TRAINING UPDATE

Lab Location:	GEC, SGMC & WAH	**Date Distributed:**	12/10/2018
Department:	Core Lab	**Due Date:**	12/18/2018
		Implementation:	**12/18/2018**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Body Fluid Analysis by Sysmex XN Series SGAH.H1019 v0

Body and Synovial Fluid Analysis, Manual Method SGAH.H09 v6

Description of change(s):

Automated Body Fluid testing is moving from the Iris to the Sysmex *– the first SOP attached is the new one for that method (very similar to CSF process)*

The fluid manual method *SOP has been revised so it is in 'sync' with the automated one (again, similar to changes made for CSF). That SOP is attached after the Sysmex one. NOTE – Process for* ***Synovial Fluids stays the SAME****, it is only performed manually.*

This is a summary of important changes:

- The new test code for fluids (except Synovial) is FLCNT.
- When a synovial fluid is ordered at GEC, the sample should be tracked and sent STAT to SGMC.
- If there is a solid clot in a body fluid, the surrounding fluid should be transferred to another tube (avoid transferring the clot). Testing may be run on the Sysmex or manually depending on the sample volume.
- All crystal exams for synovial fluid are tested at SGMC.
- TNC was changed to WBC.
- "HIDE" should be typed for "Fluid Total Cells" when count is performed manually.
- Manual differentials must be performed when TEa failures on Sysmex (difference between TC-BF and WBC-BF) exceeds the TEa of 20%.
- Order code "FPATH" is added to the Accession via REI or GUI Order Entry for a path review.

These SOPs will be implemented on December 18, 2018

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

Technical SOP

Title	**Body Fluid Analysis by Sysmex XN Series**	
Prepared by	Ashkan Chini	Date: 11/27/2018
Owner	Robert SanLuis	Date: 11/27/2018

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

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

Form revised 2/02/2007

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Cell Counts, Total RBC and Total Nucleated Cells, Body Fluid (Pericardial, Peritoneal, Pleural)	Sysmex XN Series 1000/3000	FLCNT

Synonyms/Abbreviations
Body Fluid Cell Count

Department
Hematology

Note: Synovial Fluid must be performed by manual method.

Form revised 2/02/2007

2. ANALYTICAL PRINCIPLE

The RBC detector counts the RBC via the Hydro Dynamic Focusing. The RBC is calculated as a particle count between lower and upper discriminators, which are automatically setup in the ranges of 25 – 75 fL and 200 – 250 fL. The particle size distribution is checked for abnormal relative frequencies at each discriminator level existence of more than one peak and abnormal distribution width.

Flow Cytometry is used to analyze physiological and chemical characteristics of cells and other biological particles. It is also used to analyze those cells and particles as they are passed through extremely small flow cells.

The WNR Channel uses flow cytometry to create a scatter gram and is primarily used to count the white blood cells. This scatter gram displays groups of basophil, non-basophil WBC and hemolyzed RBC.

The WDF Channel uses flow cytometry to create a scatter gram and is primarily used for classifying WBCs. This scatter gram displays groups of lymphocytes, monocytes, eosinophils, basophils and neutrophils.

The WPC Channel uses flow cytometry to create a scatter gram and is used for detecting immature WBCs such as myeloblasts and abnormal lymphocytes. This scatter gram displays groups of immature/abnormal WBCs and mature WBCs.

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	Not applicable
Other	Not applicable

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Serous fluid (Pericardial, Peritoneal, Pleural)
-Other Acceptable	None
Collection Container	EDTA tubes, plain tubes (no additives)
Volume - Optimum	2 mL
- Minimum	0.5 mL

Criteria	
Transport Container and Temperature	Transport at room temperature in collection tube
Stability & Storage Requirements	Room Temperature: 48 hours
	Refrigerated: 48 hours
	Frozen: Not Acceptable
Timing Considerations	Not Applicable
Unacceptable Specimens & Actions to Take	**Due to the nature of these specimens, do not reject unless frozen.** **Clotted specimens:** Perform counts and append the code SCLOT (*Specimen contains clots, counts may not be accurate*). **Solid Clot:** Transfer surrounding fluid to another tube (see section 8.2) but avoid transferring clot. Testing may be run on Sysmex or manually depending on sample volume. Add free text comment to results: "Solid clot noted". **Specimens received after 48 hours:** Perform counts and append the code SAGE (*Counts may not be accurate due to the age of the specimen*). **If the specimen is received frozen:** Cancel the test with the reason code SFRZ (*Specimen unsuitable for assay; received frozen*). Notify the attending nurse or physician. Note: In Cerner reason for cancellation will be "improper collection".
Compromising Physical Characteristics	Not Applicable
Other Considerations	Synovial fluid is not validated on this instrument, testing must be performed by manual methods.

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 / Kits	Supplier & Catalog Number
Cell Pack DCL	Sysmex Corporation, Cat. No. DCL-300A
Cell Pack DFL	Sysmex Corporation, Cat. No. BT965910

Reagents / Kits	Supplier & Catalog Number
Fluorocell WDF	Sysmex Corporation, Cat. No. CV377552
Fluorocell WNR	Sysmex Corporation, Cat. No. CP066715
Lysercell WDF	Sysmex Corporation, Cat. No. ZA900001
Lysercell WNR	Sysmex Corporation, Cat. No. ZA900002
Sulfolyser SLS	Sysmex Corporation, Cat. No. BJ350971

4.2 Reagent Preparation and Storage

Reagents	Cell Pack DCL, Cell Pack DFL
Storage	Store at 2 - 35°C. Avoid exposing to direct sunlight
Stability	Once in use, these remain stable for 60 days.
Preparation	None

Reagents	Fluorocell WDF, Fluorocell WNR, Lysercell WDF
Storage	Store at 2 - 35°C. Avoid exposing to direct sunlight
Stability	Once in use, these remain stable for 90 days.
Preparation	None

Reagent	Lysercell WNR
Storage	Store at 2 - 35°C. Avoid exposing to direct sunlight
Stability	Once in use, stable for 60 days.
Preparation	None

Reagent	Sulfolyser SLS 1.5 L
Storage	Store at 2 - 30°C. Avoid exposing to direct sunlight
Stability	Once in use, stable for 60 days.
Preparation	None

5. CALIBRATORS/STANDARDS

Calibration is not specific for body fluid mode. Refer to *Sysmex XN Series Operation for CBC and Reticulocytes* SOP for details on calibration.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
XN CHECK BF, Levels 1 & 2	Sysmex Corporation, Cat. No. 213516

6.2 Control Preparation and Storage

Control	XN CHECK BF
Preparation	Allow to come to room temperature, mix by manually inverting samples 4 times.
Storage	Store at 2 - 8°C
Stability	**Unopened**: manufacturer's expiration date **Opened**: 30 days when stored at 2 - 8°C after each use.

6.3 Frequency

Both levels of control must be run on all Sysmex XN instruments every 8 hours of patient testing.
QC must also be performed after shutdown, maintenance or instrument repairs.

Refer to addendum 4 "QC Instructions on Sysmex the XN" of the procedure *Sysmex XN Series Operation for CBC and Reticulocytes* to perform parallel testing for new lots of QC materials.

6.4 Tolerance Limits and Criteria for Acceptable QC

A. Tolerance Limits

The Hematology QC program is monitored in the instrument and should be set up using the Evidence-based QC Limit % Range specific for XN analyzers. These limits are provided by Sysmex and are intended to ensure reasonable error detection capability and minimal false rejection rates. Target values for each level of control will be calculated based on the data collected in the new lot evaluation.

B. Criteria for Acceptable QC

- All Controls must be within the acceptable range.
- Controls and patient data must be reviewed for acceptability and for atypical or unexpected results or trends prior to reporting patient results.
- DO NOT release results from runs with unacceptable controls or with unusual patterns, trends or distribution in patient values.

C. Corrective Action

- All rejected runs must be effectively addressed and include the following documentation:
  - Control(s) that failed and/or atypical or unexpected patient results
  - Actions taken
  - Statement of what was done with the patient samples from the affected run/batch,
  - Date and initials of the person recording the information.

- Patient samples in failed analytical runs must be reanalyzed.
- **Precision Statistics:** When there is a significant shift/bias on QC data, the root cause of the increased imprecision must be investigated and a resolution needs to be considered immediately. All of these actions must be documented including an evaluation of whether or not this affected patient care.

