

**Dignity Health
Central Coast Service Area Procedure**

Central Coast Service Area North:

- Santa Maria Campus,
Marian Regional Medical Center Arroyo Grande Campus,
Marian Regional Medical Center French Hospital Medical Center

Central Coast Service Area South:

- St. John's Pleasant Valley Hospital St. John's Regional Medical Center

SUBJECT: MANUAL BODY FLUID CELL COUNT

LAB POLICY NUMBER: 7500.H.CC.50

I. PURPOSE:

The enumeration and classification of cells from normally sterile body fluids provides clinicians with valuable information used to diagnose and treat disease. Manual cell counting using a hemocytometer is necessary when the total cells is below the linearity of the automated hematology analyzer or when the automated analyzer is unable to give accurate counts due to system flags, interferences, unusual viscosity, or low volume specimens.

II. CLIA COMPLEXITY:

High complexity

III. CLINICAL UTILITY:

The C-chip is a disposable plastic hemocytometer consisting of two enclosed chambers with the exact same surface-patterned grid as the Neubauer improved counting chamber. Each side on a hemocytometer contains 9 large squares, each of which is 1 mm square. The depth of the chamber is 0.1 mm. The same area on both sides of the hemocytometer is counted. These separate counts are evaluated to determine if the cellular distribution between the two sides is acceptable. A formula using the average number of cells counted, the dilution factor, area counted, and the volume per square is used to calculate the total cells per cubic milliliter of fluid.

IV. SPECIMEN COLLECTION:

- A. CSF samples are collected into sterile numbered tubes. Unless otherwise specified by the physician, the cell count is performed on tube #3 or #4 or the last tube collected. If an additional cell count is request, the additional cell count is performed on tube #1.
- B. Body fluid samples are collected into EDTA vacutainers tubes, evacuated containers without anticoagulants, or plastic specimen containers.
- C. Body fluid samples should be stored at room temperature and analyzed as soon as possible after collection (preferably within 1 hour). Cell counts and differentials should not routinely be performed on samples >24 hours old. Any delay in analysis could cause cell lysis, cellular degeneration, and bacterial growth which can affect the test results.
- D. All body fluid samples are closely examined for the presence of clots, fibrin, tissue, or cellular clumps before testing. Manual cell counts are not performed on any turbid samples or samples containing clots, large cellular clumps, or tissue.

- E. Because of the invasive nature in collecting body fluids, specimens submitted to the laboratory that are unlabeled, improperly labeled, submitted in inappropriate containers or outside of stability are not rejected if possible. If the specimen is improperly labeled, a nurse or physician involved in the collection may come to the lab to properly label the specimen. A detailed comment is attached to the results explaining the exception.
- F. Manual fluids cell counts may be performed on the following:
1. Cerebral Spinal Fluid
 2. Pleural Fluid
 3. Peritoneal Fluid
 4. Pericardial Fluid
 5. Synovial Fluid

V. MATERIALS:

Reagents / Media	Supplies / Materials	Equipment
<ul style="list-style-type: none"> ● Beckman Coulter DxH Diluent. ● Lyophilized hyaluronidase (stored at <-20°C). ● Streck Cell-Chex Body Fluid Controls: Store at 2-10 °C and Stable for 30 days after opening. 	<ul style="list-style-type: none"> ● Adjustable pipette and pipette tips ● C-Chip DHC-N01 disposable hemocytometer or glass hemocytometer and cover glass ● Plastic aliquot tubes 	<ul style="list-style-type: none"> ● Light microscope ● Automated Stainer

VI. CALIBRATION: N/A

VII. QUALITY CONTROL

- A. Cell Counts:
1. At least one level of manual body fluid control is counted in duplicate for each 8 hours of patient testing. If no manual body fluid count is performed, no quality control is required.
 2. The level of quality control performed is determined by the shift performing the QC.
 3. Remove the control material from the refrigerator. It is not necessary to warm the controls to room temperature before using.
 4. To mix: **(do NOT mix mechanically or vortex).**
 - a) Hold the vial vertically and roll each vial between the palms of the hands for 15-20 seconds.
 - b) Continue to mix by holding the vial by the ends between the thumb and finger, rapidly inverting the vial 20 times end-over-end using a very quick turning motion of the wrist.
 - c) Sample immediately after mixing

5. Follow procedural steps VIII.C-J.
6. Quality control counts are evaluated using the same criteria used for patient testing.
 - a) Quality control results are considered acceptable if the values correspond to the control level being used.
 - b) QC ranges can be found with their corresponding level in the product insert and in LIS.
 - c) Additional counts are needed if the results are not within range. Check the lot number on the worksheet to verify they are the same controls.
 - d) Record all quality control values on Body Fluid Cell Count Worksheet and input values into LIS. Document in LIS all actions taken with each failure/repeat of QC
7. If the QC results do not fall within the expected ranges:
 - a) Verify that an adequate area was counted for the number of cells present. Repeat testing using a larger area if necessary.
 - b) Verify that the QC material has not exceeded open stability.
 - c) Review the procedure to ensure that the QC material was appropriately mixed and calculations were performed correctly.
 - d) Perform quality control testing on additional levels of QC.
8. Do not report patient testing if quality control values are not within expected ranges.

