Document Title: Manual Body Fluid Analysis (Including CSF) SH.CP.AU.hem.0012.0004

Author (original)	Effective Date	Supersedes Procedure #
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Revised by	Version #	Date Revised	Effective Date
B. Sorensen/M. Johnson	4	8/25/2018	

Approval Signature	Approval Date
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REVISION HISTORY

Procedure #	Revision Date	Reason for Revision
SH.CP.AU.hem.0012.0001	03/09/2011	NEW
SH.CP.AU.hem.0012.0003	02/09/2012	(see previous coversheet)
SH.CP.AU.hem.0012.0004	08/25/2018	Updated format; combined CSF and Body Fluid procedures (SH.CP.AU.hem.0013); update cytocentrifuge smear preparation, dilution tables, hemocytometer diagram and QC information; add amniotic fluid and BAL information;add instructions for backup cytofuge; update reference ranges

Title: Manual Body Fluid Analysis (Including CSF)

I. PURPOSE

The purpose of this document is to provide guidance on how to perform manual body fluid analysis, including cell counts and microscopic examination. This procedure can be used on CSF, as well as other body fluids that are unable to be done on an automated platform.

II. PRINCIPLE

Manual cell counts are performed on either undiluted or diluted body fluids to determine the number of nucleated cells and erythrocytes. Brilliant cresyl blue is added to an aliquot of the sample (prior to counting) to assist in differentiating between nucleated cells and erythrocytes. A hemocytometer is used to perform the manual count. Manual differentials or microscopic review is performed to further classify nucleated cells.

Body fluids can be divided generally into the following categories:

A. Cerebrospinal fluid (CSF)
B. Synovial
C. Serous body fluids
    1. Pleural
    2. Pericardial
    3. Peritoneal (paracentesis, ascites, abdominal)
D. Peritoneal Dialysates and lavage
E. Other (includes bronchial lavage, amniotic, bile, etc.)

III. SCOPE

This procedure will be used by the UR Medicine Labs at Strong Memorial Hospital, Hematology-Chemistry Laboratory staff.

IV. RESPONSIBILITIES

Department and functional responsibilities are defined in the table below:

Group/Person	Responsibility
Quality Assurance	• Supports the development of this document
Medical Director	• Ensures that the procedure is followed • Review and approval of this document
Supervisor	• Ensures that the procedure is followed • Review and approval of this document
End User	• Follows the procedure

V. SPECIMENS

A. Specimen Collection: The provider, per proper collection protocols, collects fluids for analysis.

1. Cerebrospinal Fluid (CSF) Collection

   a. Ideally, the specimen is divided into 3 or 4 samples in sterile plastic tubes, which are labeled sequentially.

   b. Sterile CSF tubes are numbered (on the plastic). Handwritten tube numbers override the preprinted numbers.

      NOTE: Samples numbers (#1 through #4) should match the correct patient Order Number.

   c. Also acceptable: sterile cup or syringe (with no needle attached)

   d. Avoid glass tubes because cell adhesion to the glass affects the cell count and differential.

1 Tube Received	2 Tubes Received	3 Tubes Received	4 Tubes Received
Send tube to Microbiology first – remaining sample should be sent back to Hematology-Chemistry	Tube 1: Cell Count/Differential first; then Glucose/Total Protein	Tube 1: Chem/2nd Cell Count	Tube 1: Cell Count/Differential
	Tube 2: Microbiology	Tube 2: Microbiology	Tube 2: Chemistry
		Tube 3: Cell Count/Differential	Tube 3: Microbiology
			Tube 4: Cell Count/Differential

2. Synovial: For routine examination, the collection syringe should be moistened with an anticoagulant (approximately 25 U of sodium heparin/mL of synovial fluid). Oxalate, powdered ethylene-diamine-tetra-acetic acid (EDTA), and lithium heparin should **NOT BE** used because they can produce crystalline structures resembling monosodium urate crystals. Ideally, when adequate fluid is available, it should be divided into 3 samples:

   a. 5 to 10ml is collected in a sterile tube for microbiologic examination.
   b. 5 ml is collected in an anticoagulant tube (heparin or liquid EDTA) for microscopic analysis.
   c. 3-5 ml is placed in a plain (no anticoagulant) red-top tube for crystal analysis. Normal fluid does not clot.

d. If the synovial fluid is bloody, it should be placed in a sodium heparin or EDTA tube to prevent clotting.

3. Pleural Fluid: A thoracentesis is indicated for any undiagnosed pleural effusion or for therapeutic reasons when the fluid causes dyspnea. The procedure of thoracentesis consists of inserting a needle, under local anesthetic, into the pleural cavity and aspirating the fluid. Specimens for cell count can be transferred to a lavender top tube with liquid EDTA to prevent clotting.

4. Pericardial Fluid: Fluid is obtained either by pericardiotomy following limited thoracotomy or by pericardiocentesis (the sterile aspiration of fluid by a needle). Specimens for cell count can be transferred to a lavender top tube with liquid EDTA to prevent clotting.

5. Peritoneal Fluid: The procedure of removing fluid from the peritoneal cavity is called a paracentesis. The fluid submitted to the laboratory is called paracentesis fluid, peritoneal fluid, or ascites fluid. Specimens for cell count can be transferred to a lavender top tube with liquid EDTA to prevent clotting.

