TRAINING UPDATE

Lab Location:	FWMC	**Date Distributed:**	8/3/2022
Department:	Core Lab	**Due Date:**	8/22/22

DESCRIPTION OF PROCEDURE TRAINING REVIEW

Name of procedure:
SOP Title: AHC.H08 CSF Cell Count and Differential, Manual Method, **Form: AG.F12 Cell Count Worksheet**
Training Review
• Review the attached SOP and Cell Count Worksheet. • In the SOP, Focus on Section 8.2, step 2 of specimen preparation. • Review the instructions on the Cell Count Worksheet. • NOTE: Diluting fluid check MUST be recorded on the worksheet • Cell Count Worksheets are filed and maintained in the Cell Count Worksheet binder.

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

AHC.H08 CSF Cell Count and Differential, Manual Method

Copy of version 8.0 (approved and current)

Last Approval or Periodic Review Completed	8/17/2021
Next Periodic Review Needed On or Before	8/17/2023
Effective Date	9/28/2021

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Organization Adventist HealthCare

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	8/17/2021	8.0	Nicolas Cacciabeve	
Approval	Core lab approvals	8/13/2021	8.0	Robert SanLuis Robert SanLuis	
Approval	QA approval	8/9/2021	8.0	Leslie Barrett	
Approval	Lab Director	3/18/2020	7.0	Nicolas Cacciabeve	
Approval	Core lab approvals	3/17/2020	7.0	Robert SanLuis Robert SanLuis	
Approval	QA approval	3/17/2020	7.0	Leslie Barrett	
Approval	Lab Director	5/21/2019	6.0	Nicolas Cacciabeve	
Approval	Core lab approvals	5/21/2019	6.0	Robert SanLuis Robert SanLuis	
Approval	QA approval	5/17/2019	6.0	Leslie Barrett	
Approval Captured outside MediaLab	Lab Director	11/5/2018	5.0	Nicolas Cacciabeve	Recorded on 12/13/2018 by Leslie Barrett when document added to MediaLab
Periodic review Captured outside MediaLab	Designated Reviewer	11/5/2018	5.0	Nicolas Cacciabeve	Recorded on 12/13/2018 by Leslie Barrett when document added to MediaLab

Approvals and periodic reviews that occurred before this document was added to the MediaLab Document Control system may not be listed.

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
8.0	Approved and Current	Major revision	7/20/2021	9/28/2021	Indefinite
7.0	Retired	Major revision	3/17/2020	5/4/2020	9/28/2021
6.0	Retired	Major revision	5/17/2019	6/5/2019	5/4/2020
5.0	Retired	First version in Document Control	12/13/2018	11/13/2018	6/5/2019

Linked Documents

- AG.F12 Cell Count Worksheet
- AG.F87 Cell Chex Control Logs
- AG.F127 Pathologist Slide Review Request

Technical SOP

Title	**CSF Cell Count and Differential, Manual Method**	
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.		

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Cell Counts, Total RBC and Total Nucleated Cells, CSF (tube specific)	Manual/Microscopic	CT1, CT2, CT3, CT4

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	Specimens are collected in sterile tubes labeled in the order in which they are withdrawn (1, 2, 3, 4). • Tube 1 is used for color, appearance, cell count and Chemistry tests • Tube 2 is used for Serology tests • Tube 3 is used for color, appearance, cell count and diff • Tube 4 is used for Microbiology **Note**: If there is a Cytology order, process core lab testing per 3 tube protocol and use tube 4 for Cytology.

Component	Special Notations
Special Collection Procedures continued	If 3 tubes are received: • Tube 1 is used for color, appearance, cell count and Chemistry tests • Tube 2 is used for Serology tests, color, appearance, cell count and diff • Tube 3 is used for Microbiology **Note**: If there is a Cytology order, after Microbiology has taken their sample from tube 3, send remainder of tube 3 for Cytology. If less than 3 tubes are received, contact the physician for specific tests to be performed.
Other	Not applicable

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Tube #1 and #3 (See section 3.1 if less than 4 tubes) 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:	Process Immediately. Rapid deterioration and cell lysis occurs on prolonged standing in CSF.
	Refrigerated:	Same as above.
	Frozen:	Unacceptable
Timing Considerations	Not Applicable	
Unacceptable Specimens & Actions to Take	**Clotted specimens:** Perform counts and append the code SCLOT (*Specimen contains clots, counts may not be accurate*). **Specimens received after 24 hours:** Perform counts and append the code SAGE (*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 reason code SFRZ (*Specimen unsuitable for assay; received frozen*). Notify a caregiver and document in the LIS.	
Compromising Physical Characteristics	None defined	
Other Considerations	None defined	

