TRAINING UPDATE

Lab Location:	SGMC, WAH & GEC	**Date Distributed:**	3/21/2017
Department:	Core Lab	**Due Date:**	4/11/2017
		Implementation:	**4/11/2017**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Body and Synovial Fluid Analysis SGAH.H09 v5

Note: this has been converted to a system SOP

Description of change(s):

Section	Reason
3.2	Edit comments for samples with clots
4,6	Remove individual section labeling instructions and add general one
8.4	Specify albumin added before diluted sample
10.5	Move patient review from section 6
10.7	Remove reporting intra or extracellular *(make SOP match practice)*
11.1	Add ranges for synovial fluid diff
15	Update to new standard wording
16	Add Fluid Keyboard SOP and Path Review form

This revised SOP will be implemented on April 11, 2017

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

Technical SOP

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

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

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

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1. TEST INFORMATION

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

Note: For CSF, refer to procedure 'Cell Count and Differential, CSF'

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

Department
Hematology

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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

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Criteria		
-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	Since body fluid specimens are more difficult to obtain and are frequently "one time only" samples, the criteria for an acceptable sample should not be as rigid as those for peripheral blood. Samples with small clots should not be rejected, but the presence of clot should be noted.	
	Condition	**Then**
	Small clots	Perform test, append results with free text comment: *Small clots noted* ~~*Cell count may be inaccurate due to presence of clots in sample*~~
	Solid clot	In the presence of a solid clot a cell count cannot be performed. A slide can be made on the surrounding fluid and an examination should be made for cellular content. (ie many RBC, few WBC noted). Add a free text comment to results: *Solid clot noted*
	Age of specimen (>48 hrs)	Since each specimen deteriorates at unpredictable ranges, aged specimens are to be tested and evaluated for significant deterioration of TNC. Append results with free text comment: *Count performed on specimen #-days old, appearance of cells may be affected.* Only results deemed valid will be reported. Unacceptable results cannot be reported and the test should be cancelled. Result the test as: *Unsuitable due to the age of the specimen. Test has been cancelled.* Perform CRW to credit the test. Notify a caregiver
Compromising Physical Characteristics	None defined	

Criteria	
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	Wescor, SS-035A
Thiazin	Wescor, SS035/049B
Eosin	Wescor, SS-035C
Methanol	Wescor, SS-MEOH
Aerofix	Wescor, SS-148
0.9% Saline	Thermo 0.9% Saline cat # 23535435
22% Albumin (Obtain from Blood Bank)	Immucor CE 0088
Diff Quick Stain Pak (GEC only)	Siemens
0.005% Methylene Blue Diluting Fluid	Chantilly reagent room

4.2 Reagent Preparation and Storage

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

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

Reagent	**Wescor Aerospray Eosin**
Container	Plastic Bottle
Storage	Room temperature

Stability	Manufacturer's expiration date
Preparation	Ready to use

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

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

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

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

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

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

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

6.2 Control Preparation and Storage

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

6.3 Frequency

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

6.4 Tolerance Limits 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.

- If both QC values are within 2 SD, patient results may be released.
- If a control value is >2SD, repeat the control before running patient samples. If the repeat control is within 2SD, patient samples may be run.
- If the repeat of the control value is still >2SD, further investigation is required before running patient samples.

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

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

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

e) **Review of QC**
- Refer to SOP 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

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

7.3 Supplies

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

8. PROCEDURE

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

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

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

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

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

8.3 Concentration

Step	Specimen Preparation
1.	Place a drop of 0.005% Methylene Blue diluting fluid on a slide and coverslip. Examine under 100X for contamination with artifacts, crystals or bacteria, replace fluid if necessary. Record the examination on the Cell Count Worksheet. If the diluting fluid is acceptable to, proceed to specimen dilution.