XI. PROCEDURE

- A. All body fluids specimens are processed immediately. Cell counts and differentials should be performed as soon as possible, preferably within 1 hour.
- B. Perform a visual examination of the fluid.
 1. Record the total volume if possible.
 2. Document the color and clarity of the unspun specimen.
 3. For CSF specimens, record the color and clarity of the supernatant if the unspun sample is bloody.
 4. Inspect the sample for the presence of clots, fibrin, large cellular clumps, or tissue.
 5. Body fluid which appears to contain tissue is referred to the pathology department.
 6. Synovial fluids can be pretreated with hyaluronidase to reduce sample viscosity. Add approximately 5 mg (enough to coat the end of a wooden applicator stick) of lyophilized hyaluronidase to a well-mixed 1 mL aliquot of fluid. Mix and let sit for 5 minutes.
- C. Pipette 10 μ L of well mixed patient sample into each side of a hemocytometer. Use caution not to introduce bubbles.
- D. Allow the slide to remain undisturbed for several minutes to allow the cells to settle.
- E. Scan the gridded areas on both sides of the chamber on 10x power to confirm good cell distribution and to determine an approximate cell concentration. If cells are overlapping, the specimen should be diluted.
 1. Obtain Beckman Coulter diluent.

2. Perform a microscopic exam of the diluent to verify that the diluent is free from cells or other contaminants.
 3. Perform a dilution using the lowest possible dilution factor necessary to obtain good cell distribution in the counting chamber. The greater the dilution factor, the more error that may be introduced.
- F. The number of squares (area) to be counted is determined by the concentration of cells present. In general, the lower the cell concentration, the more squares counted. If the cell concentration is different for red blood cells versus white blood cells, it may be necessary to count one area for one cell type and a different area for the second cell type.
- G. Common sense judgement is used to determine the area counted. As a general rule, all 9 squares should be counted if the total cells seen are less than 200 in the entire ruled area.
- H. Count and record the number of white blood cells in a predetermined area on each side of the hemocytometer. Count and record the number of red blood cells in a predetermined area on each side of the hemocytometer.
1. Red blood cells will appear smooth, refractile, and may have a yellowish or reddish tinge. They may be round or crenated.
 2. White blood cells will have a rough or grainy surface and are less refractile. They may have a bluish or grayish tinge. The shape is generally round but may have rougher or more irregular outer edges.
 3. A methylene blue stain can be used to improve differentiation of nucleated cells from non-nucleated cells.
 4. All cells that lie within the square and those touching the left or upper boundary are counted. Cells touching a right or lower boundary are not to be counted, even if they lie mostly in the square.
 5. When double lines separate the counting areas, the outer line is considered the boundary.
 6. When triple lines divide the counting areas, the middle line is considered the boundary.
- I. Duplicate counts are evaluated for acceptability:
1. If the number of cells counted per side is ≤ 50 , the two counts must agree ± 5 cells of the mean.
 2. To calculate the mean add the two counts and divide by 2.
 3. If the number of cells counted per side is > 50 , the 2 counts must agree within 10% of the mean.
 4. To calculate 10% of the mean, multiply the mean by 0.1 or multiply by 10%. Additional counts are performed if the agreement between the two sides is unsatisfactory.
- J. Record all values on Body Fluid Cell Count Worksheet. Completed worksheets are retained for two years after they have been reviewed.
- K. Make at least one cytospin slide on each body fluid specimen, regardless of the cell count.

- L. Stain the cytopsin slide according to procedure and scan each slide on low power. The scan is performed regardless of cell count.
- M. Scan each slide looking for malignant cells. Confirm the accuracy of the manual count by comparing the cell concentration observed on the cytopsin slide to the calculated manual count.
- N. Perform a 5-part differential on all body fluid specimens in which the calculated WBC count is >5 cells/mm³.