NOTE: The laboratory director or designee may override rejection of partial or complete runs. Justification for the override must be documented in detail.

6.5 Documentation

- QC tolerance limits are programmed on the instrument; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Lead Technologist or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance

- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- QC is submitted to Sysmex for peer group comparison as it is run
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Sysmex XN Series 1000/3000

7.2 Equipment

Refrigerator

7.3 Supplies

Pipettes
12 x 75mm disposable culture tubes
Glass Micro cups

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	QC Run
1.	Verify the indicator LED light is solid green (not flashing)
2.	Press the mode switch, the tube holder slides out forward
3.	Select the **Change Analysis Mode** and choose **Body Fluid**, the instrument will automatically perform a background check. Wait until the background check is completely finished before moving on to the next step. **Note:** When the instrument is in the Body Fluid mode, background checks are done before and after each sample. Operator does not need to manually initiate a background check. The instrument automatically verifies background checks and if these are not acceptable it will repeat the background check until it passes.
4.	Select **Manual Analysis** button
5.	Click the **Read ID** box
6.	Ensure the **Cap Open** box is not checked. Run QC with the cap on. Only check this box if QC gets down to 1 mL in the vial, at that time remove the cap on the vial.
7.	Mix the QC vial by inverting it 4 times and then place the vial in the tube holder on the instrument.
8.	Press the **Start** switch on the analyzer

8.2	Test Run
1.	Verify the indicator LED light is solid green (not flashing)
2.	Press the mode switch, the tube holder slides out forward
3.	Select the **Change Analysis Mode** and choose **Body Fluid**, the instrument will automatically perform a background check. Wait until the background check is completely finished before moving on to the next step. **Note:** When the instrument is in the Body Fluid mode, background checks are done before and after each sample. Operator does not need to manually initiate a background check. The instrument automatically verifies background checks and if these are not acceptable it will repeat the background check until it passes.
4.	Select the **Manual Analysis** button
5.	Click the **Read ID** box, and scan the patient barcode so that the accession number populates. If the sample does NOT have a bar code, then click **Query to Host** and manually type the accession number into Sample ID field.
6.	Choose (click) the **Cap Open** box

8.2	Test Run
7.	• Label a 12 x 75 mm tube with the patient label and confirm ID by matching to the original sample. • Mix the sample in its original container and then pipette 0.5 mL into the labeled 12 x 75 mm tube. • Place the 12 x 75 mm tube in the tube holder on the instrument. **Note**: For small volume samples, label a micro cup with an LIS small label (foot) and pipette 200 µL into it. Place the cup on the instrument.
8.	Press the **Start** switch on the analyzer

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

All calculations will be performed by Data Innovations (DI).

TC-BF# count from the Sysmex is reported as (number) x 10^3 cells/µL.
Count must be converted to cells/µL (DI will multiply Sysmex result by 1,000).

Examples:

a. TC-BF# count (Sysmex) = 20.5 x 10^3 cells/µL
20.5 x 1000 = 20,500 cells/µL

b. TC-BF# count (Sysmex) = 0.5 x 10^3 cells/µL
0.5 x 1000 = 500 cells/µL

RBC-BF# count from the Sysmex is reported as (number) x 10^6 cells/µL.
Count must be converted to cells/µL (DI will multiply Sysmex result by 1,000,000).

Example:

a. RBC-BF# count (Sysmex) = 0.004 x 10^6 cells/µL
0.004 x 1000,000 = 4,000 cells/µL

WBC-BF# count from the Sysmex is reported as (number) x 10^3 cells/µL.
Count must be converted to cells/µL (DI will multiply Sysmex result by 1,000).

Examples:

a. WBC-BF# count (Sysmex) = 20.5 x 10^3 cells/µL
20.5 x 1000 = 20,500 cells/µL

b. WBC-BF# count (Sysmex) = 0.5 x 10^3 cells/µL
0.5 x 1000 = 500 cells/µL

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

TC-BF (total nucleated cell count, body fluid) is the total cell count in a body fluid; this parameter includes WBCs and high-fluorescing non-WBCs. TC-BF value has taken the WBC count and added in the unknown larger cells that Sysmex has seen in the sample. The instrument may see some high fluorescent cells that it cannot identify; they are NOT WBCs but typically are malignant cells, tumor cells, mesothelial cells, and etc.; those large unknown cells that are seen in body fluids. The instrument provides the known WBC count (the true white blood cells) and then adds in any of these unknown cells to calculate the Total Nucleated cell count (TC-BF).
If the TC-BF count and the WBC-BF count are significantly different it will alert the physician that there is some other type of cells present in the body fluid besides just white blood cells.

When the difference between TC-BF and WBC-BF exceeds the TEa (see table below), a manual differential count will be required. TEa will be calculated by DI.

Fluid	TEa
Pericardial	20 %
Peritoneal	20 %
Pleural	20 %

WBC-BF (white blood cell count, body fluid) this parameter includes WBCs.

RBC-BF (red blood cell count, body fluid) this parameter includes RBCs.

PMN is the polymorphonuclear (Granulocytes: Neutrophil, Eosinophil, and Basophil) cell count in a body fluid. These cells are consistent with acute inflammatory conditions. PMN is reported as both whole number and percent.

MN is the mononuclear (cells with a single granulated cytoplasm: Lymphocyte and Monocyte) cell count in a body fluid. These cells are consistent with chronic inflammatory response. MN is reported as both whole number and percent.

10.2 Rounding

Any result rounding is performed at the interface level.

10.3 Units of Measure

Cells	Result from Sysmex	Final result in LIS
TC – BF#	10^3/ µL	cells / µL
WBC – BF#	10^3/ µL	cells / µL
RBC – BF#	10^6/ µL	cells / µL
MN #	10^3/ µL	cells / µL
MN %	%	%
PMN #	10^3/ µL	cells / µL
PMN %	%	%

10.4 Analytical Measurement Range (AMR)

Parameter	Sysmex XN Series	LIS Range
TC – BF#	0.003 – 10.000 x 10^3/µL	3 – 10,000 cells/µL
WBC – BF#	0.003 – 10.000 x 10^3/µL	3 – 10,000 cells/µL
RBC – BF#	0.002 – 5.000 x 10^6/µL	2,000 – 5,000,000 cells/µL
MN #	0.003 – 10.000 x 10^3/µL	3 – 10,000 cells/µL
PMN #	0.003 – 10.000 x 10^3/µL	3 – 10,000 cells/µL
MN %	0.0 – 100.0 %	0 – 100 %
PMN %	0.0 – 100.0 %	0 – 100 %

10.5 Review Patient Data

- Review patient results for unusual patterns, trends or distribution.
- Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.

10.6 Repeat Criteria and Resulting

Parameters to be reported are listed in section 10.3.

IF the TC-BF# or WBC-BF# result is…		THEN…
From Sysmex	From DI/LIS	
<0.003 x 10^3/µL	<3 cells/µL	Report the result as <3 cells/µL
>10.000 x 10^3/µL	>10,000 cells/µL	Make a 1:10 dilution with Cellpack DCL to obtain a number within the reportable range and then multiply the result by the dilution factor (10). If the result remains >10.000 x 10^3/µL after dilution, report as >10,000 cells/µL Dilution factor is entered in DI.

IF the RBC-BF# result is …		THEN…
From Sysmex	**From DI/LIS**	
<0.002 x 10^6/µL	<2,000 cells/µL	Report the result as <2,000 cells/µL
>5.000 x 10^6/µL	>5,000,000 cells/µL	Report as >5,000,000 cells/µL

IF the MN# and/or PMN# result is …		THEN…
From Sysmex	**From DI/LIS**	
<0.003 x 10^3/µL	<3 cells/µL	Report the result as <3 cells/µL

Manual Differential:
A manual differential must be performed if difference between TC-BF and WBC-BF exceeds the TEa of 20%. Refer to the procedure *Body and Synovial Fluid Analysis, Manual Method* for detailed instructions.

11. EXPECTED VALUES

11.1 Reference Ranges

Parameter / Units of Measurement	Reference Range
Color	Straw
Appearance	Clear
Cell counts and differential (TC-BF#, WBC-BF#, RBC-BF#, MN #, PMN #, MN %, PMN %)	Not established

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Serous fluid analysis is ordered by physicians to diagnose infections, hemorrhages, malignancies and other disorders. Cell count determination is part of the analysis.

