Manual Body Fluid Analysis

Manual Body Fluid Analysis

Purpose

This procedure provides instructions for performing manual Body Fluid Cell Count (Red Blood Cells and Total Nucleated Cells) and Differential Count. Analysis of these fluids is performed to determine the presence of infection, malignancy or hemorrhagic processes.

Scope

This procedure is intended for Clinical Laboratory Scientists performing microscopic analysis for body fluids in the Hematology department.

Specimen sources

This procedure applies to these following body fluids:

- Abdominal/Peritoneal fluid
- Ascites fluid
- Bronchial Lavage
- CSF
- Gastric fluid
- Pericardial fluid
- Pleural/Thoracentesis fluid
- Synovial fluid

Specimen collection

- The specimens can be collected in sterile screw-cap containers or aliquots placed in green-top (heparinized) tubes. **Note**: use lavender-top (EDTA) tube for synovial fluid.
- If received in large containers, the body fluid must be well mixed and a small portion (~5 ml) aseptically removed and placed in a 5 ml tube with the appropriate anticoagulant.

Note: Observe specimen for blood clots, fibrin clots and pellicle formation.

- If small blood or fibrin clots are detected, perform the test and include a comment in the report stating that results may not be accurate due to blood or fibrin clot formation.
- For synovial or joint fluids, a dab of hyaluronidase on an applicator stick may be added to the aliquot to break up mucous.

Specimen rejection

- Grossly clotted specimen

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Manual Body Fluid Analysis, Continued

Equipment

- Microscope
- Cytospin

Note: Except for BAL, pericardial and amniotic fluids, validated types of body fluids can be analyzed using a validated Sysmex XN analyzer. Results may still be entered manually for the body fluids in scope.

Reagents and supplies

The following is the list of reagents and supplies required.

- 3% acetic acid
- Acidified crystal violet stain
- Saline
- Sysmex DCL Cellpack (for dilutions)
- Hemocytometer
- Hemocytometer coverslip
- Hemocytometer C-Chip
- Calibrated pipettes with tips
- Test tubes (for dilutions)
- Hyaluronidase

Safety or special safety precautions

All staff members performing these procedures must adhere to regional and local workplace safety policies. These will include but may not be limited to:

- Equipment safety, proper body mechanics, sharps exposure
- Use of Biological Safety Cabinet when processing body fluids
- Proper use of gloves, protective eyewear and suitable laboratory attire when performing these procedures
- Exposure to body fluids
- Proper handling of regular and biohazardous waste
- Handling of regular and infectious waste
- Proper cleaning of work area
- Proper hand washing
- Proper storage and disposal of chemical hazardous waste

Quality Control

Refer to local quality control procedures.

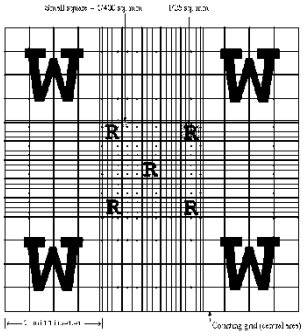
Before you begin

- Always use sterile technique when handling body fluid specimens.

Performing manual cell count

Follow the steps below to complete the cell count for body fluids.