X. INTERPRETATION OF RESULTS (FOR QUANTITATIVE TESTS):

O. CSF normal ranges:

- 1. 0-29 days old:
 - a) WBC 0-19 cells/mm³
 - b) RBC 0-5 cells/mm³
- 2. ≥ 30 days old:
 - a) WBC 0-5 cells/mm³
 - b) RBC 0-5 cells/mm³

P. CSF WBC critical ranges:

- 1. 0-29 days old: >19 cells/mm³
- 2. ≥ 30 days old: >5 cells/mm³

Q. A CSF WBC count of >5 is considered critical. Refer to Critical Value Laboratory Notification policy for information on reporting critical values.

R. Body fluids other than CSF do not have normal/critical ranges in Cerner. Results should always be interpreted in light of the total clinical presentation of the patient including clinical history, data from additional tests, and other appropriate information.

S. Any body fluids with suspected malignancy, abnormal/suspicious cells or unidentified cells are sent to pathology for review. Refer to Submission of Slides for Pathology Review for instruction on submitting slides for pathology review.

T. Body fluid(s) which appear to contain tissue should be referred to the pathology department.

U. Cell counts are not routinely performed on body fluid specimens containing clots, fibrin, or large cellular clumps as the results will be inaccurate. A differential should be performed if possible.

V. Cell count calculations are performed using the following formula.

Mean of 2 counts	<input style="width: 50px;" type="text"/>	x	Dilution	<input style="width: 50px;" type="text"/>	x	Depth Factor	<input style="width: 50px;" type="text" value="10"/>
cells/mm³							
=	<input style="width: 100px;" type="text"/>						
	Number of Large Squares counted (Area in mm²) per side						

XI. RESULT REPORTING:

- A. In Accession Result Entry, scan the patient/QC barcode or manually enter the accession number.
- B. In the appropriate fields, select the body fluid type, color, and clarity from the drop-down options.
- C. For CSF fluids, enter the total volume of fluid submitted, the tube number that the count was performed on, and post-spin color and clarity if applicable.
- D. Enter the calculated CSF WBC or TNC and RBC counts from the worksheet.
- E. For body fluids with a CSF WBC or TNC count of ≤ 5 cells/mm³; under comment enter 'slide reviewed no abnormal or immature cells seen' or 'slide reviewed, atypical or immature cells seen, sent for review'
- F. For body fluids with a CSF WBC or TNC of >5 cells/mm³; report 5-part differential (mesothelial, synovial lining cells, etc if present are included in the differential).
- G. Review results to ensure accurate manual entry. If necessary, add a Result Comment or Result Note to document collection or processing problems and/or communications with nurses or physicians.
- H. Select VERIFY when complete.

XII. LIMITATIONS OF PROCEDURE:

- A. Cell lysis may begin shortly after the collection of a body fluid. Cell counts should be performed as soon as possible after collection preferably within one hour.
- B. A fluid is considered clotted if one or more bloody or gelatinous clots can be seen in the fluid. If the specimen is clotted, no cell counts should be reported. Tests are result as TNP (test not performed) and a comment is added explaining the reason that the count cannot be performed. Manual estimations of FEW, MODERATE or MANY WBCs and RBCs are noted in the comment. WBC differentials are performed and result as.
- C. In cases where small clumps/clusters of cells are seen on the hemocytometer, a comment is attached to the cell count indicating that the cell count may be inaccurate due to the presence of clumped cells.
- D. When performing a cell count on bloody fluids, only intact red cells should be counted. If present, a comment is added explaining the presence of ghost cells in the fluid. Ghost cells seen in CSF usually indicate a previous or "old" bleed.
- E. No cell counts should be reported on bronchial lavage or wash. Counts are result as TNP (test not performed) and a comment is added explaining the reason that the count cannot be performed. Manual estimations of FEW, MODERATE or MANY WBCs and RBCs are noted in the comment. WBC differentials are performed and result as.

XIII. REFERENCES:

Brunzel, N. A. (2013). *Fundamentals of Urine & Body Fluid Analysis*. St. Louis: Elsevier Saunders.

College of American Pathologists, Hematology and Coagulation Checklist. Northfield, IL, Current Revision.

Harmening, D. M. (2009). *Clinical Hematology and Fundamentals of Hemostasis* (Fifth ed.). Philadelphia: F.A. Davis Company.

iN Cyto. (2010). Retrieved May 20, 2016, from NHC-N01 (Neubauer Improved) Product Information: http://www.incyto.com/product/product02_detail.php

Munson Ringsrud, K., & Jorgenson Linne, J. (1995). *Urinalysis and Body Fluids, a ColorText and Atlas*. St. Louis: Mosby, Inc.

XIV. ASSOCIATED DOCUMENTS:

ATTACHMENT 1: MANUAL BODY FLUID WORKSHEET