13. PROCEDURE NOTES

- **FDA Status:** Approved/Cleared
- **Validated Test Modifications:** None

This section explains the Interpretive Program (IP) message generated by the Sysmex XN analyzer and the corrective action.

13.1	WBC Abn Scattergram
Cause: Clustering in the WDF scattergrams is abnormal; meaning analyzer cannot separate the cell population with confidence.	
Corrective Action: 1. If dashes or asterisk appear in place of data: a. Repeat the sample b. If dashes or asterisk still remain, perform a manual differential and cell count	

14. LIMITATIONS OF METHOD

14.2 Precision

Precision is assessed by analysis of body fluid. The data appears consistent and all parameters have a low CV%.

14.3 Interfering Substances

None

14.4 Clinical Sensitivity/Specificity/Predictive Values

None

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

- Safety Data Sheets
- Sysmex XN Reference Manual
- Quality Control Program policy
- Quest Diagnostics Records Management Program
- Laboratory Safety Manual
- Data Innovations Instrument Manager; Laboratory Policy

- Body and Synovial Fluid Analysis, Manual Method; Hematology procedure
- Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls

17. REFERENCES

1. Quest Diagnostics Best Practice Sysmex XN Series Operation for CBC SOP, revised 04/03/2017
2. Quest Diagnostics Best Practice Sysmex XN Series Operation for Automated Nucleated Cell Counts in Body Fluid, revised 12/2017
3. Sysmex Hematology Analyzer XN Series Instruction for use, revised 07/2015
4. Sysmex XN – 3000 Automated Hematology System Quick Guide, revised 01/2013
5. Sysmex XN Check BF Quality Control Package Insert, revised 10/2016

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

Addendum	Title
A	DI (Data Innovations) Actions

Addendum A

DI (Data Innovations) Information and Actions

A. Instrument and DI/SQ Body Fluid Test Code Translation

Description	Instrument Code	DI/SQ Codes
		Body Fluid Tube
Color	N/A	FLCOL
Appearance	N/A	FLAPP
White Blood Cells	WBC-BF	FLWBC
Red Blood Cells	RBC-BF	FLRBC
Mononuclear Cells Absolute	MN#	FLMN2
Mononuclear Cells Absolute	MN%	FLMNP
Polymorphonuclear Cells Percent	PMN#	FLPMN
Polymorphonuclear Cells Percent	PMN%	FLPMNP
Total Nucleated Cell Count Absolute	TC-BF#	FLTC
Cells, Other	N/A	FCOMM

B. Available Cell Counters

Body Fluid Cell Counters
SGMC Fluid Cell Counter
WAH Fluid Cell Counter
GEC Fluid Cell Counter

C. To adjust the diluted result by the dilution factor:

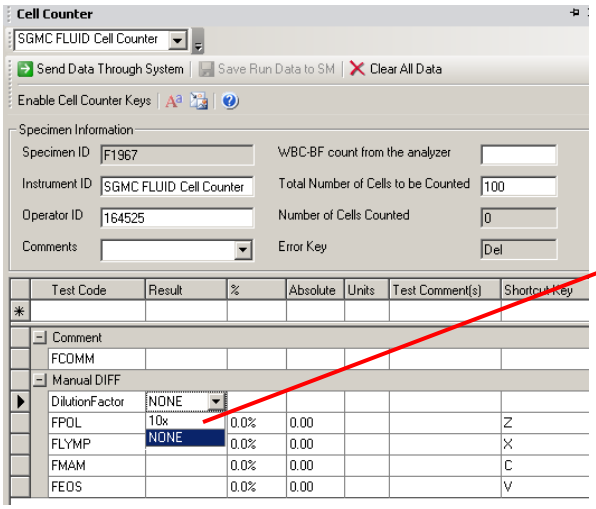
1. Access the Body Fluid Cell Counter and select the dilution factor used from the drop down menu.
    a. If the specimen is diluted at 1:10 dilution, select “10x”.
    b. If the specimen is not diluted, select “None.”
    c. If there is no dilution factor selected, DI will display “Dilution Factor Required”
2. Perform the differential count if needed.
3. Send the data through the System.
4. The adjusted results will display on the Run Worksheet as a new run. The color and appearance will need to be resulted. DI will add an error code of “Check Dilution” and “Dilution Factor Applied” to the WBC.

Examples:

Diluted results before the dilution factor is applied

Test Name	Test St...	Result (5)	Units (5)	Reference ...
Body Fluid				
FTYP23	Held fo...			
FLAPP	Held fo...			
FLCOL	Held fo...			
FLTC	Held fo...	2863	cells/uL	None -
FLWBC	Held fo...	2818	cells/uL	0 - 5
FLRBC	Held fo...	10000	cells/uL	None -
FLMNP	Held fo...	53.1	cells/uL	60 - 100
FLPMN	Held fo...	1322	cells/uL	0 - 0.06
FLPMNP	Held fo...	46.9	cells/uL	0 - 6
FLMN2	Held fo...	1496	cells/uL	0.6 - 1.0

Diluted result with the dilution factor applied

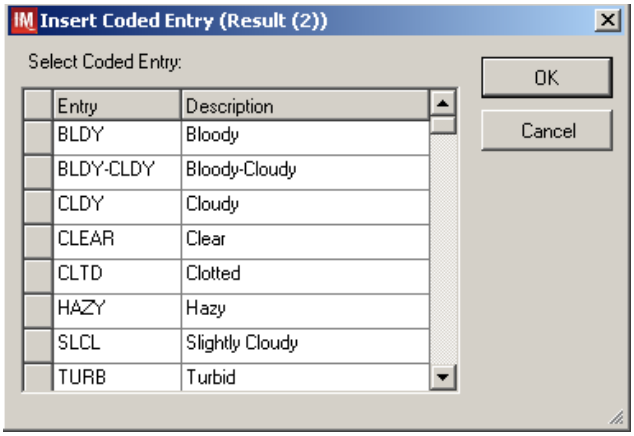


Test Name	Test St...	Result (6)	Error Code(s) (6)	Error Name(s) (6)
Body Fluid				
FTYP23	Held fo...			
FLAPP	Held fo...			
FLCOL	Held fo...			
FLTC	Held fo...	28630	Check Dilution	Dilution Factor Applied
FLWBC	Held fo...	28180	Check Dilution	Dilution Factor Applied
FLRBC	Held fo...	100000	Check Dilution	Dilution Factor Applied
FLMNP	Held fo...	53.1		
FLPMN	Held fo...	13220	Check Dilution	Dilution Factor Applied
FLPMNP	Held fo...	46.9		
FLMN2	Held fo...	14960	Check Dilution	Dilution Factor Applied
Manual DIFF				
DilutionFactor	Held fo...	10x		
FCOMM	Held fo...	SCYT		
FEOS%	Held fo...	1		
FLYMP%	Held fo...	34		
FMAM%	Held fo...	55		
FPOL%	Held fo...	10		

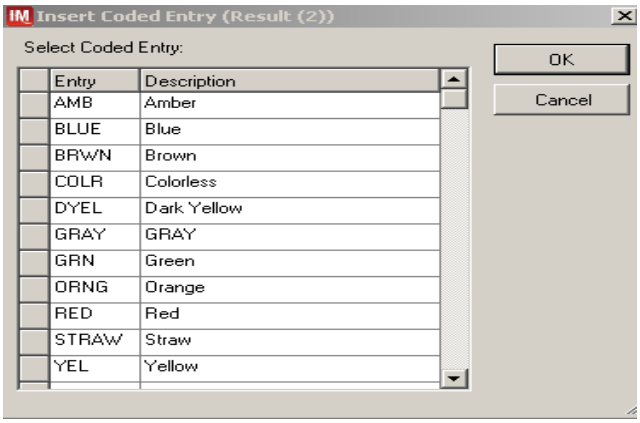
D. Resulting Fluid Type, Appearance and Color

1. Fluid type is reported in Sunquest. **It is not available for viewing in DI.**

2. Select **FLAPP** and right click. Select the **Insert Coded Entry**.
3. Select the appropriate appearance and press **OK**.