6. Other fluids: amniotic, bile, bronchial alveolar lavage fluid (BAL), abscess

B. Collection Container

Common Types of Fluids	Preferred Specimen Collection Tube
Cerebrospinal fluid (CSF)	Sterile plastic tube(s) or sterile cup or syringe
Synovial fluid (cell count only)	EDTA vacutainer**
Pleural fluid	EDTA vacutainer
Pericardial fluid	EDTA vacutainer
Peritoneal fluid	EDTA vacutainer / Sterile cup

**Red top tubes are ideal for crystal analysis. Refer to the Synovial Fluid Crystal Analysis Procedure SH.CP.AU.hem.0015.

Other acceptable collection tubes include: sterile red top (no additive vacutainer tubes), sterile cups, and needleless syringes.

C. Volume (all fluids):

1. Optimal: 3-5ml

Minimum: 1 ml (suggested)

2. Lab Staff will make an attempt to contact the provider to prioritize tests if an inadequate sample volume to complete all ordered tests is received.

D. Stability:

1. Manual cell counts and smear preparation of body fluids are performed as soon as possible after receipt due to lysis of the cells.
2. CSF: Should be processed within four hours of collection. If > 4 hours old and cell differential is needed, a comment should be added to CCOM2 field:

   @SCDEG: Interpret CSF differential with caution – count may be inaccurate due to age of sample (> 4 hrs)
3. Specimens > 8 hours old from collection time: Footnote the results with a comment saying “Interpret results with caution: specimen greater than 8 hours old when tested in the lab.”

E. Storage

1. After cells counts and differentials/descriptions are completed, fluids are stored by date of collection in the refrigerator (2-8ºC) for 1 week.

VI. SPECIAL SAFETY PRECAUTIONS

A. The Standard Universal Precautions recommended by the Centers of Disease Control should be followed whenever blood or body fluids are handled. These precautions include wearing gloves (and other personal protective equipment, if appropriate).

B. Refer to Safety Data Sheets for safety information concerning laboratory reagents or materials.

C. Quality control materials are for In Vitro diagnostic use only.

D. Creutzfeldt-Jakob Disease (CJD or Mad Cow):

1. For complete information, refer to the Lab Safety General Procedure number (SH.CP.AU.gen.0005) for handling specimens from known or suspected CJD Patients.

   a. CJD is a prion disease, a progressive neurologic disorder with a long incubation period.

   b. CJD is transmissible, fatal, and resistant to usual methods of sterilization and disinfection.

c. Brain, spinal cord, and eye have high infectivity.
d. CSF, kidney, liver, lung, lymph nodes, spleen, and placenta have low infectivity.
e. Blood, other tissue, body fluids, excretions, and secretions have no detectable infectivity for humans.
f. The main precaution is to avoid puncture of the skin.

2. For processing **ALL** CSF samples from known or suspected CJD patient:
   a. Lab coat, gloves, full face protection (shield), and disposable counter cover are required.
   b. Only disposable supplies are used.
   c. Dispose of samples, supplies, and personal protective equipment in orange CJD biohazard bags and sharps containers immediately after processing the sample.
   d. Cleanup of any spills is performed immediately.
3. For **known** CJD or R/O CJD samples:
   a. Double gloves and disposable lab coats are required.

VII. MATERIALS

A. Equipment

1. Microscope: Bright light (10x, 20x, 40x, and 50x and 100x oil immersion)
2. Cytocentrifuge
3. Cell counter

B. Supplies

1. Microhematocrit tubes (plain)
2. Disposable Hemocytometer
3. Certified MLA pipettes (10-1,000µl)
4. Applicator sticks
5. Falcon tubes and caps
6. Kim wipes
7. Gauze
8. Plastic transfer pipettes
9. Microscope slides (75x25x1mm)
10. Single cytology funnel
11. Cytocentrifuge clips
12. Wax pencil
13. Immersion oil

C. Reagents

1. Hyaluronidase – Store in freezer (<0ºC). This is stable for 2 years from the open date of use. It is used to break down hyaluronic acid in synovial fluid.
2. Diluting fluid – Sysmex Cellpack diluent (replaced weekly)
3. Alcohol pads – 70% isopropyl alcohol
4. 22% Bovine Albumin
5. Brilliant Cresyl Blue Reticulocyte Stain
6. Wright's Giemsa Stain
7. 10% bleach

VIII. QUALITY CONTROL

A. Cell-Chex Commercial Controls

Cell-Chex is control material that monitors technologist accuracy in quantitation of red and nucleated cells in patient CSF and other body fluid samples including pleural, pericardial and synovial fluid. One of the three levels of Cell-Chex controls (Level 1-CC, Level 1-UC and Level 2) is counted each shift or every eight hours.

1. Storage and Stability

   a. Cell-Chex is stable through the expiration date when stored at 2º to 10ºC.
   b. After opening, Cell-Chex is stable for 30 days when stored at 2º to 10ºC.

2. Indication of product deterioration

   a. Inability to obtain expected values for total cell counts may indicate product deterioration. If the recovered values are not within the expected ranges:

      1) Review the open and expiration date of the material. If expired, discard material and open a new vial for analysis.
      2) If not expired, but has been repeated many times. Open a new vial and recount. If the values are still outside the reportable range, inform the supervisor or technologist in charge.

B. Cell-Chex Instructions for Use:

1. Remove the controls from the refrigerator. It is not necessary to warm the controls to room temperature before using.

2. To mix: **DO NOT MIX MECHANICALLY**

   a. Hold vial horizontally between the palms of the hands and roll the vial back and forth for 30 seconds.

b. Hold vial on cap end and mix by rapid inversion, **using 20 quick flicks of the wrist**, to ensure the cells are completely suspended.
c. Invert the vials 8-10 times before sampling.