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Rinse	ELITechGroup SS-071 A
Thiazin	ELITechGroup SS-071 B
Eosin	ELITechGroup SS-071 C
Methanol	ELITechGroup SS-MEOH
Aerofix (Additive for Methanol)	ELITechGroup SS-148
0.9% Saline	Thermo 0.9% Saline cat # 23535435
22% Albumin (Obtain from Blood Bank)	Immucor CE 0088
RAL Diff-Quik Stain Pak	RAL Diagnostics #720555-0000

4.2 Reagent Preparation and Storage

Reagent A	**ELITechGroup Aerospray Rinse**
Reagent B	**ELITechGrout Aerospray Thiazin**
Reagent C	**ELITechGroup Aerospray Eosin**
Container	Plastic Bottle
Storage	5-30°C
Stability	Manufacturer's expiration date
Preparation	Ready to use

Reagent	**Wescor Aerospray Aerofix**
Container	Plastic Bottle
Storage	15-30°C
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	15-30°C
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 - 10C for long term storage
Stability	Stable until expiration date on the bottle. If turbid, discard.
Preparation	Ready to use

Reagent	**RAL Diff-Quik Stain Pack**
Container	Plastic Bottle
Storage	15 - 25C
Stability	**Unopened:** Until expiration date on box label. **Opened:** Remains stable 2 months after opening. Keep away from light.
Preparation	Ready to use

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Control	Supplier & Catalog Number
Cell-Chex L1-UC, L1-CC and L2 (2mL each)	Streck Laboratories, Inc. Cat # 212431
Cell-Chex L1-UC, L2	Streck Laboratories, Inc. Cat # 212420
Cell-Chex L1-CC	Streck Laboratories, Inc. Cat # 212430

6.2 Control Preparation and Storage

Control	Cell-Chex Level L1-UC, L1-CC and L2
Preparation	None required. It is not necessary to warm the controls to room temperature before using**.**
Storage/Stability	• Store upright at 2-10°C • 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
L1-CC perform a cytospin differential
L2 perform cell count

- **Automated or Manual stain method** comparison 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 for contamination each day of use and documented on the Cell Count Worksheet. Refer to section 8.2 for further details.**

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.

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 Documentation

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

6.6 Quality Assurance Program

The laboratory participates in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Not applicable

7.2 Equipment

Microscope
Aerospray Cytocentrifuge
CytoTek centrifuge (GEC only)

7.3 Supplies

Disposable Pipettes
Hemacytometer (disposable) C-CHIP
MLA pipette and tips
Disposable tubes
Cover glass
Microscope slides
Petri dish
Applicator sticks
Cytopro
Cyto-Tek 2500 (GEC)

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

<table>
<tr><th>Step</th><th>Examination for Appearance and Color</th></tr>
<tr><td>1.</td><td>Examine the CSF for appearance and color.</td></tr>
<tr><td>2.</td><td>Appearance: Indicate what the fluid looks like before centrifugation; use the following codes:
<table>
<tr><th>Description</th><th>Code</th><th>Description</th><th>Code</th></tr>
<tr><td>Clear</td><td>CLEAR</td><td>Turbid</td><td>TURB</td></tr>
<tr><td>Cloudy</td><td>CLDY</td><td>Bloody</td><td>BLDY</td></tr>
<tr><td>Slightly Cloudy</td><td>SLCLDY</td><td></td><td></td></tr>
</table>
</td></tr>
</table>

Step	Examination for Appearance and Color
3.	**Color**: Centrifuge an aliquot for the time and speed posted on centrifuge to remove the cellular elements. Examine the supernatant and report the color using the following descriptions and codes:

Description	Code	Description	Code
Colorless	COLR	Brown	BRWN
Yellow	YEL	Red	RED
Pink	PINK		

8.2 Concentration

Step	Specimen Preparation
1.	Inspect specimen to determine the appropriate dilution. The sample can be assayed without diluting if the cell count is low. Follow steps for un-diluted sample.
2.	If dilution is to be performed, place a drop of diluting fluid (0.9% saline) 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, proceed to specimen dilution.
3.	Mix specimen well and make the appropriate dilution. Refer to dilution tables below.

Step	Un-diluted Sample
1.	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.
2.	If the hemacytometer is overcharged, it must be discarded and a fresh one used.
3.	Place the charged hemacytometer in a humidified Petri dish for 10 minutes to allow the cells to settle.
4.	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 and less than 200 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%.

