20 (2 drops CSF + 40 drops Isoton</td></tr>
<tr><td colspan="3">Mix dilution well and place 3-5 drops into the Cytospin funnel. Add 1 drop of albumin.</td></tr>
<tr><td>3.</td><td colspan="3">Centrifuge Sample:
See procedure Aerospray Hematology Slide Stainer Cytocentrifuge (SGAH/WAH) or Cytospin CSF/Body Fluid Slide Preparation (GEC) as appropriate.</td></tr>
<tr><td>4.</td><td colspan="3">Stain slide using the Aerospray stainer or Diff Quick Stain Pack as appropriate</td></tr>
</table>

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.

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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.**

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
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.

Predominant Cells Seen in CSF		
Type of Cell	**Major Clinical Significance**	**Microscopic Findings**
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.
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.

Found revised 8/31/00

13. PROCEDURE NOTES

- **FDA Status:** LDT without message
- **Validated test modifications:** not applicable.

- Perform cell counts as soon as possible since cells deteriorate with time.
- Low power scanning should be performed on smear to evaluate cell distribution and evaluate for presence of malignant cells.

14. LIMITATIONS OF METHOD

Not applicable

15. SAFETY

You, the employee, have 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 the learn 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.

Report all accidents and injuries to your supervisor or the Environmental, Health and Safety Coordinator.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Hematology Slide Stainer Cytocentrifuge, Wescor Aerospray Model 7150, WAH Hematology SOP
3. Hematology Slide Stainer Cytocentrifuge, Wescor Aerospray Model 7120, SGAH Hematology SOP
4. Cytospin CSF/Body Fluid Slide Preparation, GEC Hematology SOP
5. Diff Quick Stain Kit, Hematology SOP

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

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

19. ADDENDA

A. Cell Count Worksheet (See Attachment Tab of Infocard)
B. Cell Chex Control Log (See Attachment Tab of Infocard)