3. Samples must be removed using a clean capillary tube. The vial must be closed immediately after sampling is complete. Care should be taken to prevent cross contamination of the control.

a. For hemocytometer counts and calculations please refer to below procedure.
b. Cell-Chex is also used as a synovial fluid control for crystal analysis.

4. After sampling, wipe the threads of both the vial and cap before replacing and returning to refrigeration for maximum open-vial stability.

5. Enter results in Unity QC.

C. Body Fluid Differential/description: In order to maintain staff competency, body fluid images and test questions will be uploaded to University of Washington website Medtraining.org. Staff will be tested minimally once a year. Unacceptable results will be reviewed with staff, if necessary. If unacceptable results occur again, remedial training may be necessary.

IX. PROCEDURE – (STEP/ACTION)

A. Hemacytometer cell counts

1. Place the fluid tube on a horizontal rocker to ensure proper mixing.

2. Place a patient label on the Fluid Counts log sheet SH.CP.AU.frm.0210.

3. Record the fluid type and tube number, if appropriate, on the Fluid Counts log sheet.
   a. If fluid is a CSF, make sure CSF sample order number matches the correct tube number in Soft.
   b. If a number is handwritten on a CSF label, the handwritten number supersedes the preprinted number.
   c. If a pair of CSFs have the same collection time, contact SMS about changing one of the collection times (this affects how providers see results in eRecord).
4. Macroscopically, examine the fluid for: volume, color, appearance, clots, clumps or debris and record results on the fluid log sheet.

NOTE: If clots, clumps or debris are present see the Limitations section of this procedure.

a. Color is reported as colorless, pink, red, brown, yellow, light brown, light yellow, green, orange or white.
b. Appearance is reported as clear, hazy, cloudy or bloody.

5. Synovial fluids require addition of hyaluronidase prior to analysis:

a. Hyaluronidase (by breaking down hyaluronic acid) changes the viscosity of the sample to allow better analysis.
b. Aliquot off a portion of the synovial fluid, if possible.
c. Dip an applicator stick into the powdered hyaluronidase; transfer the powder to the synovial fluid. Allow the sample to mix for 5 minutes.
d. Additional hyaluronidase may be added to the sample if the viscosity is not changed sufficiently.

6. If the fluid is clear and colorless, prepare an undiluted sample for counting. If a fluid is bloody or cloudy due to high cellularity, a dilution is made prior to staining. Refer to Appendix 1: Preparing Fluid Dilutions.

a. Hold a plain microhematocrit tube almost horizontally. Using capillary action, allow the inside of the microhematocrit tube to coat with brilliant cresyl blue stain. **DO NOT** allow the stain to completely fill the tube (leave the non-filling end clear of stain).
b. Expel the stain from the microhematocrit tube by placing the filling end against a piece of gauze. Capillary action will cause the excess stain to be removed from the microhematocrit tube.
c. Using the unstained end of the microhematocrit tube, fill the microhematocrit tube with the fluid to be counted.
d. Mix the stain and fluid by gentle inversion and rolling.

7. Carefully charge a disposable hemocytometer with stained fluid.

a. Using a small microhematocrit tube, fill the counting area on both sides of the hemocytometer by capillary action.
b. Fill the counting area completely but **DO NOT** allow the counting areas to overflow.

8. Place the hemocytometer in a petri dish containing moistened gauze. Allow the charged hemocytometer to sit for at least 5 minutes. This allows the nucleated cells to be stained and the fluid to settle into one plane.

9. Using the charged hemocytometer and the 20X objective, perform a nucleated cell and red blood cell count.
See Appendix 2: Using a Hemocytometer for additional instruction.

a. Scan the entire hemocytometer chamber to assess the distribution and dilution of the cells. Cells should appear in monolayer. If cells are overlapping, refer to Appendix 1: Preparing Fluid Dilutions.

b. Count at least 100 nucleated and 100 red blood cells on each side of the hemocytometer.

c. An equal counting area is used for each cell type (nucleated cells or red blood cells) on both sides of the hemocytometer. Refer to Appendix 2 of Body Fluid Procedure SH.CP.AU.hem.0012 (Using a Hemocytometer) for diagram.

1) Less than 100 cells of a type (nucleated or RBC) may be counted in 9 hemocytometer squares on a low cellularity fluid that is not diluted prior to analysis.
2) High cellularity fluids may require less than 9 squares to be counted on each side in order to reach at least 100 cells of each type i.e. 1/5 mm^2 or 1/9 mm^2.
3) Different dilutions or different counting areas may be required for each cell type.
4) Lysis of RBCs: If lysis of red blood cells is required to determine either the nucleated cell count or presence of yeast, **DO NOT** add acetic acid (component of Turk's solution) to synovial fluid (doing so will form a mucin clot). Ammonium oxalate may be used to lyse red blood cells in synovial fluids. (see Manual Platelet procedure for Leukocheck reservoir that contains ammonium oxalate)
5) Perform any manual dilutions in duplicate.

10. Record the counting information on the Fluid Counts log sheet.

a. Nucleated cell count from side #1 and #2*
b. Red blood cell count from side #1 and #2*
c. Number of large squares ($1 mm^2$) counted for nucleated cell and red blood cell count.
d. Dilution factor

*Counts from each cell type must agree within 2 standard deviations of each other. Refer to the Calculations section.