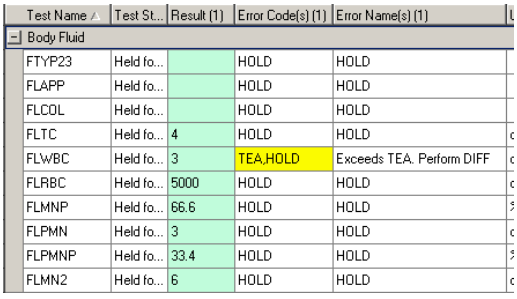


4. Select **FLCOL** and right click. Select the **Insert Coded Entry**.
5. Select the appropriate appearance and press **OK**.



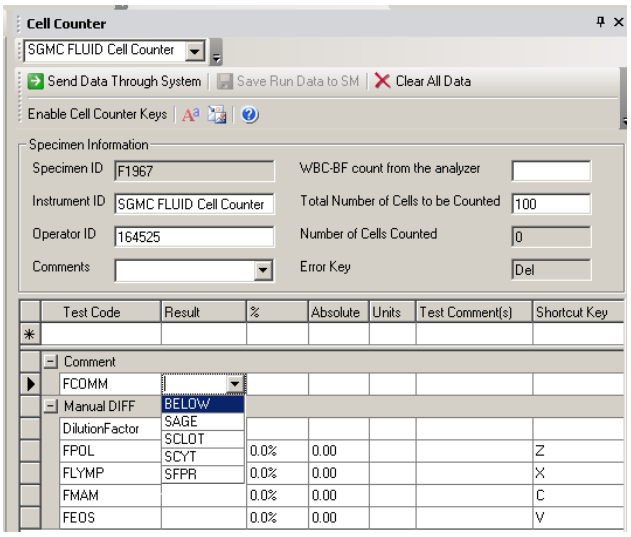
E. TEa and Manual Differential

DI will display an error of “Exceeds TEa, Perform DIFF” whenever the difference between TC-BF and WBC-BF exceeds the TEa. A manual differential count is required whenever error code “TEA HOLD” is seen.



Test Name	Test St...	Result (1)	Error Code(s) (1)	Error Name(s) (1)
Body Fluid				
FTYP23	Held fo...		HOLD	HOLD
FLAPP	Held fo...		HOLD	HOLD
FLCOL	Held fo...		HOLD	HOLD
FLTC	Held fo...	4	HOLD	HOLD
FLWBC	Held fo...	3	TEA,HOLD	Exceeds TEA. Perform DIFF
FLRBC	Held fo...	5000	HOLD	HOLD
FLMNP	Held fo...	66.6	HOLD	HOLD
FLPMN	Held fo...	3	HOLD	HOLD
FLPMNP	Held fo...	33.4	HOLD	HOLD
FLMN2	Held fo...	6	HOLD	HOLD

F. Body Fluid Cell Counter



The following coded entries are available for FCOMM (Comment) field:

Code	Interpretation
SAGE	Counts may not be accurate due to specimen age
SFPR	Submitted for Path Review
SCLOT	Specimen contains clots, counts may not be accurate
SCYT	See Cytology Report

For use of Cell Counter, refer to DI SOP.

G. Order of Release

Body Fluid Count and diff reporting may consist of three (3) groups in DI. They are Body Fluid (includes automated diff), Comment and Manual Diff. Below is the order in which they need to be released in DI to ensure proper filing into Sunquest.

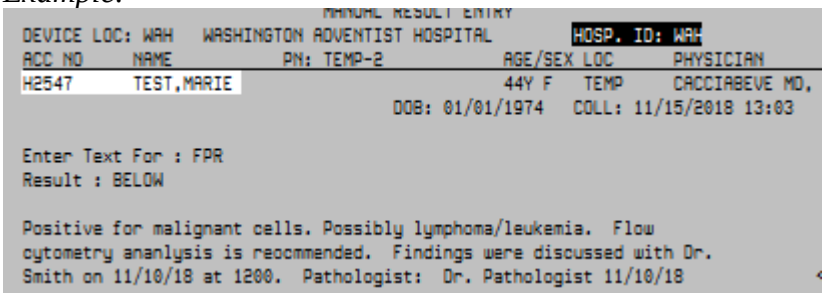
- Release the Body Fluid group
- Release the Comment group
- Release the Manual Diff group

H. Pathologist Review Process

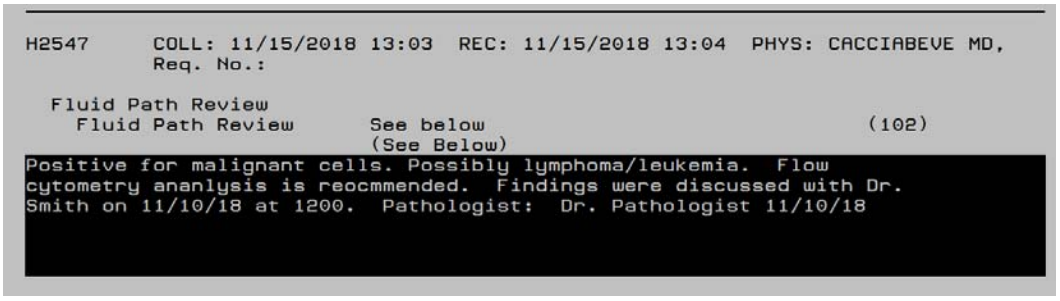
1. To submit slides for path review -
   - Add order code FPATH to the Accession via REI or GUI Order Entry.
   - Complete Pathologist Slide Review Request form.
   - Give slide(s) and review form to the pathologist.

2. 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. The original reported diff does NOT need to be corrected.

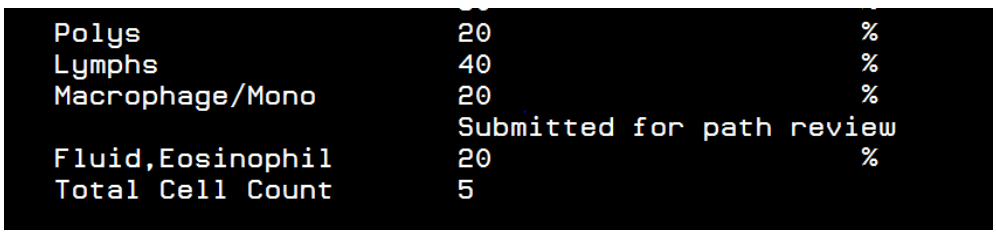
   When resulting, at the Result: prompt type in **BELOW#** then press ENTER.
   Example:



   Example of display in Sunquest Inquiry for Fluid Path Review



   Example of display in Sunquest Inquiry for manual diff with comment attached denoting sent for path review



Technical SOP

Title	**Body and Synovial Fluid Analysis, Manual Method**
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**

Form revised 7/01/01

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	Hemacytometer, Microscope	FLCNT for all Body fluids EXCEPT Synovial (see below)
Cell Count and Diff, Synovial Fluid to include Crystal exam (SGMC & WAH perform counts, crystals are SGMC only)		SFCC

Note: Refer to CSF specific procedures for that specimen type

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 RBC and 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 (syringe) and then aliquoted as needed. **Process for Synovial Fluid specimens at Germantown Emergency Department ONLY:** **Germantown:** 1. Record Total Volume onto original specimen label and lavender top tube and then aliquot specimen into appropriate containers: • 3mL into Lavender Top (EDTA) for cell count • 1mL into plastic vial unpreserved for crystal analysis • 1mL into sterile container for culture and gram stain • 2mL into plastic aliquot tube to be sent to Chantilly (by core lab processors) for chemistry analysis. 2. Inspect the sample prior to sending to SGMC for testing. If solid clots are found, notify the caregivers of the extent of testing that can be performed on the sample. 3. Track specimen to SGMC using the template GLAB and send to SGMC via STAT courier. 4. KEEP some of the original sample at GEC.
Other	Not applicable

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	*Site specified on collection* 3 mL fluid in EDTA for Count, Diff 1 mL fluid (unpreserved) for Crystal
-Other Acceptable	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
Unacceptable Specimens & Actions to Take	**Due to the nature of these specimens, do not reject unless frozen.** **Clotted specimens:** Perform counts and append the code SCLOT (*Specimen contains clots, counts may not be accurate*). **Solid Clot:** Transfer surrounding fluid to another tube (see section 8.2) but avoid transferring clot. Testing may be run on Sysmex or manually depending on sample volume. Add free text comment to results: "Solid clot noted" **Specimens received after 48 hours:** Perform counts and append the code SAGE (*Counts may not be accurate due to the age of the specimen*). **If the specimen is received frozen:** Cancel the test with the reason code SFRZ (*Specimen unsuitable for assay; received frozen*). Notify the attending nurse or physician. Note: In Cerner reason for cancellation will be "improper collection".
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
0.005% Methylene Blue Diluting Fluid	Chantilly reagent room

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

Reagent	**0.005% Methylene Blue Diluting Fluid. Obtain when needed from the reagent room in Chantilly.**
Container	Brown Glass Bottle
Storage	15-30°C
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 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 and crystal exam
  L1-CC perform a cytospin differential and a crystal exam
  L2 perform cell count only
  Note: crystal exam only performed at SGMC
- **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 daily for contamination and documented. Refer to section 8.3

6.4 Tolerance Limits and Criteria for Acceptable QC

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) **Crystal exam (SGMC only)**
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 Quality Control Program for more details.
- Upon weekly and monthly review of QC, if the results exceed allowable ranges, then verify investigation and corrective actions were documented.