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 CSF to 100µl of 0.9% saline. 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 and less than 200 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 CSF to 900µl of 0.9 % saline. 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 5 through 7 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 CSF to 950µl of 0.9% saline. 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 5 through 7 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 CSF to 980µl of 0.9% saline. 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 5 through 7 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 CSF to 990µl of 0.9% saline. 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 5 through 7 for 1:2 Dilution

8.3 Differential Count

IF	THEN
Cell count is ≤5	Do not perform differential. Result with **NOTP-;**due to an insufficient number of cells in the sample.
Cell count is >5	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. If abnormal or immature cells are noted, a second technologist must also perform a differential and then refer slide(s) to a Pathologist for review.

8.4 Cytospin

Step	Cytospin		
1.	Assemble sample chamber and glass microscope slide in the 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 (SGMC/WOMC) 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

Perform Correlation Check by verifying the number of nucleated cells and non-nucleated cells from the differential correlate with the cell count. *Example*: If rare RBCs are seen on the differential, then there should be a "low" number of RBCs for the count.

- If acceptable, circle YES on the manual fluid worksheet.
- If not acceptable, circle NO on the manual worksheet and repeat count or differential.

10.2 Rounding

Results for cell counts are rounded to whole numbers.

10.3 Units of Measure

Parameter	Units
RBC	Cells/µL
TNC (WBC-BF)	Cells/µL
Differential Counts	%

10.4 Clinical Reportable Range

Not applicable

10.5 Review Patient Data

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

10.6 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 tech 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.

Pathology Review:
All fluids needing a pathology review are to be taken to the pathologist on call for Hematology. Slides submitted for pathology review are accompanied by an Interim Report by Accession (FUNC: IRA in SmartTerm) and the Pathologist Slide Review form.

Resulting:
Refer to the addendum *Fluid Keyboard: Accessing Differential Result Entry for CSF* for details to result via the SQ keyboard.

Note: Manual differentials performed due to TEa failures on Sysmex (difference between TC-BF and WBC-BF exceeds the TEa of 20% during Sysmex testing) must be reported via the CSF Cell Counter in DI.

11. EXPECTED VALUES

11.1 Reference Ranges

Parameter / Units of Measurement	Both Male and Female	
	Neonate	Adult
Color	Colorless	
Appearance	Clear	
RBC - BF cells/µL	None	None
WBC - BF cells/µL	0 - 30	0 - 5
Lymphocyte %	<70	<70
Monocyte %	<30	<30
Eosinophil %	<10	<10

Note: TNC are reported in LIS as 'WBC-BF' to align with automated method.

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

CSF Appearance		
Appearance	**Cause**	**Most Significance**
Crystal Clear		Normal
Hazy, turbid, cloudy	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 (color)	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

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, lymphocytes, 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.

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.

14. LIMITATIONS OF METHOD

Not applicable

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Hematology Slide Stainer Cytocentrifuge, Aerospray® Model 7151, SGMC / WOMC Hematology SOP
3. Cytospin CSF/Body Fluid Slide Preparation, GEC Hematology SOP
4. RAL Diff-Quik Stain Kit, Hematology SOP
5. Cell Count Worksheet (AG.F12)
6. Cell Chex Control Log (AG.F87)
7. Pathologist Slide Review Request (AG.F127)

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
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
3	3/30/16		Change SGAH to SGMC throughout	L Barrett	R SanLuis
3	3/30/16	8.1	Replace specific centrifugation instruction with referral to posted instruction	L Barrett	R SanLuis
4	10/15/18	Header	Add other sites, update title to include method	L Barrett	R SanLuis
4	10/15/18	1	Update assay name & codes to match LIS	L Barrett	R SanLuis
4	10/15/18	3.1	Revise tube processing order to match standard protocol	L Barrett	R SanLuis
4	10/15/18	3.2	Revise tube numbers, add codes for comments	L Barrett	R SanLuis
4	10/15/18	4,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
4	10/15/18	4	Update automated stain and Diff-Quik info	D Collier	R SanLuis
4	10/15/18	6	Update product numbers & storage temp	D Collier	R SanLuis
4	10/15/18	8.3	Add second tech review for abnormal cells	L Barrett	R SanLuis
4	10/15/18	10.5	Moved review from section 6	L Barrett	R SanLuis
4	10/15/18	10.6	Added reporting section	L Barrett	R SanLuis
4	10/15/18	11.1	Updated RBC & WBC to match automated method	L Barrett	R SanLuis
4	10/15/18	12	Updated appearance to match reporting practice, Removed extraneous info	D Collier	R SanLuis

