I. PRINCIPLE:

Body fluids other than urine and blood are analyzed for enumeration and differentiation of cells. Cell counts are performed by standard chamber count technique. Hematocrits on grossly bloody fluids are performed following microhematocrit procedure. Differentials are performed on Wright stained cytocentrifuge or pulled smear preparations.

II. SPECIMEN:

Body fluids other than blood and urine are classified under the general heading of Body Fluids. These include cerebrospinal, pleural, peritoneal, pericardial, peritoneal dialysis, peritoneal lavage (BAL), amniotic, synovial fluids, and drainage fluids (usually Jackson Pratt maybe cyst or abscess, etc). Fluids are processed regardless of the presence of clots; it must be noted in the report that clots are present in the specimen and cell count will be compromised. If the fluid is a solid clot, it will not be processed further and the floor notified. The laboratory should check if there are other specimen tubes (shared samples from Chemistry or Microbiology) that would be acceptable for analysis. Cerebrospinal fluid should be processed within eight hours of collection. Other fluids should be processed within twenty-four hours of collection.

III. REQUIRED MATERIALS:

A. Gilson adjustable pipet (0-1000 ul and 20 - 200 ul) and pipet tips
B. 12 x 75 mm test tubes
C. Neubauer counting chambers and coverslips
D. Disposable plastic and glass pipets
E. Disposable Cytocentrifuge chambers, Thermo Fisher Scientific
F. 3 x 1 inch glass slides
G. Sterile saline - Change daily working tube
H. 22% Albumin
I. Cytocentrifuge
J. Hematek 2000 stainer
K. Microscope
L. Streck Cell-Chex levels 1 and 2
M. General lab centrifuge
N. Microhematocrit tubes/Microhematocrit centrifuge

IV. QUALITY CONTROL:

A. Daily in A.M

Note: both saline and albumin must be organism and particle free to be used; if first review is unacceptable (note in log), change reagent and review. If any problems still exist a supervisor must be informed and reagent not used.

1. Saline:

   a. Fill a clean dry tube with saline
   b. Date tube
   c. Remove 1 drop of saline and place on a 3xl-inch glass slide and coverslip.
   d. Review prep under microscope for presence of organisms and particles.
   e. If prep is particle free and organism free, saline may be used; if particles or organisms are seen, discard saline and open new bottle of saline and repeat, review.
   f. Acceptable review is particle and organism free
   g.. Document review in "saline/albumin" log.

2. Albumin:

   a. Remove albumin from refrigerator.
   b. Place 1 drop of albumin on a 3xl-inch glass slide and coverslip.
   c. Review prep under microscope for presence of organisms and particles.
   d. If particles or organisms are seen, discard albumin and open a new bottle and repeat review.
   e. Acceptable review is particle and organism free.
   f. Document review in "saline/albumin" log

3. Streck Cell -Chex fluid controls (Level l and 2) (stored in Fluid refrigerator. Unopened control material stable until outdate. Open vials stable for 30 days. Expiraton dates should be on control vials.

   a. Mix vials thoroughly by rolling between hands 20 - 30 seconds, then rapid inversion to resuspend and then gently invert 8 - 10 times (does not need to come to room temp).
   b. Plate both sides of hemocytometer with level 1 and a second chamber with level 2.

c. Count WBC's and RBC's on both sides of sides of chambers.
d. Average the WBC and RBC counts and multiply by chamber count factor.
e. Order fluid Q.C. in Soft and enter results. If counts are not within posted ranges, replate and repeat counts. If still unacceptable, notify a supervisor immediately.

V. CELL COUNT PROCEDURE:

A. Mix fluid.
B. Observe fluid for presence of clots and abnormal color; note in computer if present.
C. Plate both sides of Neubauer counting chamber and allow cells to settle.
D. Examine chamber under low power to evaluate cellularity and distribution.

E. Method of Counting Fluids:

1. Clear or low cellular fluids: Count undiluted fluid
2. Cellular (counts>400/cumm):
3. Dilute with saline using the following table as a guide.

Dilution	Fluid Volume	Saline Volume
1:2	0.2 ml	0.2 ml
1:5	0.1ml	0.4 ml
1:10	0. lml	0.9 ml
1:20	0.1ml	1.9 ml
1: 100	10 u1	0.99 ml
1:200	10 ul	1.99 ml

F. Alternate Methods for Counting bloody fluids and cellular joint fluids:

1. Glacial Acetic acid(See Appendix E) dilution should be done on bloody Fluid when nucleated count is low. Brown bottle in fluid area 3% glacial acetic acid. 1:20 – 50 ul of fluid diluted with .950 ml of 3% glacial acetic acid in separate tube.