11. For details on when and how to report CSF differentials, refer to Section XIII, Result Reporting.

B. Prepare a cytocentrifuge smear with the sample. Refer to Appendix 3: Guidelines for Preparing Cytocentrifuge Smears

1. Label a frosted glass slide with the appropriate patient label.

2. Using a wax pencil, circle the area on the back of the slide where the cytospin hole aligns with the slide. Be careful not to make the circle too small.
3. The labeled slide and the cytology funnel are secured with the two retaining hooks against the cytoclip slide clip.
4. Ensure that the funnel, slide and clip are properly aligned.
5. Remove the sealed head from the cytospin. **Never open/close the sealed head when it is in the instrument.**
6. Open the sealed head and place the assembled cytoclip(s) in the cytospin head making sure that the clips are balanced. The reason that the clip assembly tilts is so that when the sample is placed in the chamber it does not run onto the slide until centrifugation.
7. Place fluid in the appropriate sample chamber that is loaded in the cytospin head. Refer to Appendix 3 for guidelines on the appropriate amount (drops) of fluid to add based on fluid appearance.
8. Add one drop of albumin to the funnel. This helps keep cells intact during centrifugation.
9. Cap the funnel and seal the cytospin head. **Never open/close the sealed head when it is in the instrument.**
10. Place the cytoclip head into the cytocentrifuge.
11. Gently close the cytocentrifuge lid.
12. Press “Start” to cytocentrifuge the sample for 5 minutes at 1200 RPM.
13. Immediately after spinning ceases, remove the sealed head from the centrifuge and the slides from the cytoclip.
14. Remove the cytology funnel from the slide carefully, so that the smear is not disturbed. Air dry the smear(s) for 5 minutes.
15. Stain the smears according to the Midas Stainer procedure (SH.CP.AU.hem.0017).
16. Refer to Shandon Cytospin 4 Operator Guide for additional information.
17. If the Shandon Cytospin 4 cytocentrifuge is not operational, the Cytospin2 Statspin centrifuge should be used. Refer to Appendix 4 for operational information.

NOTE: Occasionally a BALD (Bronchoalveolar Lavage Differential) will be ordered by Cytology. Stain the slide(s) in accordance with step 15 above and follow the process for reporting as outlined in SH.CP.AU.jad.0176 BAL Differential Processing.

C. Pediatric Hem/Onc Protocol

1. Pediatric Hematology providers require a differential on their CSF samples regardless of the nucleated cell count.
2. In the majority of cases, the LIS should add the CDIFF automatically if the provider is on the list for “Ped Hem Onc” providers.

3. These samples may also be labeled: “PEDIATRIC HEM/ONC LEUKEMIA PROTOCOL” on the CSF tubes and requisition. If the sample is labelled like this, a CDIFF should be done regardless of the nucleated cell count.

D. Microscopic Review of the Stained Cytocentrifuge Smear:

1. CSF - Differentials performed on:

   a. CSF samples with nucleated count > 5 cells/µL.
   b. Pediatric Hem/Onc Leukemia Protocol samples.
   c. By specific request from the provider. If requested by provider, add a CDIFF (CSF Differential) in order entry.

2. Verify proper slide identification.
3. Using the 10x, 50x and 100x objective as appropriate, perform an evaluation of the smear. Examine the body fluid smear for:

   a. Uniform cellular distribution: If presence of a large number of smudge cells is noted and additional fluid is available, a new smear should be attempted.
   b. Appropriate dilution (if required) of sample to prepare smear.
   c. Proper staining
   d. Adequate cell yield and appropriate proportion of nucleated cells vs. red blood cells (compared to the cell counts).
   e. WBC/RBC phagocytosis
   f. Hemosiderin and hematin crystals
   g. Immature hematopoietic cells
   h. Atypical, abnormal, or malignant cells
   i. Presence of microorganisms (intra or extracellular)

   NOTE: Presence of microorganisms (intra or extracellular) in a CSF is considered a **CRITICAL VALUE.** Refer to Results Reporting section.

4. **CSF Slide review**: Perform full differential

   a. Using a 50x or 100x (oil immersion) lens, perform a 100 cell differential. If 100 nucleated cells are not present on the smear, count as many nucleated cells as possible. Refer to the Manual Differential Procedure SH.CP.AU.hem.0007 for classification of cell types.

   b. ANY abnormal/atypical cells (including malignant or LE cells) or findings (microorganisms/crystals) are described and marked for pathologist review.

   c. Presence of microorganisms (intra or extracellular) in a CSF is considered a **CRITICAL VALUE**. Refer to Results Reporting section.

d. Add a comment to the results if a significant number of smudge or necrobiotic cells are present. If a large enough number of smudge cells are present, the slide should be remade, if possible.

e. Do not include choroid plexus, ependymal, or epithelial cells in any differentials. The presence of choroid plexus cells or ependymal can be noted in a comment, if applicable.

5. **Ascites-abdominal-peritoneal-paracentesis Fluid**:

   a. Perform a "% segs" and a cell description on all fluids identified as peritoneal, abdominal or ascites or PD (peritoneal dialysis).

   b. % segs. Classify (as a %) 100 nucleated cells as either seg or other using the diff keypad.

   c. If less than 100 cells are counted, convert the counts into percentages. The percent segs will automatically be reported and the absolute neutrophil count will be calculated and reported by the computer.

   d. Begin the description with the predominant cell type and go through to moderate numbers of cells present (example: predominantly segmented neutrophils, many lymphocytes, and moderate macrophages).

   e. Rare, normal cells do not need to be mentioned.

   f. ANY abnormal/atypical cells (including malignant and LE cells) or findings (microorganisms/crystals) are described and marked for pathologist review.

   g. Footnote results if a significant number of smudge or necrobiotic cells are present.