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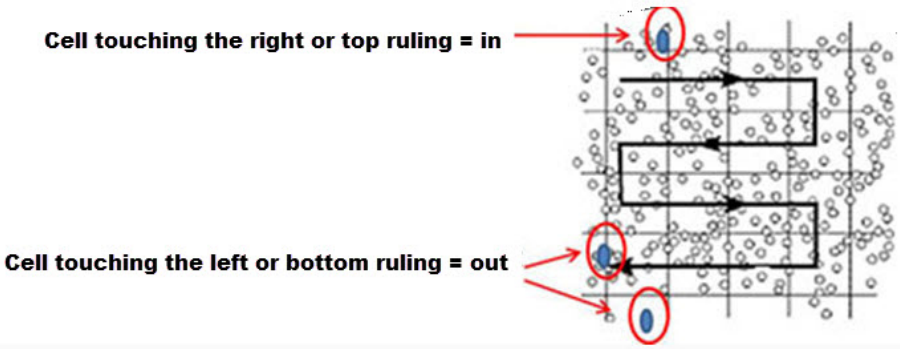
Step	Specimen Preparation
2.	Inspect specimen to determine the appropriate dilution. a. All specimens will be diluted with 0.005% Methylene Blue Diluting fluid. b. The minimum dilution is 1:2. This will ensure distinction between RBC and TNC. Red Cells will not pick up the methylene blue stain and will 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.
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 

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Step	1:2 Dilution
	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.
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

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

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 ~~3-5~~ drops of fluid plus 1 drop of albumin into a disposable cytofunnel and place into the Cytospin centrifuge. The albumin is used to make the cells adhere to the slide better before the staining procedure. Synovial fluids do not require albumin added.	
	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)

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Step	Cytospin	
		Mix dilution well. Place one (1) drop of albumin into the Cytospin funnel and then add 3-5 drops of the diluted sample ~~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/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 and WAH 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

Step	Crystal Examination
	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:

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

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

All results are rounded to whole numbers.

10.3 Units of Measure

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

10.4 Clinical Reportable Range

None defined

10.5 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%.

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.

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

10.7 Crystal Resulting

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

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

Parameter/Units of Measurement	Reference Range
Color	Pleural Fluid – Pale Yellow Peritoneal Fluid – Pale Yellow/Straw Pericardial Fluid – Pale Yellow/Straw Synovial Fluid – Pale Yellow/Straw
Appearance	Clear
Red Blood Cells / µl	Not Established

Parameter/Units of Measurement	Reference Range	
	Body Fluid	Synovial Fluid
Total Nucleated Cells / µl	Not Established	10 - 200
Neutrophils / %	Not Established	15 - 45
Lymphocytes / %	Not Established	40 - 80
Monocyte/Macrophage / %	Not Established	15 - 45
Eosinophils / %	Not Established	
Crystal (synovial fluid only)	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
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

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% 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, Wescor Aerospray® Model 7151, SGMC / WAH Hematology SOP
3. Cytospin CSF/Body Fluid Slide Preparation, GEC Hematology SOP
4. Diff Quick Stain Kit, Hematology SOP
5. Fluid Keyboard: Accessing Differential Result Entry on Fluids, Hematology SOP
6. Cell Count Worksheet (AG.F12)
7. Cell Chex Control and Cell Chex Differential Control Log (AG.F87)
8. 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
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

19. ADDENDA

A. Polarizer Reference Manual for WAH

B. Polarizing Attachment Instructions for SGMC
C. Crystals in Synovial Fluid

Addendum A **Polarizer Reference Manual for WAH**

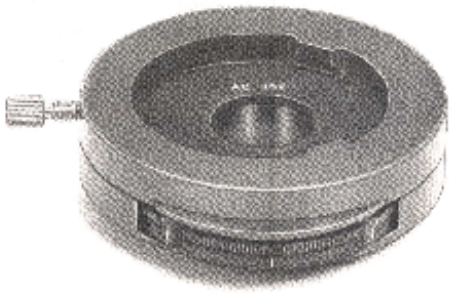
REFERENCE MANUAL

1152 AND 1991 ANALYZER TURRET And 1153 POLARIZER/FULL WAVE PLATE

Installation And Operation On Series Ten, One-Ten, One Twenty and Four-Ten MICROSTAR® Microscopes, DIASTAR SERIES Microscopes

I. INTRODUCTION

The addition of an 1152 or 1991 Analyzer Turret and 1153 Polarizer/Full Wave Plate to your 110, 120, 410 or DIASTAR microscope enables quick yet accurate differentiation of Gout/Pseudo Gout. Components and installation are slightly different on the Series 10 Microscope. Procedure using the 110/120/410/ DIASTAR is described first; a section covering the Series 10 follows.