- OR –

2. Dilution using Methylene Blue instead of saline if low volume of fluid or nucleated cell count is low. \
3. Dilution with 1:1 saline-Methylene blue solution can be used for cellular joint fluids. The Wbcs will stain pale blue.

NOTE: Since joint fluids tend to be very viscid, a 1:2 or 1:10 dilution should be made to facilitate counting and preparing the cyto smear.

G. Count and differentiate rbc from nucleated cells on 40X power on both sides of counting chamber and calculate the red and nucleated cell counts using the formula below. Agreement of counts is within or equal to 10%. If discrepant, fluid must be recounted.

H. To determine what area of the counting chamber is counted, the large square on upper left hand corner is evaluated for cellularity.

I. Counting cells:

Cells/ruled chamber (large square)	# Large squares to count
0- 24	9
25_-99	4
100 - 200	1
>200	dilute or ten small center squares

Formula used for undiluted and diluted fluids.

See counting chamber illustration next page

J. Calculating chamber count factor:

1. Depth x dilution x area counted (area corrected to 1 sq mm)

2. Counting chamber calculation factors for undiluted fluid:

# Large squares counted	Factor
9	10/9 x count
4	10/4 x count
1	10/1 x count
10 small squares	25 x count

Examples:
All 9 large squares counted:
9 RBCs x 10/9 = 10 cells/cu mm RBCs
2 nucleated cells x 10/9 = 2 cells/cumm nucleated cells

1:10 dilution with 10 small center squares counted:
95 RBCs x 25x 10 =23,750 cells/cumm RBCs
108 nucleated cells x 25x10 =27,000 cell/cumm Nucleated cells

K. When counting, count cells within grid lines (top side and left side to avoid counting cells twice). Record both counts and calculations per each side on log sheet.

Note: Cells counted x calculation factor x dilution factor= cells/cu mm

VI. SMEAR PREPARATION:

A. Smear preparation

1. Cytocentrifuge Operation and Preparation:

a. Remove centrifuge head and shield of CytoSpin 3 in order to load slides and specimens. Position labeled (accession #) slides into disposable cytofunnel and insert into centrifuge head. Pipette the sample into double-headed cytofunnel.

b. Clear fluids- pipet 0.1ml of fluid in one side of double-headed cytofunnel and 0.2 ml in other side.

c. If tinged with blood or slightly cloudy, cyto smear is made according to the following: pipette 0.1 ml of fluid into labeled tube add 0.5 ml of saline and mix.

d. For CSF, dialysate, and peritoneal fluids only, add 1 drop of 22% albumin to the chambers before adding fluids. Other fluids do not require albumin to "cushion" the cells.

e. Place cover on centrifuge head and install head into centrifuge. Close lid and press START on pad of cytocentrifuge. Spin 10 minutes at speed of 1100 RPM. .

f. When centrifuge stops, remove centrifuge head then fluid slides and mark fluid spot on the reverse side of the slide.

g. Air dry slides and stain with Wright stain.

2. Drop smears must be made if fluid is bloody or cellular or question yeast or bacteria.
   a. Centrifuge specimen at 1200 RPM.
   b. Remove supernatant to clean, labeled tube (use extra printer label and note on tube supernatant)
   c. Resuspend sediment in 1 drop of supernatant.
   d. Gently smear one drop of sediment using a pipette onto a 3 x 1 inch slide.
      Cellular fluid prepare a slide from the sediment as you would make a peripheral smear.
   e. Label slides with accession number
   f. Air dry slide completely and Wright stain.

3. Hematocrits on bloody fluids- follow microhematocrit procedure in Main Lab manual p.H-39

VII. NUCLEATED CELL DIFFERENTIAL:

A. Cells are identified from Wright stained slide only:

Differentiation of nucleated cells is made using the following classification of cells.
Granulocytes
Lymphocytes
Monocytoid (CSF)
Tissue Cells (Body fluids- includes histiocytes and mesothelial cells)
Others - includes eosinophils, basophils, nucleated red cells and blasts.