Step	Action
1	Mix the specimen well by rotating in an automated mixer for a maximum of two to five minutes or hand mix by inverting the tube ten to 15 times. **Caution: excessive rocking may damage cells**
2	Specimens are usually counted undiluted, unless they are bloody or cloudy. Typical dilutions for any fluid can range from 1:10 to 1:200 or higher, depending on the turbidity of the specimen. Note: Different diluents can be used to dilute the fluids. Isotonic saline can be used for both white and red cell dilutions, while acetic acid or hypotonic saline may be used to lyse red cells for white cell dilutions.
3	• Prepare and charge the Hemocytometer. 1) Verify that lines in all counting chambers or optical grids are bright and free from scratches, dirt, or debris. 2) Place a coverslip on the hemocytometer. 3) Place the hemocytometer in a Petri dish lined with moist paper. 4) Elevate the hemocytometer on two sticks so it does not come in direct contact with the moist paper 5) Fill both sides of the hemocytometer, being careful not to overfill. Enough liquid should be introduced so that the mirrored surface is just covered. 6) After the hemocytometer is loaded, allow the cells to settle for 5-10 minutes (the amount of time required for the cells to settle depends on the cellularity of the specimen. **Note:** If a single-use C-Chip is in use, skip steps 2-4 above and load 10uL of sample into the sample injection area so it fills the chamber by capillary action. Be careful not to make air bubbles. • The following guidelines are recommended for counting areas: 1) If less than an estimated 200 cells are present in all nine squares, count all nine squares. This area counted is 9 mm^3. 2) If more than an estimated 200 cells are present in all nine squares, then count the four corner squares. This area counted is 4 mm^3. 3) If more than an estimated 200 cells are present in one square, then count five of the squares within the Cerner square for an area of 0.2 mm^3.

4	• Place the hemocytometer under the microscope, using low power only (10x), and adjust to see the cells. Scan the large squares. For accuracy, there should be even distribution of cells (approximately no more than ten cells variation in the large squares). Cells should not overlap. For diluted samples, a minimum of 200 cells should be counted. • Then, switch to high power magnification (40x). The count is performed under high power. Depending on the number of cells present, an appropriate number of squares should be counted. The more cells present, the smaller and fewer the numbers of squares that need to be counted.
5	Calculate cells per chamber using the following:  Large Square (W) = 1 mm^3 or 0.1μL Small Square (R) = 0.004 mm^3 or 0.004μL Formula: **Cells/μL** = #of cells counted x dilution factor / # of square mm counted x chamber depth (0.1mm)
5	The number of cells from each side of the chamber must agree within 20% or the count must be repeated. For counts with ≤10 cells/μL, agreement must be within +/- 2 cells/μL.
6	After the count is completed, thoroughly clean the hemocytometer with alcohol and store dry in the covered petri dish. If using a disposable hemocytometer, discard as appropriate.

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Performing manual differential count

Follow these steps to perform manual differential count.

Step	Action
1	Prepare a cytospin smear for differential count following a cytospin smear preparation procedure, air dry, fix and stain with Wright stain. Perform a 5-part differential on the stained smear.
2	The quality of body fluid smears must be satisfactory (i.e., uniform cell distribution, appropriate dilution so cells are not crowded, properly stained, adequate cell yield, ready recognition of cell types that are reported). Check slide stain quality and document results using the following acceptance criteria: • Red blood cells: red to pink • Neutrophils: dark purple nuclei, pale pink cytoplasm, reddish-lilac small granules • Eosinophils: blue nuclei, pale pink cytoplasm, red to orange-red large granules • Basophils: purple to dark blue nucleus, dark purple, almost black large granules • Lymphocytes: dark purple to deep bluish-purple nuclei, sky blue cytoplasm • Monocytes: purple to dark blue nucleus, blue-gray cytoplasm • Platelets: violet to purple granules Refer to Blood Cell Morphology Atlas for more details.
3	Count 100 WBCs and perform a 5-part differential.
4	Look for and note any abnormal or suspicious cells. If unable to identify a cell confer with another CLS, Lead CLS, supervisor, or with a pathologist before reporting results.
5	If abnormal or suspicious cells are present, initiate appropriate pathologist review process.
6	Follow local retention period to save the slide.
7	Enter results into Cerner using Accession Result Entry (ARE).

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Resulting body fluid counts

Follow the steps below to complete the reporting of the body fluid counts:

Step	Action
1	Except for BAL, amniotic, peritoneal, ascites and abdominal fluids sources, all body fluid results must be entered using instrument middleware. Exempted fluid counts are manual entries in Cerner. **NOTES:** 1) Manually enter results in Cerner when instrument middleware is down. 2)Follow appropriate verification process of manually entered results prior to release.