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.

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

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

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

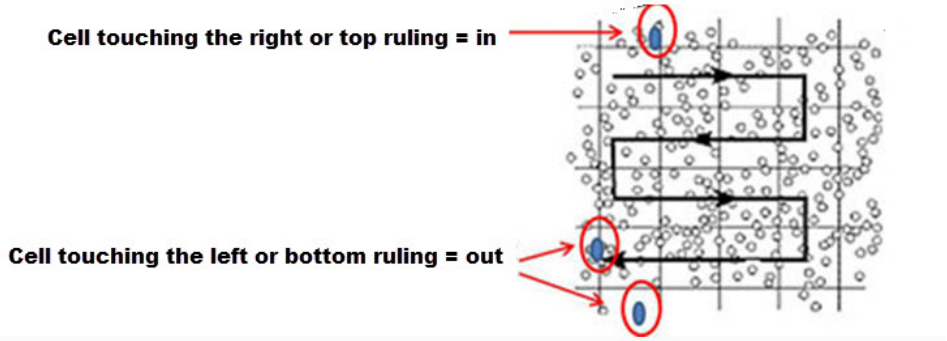
IF	THEN	IF	THEN
Bloody	BLDY	Clotted	CLTD
Bloody, cloudy	BLDY-CLDY	Hazy	HAZY
Clear	CLEAR	Turbid	TUR
Cloudy	CLDY	Slightly Cloudy	SLCL

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, 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 sample injection area. The chamber will fill by capillary action if the hemacytometer is clean.

Form revised 7/01/01

Step	1:2 Dilution
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. • Move the hemacytometer in a zigzag pattern as show below. For cells that overlap the outside lines, count it as "in" if it overlaps the top or right line, and "out" if it overlaps the bottom or left  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	Other Dilutions
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. Following the chart below, add specified amount of body fluid to specified amount of Methylene Blue Diluting Fluid. Mix dilution well. Let sit 10-15 minutes.

Dilution	Body Fluid volume	Methylene Blue fluid volume	Dilution Factor
1:10	100µL	900µL	10
1:20	50µL	950µL	20
1:50	20µL	980µL	50
1:100	10µL	990µL	100

Step	Other Dilutions
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 2-3 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.	
	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. Place one (1) drop of albumin into the Cytospin funnel and then add 3-5 drops of the diluted sample.	
3.	**Centrifuge Sample**: See procedure Aerospray Hematology Slide Stainer Cytocentrifuge (SGMC/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 (SGMC only)

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.
2.	Refer to the appropriate addenda for polarizer instructions based on your site.
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).
4.	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.
5.	With the small handle (red compensator) to the left of the front slot opening, thus separating the light according to components of slow and fast vibration, the crystal can be identified. With the above setting, the direction of vibration is the slower component. 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 crystal turns yellow and the negatively birefringent crystal turns blue. **Monosodium Urates exhibit a Negative birefringence** with the red compensator; **Calcium Pyrophosphates exhibit a Positive birefringence** with the red compensator.
6.	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.
7.	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.
8.	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:

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

Form revised 7/01/01

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
RBC	Cells/µl
WBC	Cells/µl
Differential Counts	%

10.4 Clinical Reportable Range

None defined

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 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 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 that need 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.

Resulting:

Fluid Type is entered during the accessioning process.

Cell Counts, Color and Appearance -

Manually enter into SQ using the appropriate worksheet (SGMC is SHE, WAH is WHE, GEC is GHE).

"HIDE" should be typed for "Fluid Total Cells" when count is performed manually.

```
Fluid Count
  Fluid Type            Pleural Fluid
  Fluid Apperance       SlCloudy
  Fluid Color           Yellow
  Fluid Total Cells     <do not report>      cells/mcL
  FLUID WBC             15000                cells/mcL
  FLUID RBC             100                  /uL
```

Differential -

Refer to the addendum *Fluid Keyboard: Accessing Differential Result Entry for Body Fluid* for details to result via the SQ keyboard and documentation of pathology review.

Note: Manual differentials must be performed when TEa failures on Sysmex (difference between TC-BF and WBC-BF) exceeds the TEa of 20%.

10.7 Crystal Resulting

Report the presence or absence of crystals seen under high power using these codes:

LIS Code	Translation
CAPYCS	Calcium Pyrophosphate crystals seen
MURACS	Monosodium Urate crystals seen
CHOLCS	Cholesterol crystals seen
NONES	None seen

11. EXPECTED VALUES

11.1 Reference Ranges

Pericardial, Peritoneal, Pleural Fluid

Parameter / Units of Measurement	Reference Range
Color	Straw
Appearance	Clear
Red Blood Cells / µl	Not established
White Blood Cells / µl	Not established
Differential / %	Not established

Synovial Fluid

Parameter / Units of Measurement	Reference Range
Color	Straw
Appearance	Clear
Red Blood Cells / µl	Not Established
White Blood Cells / µl	10 - 200
Neutrophils / %	15 - 45
Lymphocytes / %	40 - 80
Monocyte/Macrophage / %	15 - 45
Eosinophils / %	Not Established
Crystal	None Seen

11.2 Critical Values

None established

11.3 Standard Required Messages

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 bacterial 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

Disease Groups	Joint Disorders
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

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 / WAH 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 and Cell Chex Differential Control Log (AG.F87)
7. Pathologist Slide Review Request (AG.F127)

17. REFERENCES

Body Fluid Analysis procedure, Hematology BPT, QDHE749 v1.2
Synovial Fluid Analysis procedure, Hematology BPT, QDHE748 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
2	6/17/14	1, 8.6	Specify synovial fluid testing sites	L Barrett	R SanLuis
2	6/17/14	3.1	Add instruction for sending synovial fluid from GEC to SGAH	L Barrett	R SanLuis
3	11/16/14	8.3	Remove coverslip, add zigzag counting, reformat to add dilution chart	L Barrett	R SanLuis
3	11/16/14	8.6	Add polarizing light instruction	L Barrett	R SanLuis
3	11/16/14	10.5	Remove synovial fluid under GEC instruction	L Barrett	R SanLuis
3	11/16/14	10.6	Add LIS codes	L Barrett	R SanLuis
3	11/16/14	17	Add BPT synovial fluid SOP	L Barrett	R SanLuis
3	11/16/14	19	Add polarizer information and crystal descriptions	L Barrett	R SanLuis