B. The number of cells counted in the differential is guided by the number of nucleated cells/cumm. The minimum number of cells in the differential should be:

Nucleated cells/cumm	Cells in diff
5	5
10	10
25	25
>25	100

C. All differentials counts are reported as % regardless if less than 100 cells are counted.

VII. PROCEDURAL NOTES:

Process only one type of fluid per patient per 24 hours.
All fluids are checked for microorganisms as part of the analysis.

A. Peritoneal Dialysate Fluids:
1. No differentials are done on peritoneal dialysis fluids with nucleated cell counts <l00/cumm
2. Cytopreps and drop smears should be made and scanned for microoganisms
3. Only 1 dialysate fluid/day (midnight to midnight) will be analyzed.

B. CSF Specimens:
1. Pedi hem/onc slides are checked for bacteria and reviewed for blasts and abnormal cells by competent techs or supervisor. (See policy for techs passing annual fluid competency.)
2. Bloody CSF specimens are always centrifuged to evaluated supernatant for xanthochromia and hemolysis. If either present, enter canned comment "sample hemolyzed" or " sample xanthochromic" on patient specimen in computer.
3. Presence of ghost cells- only count intact rbcs and put comment against rbc count, "cell count maybe inaccurate due to presence of ghost cells."
4. If 2 tubes of CSF received, count lowest number tube first. If cell count within normal limits <5 rbc and <10 nucleated cells), the second tube is not counted. Report count and differential on the first tube, and "Cancel" cell count on the second tube and comment why count cancelled..

C. Ascites, Peritoneal and Dialysates:

1. Absolute granulocyte counts (ANC) must be calculated for ascites fluids.

Formula:

a. Total Wbc(nucleated count) x decimal form of polymorphonuclear cells(segs)= ANC.

Example: Total WBC= 3,344 x .84(84%) granulocytes(segs)= 2880/ul(ANC), this is considered a critical value, > or = 250/ul. When fluid diff is less than 100 cells, % polys is determined by: # of polys in diff divided by total # of cells counted in diff. That number in decimal form times by total Wbc= ANC.

D. BAL (bronchial lavage):

1. Differential for BAL: if > 50% of cells on cyto smear are degenerated , differential is not reported and comment put into computer; " unable to report diff. due to marked degenerated cells".

E. Wound drainage, abscess fluid, cyst fluid or other surgical drainages ex. (Jackson Pratt)

1. Chamber cell counts will not be performed. Should be cancelled with a comment. A spun Hematocrit will be ordered and performed. If fluid is bloody, report %. If fluid is clear, a spun Hct will be reported as < 2%.
2. A pulled smear will be made, stained and examined for significant and/or abnormal findings. Significant findings are rbcs, wbcs, bacteria, yeast and other organisms. If cellular, perform differential. These findings will be commented against the spun Hct. No noted findings: report " No significant or abnormal findings seen on smear" in computer.

F. Joint (Synovial) Fluids:

1. In the cases of markedly bloody joint/synovial fluids, resembling peripheral blood, a spun Hct is performed and the Rbc cell count is cancelled.
2. F4 the Rbc count and comment-"Due to markedly bloody fluid, manual Rbc count is not performed." In addition comment against the "Fluid Type," spun Hct %.
3. Perform wbc count per procedure.

G. All fluid specimens:

a. Bacteria and fungi seen on cyto smear
prepare drop or pull smear and review
check computer to see if new finding.

(1.) New finding:
notify supervisor (or lab resident off shift)
check to see if micro has paired specimen and results
call caregiver and note in computer who and when notified
leave for supervisor/resident director review

(2.) Old finding:
enter comment into computer
do not leave for review

b. Abnormal Cells/ Critical Findings(first time)

any fluid with blasts, malignant or "atypical" cells must be referred to supervisor directly and reviewed by lab resident/director prior to reporting. These abnormal smears are retained in some cases for staff education.

Flint(fluid interpretation) Criteria for Csfs and Body Fluids:
First Time findings:

1. Bacteria, Yeast and other organisms
2. blasts
3. Questionable abnormal/immature/malignant cells
4. Suspicious tissue cell clumps
5. High percentage of signet rings
6. Any questionable significant abnormal findings
7. Questionable bone marrow contamination, especially in pediatric csfs.
8. Reactive lymphs and tissue cells
9. For Csfs= > 100/ul total count and >75% lymphs(both must be present).
10. Cells identified as Others- use fixed comment, " Cell ID referred to physician interpretation." Reporting Others

on subsequent samples-also use fixed comment, " Cell ID referred to physician interpretation" with the addition of the date of the interp.