2	Body fluid results to be entered are:
	• Appearance: Bloody, Cloudy, Clear, Clotted, Hazy, Slightly Hazy
	• RBC (cells/mm^3) count Manual
	• TNC (cells/mm^3) count Manual
	• Differential
	  ○ Segs pct
	  ○ Lymph pct
	  ○ Mono/Macro pct (include macrophage in the count)
	  ○ Eos pct
	  ○ Baso pct
	  ○ Mesothelial cells
	  ○ Other nucleated cells *(enter in result comment of this field the name of cells identified, include count if multiple types of cells observed)*
	• WBC Cnt Manual- Calculation subtracting the percentage of Mesothelial cells + Oth nucleated cells.
	Comments: Indicate in the result comments the presence of cell clumps or if differential count was performed under 100 cells.

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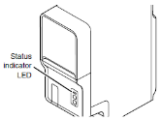
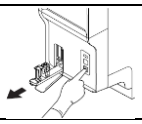
Manual Body Fluid Analysis, Continued

Cell count using Sysmex XN analyzer

For **peritoneal, ascites and abdominal fluids sources,** follow the steps below to use the Sysmex XN analyzer for specimen analysis.

Step	Action
1	Check the status of the analyzer. Check the Status indicator LED on the analyzer to confirm analyzer is in **READY** state. 
2	Press the mode switch to eject the tube holder. 
3	Select the Change Analysis Mode button on the control menu.
4	Select analysis mode **[BODY FLUID],** then select **[OK]**
5	Analyzer automatically performs a Background Check on the diluent fluid and lysing agent to check for contamination that will affect cell counts. **Note**: The analyzer will automatically perform a background check up to three times (3X) to achieve an acceptable background check value.
6	Ensure **Background Check** passes, then proceed to sample or QC analysis. Acceptable Background Limits are as follows: WBC-BF 0.001 x 10^3 / µL or less RBC-BF 0.003 x 10^6 / µL or less
7	Place a well-mixed patient body fluid in a vial with the correct sample barcode for analysis in the sample tube holder. Note: There are two sample tube holders. When performing test on a micro collection tube, insert the tube all the way in, so that the bottom of the tube contacts the bottom of the holder.
8	Click the **[Manual Analysis]** button in the analyzer Control Menu.

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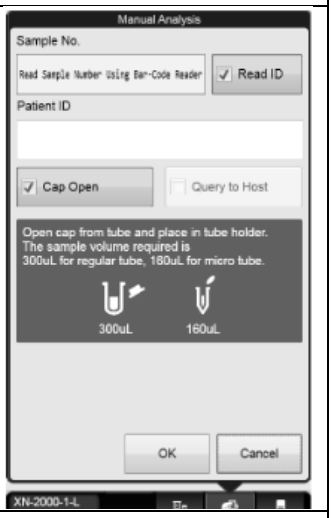
Cell count using Sysmex XN analyzer, Continued

9	Input the Sample ID in the Patient ID field or select **[READ ID]** to read the barcode.	
10	If sample tube is uncapped, check the **[CAP OPEN]** box**.** If sample tube is capped ensure the **[CAP OPEN]** box is unchecked	
11	Click **[OK]** and press the start switch (Blue Button). **Perform AUTORINSE between sample runs.**	
12	Cancel the order in instrument middleware.	
13	Manually input TNC, RNC result in Cerner with a comment "Results obtained using Sysmex XN".	
14	If dilution is needed use DCL Cellpack as diluent. Run DCL as patient to ensure that DCL is not contaminated and manually calculate the final result after multiplying with the dilution factor.	
15	Keep copy of XN print out and any dilution workout in the manual or abdominal fluid binder.	