Version	Date	Section	Reason	Reviser	Approval
4	10/15/18	15	Update to new standard wording	L Barrett	R SanLuis
4	10/15/18	16	Update SOP title	L Barrett	R SanLuis
4	10/15/18	19	Added keyboard steps for reporting	L Barrett	R SanLuis
5	5/16/19	2	Updated local test codes	L Barrett	R SanLuis
5	5/16/19	8.1	Specify reporting for appearance & color	H Genser	R SanLuis
5	5/16/19	8.3	Add no diff performed if count is less than 5	L Barrett	R SanLuis
6	3/13/20	Header	Changed WAH to WOMC	L Barrett	R SanLuis
6	3/13/20	4.1, 4.2	Deleted methylene blue diluting fluid	H Genser	R SanLuis
6	3/13/20	6.3	Deleted diluting fluid check	H Genser	R SanLuis
6	3/13/20	8.2	Changed diluent to 0.9% saline, added steps for undiluted sample	H Genser	R SanLuis
6	3/13/20	10.1	Added correlation check	H Genser	R SanLuis
7	8/6/21	Header	Changed the Site to All Laboratories	D Collier	R SanLuis
7	8/6/21	Footer	Changed number prefix from SGAH to AHC	D Collier	R SanLuis
7	8/6/21	6.3	Added diluting fluid check	D Collier	R SanLuis
7	8/6/21	8.2	Added instructions for dilution fluid check	D Collier	R SanLuis
7	8/6/21	10.6	Update wording to define Function IRA	D Collier	R SanLuis
7	8/6/21	Addendum A 9.b	Removed references to “not needing path review if cytology is ordered” added instructions for documenting cytology orders and interpretation on Pathology Slide Review form.	D Collier	R SanLuis

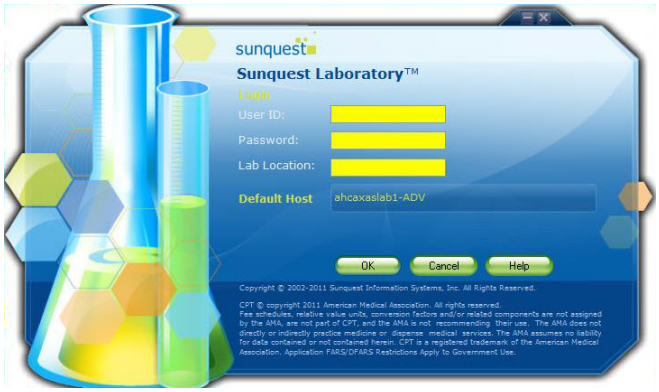
19. ADDENDA

A: Fluid Keyboard: Accessing Differential Result Entry for CSF

Addenda A

Fluid Keyboard: Accessing Differential Result Entry for CSF

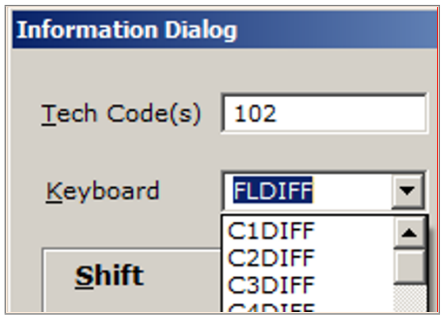
1. Log into the Sunquest GUI application.



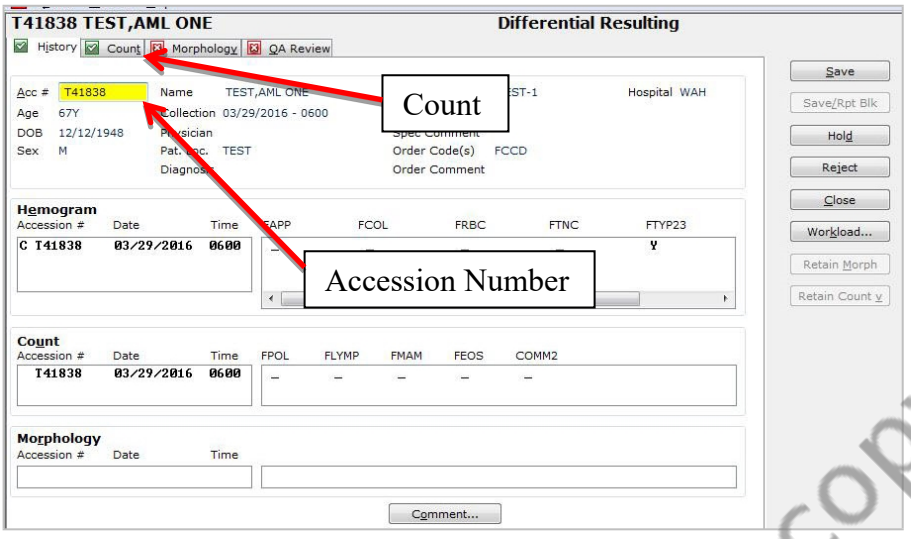
2. Click on **Differential Result Entry**.