Note: CSF- Nucleated cell count > 1000/ml must be referred to lab resident.
ANC of > or = to 250/ul in ascites is a critical value- notify caregiver and/or lab resident if multiple times.

c. All Body Fluids (other than CSF):
Differentials are sent out by competent techs or supervisor.(Refer to policy of guidelines to fluid reporting).

d. Fluid Specimen Storage:
1. Specimens and cyto smears are retained for 7 days.
2. Completed fluids forwarded to other labs must be noted in " sign-out" log.
3. All dilution tubes must be discarded after count completed.
4. Label all remaining specimen tubes and identify as supernatant, sediment or original specimen.

IX. NORMAL RANGES:

CSF:
WBC < 6 cells/ul
RBC < 5 cells/ul
Lymphs 0 – 10 (Child)
0 - 6 (Adult)
See table for significance

A. Pleural Fluid:
1. Normal volume: < 20 mI. (Normally not tapable.)
2. Appearance:
a. Normal - clear, pale yellow
b. Chylous - milky, white
c. Inflammatory - cloudy, turbid (increased leukocytes)
3. Differential on nucleated cells may be useful to differentiate between mycobacterium vs bacterial infection.

B. Pericardial Fluid

1. Normal fluid: 10 - 50 ml
2. Appearance: interpretation similar to Pleural Fluid.
3. Differential on nucleated cells:
   a. Over 1000 leukocytes/ul with a predominance of neutrophils suggests bacterial infection.
   b. Viral pericarditis, tuberculosis, and post-myocardial infarction syndrome usually show a predominance of lymphocytes.

C. Peritoneal Fluid:
1. Normal volume: < 50 ml
2. Appearance: Similar to Pleural Fluid. Green, bile-stained fluid is occasionally seen;
   Confirm with spot test for bilirubin (IctoTest).
3. Cell count and differential:
   a. < 300 leukocytes/ul considered normal.
   b. < 25% neutrophils is considered normal.
      (1.) Absolute granulocyte counts over 250/ul correlates strongly with bacterial peritonitis and is a critical value which needs to be communicated to a caregiver.
      (2.) High percentage of lymphocytes may suggest TB.

D. Synovial Fluid:
1. Normal volume: <3.5 ml
2. Appearance: clear, yellow fluid.
3. Cell count and differential:
   a. < 200 leukocytes/ul considered normal.
   b. < 25% neutrophils considered normal.
   c. Crystals: None is normal (See "Examination of Crystals in Synovial Fluid")

E. Amniotic Fluid:
1. Appearance: Colorless to pale straw; clear to slightly cloudy
2. Cell count and differential:
   a. No granulocytes or bacteria should be present.
   b. Squamous cells are considered normal and not counted in the differential.

X. REFERENCES:

1. Kjeldsberg, Carl and Knight, Joseph. Body Fluids. Laboratory Examination of Amniotic. Cerebrospinal. Seminal. Serous and Synovial Fluids. ASCP Press, 3rd edition. 1993.
2. Spriggs and Boddington. The Cytology of Effusions. Grone and Stratton, 1968.
3. Todd, Sanford, Davidsohn. Clinical Diagnosis and Management by Laboratory Methods. Saunders, 1979. Chapter 18, pp. 635 - 679.
4. Romero R, Emamian M, Quintero R, et al." The Value and Limitations of the Gram Stain in the Diagnosis of Intraamniotic Infection." Am J Obstet Gynecol. 1988; 159: 114-119.
5. Cytospin 3 manual

X. HISTORY:

H-1 This procedure was written by N. Ortoli-Drew on 11-1999.
H-2 This procedure was revised by N. Ortoli-Drew on 9-2005.
H-3 This procedure was revised by D. Fico on 9-2007.
H-4 This procedure was revised by D.Fico on 11-2010.
H-5 This procedure was revised by D. Fico on 3-14-2011.
H-6 This procedure was revised by D. Fico on 9/2012.
H-7 This procedure was revised by D.Fico on 3/2013.
H-8 This procedure was revised by D. Fico on 1/2014.

Appendix E

3% Glacial Acetic Acid- Reagent for counting bloody body fluids to lyze rbcs to facilitate enumerating nucleated cells.