6. **All other fluids:** Perform a cell description

   a. Using a 50x or 100x (oil immersion) lens, describe the cells present in a paragraph type form. Refer to the Manual Differential Procedure SH.CP.AU.hem.0007 for the classification of cells.

   b. Begin the description with the predominant cell type and go through to moderate numbers of cells present (example: predominantly segmented neutrophils, many lymphocytes, and moderate macrophages).

   c. Rare, normal cells do not need to be mentioned.

   d. ANY abnormal/atypical cells (including malignant and LE cells) or findings (microorganisms/crystals) are described and marked for pathologist review.

   e. Footnote results if a significant number of smudge or necrobiotic cells are present.

7. Bronchoalveolar lavage differential (BALD); Refer to SH.CP.AU.jad.0176.

8. All fluid cytocentrifuge smears that are reviewed by laboratory personnel are placed in the pathologist's folder for supervisor or pathologist review.

X. LIMITATIONS

A. Sub-Optimal Specimen Integrity and Unacceptable Specimens:

1. Partially Clotted: Perform analysis and add a comment to the report stating "Interpret with caution, cell count may be inaccurate due to the presence of fibrin clot(s)." Use F5 comment field in LIS ("small clots in specimen") as appropriate.

2. Completely Clotted: Cell counts are not performed. If possible, prepare and stain a cytocentrifuge smear for possible review (if requested).

3. Clumping: Footnote results with a comment: "Interpret results with caution. Counts may be inaccurate due to the presence of macroscopic (or microscopic) clumping."

4. Debris: Footnote the results with a comment: "Interpret results with caution. Counts may be inaccurate due to the presence of debris." Use F5 comment field in LIS ("abundant debris in fluid") as appropriate.

XI. CALCULATIONS

A. The number of cells counted on separate sides must fall within (plus or minus) 2 standard deviations of their mean.

1. Mean

$$\text{Mean} = \frac{\text{CountSide1} + \text{CountSide2}}{2}$$

Example: Side 1 = 16 cells and Side 2 = 34 cells

$$\frac{16+34}{2} = 25$$

2. Two Standard Deviations (2 SD)

$$2\text{SD} = \sqrt{mean} \times 2$$

Example:

$$\sqrt{25} \times 2 = 10$$

3. Acceptable Range (2 SD from the mean)

$$\text{mean} - 2\,\text{SD} \text{ to } \text{mean} + 2\,\text{SD}$$

Example: Mean = 25 and 2 SD = 10

25 – 10 = 15
25 + 10 = 35
Acceptable Range = 15 to 35
So: 16 and 34 are within the acceptable range

4. Cell count/µL

$$\text{Cells}/\mu\text{l.} = \frac{\text{mean \# of cells counted x dilution x 10}}{\text{\# of large grids counted (one side)}}$$

Example: 25 cells counted with no dilution in 9 large grids

$$\frac{25 \text{ x } 1 \text{ x } 10}{9} = 28 \text{ cells/µL}$$

NOTE: Cell count standard deviations and calculation can also be performed using the LIS by entering the number of cells counted on each side, dilution factor and number of large squares counted.

XII. INTERPRETATION

A. CSF

1. Appearance:
   a. Normal: Clear and colorless with a viscosity similar to water.
   b. Abnormal: The fluid may appear cloudy, turbid, bloody, viscous, or clotted. Cloudy or turbid fluid may be due to pleocytosis (leukocyte count greater than 200/uL), the presence of microorganisms or RBCs (more than 400/uL), or an increased protein level. Following injection of radiographic contrast medium, the CSF may have an oily appearance. A green tinge may be seen in grossly purulent CSF. A fat embolism in the brain may be associated with fat globules of varying sizes in the CSF. Pink-red CSF usually indicates the presence of blood, a condition that may have resulted from subarachnoid hemorrhage, intracerebral hemorrhage, infarct, or traumatic tap.
2. Red Blood Cell Counts:
   a. Red cell enumeration is used for evaluating the possibility of hemorrhage into the central nervous system.
   b. Traumatic tap vs. pathologic bleeding:
      1) Traumatic taps are more common in infants.

2) A traumatic tap shows a maximum amount of blood in the first sample, with a progressive decrease in subsequent samples. Supernatant is clear. A clot may be present in the sample.
3) Subarachnoid hemorrhage, the amount of blood is generally the same in all tubes collected. Supernatant is xanthochromic (pink or yellow due to breakdown of hemoglobin). Samples are not usually clotted.
4) The presence of crenated RBCs is not useful in differentiating traumatic tap from pathologic bleeding.

3. White blood cell differential

Type of Cell	Clinical Significance
Lymphocyte	Viral, tubercular, and fungal meningitis, bacterial meningitis (occasionally), Multiple sclerosis
Neutrophil	Bacterial meningitis, early viral, tubercular, and fungal meningitis, Intracranial hemorrhage, Intrathecal injections, meningeal malignancy
Mixed cellular reaction (lymphocyte, neutrophil, monocyte)	Partially treated bacterial meningitis, chronic bacterial meningitis, cerebral abscess, tubercular meningitis, fungal meningitis, amebic meningitis
Eosinophil	Parasitic infections, Allergic reactions, Intracranial shunts
Macrophage	Chronic meningitis, treated bacterial meningitis, Intrathecal injections, Intracranial hemorrhage
Erythrophage (macrophage containing RBCs)	Hemorrhage (12 hours – 1 week)
Siderophage (macrophage containing hemosiderin)	Hemorrhage(2 days – 2 months)
Hematoidinophage (macrophage containing hematin crystals)	Hemorrhage (2-4 weeks)
Lipophage (macrophage containing fat)	Brain necrosis, infarct, anoxia or trauma
Plasma cells	Subacute and chronic inflammatory reactions, multiple sclerosis
Malignant lymphoid cells	Lymphoma, leukemia
Blasts	Leukemia, Lymphoma
Other malignant cells	Primary brain tumor, metastatic tumor
Ependymal/choroid plexus cells	Trauma, surgery, ventricular shunts, neonate, intrathecal injections
Cartilage cells	Traumatic puncture
Bone marrow cells	Traumatic puncture
Primitive cell clusters (blast-like)	Intracranial hemorrhage in premature infant, neonate; possibly of germinal matrix origin