No. 1152 ANALYZER

II. INSTALLATION

Installation of the 1152 or 1991 Analyzer Turret and the 1153 Polarizer/Full Wave Plate is a simple, straightforward operation. Care should be taken, however, to ensure proper alignment of the parts relative to one another and the microscope stage. Filters and accessories such as Vertical Fluorescent Illuminators, Aperture Viewers or Dual Viewing Attachments should be removed from the microscope prior to installing the Analyzer Turret.

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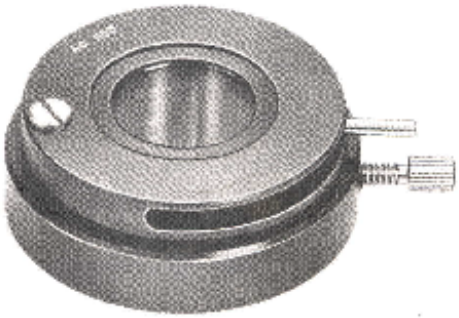

No. 1153 POLARIZER

A. 1152 or 1991 Analyzer Turret

1. Remove Body and place Turret on stand as shown in Figure 1.
2. Orient turret so that Filter Wheel faces front of microscope (Figure 1). Tighten Arm Locking Screw.
3. Mount Body on turret with eyetubes facing front of microscope. Tighten Locking-Screw.

B. 1153 Polarizer/Full Wave Plate

1. Raise Condenser to upper stop position. If auxiliary condenser is attached, rotate it out of light path.
2. Place 1153 Polarizer on Illuminator Window Assembly (Figure 1).
3. Orient analyzer assembly approximately as shown in Figure 2. (Final positioning is described as follows.)

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C. Orienting 1153 Polarizer/Full Wave Plate

1. Rotate Filter Wheel to "1" position.
2. Rotate Full Wave Plate out of light path (dotted line in Figure 2).
3. Turn on illuminator.
4. Field should be nearly (or totally) black.
5. **Rotate 1153 until maximum extinction (blackest field) is achieved. This should require only slight movement in one direction or another. If not, double check original mounting position of 1153 (Figure 2).**
6. Tighten Locking-Screw.

III. OPERATION

A. Brightfield

For brightfield viewing, turn Analyzer Turret to "0" position. Cat. No. 1153 Polarizer can be left in position, but be sure Full Wave Plate is swung out of light path.

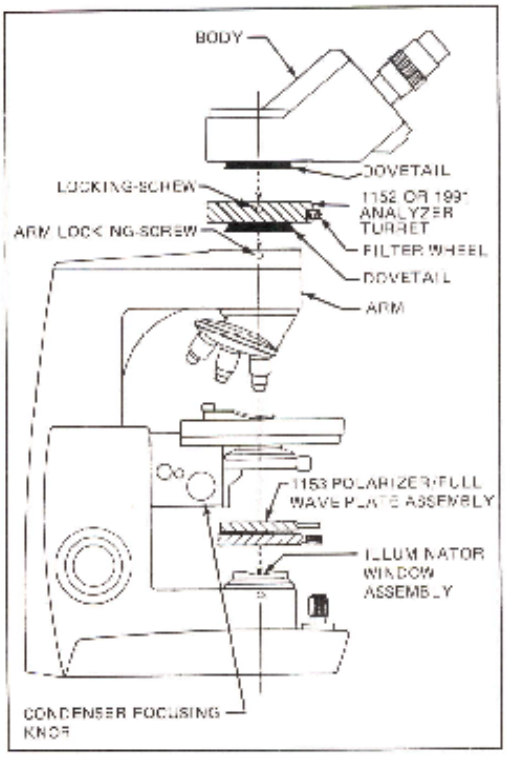


Figure 1.

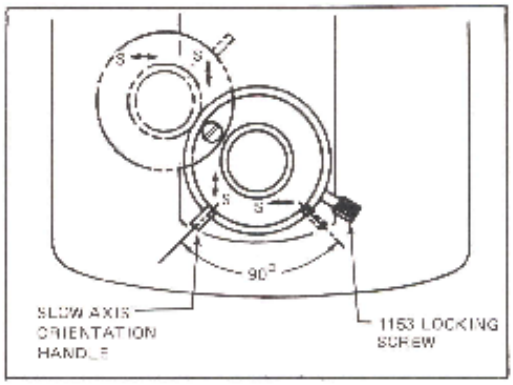


Figure 2.

B. Normal Polarization

Once Polarizer is correctly oriented (Section II, C), the microscope is set up for polarized light. Merely swing Full Wave Plate out of light path and set Analyzer Turret to "1" position.