Preparation: 15 ml conc. Glacial Acetic Acid(kept in acid cabinet in Spec. Heme) diluted into 485 ml of distilled water.

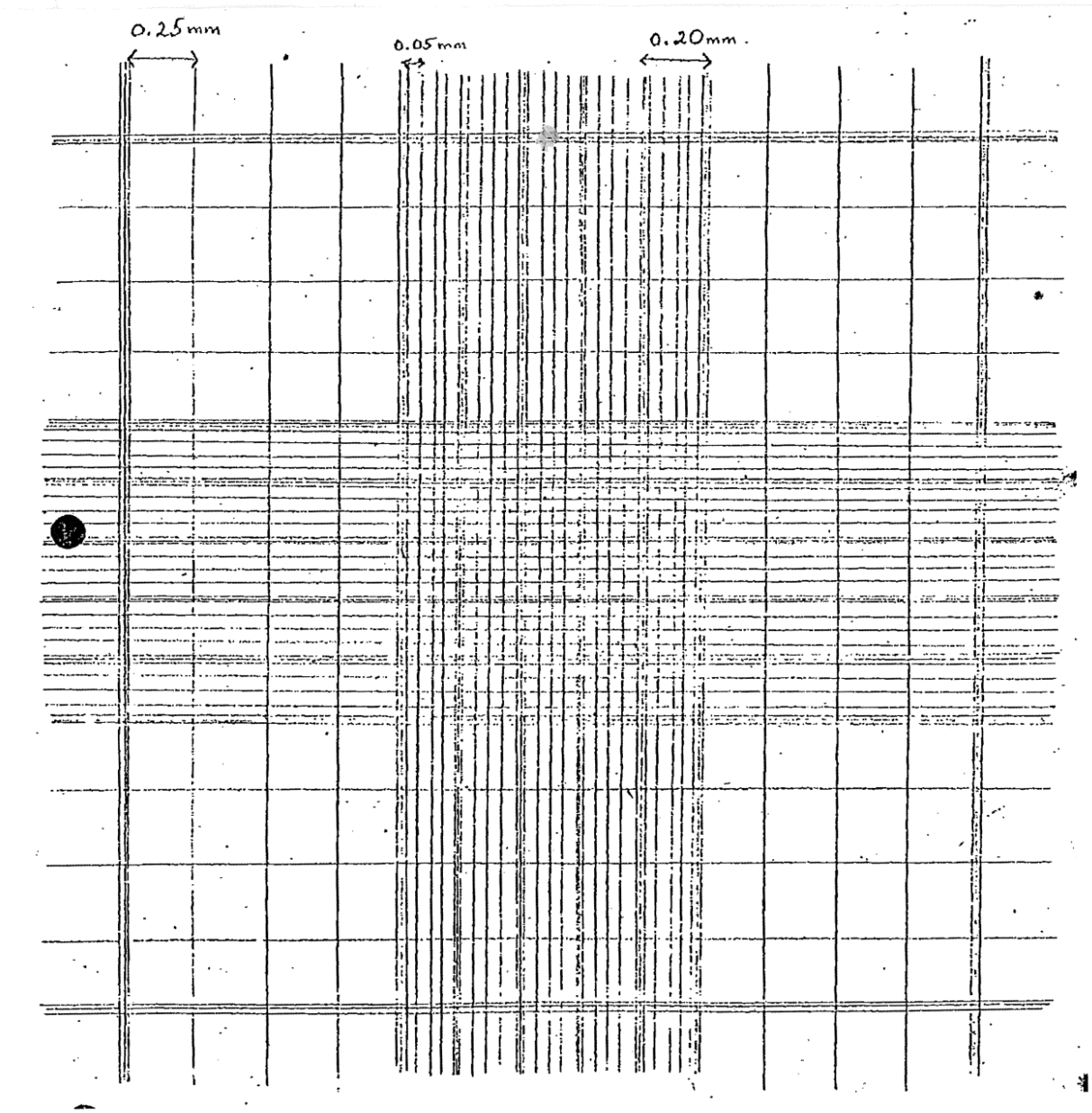
Stability: Indefinitely

Reagent needs to be properly labeled.

Daily Saline Albumin Review Log

Date	Saline	Albumin	Tech	Date	Saline	Albumin	Tech

Refer to Body Fluid Procedure (H-08-003) Section IV Appendix B



1 mm

3 mm x 3 mm Rectangle

Depth: 0.1 mm