B. Other Body fluids:

1. Body fluid analysis is a diagnostic tool that is especially useful in determining most inflammatory processes, traumas, malignant neoplasms, and other disease processes. Most commonly, analysis is performed on synovial and serous (pleural, pericardial, and peritoneal) fluids. Manual differentials (and microscopic cell descriptions) are the foundation for the diagnosis of hematopoietic disorders.
2. Red cell enumeration can also be used to assist in determining the amount of peripheral blood contamination or the need for an exploratory laparotomy following trauma to the abdomen.

C. For more information on interpretation of serous and synovial fluids, refer to References section.

XIII. RESULT REPORTING

A. Record fluid appearance and cell counts (nucleated and RBC) on the Fluid Counts log sheet.

B. Enter the appearance, nucleated cell count, the red blood cell count, number of large squares and dilution factor into the LIS.

1. Cell counts are reported as cells/µl
2. Numerical results are reported to the nearest whole number.
3. Footnote results that are suspect due to a delay in time, clumping, clotted, or debris. Refer to the Limitations Section.

C. Amniotic fluid: Amniotic fluid usually contains epithelial cells. These cells should be counted as nucleated cells for the cell count and described in the fluid differential description.

D. CSF Differential Reporting:

1. Nucleated count of less than 6: Differential is not performed unless specifically requested by a unit or physician or Pediatric Leukemia Protocol. File the stained slide in case a differential is requested in the future. In the CSF remark field the computer automatically enters a comment which says, “CSF differential not performed. Nucleated cell count is within the normal range. Cytospin slide available for review. Call x5-9690 to request a differential if clinically indicated.”
2. Differential: <5 Nucleated Cells seen: The differential is not reported in percentages. Manually enter the cells seen in the fluid remark field i.e. two lymphs and one neutrophil seen on cytospin.
3. Diff: 5-99 Nucleated Cells: Report the differential in percentages. The total number of cells counted is also reported.
4. Diff: 100 Nucleated Cells: Report the differential in percentages.

5. Cells begin deteriorating in just a few hours particularly in body fluids with low protein content such as CSF. If there is prolonged delay in preparing cytocentrifuge slides (more than four hours for CSF), the report should include a statement that the differential count may be inaccurate due to cellular degeneration. Add the following comment to the CCOM2 field:
   @SCDEG: Interpret CSF differential with caution – count may be inaccurate due to age of sample (> 4 hrs)
6. The presence of microorganisms (intra or extracellular) in a CSF is considered a **CRITICAL VALUE**. Report the finding to the unit/physician immediately. Enter the finding in the fluid remark field and then mark the sample for Pathologist review. Refer to policy VI-14 Clinical Laboratory Critical Value Immediate Notification Policy for reporting and documentation.

E. Count the % segs if applicable. Refer to Section IX.D.5

F. Perform cell description as applicable. Refer to Section IX.D.6

G. Quality control results are entered into Unity QC. We follow the manufacturer's expiration date and open vial stability.

1. A new lot cross over work up has to be completed before using a new lot of Cell-Chex QC material.
2. QC material should be labeled with QC label to ensure the correct lot number is in use.

H. Normal Reference Ranges (CSF):

Test	Age	Reference Interval
CSF RBC	0-30 days	0-50 cells/µL
	>30 day	0-5 cells/µL
CSF Nucleated Cells	0-30 days	0-27 cells/µL
	1 month to 16 yrs	0-7 cells/µL
	Adult	0-5 cells/µL
Leukocyte Differential		
Segmented Neutrophils	0-30 days	0-8%*
Lymphocytes	0-30 days	2-38%
Monocytes	0-30 days	50-94%
Macrophages	0-30 days	1-9%
Segmented Neutrophils	Adults	0-2%
Lymphocytes	Adults	63-99%
Monocytes	Adults	3-37%
Macrophages	Adults	0-1%

*may be increased in high-risk neonates without meningitis[C]

I. Normal Reference Range – Nucleated cells - other body fluids:

Synovial fluid:	10-200/ µL
Pleural fluid:	0-1000/ µL

XIV. TRAINING

A. Staff are trained by a laboratory designated trainer and a training record is completed and signed by both trainer and staff (trainee).

XV. REFERENCES

A. Kjeldsberg, C, Knight, J. Body Fluids, Third Edition, p. 269.ASCP Press 1993.

B. Henry, John, Clinical Diagnosis and Management by Laboratory Methods, Nineteenth Edition, 1996. pp. 457-479.

C. CLSI Infobase, Body Fluid Analysis for Cellular Composition, H56-A, June 2006.

D. Hycor Biomedical, Kova Glasstic Slide 10 with Grids Package Insert, P/N 91064-07 8/02.

E. Bertholf, Kao. Disposable Plastic and Reusable Glass Hemocytometers for Cell Counts. Laboratory Medicine Vol. 22, No. 12. December 1991.