Version	Date	Section	Reason	Reviser	Approval
4	3/7/17	Header	Add other sites	L Barrett	R SanLuis
4	3/7/17	3.2	Edit comments for samples with clots	L Barrett	R SanLuis
4	3/7/17	4, 6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
4	3/7/17	8.4	Specify albumin added before diluted sample	L Barrett	R SanLuis
4	3/7/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
4	3/7/17	10.7	Remove reporting intra or extracellular	L Barrett	R SanLuis
4	3/7/17	11.1	Add ranges for synovial fluid diff	L Barrett	R SanLuis
4	3/7/17	15	Update to new standard wording	L Barrett	R SanLuis
4	3/7/17	16	Add Fluid Keyboard SOP and Path Review form	L Barrett	R SanLuis
5	12/4/18	Header	Update title to include method	L Barrett	R SanLuis
5	12/4/18	1	Update order code, delete 'fluid, other', change crystal exam to SG only	L Barrett	R SanLuis
5	12/4/18	3.2	Add comment codes & instruction for solid clot	L Barrett	R SanLuis
5	12/4/18	4	Update automated stain and Diff-Quik info	L Barrett	R SanLuis
5	12/4/18	6	Update product numbers & storage temp	L Barrett	R SanLuis
5	12/4/18	6.4	Update QC review to match practice	L Barrett	R SanLuis
5	12/4/18	8.1	Remove pale yellow as color choice	L Barrett	R SanLuis
5	12/4/18	10.6	Add reporting section	L Barrett	R SanLuis
5	12/4/18	11.1	Update serous fluid to match automated method, Separated chart for synovial fluid & changed TNC to WBC	L Barrett	R SanLuis
5	12/4/18	19	Add keyboard instructions, delete WAH polarizer	L Barrett	R SanLuis

19. ADDENDA

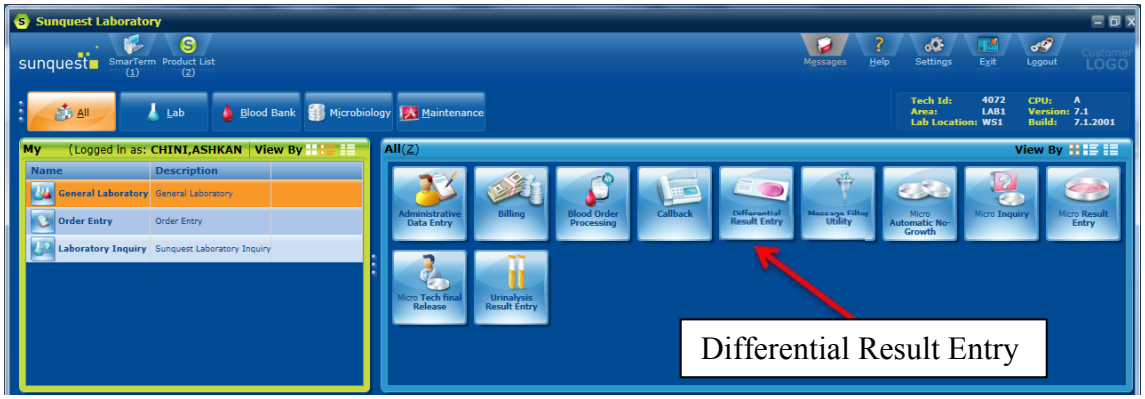
A. Fluid Keyboard: Accessing Differential Result Entry for Body Fluid
B. Polarizing Attachment Instructions for SGMC
C. Crystals in Synovial Fluid

Addenda A

Fluid Keyboard: Accessing Differential Result Entry for Body Fluid

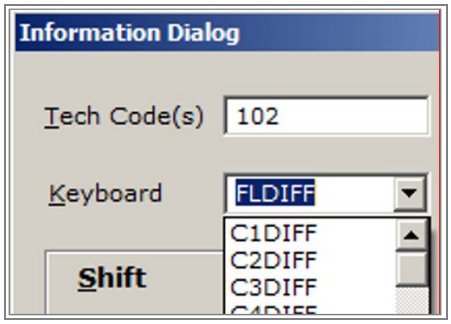
1. Log into the Sunquest GUI application.

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



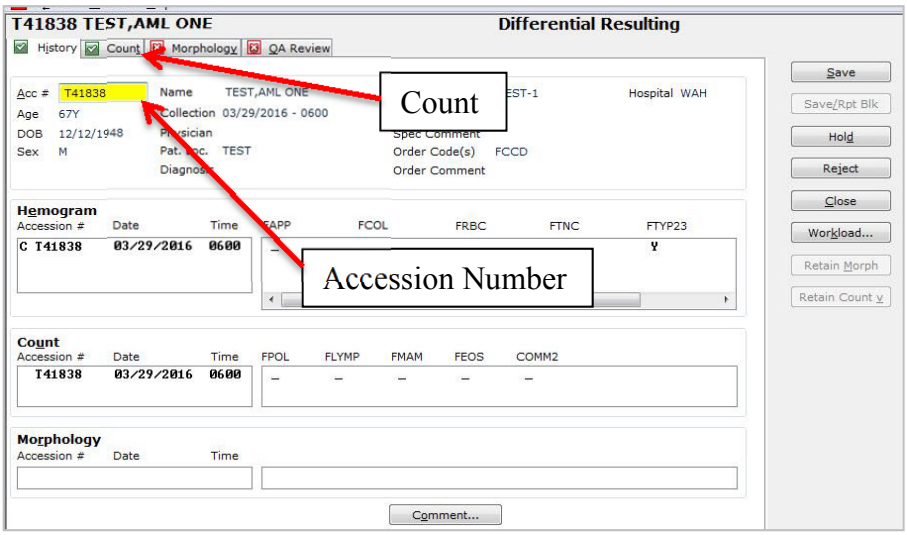
3. Under "Information Dialog" screen, click on the down arrow and select the type of fluid.

   Note: Select the FLDIFF keyboard.

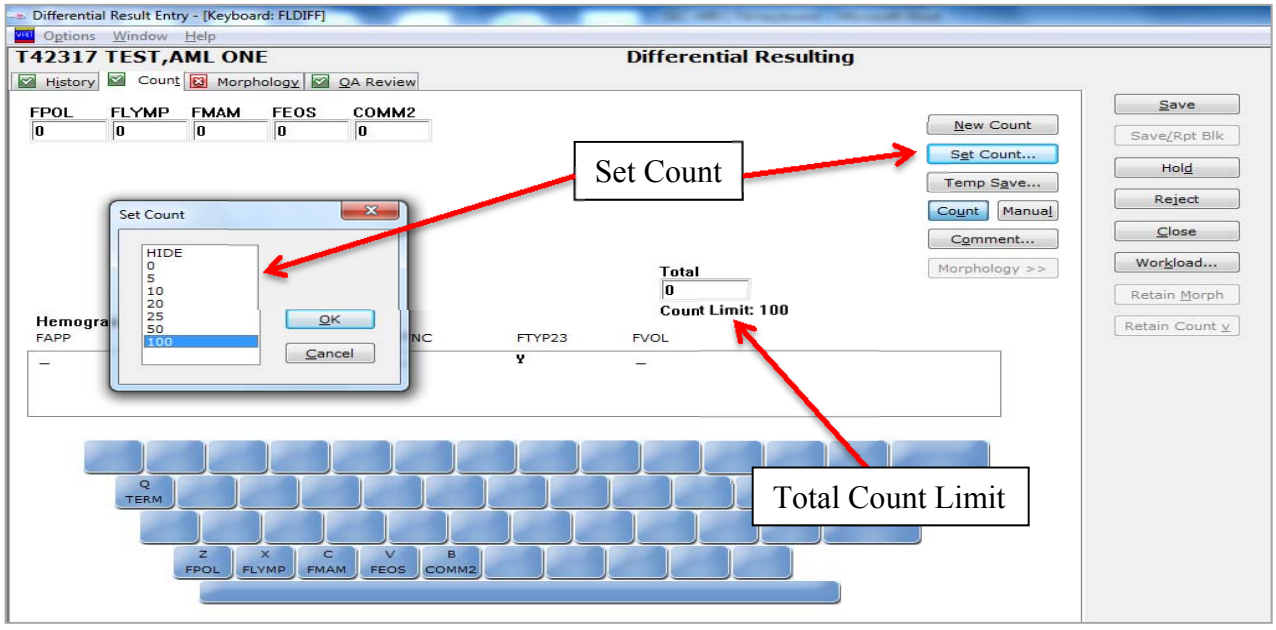


Form revised 7/01/01

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**.