C. Differentiation of Gout/Pseudo Gout

The following section explains the basic procedure for Gout/Pseudo Gout differentiation. Basically, this test is made possible due to the negative birefringence of urates and positive birefringence of pyrophosphates. If you would like additional information on theory, please consult the reference listed at the bottom of page 3.

Both Gout (Monosodium Urate) and Pseudo Gout (Calcium Pyrophosphate) crystals tend to be needle shaped. However, many crystals may be broken and/or irregular. To do the test, it is necessary to find at least one intact crystal oriented North-South (i.e., vertically) and one East-West (horizontally) in the field of view. Use of 40X objective is recommended.

Following is the procedure for identification of Gout. **To insure the test is being done correctly, a slide of known Monosodium Urate crystals should be used initially.**

1. Swing Full Wave Plate out of light path.
2. Place slide on stage and bring crystals into sharp focus. The needle shaped crystals will appear white regardless of orientation.
3. Swing in Full Wave Plate and put Orientation Handle in extreme left position. Crystals with long dimension in the N-S direction should appear yellow, those E-W blue.
4. Move Orientation Handle to extreme right position. Now N-S crystals should be blue, E-W yellow. (See Figure 3.)

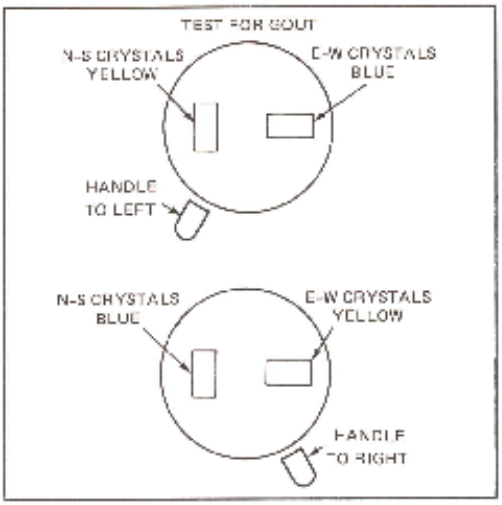


Figure 3.

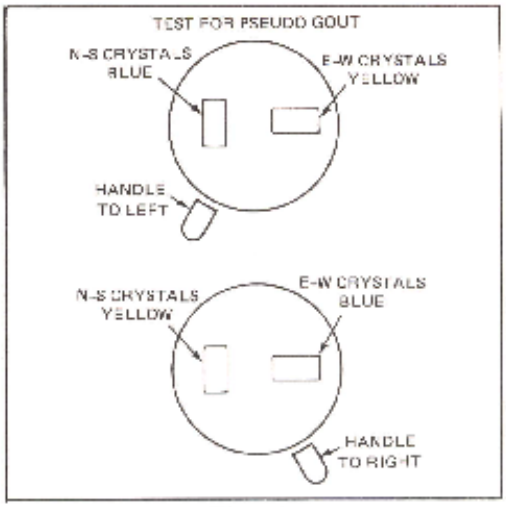


Figure 4.

Be sure to test crystals with Orientation Handle in each position to insure positive identification.

The test for Pseudo Gout is done identically to that described above. However, the color change is opposite that in Gout. That is, with the handle at the left extreme, N-S crystals are blue, E-W yellow, and vice versa with the lever at the right side. (See Figure 4.)

IV. INSTALLATION OF 1114 ANALYZER TURRET AND 1153 POLARIZER/FULL WAVE PLATE WITH ADAPTER ON SERIES 10 MICROSCOPES

A. Installing and Aligning the 1114 Analyzer Turret

1. Position the 1114 Analyzer Turret so that the Filter Wheel faces left or right (90° from front of scope) and lock by tightening screw.

2. Mount the Body on the 1114 Analyzer Turret with the eyepieces facing front and lock it in place by tightening the Locking-Screw.

B. Installing the 1153 Polarizer/Full Wave Plate and Adapter

The 1153 Polarizer/Full Wave Plate and Adapter 1153A are mounted on the Illuminator Window Assembly Mounting Flange as shown in Figure 5. (Orient as per Figure 2.) Rotate 1114 Filter Wheel to "1" position. Proceed with installation instructions Section II, C, Steps 2 6.