F. InCYTO C-CHIP DHC-N01, Disposable Hemacytometer Instructions.

G. Operator's Manual, Cytofuge 2 Cytocentrifuge System, Model M801-22, 54-004385-006 REV B.

H. Filter Concentrators, Cytofuge, FF01/FFR1/FF01-B, Package insert.

Appendix 1: Preparing Fluid Dilutions

1. View the fluid microscopically, unstained, on a wet prep to estimate the fluid's cellularity and then prepare the appropriate dilutions for the nucleated and RBC count from the dilution chart.

2. A different dilution for the nucleated cell and red blood cell count may be required.

3. Even if a fluid is cloudy the cell count may be low. Crystals, fat globules, or contrast media may cause cloudiness.

Quantity of Fluid – Sufficient volume sample		
Fluid (µL)	**Cell Pack/Cresyl Blue Mixture (µL)**	**Final Dilution**
100	900	1:10
100	1900	1:20
20	980	1:50
20	1980	1:100
10	1990	1:200

Quantity of Fluid – Low volume sample		
Fluid (µL)	**Cell Pack/Cresyl Blue Mixture (µL)**	**Final Dilution**
20	180	1:10
20	380	1:20
20	980	1:50
20	1980	1:100
10	1990	1:200

Appendix 2: Using a Hemacytometer

Using the charged hemocytometer and the 20x objective, perform a nucleated cell and red blood cell count.

1. Scan the entire hemocytometer chamber to assess the distribution and dilution of the cells. Cells should appear in monolayer. If overlapping of cells is seen refer to Appendix 1: Preparing Fluid Dilutions.
2. Count at least 100 nucleated cells and 100 red blood cells on each side of the hemocytometer.
3. Count all cells within the specified counting area, remembering to count the cells on 2 of the outside margins and therefore excluding those lying on the other 2 outside margins.
4. An equal counting area is used for each cell type (nucleated cells or red blood cells) on both sides of the hemocytometer.
    a. Less than 100 cells of a type may be counted in 9 large squares on a low cellularity fluid that is not diluted prior to analysis.
    b. High cellularity fluids may require 1 to 9 large squares to be counted on each side of the hemocytometer to reach at least 100 cells of each type.

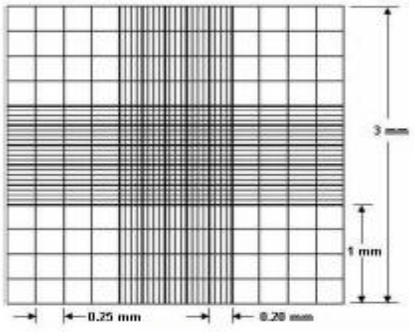


Figure 1. Grid pattern

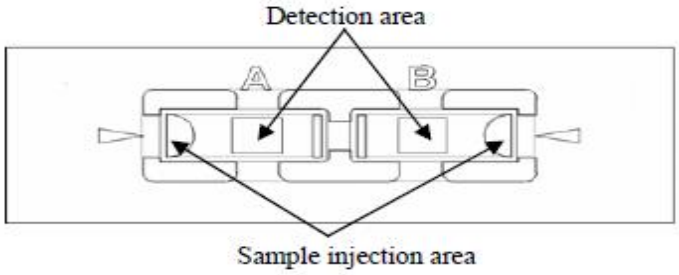


Figure 2. DHC-N01

Chamber Volume:	6.6 µL	Volume Within Grid:	0.9 µL
Chamber Depth:	0.1 mm	Large Grid Size:	1 mm x 1 mm (1 mm^2)
Outer Grid Dimension:	3 mm x 3 mm (9mm^2)	Large Grid Volume:	0.1 µL

Appendix 3: Guidelines for Preparing a Cytocentrifuge Smear

Guidelines for the number of fluid drops to add to the cytocentrifuge chamber based on fluid appearance:

Fluid Appearance	Number of Drops of Fluid*
Clear	5-6
Slightly Cloudy	2-3
Moderately Cloudy	1-2
Very Cloudy	Dilute with Cellpack diluent until moderately cloudy – then add 1-2 drops

*Each fluid cytospin slide also requires one drop of albumin.

The most representative distribution is obtained by the preparation of a cytocentrifuge smear. If unable to obtain an adequate cytocentrifuge smear on a cellular fluid, a push smear may be prepared. This is particularly helpful on bloody fluids.

Setting Parameters on the Cytocentrifuge Control Panel

Program 1: 1200 RPM for 5 minutes (for most serous fluids and CSF)

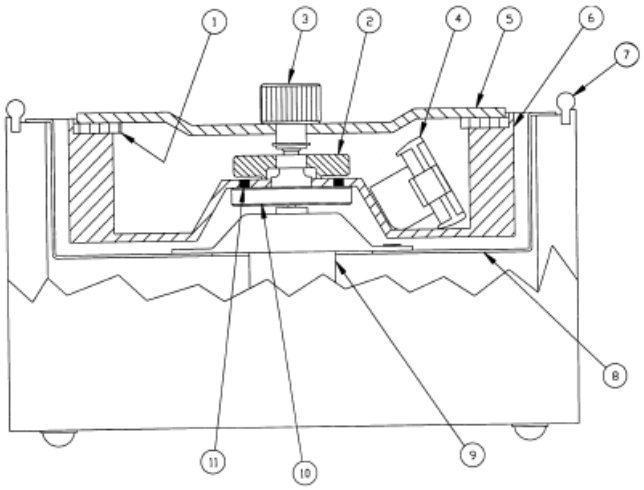
If a fluid sample contains cells that are particularly fragile i.e. the cells appear smudged when slide is scanned, a different speed or time can be used to spin the slide. If a different speed or time is desired for slide preparation, the cytocentrifuge may be programmed as follows:

1. Press “set time”
2. Press desired time in minutes
3. Press “enter”
4. Press “set speed”
5. Press desired speed in RPM
6. Press “enter”
7. Set acceleration to “High”

Appendix 4: Operation of the Cytofuge 2 Cytocentrifuge
(back up for other cytocentrifuge)

Access the Rotor

Access the rotor cover by turning the knurled knob 3 (cover nut) counterclockwise while holding the rotor itself to prevent turning. (See Figure 1.)



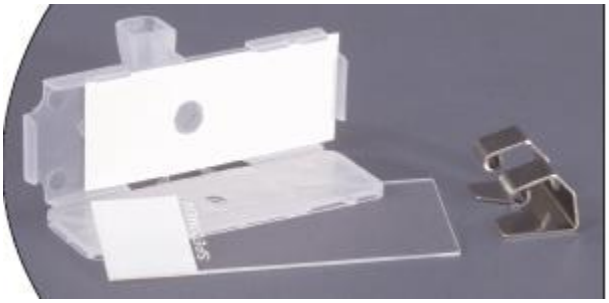
1. Rotor Gasket
2. Rotor Hold-Down Nut (3-lobed)
3. Cover Nut
4. Cell Concentrator (shown in rest position)
5. Rotor Cover
6. Rotor
7. Bowl Gasket
8. CytoFuge Bowl
9. Motor
10. Rotor Mount
11. Rotor Indexing Pin

Remove the Rotor

1. Hold the rotor with one hand and remove the rotor cover.
2. Remove the three-sided nut by turning it counterclockwise.
3. Lift the rotor straight out from the centrifuge bowl.

Add Sample to the Cell and Filter Concentrator

1. Unhinge the Cell and filter concentrator. Align the Filter card hole with the concentrator hole.
2. Place the microscope slide on the back side of the filter concentrator.
3. Gently compress the assembly with one hand and slide the Steel Clip over the bottom of the concentrator.



4. Place assembled Cell and Filter Concentrators in the CytoFuge rotor in a balanced arrangement before loading sample.
5. The filter concentrators should tilt toward the center of the rotor.

6. Slowly add sample to the bottom of the funnel. Avoid getting droplets onto the walls of the funnel. Avoid exposing the filter material or the microscope slide to sample during the loading process. DO NOT OVERFILL THE DEVICE.
7. Secure the lid on the rotor.
8. Process the specimen as quickly as possible following sample addition to prevent cells from settling.

NOTE: In a properly loaded Concentrator, while in the "rest" position, the fluid does not contact the slide. During the first few rotations the Concentrators tilt to a vertical orientation, (noted by an audible click) bringing the fluid in contact with the microscope slide. The Concentrators remain upright until rotation has nearly stopped at which time they shift back to the rest position.

CAUTION - Addition of excess amounts of liquid (overfilling) to Cell or Filter Concentrator will result in the fluid being "spun out" during centrifugation

Replace the Rotor

1. Place the rotor on the two rotor indexing pins, aligning the corresponding holes.
2. Tighten the rotor hold-down nut by turning it clockwise.

CAUTION - *Be careful when replacing the rotor. Make sure the two locator pins are in the corresponding holes in the rotor. Do not over tighten the hold-down nut. Make certain it is replaced with the flat surface facing up.*

CAUTION - *NEVER use any tool to tighten either the rotor nut or the cover nut.*

Spin the Sample

CAUTION *- Never operate the CytoFuge 2 without the rotor cover in place.*

1. Screw on the rotor cover.
2. Close and latch the centrifuge cover.
3. Select the *time (5 minutes)* and *speed (1300 RPM)*.
4. Press *start* button.
5. When the timed cycle is complete, the rotor stops; three beeps sound; and the interlock mechanism releases. The cover latch can then be squeezed to open.
6. Unscrew the rotor lid.
7. Remove the Steel Clip and unhinge the concentrator(s).
8. Carefully remove the slide(s) for further processing.

Manual Body Fluid Analysis (including CSF) – SH.CP.AU.hem.0012.0004

Knowledge Check

In the event of a question answered incorrectly: Single-line through the incorrect answer, initial & date, then select the correct answer.

ALWAYS HAVE CHANGES INITIALED BY YOUR TRAINER.

Circle True or False for each of the following statements.

1	True or False	If CSF is > 4 hours old and cell differential is needed, it is OK to process and release the results without a comment.
2	True or False	An equal counting area is used for each cell type (nucleated cells or red blood cells) on both sides of the hemocytometer.
3	True or False	The normal reference range on a CSF nucleated cell count for a neonate 0-30 days old is 0-5 cells/uL.
4	True or False	When resuspending Cell-Chex controls, it is acceptable to place the vials on the rocker to mix them.
5	True or False	Adding one drop of albumin to the cytofunnel instead of using albuminized slides helps keep cells intact during centrifugation.

Any incorrect answers I may have initially written have been discussed and corrected. I now understand the answers I may have gotten wrong.

PASSING GRADE IS 80% OR GREATER

_____
Employee name (print)

_____ _____
Employee signature **(Date)**

_____
Supervisor/Manager name (print)

_____ _____
Supervisor/Manager signature **(Date)**