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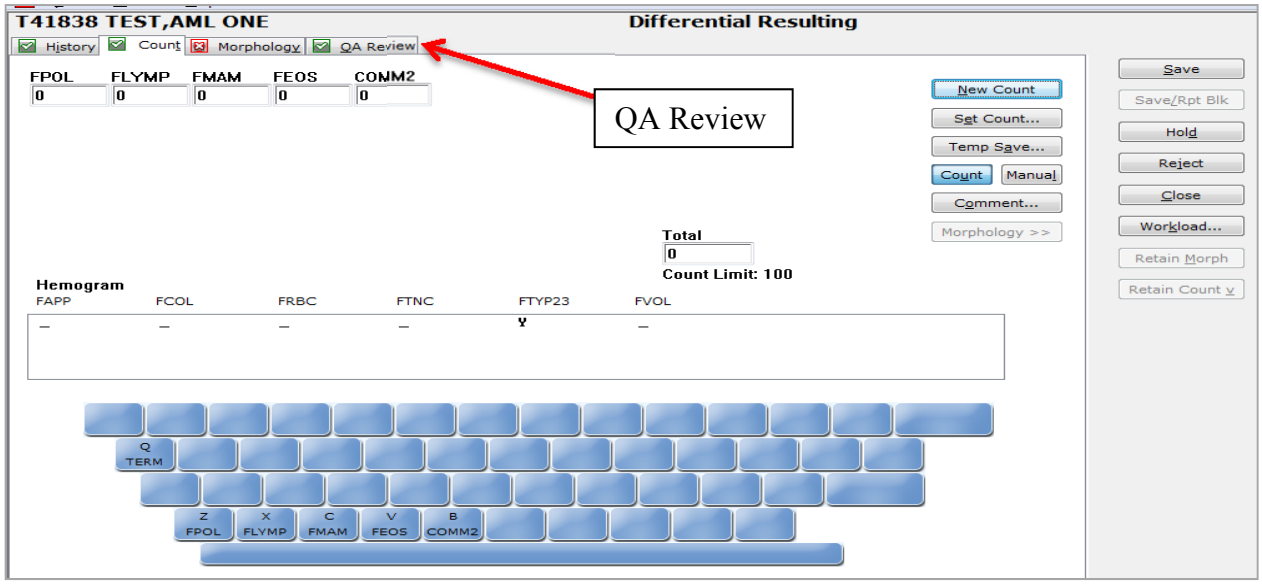

7. Review the QA report, then click **SAVE**.

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.

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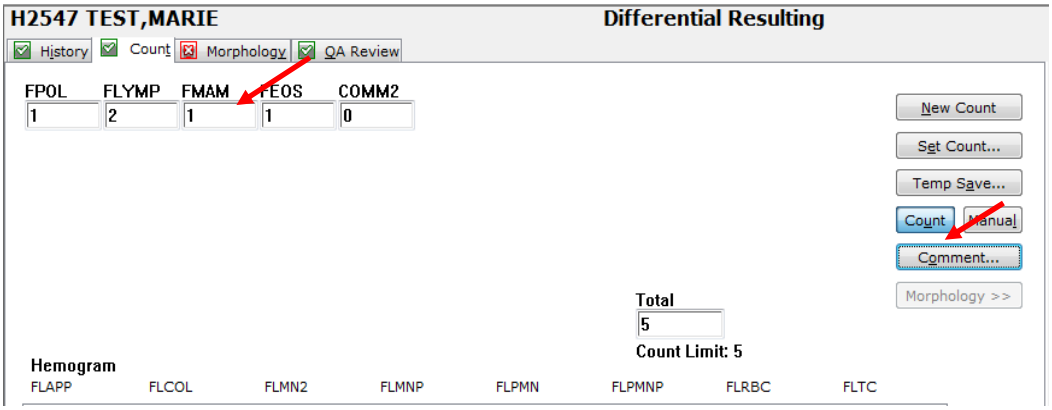
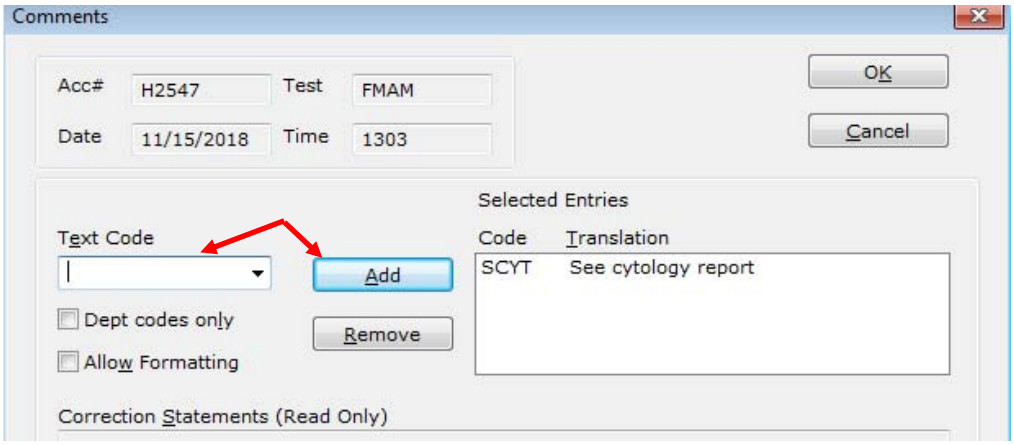
9. If the slide requires a pathologist review, then check to see if there is a Cytology order.
   a. If there is a cytology order
      - The slide does NOT need to be submitted to pathology for review.
      - Append English Text code **SCYT** (translates to See Cytology Report) to one of your cells 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:
      Click in the FMAM count box and then click on the **Comment** button.



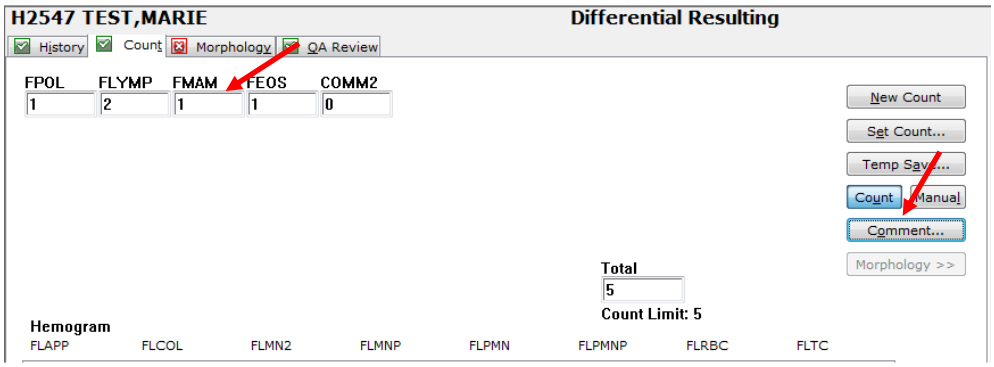
Another window opens. In the Text Code box type in **SCTY** and then click **ADD**. Text Code is translated in the box to the right. Click **OK** to save.



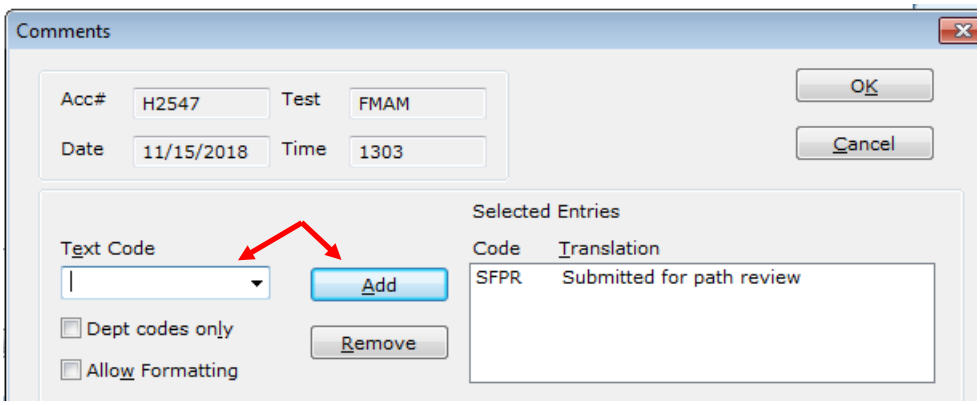
Note: The comment will append to the cell type you selected and can be seen in the QA Review tab.

b. If there is no Cytology order -
   - The slide will need to be submitted to pathology for review. The next steps describe how to document sending it for review, sections 9.c and 9.d outline how to order Path Review and 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.