3. Under "Information Dialog" screen, click on the down arrow and select the type of fluid.

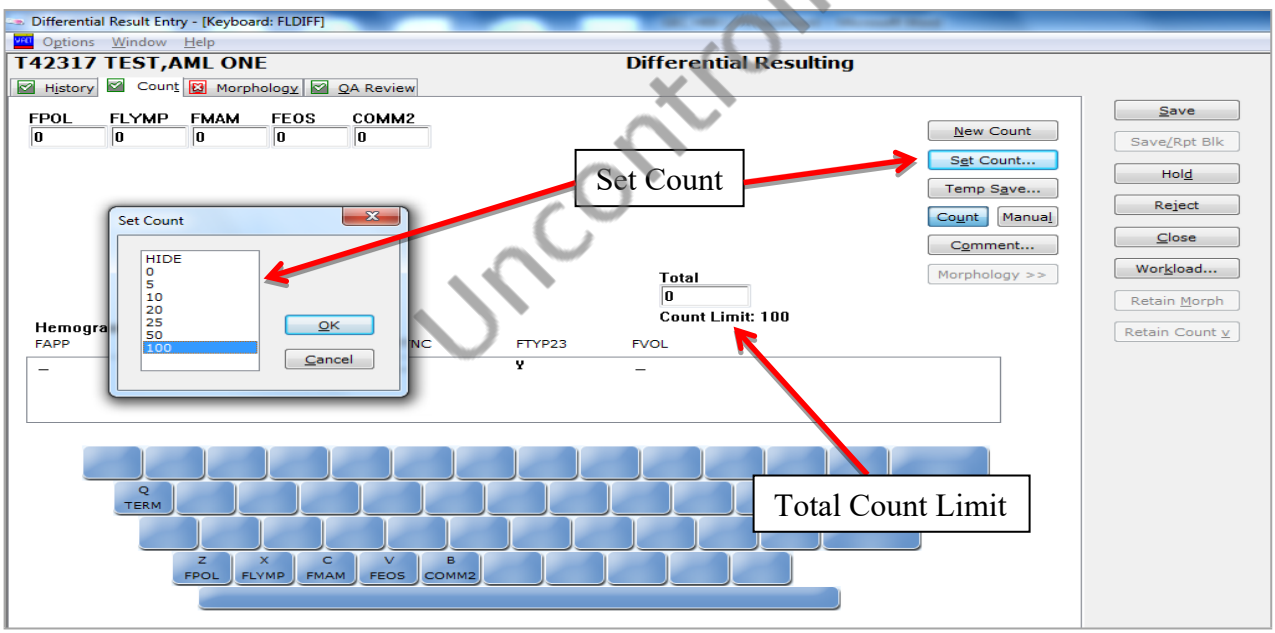
   Note: For CSF select the keyboard that is associated with the CSF tube number. *Example*: if diff is being performed on tube 1 then select C1DIFF.