C. Follow Section III, on Operation.

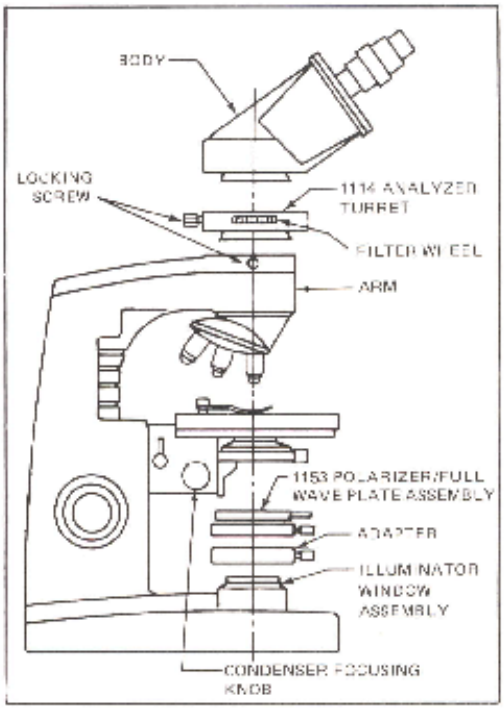


Figure 5.

Reference: P. Phelps, A.D. Steel, and D.J. McCarty, Jr., "Compensated Polarized Light Microscopy: Identification of Crystals in Synovial Fluids from Gout and Pseudogout," JAMA, 203, No. 7 (1968), 166–70.

Addendum B **Polarizing Attachment Instructions for SGMC**

M328J/E 04.9.NF-1

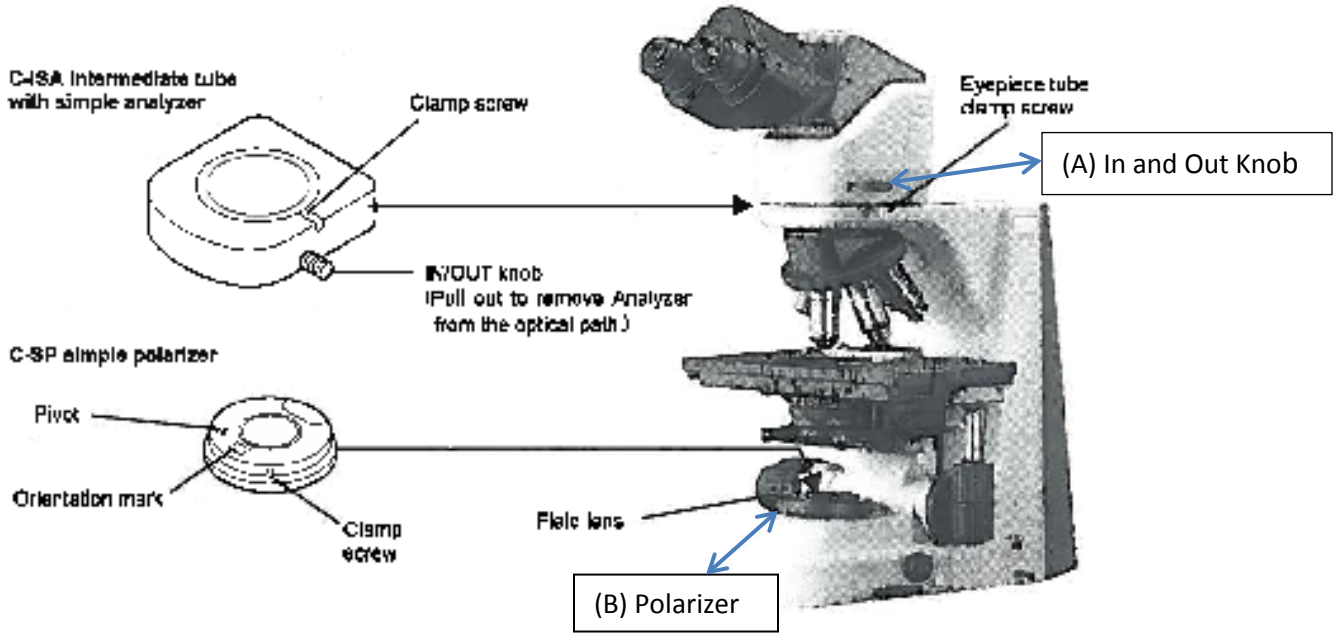
Nikon

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'

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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.