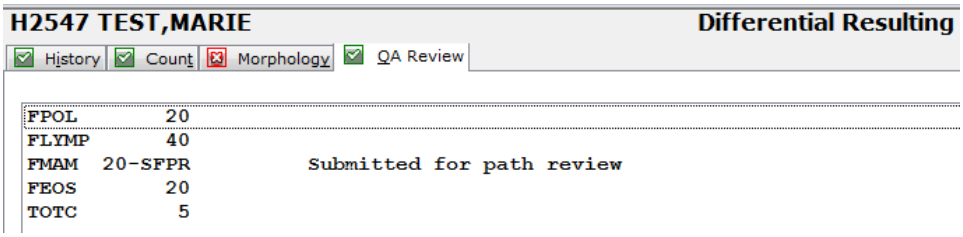
   Example:
   Click in the FMAM count box and then click on the **Comment** button.



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:*



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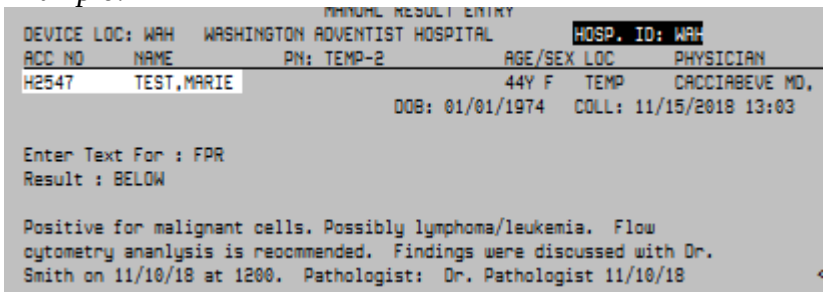
c. To submit slides for path review -
   - Add order code FPATH to the Accession via REI or GUI Order Entry.
   - Complete Pathologist Slide Review Request form.
   - Give slide(s) and review form to the pathologist.

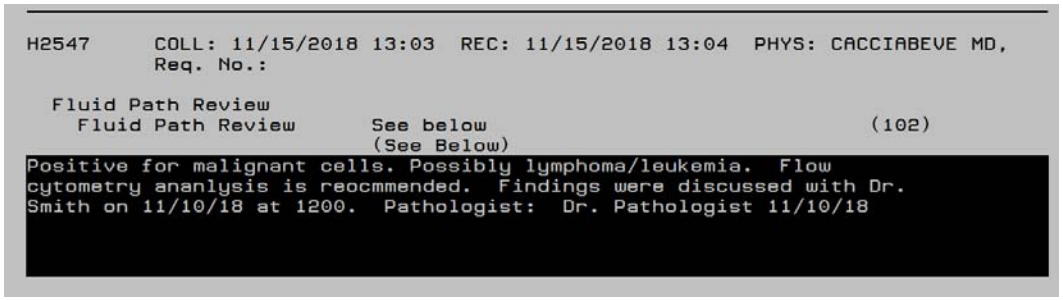
d. When the Pathologist Slide Review form and slide(s) are returned to the lab, enter results into the LIS via SmartTerm. Note: This should also include the pathologist's comments or assessment regarding the diff count which has already been reported in SmartTerm. The original reported diff does NOT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

   When resulting, at the Result: prompt type in **BELOW#** then press ENTER.

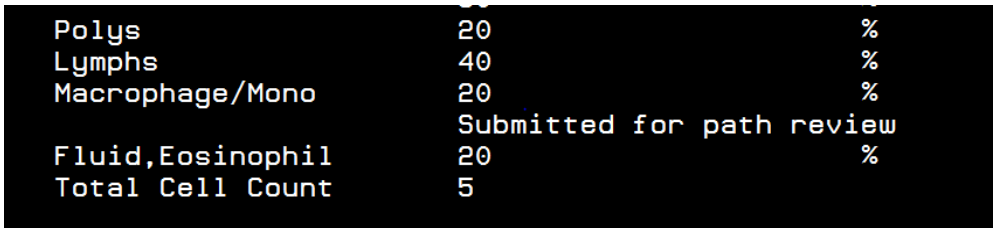
   Example:



Example of display in Sunquest Inquiry for Fluid Path Review



Example of display in Sunquest Inquiry for manual diff with comment attached showing sent for path review



Addendum B **Polarizing Attachment Instructions for SGMC**

M328J/E 04.NF-I

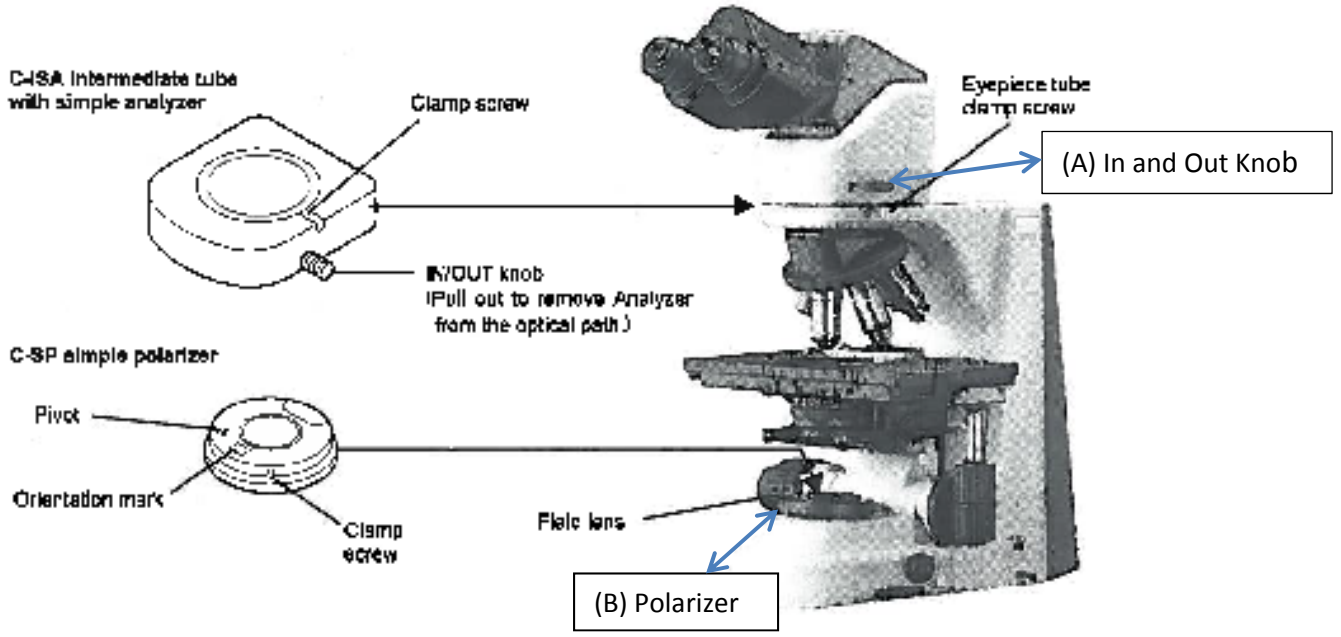
Nikon

ECLIPSE i Series Simple Polarizing Attachment Instructions

Thank you for purchasing the Nikon product.

This manual is written for the users of the Nikon Simple Polarizing Attachment for ECLIPSE i series. To ensure correct usage read this manual together with the instruction manual supplied with the microscope.

When retardation measurement or precise polarizing microscopy is necessary, use the polarizing microscope specifically designed for that purpose.



To view crystals:

1. Push in knob as shown above in picture (A)
2. Slide the polarizer on the field lens (B)
3. Slide the silver tab on polarizer from Z' to Z'

Form revised 7/01/01

Addendum C

Crystals in Synovial Fluid

Types of Crystals Reported by Adventist Hospital Labs

CRYSTAL	SHAPE	BIREFRINGENCE	COMMENTS
Monosodium Urate	Needle, rod-like with parallel straight edges. Usually 8-10µ long	Strong (Neg)	Gout, intracellular crystals in acute attack
Calcium Pyrophosphate	Often rhomboid, may be rod-like, diamond or square. Usually <10µ long	Weak (Pos)	Pseudogout or articular chondrocalcinosis, intracellular in acute attack
Cholesterol	Flat, plate-like, with notch in corner. Often >100µ long. Occasionally needle-like	Strong (needles are positive)	Never phagocytosed. Present in chronic effusions, particularly rheumatoid arthritis.