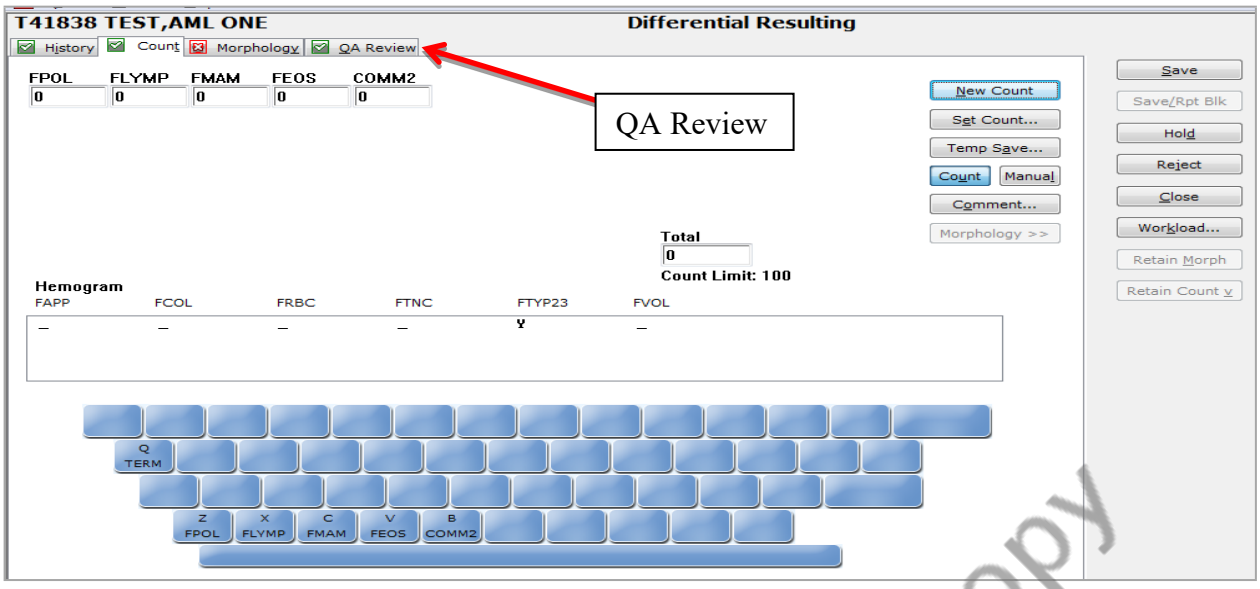
4. Enter the accession number and press enter. When patient information is displayed, verify it matches the specimen tested. Once patient identification is confirmed, click on **Count**.



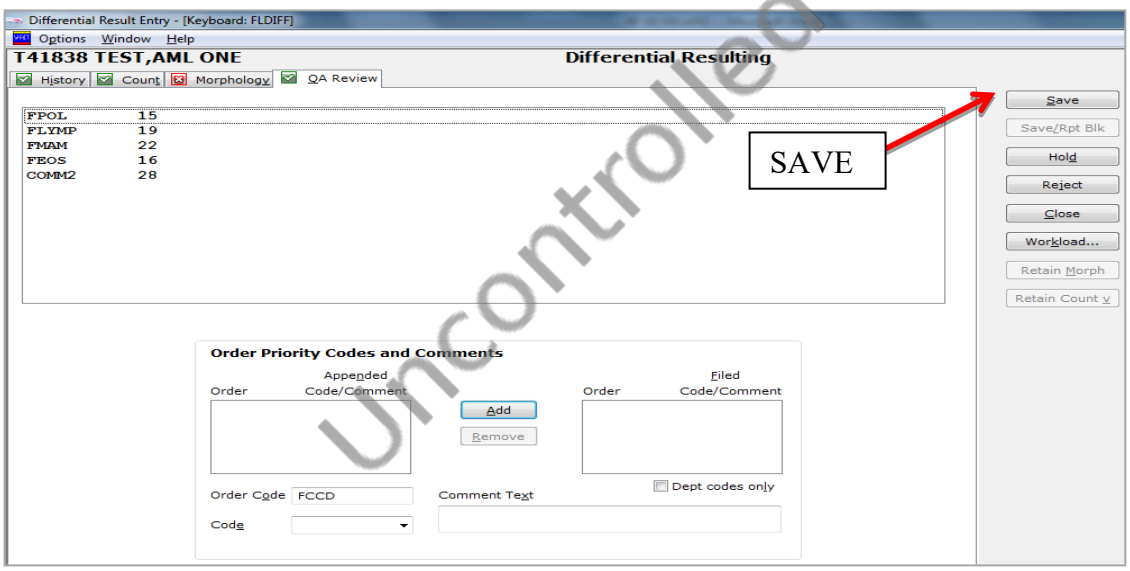
5. Examine the slide and adjust the "Total Count Limit" accordingly. To adjust the Total Count Limit, click on **Set Count** and then choose one of the options.



6. Enter the differential count. When finished click on **QA Review**.



7. Review the QA report, then click **SAVE**.



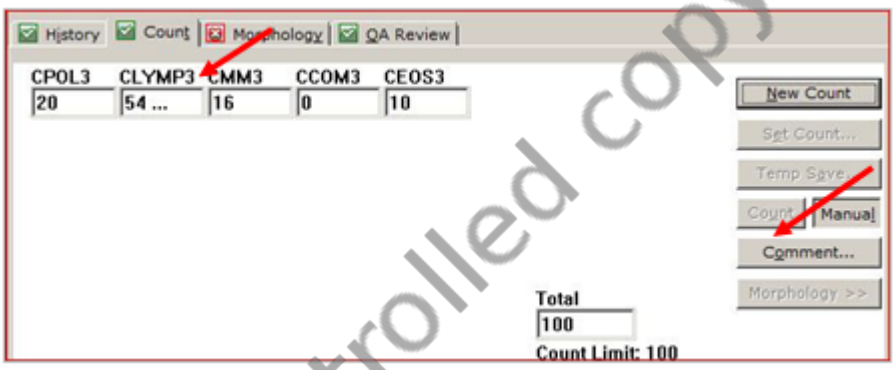
8. If the slide requires a second tech review, click on **HOLD**.
   a. A second tech will perform the differential using a manual cell counter or the off-mode in Sunquest and record results on the Pathologist Slide Review Request form.
   b. The criteria specified on the Automated Stainer Differential Comparison and Stain Quality Log is used to compare results.
      - If the result comparison meets the criteria, then the original differential is reported.
      - If the differential results do NOT correlate, then supervisor/tech in charge will review the diff and decide which results to report.

9. If the slide requires a pathologist review:

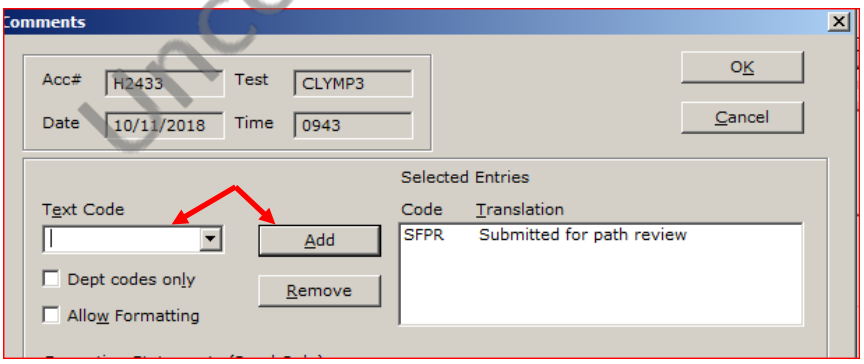
   - The slide will need to be submitted to pathology. The next steps describe how to document sending it for review, sections 9.b and 9.c outline how to order the Path Review and how to result it.
   - Append English Text code **SFPR** (translates to Submitted for Path Review) to one of your cells counts. Choose a cell type that was observed because the English Text code will not post if you append it to a cell count with a result of 0.

Example:

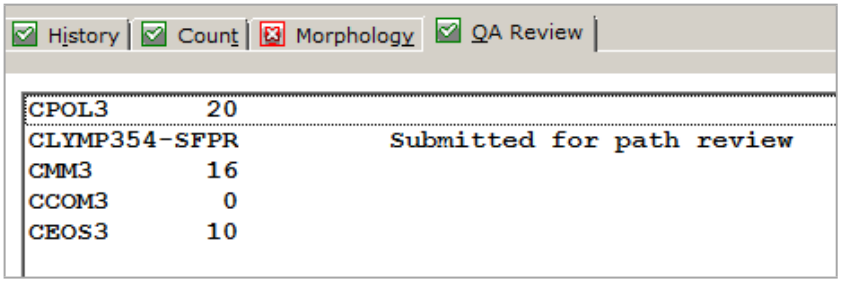
a. Click in the CLYMP3 count box, and then click on the **Comment** button.



Another window opens. In the Text Code box type in **SFPR** and then click **ADD**. Text Code is translated in the box to the right. Click **OK** to save.



Note: The comment will append to the cell type you selected and will be seen in the QA Review tab. *Example:*



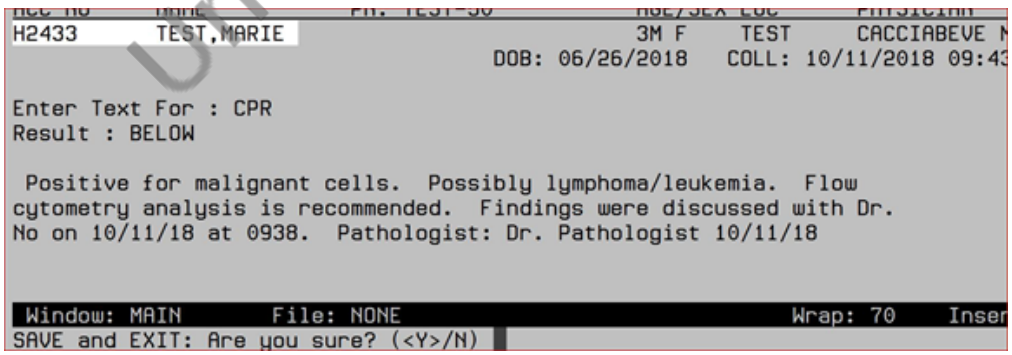
b. To submit slides for path review –

- Add order code CPATH to the Accession via REI or GUI Order Entry.
- Complete Pathologist Slide Review Request form.
- Check to see if patient has orders for a Cytology workup. If so, you MUST document this by checking the box on the Pathologist Slide Review Request form. The Pathologist will then compare the data from both areas and document on the form.

- Give slide(s) and review form to the pathologist.

c. When the Pathologist Slide Review form and slide(s) are returned to the lab, enter results into the LIS via SmartTerm. Note: This should also include the pathologist's comments or assessment regarding the diff count which has already been reported in SmartTerm. There original reported diff does NOT need to be corrected.

Example:



Example of display in Sunquest Inquiry:

H2433 COLL: 10/11/2018 09:43 REC: 10/11/2018 09:53 PHYS: CACCIABEVE MD,
Req. No.:

CSF Path Review
CSF Path Review See below
(See Below)
Positive for malignant cells. Possibly lymphoma/leukemia. Flow cytometry analysis is recommended.
Findings were discussed with Dr. No on 10/11/18 at 0938. Pathologist: Dr. Pathologist 10/11/18

CSF Tube 3

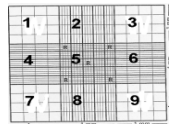
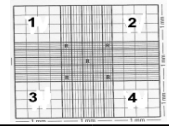
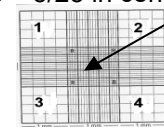
Apperance tube 3	Cloudy	[CLEAR]	
Color tube 3	Colorless	[COLR]	
CSF WBC Tube 3	2	[0-5]	cell/mcL
CSF RBC Tube 3	12		cells/mcL
CSF Polys tube 3	20	%	
CSF Lymph tube 3	54	%	
Submitted for path review			
CSF Macro/Mono tube 3	16	%	
CSF EOS tube 3	10	%	

Adventist HealthCare

Cell Count Worksheet

☐ Germantown Emergency Center
☐ Shady Grove Medical Center
☐ White Oak Medical Center
☐ Fort Washington Medical Center

Patient Name: _____ **Med. Rec. #** _____
Specimen #: _____ **Tech Code** _____
Date: **Correlation Check: Acceptable? YES or NO (circle one)**
Automated Count ☐ **Diluting Fluid Check: Acceptable? YES, NO or NA (circle one)**

Manual	1		2		3	
# of cells in 1 Square	**<20**- Count all 9 squares 		**>20**- Count 4 corner squares 		**>200**- 5/25 in center square 	
	RBC	**TNC**	**RBC**	**TNC**	**RBC**	**TNC**
Chamber 1						
Chamber 2						
Average						
Correction Factor	**X 1.1**	**X 1.1**	**X 2.5**	**X 2.5**	**X 50**	**X 50**
Multiply by correction factor			☐			
Multiply by Dilution	**X** _____	**X** _____	**X** _____	**X** _____	**X** _____	**X** _____
Final result						

Tube # _____ **Volume** _____ **Crenated RBCs:** _____
Color _____ **Appearance** _____
Fluid Type

Second Tech Review_____

Diff:

Poly	
Lymph	
Mono/Macro	
Eos	
Other Cells	
Crystals	

Master Formula

$$\frac{\text{cells}}{\text{\# of squares counted}} \times 10 \times \text{dilution} = \text{Cells}/\mu L$$

Instructions for use:

1.	Place a drop of diluting fluid on a slide with coverslip. Observe 10 fields under high power for the presence of debris or bacteria. Document on this worksheet. If not performing a dilution, select N/A.
2.	Evaluate the number of cells in one square. If < 20 cells, record all results on column #1. If > 20 cells are observed, record all results on column #2. If > 200 cells are observed, record all results on column #3.
3.	Count the RBC's and TNC's for each chamber separately. Record the results in the corresponding location.
4.	Average the result. Add the number of cells counted in both chambers and divide by 2. Record this number in the column for the average number of cells observed.
5.	Multiply the average number of cells by the Correction Factor. Multiply by the dilution (if applicable).
6.	Record the final count in the appropriate square for the cell type, i.e. RBC's or TNC's.
7.	Perform Correlation Check (verify the number of nucleated cells and non-nucleated cells from differential correlate against the cell count) If Acceptable, circle YES on manual fluid worksheet. If not acceptable, circle NO on manual worksheet and repeat count or differential.
8.	Record the results in the LIS, have second tech review before accepting.
9	Keep this sheet in the Cell Count Worksheet binder.