Lower Limit of Detection by Sysmex XN

Perform the cell count manually whenever:

Body Fluid Total Count result is $\leq 0.003 \times 10^3$ µL (≤3 µL), and/or
Body Fluid RBC result is $< 0.002 \times 10^6$ µL (<2000 µL)

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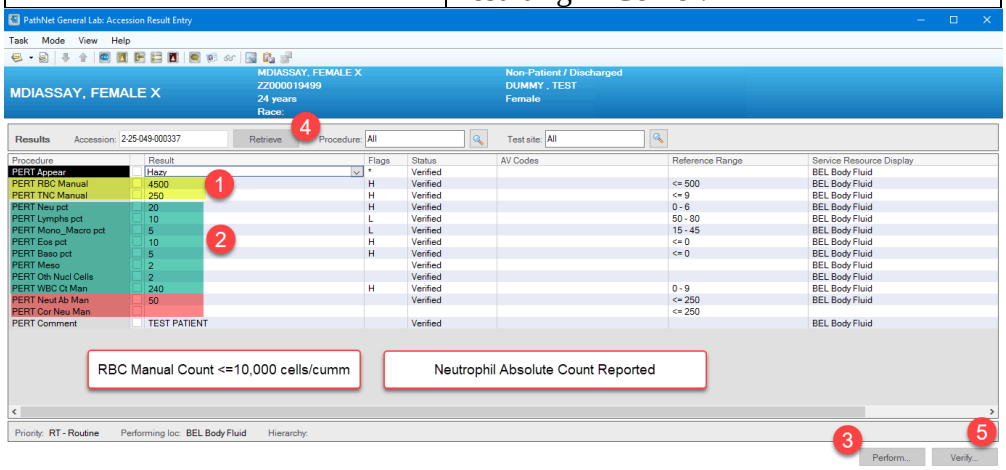
PMN correction for peritoneal, ascites and abdominal fluids

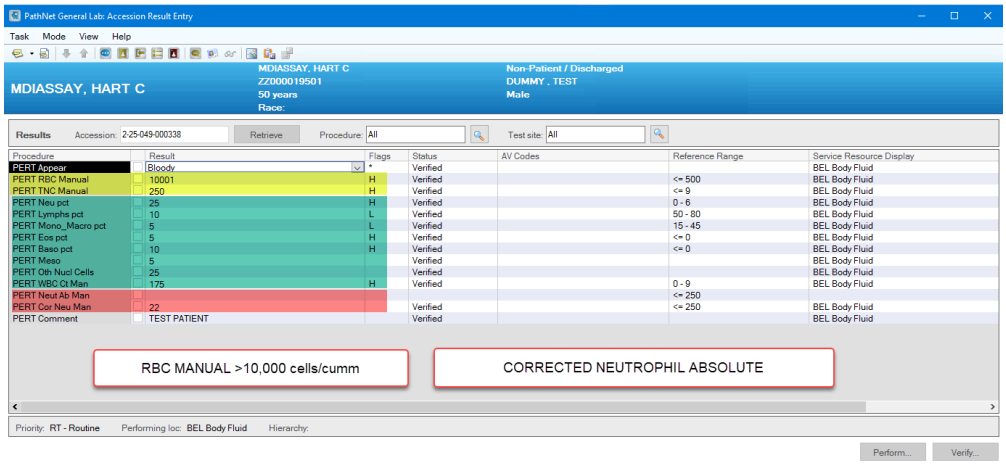
A corrected absolute neutrophil count (PMN) is applied to peritoneal, ascites and abdominal fluids when the RBC count exceeds 10,000 cells/mm^3. *A corrected PMN count greater than 250 cells/mm^3 is diagnostic of Spontaneous Bacterial Peritonitis.*

Follow these steps in Cerner for peritoneal, ascites and abdominal fluids:

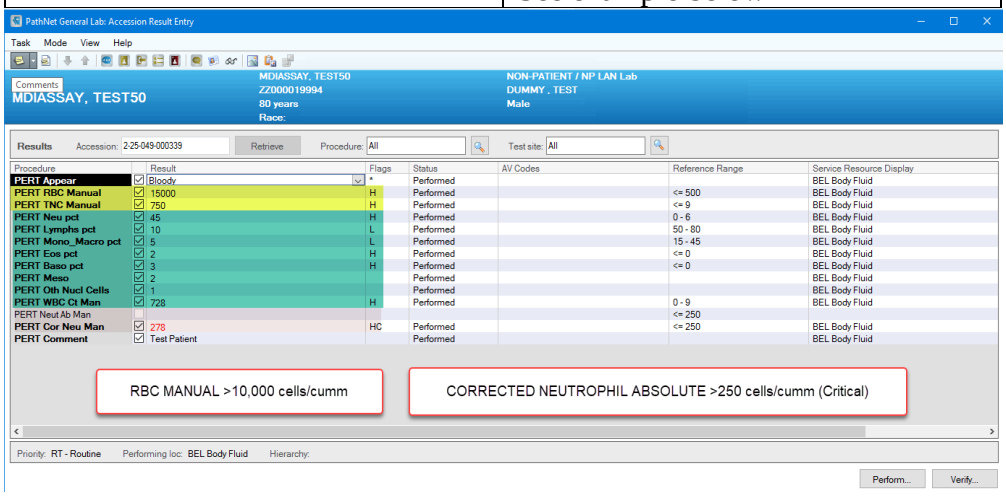
If	Then
RBC count <=10,000 cells/mm^3	Report the neutrophil absolute count.
	Follow the numbered steps below for resulting in Cerner.



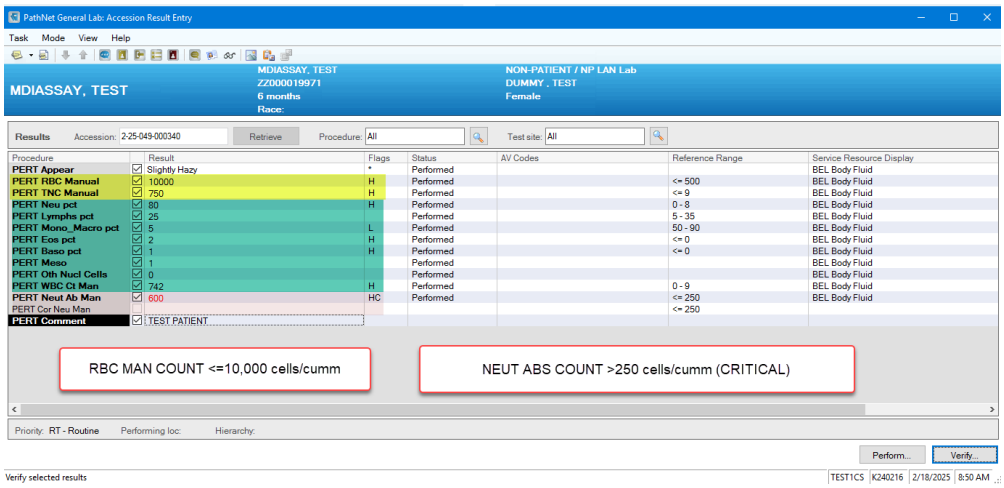
If	Then
RBC count >10,000 cells/mm^3	Report the corrected neutrophil absolute count. Equation applied in Cerner: Corrected Neut Abs Count = TNC * (Neut pct /100) – (RBC Count / 250)
	See example of Cerner corrected result below (second result field in red)



If	Then
RBC count >10,000 cells/mm^3 and the corrected neutrophil absolute count exceeds 250 cells/mm^3	Critical flagging is triggered in Cerner
	See example below



If	Then
RBC count <=10,000 cells/mm^3 and the corrected neutrophil absolute count exceeds 250 cells/mm^3	Critical flagging is triggered in Cerner
	See example below



Calculations for peritoneal, ascites and abdominal fluid PMN correction

- RBC count <=10,000 cells/mm^3
  Neutrophil Abs Man = TNC Man Count * (Neutrophils pct/100)

- RBC count >10,000 cells/mm^3
  Corrected Neut Abs Count = TNC * (Neut pct /100) – (RBC Count / 250)

Alert Values

Corrected neutrophil absolute count exceeds 250 cells/mm^3

Controlled Documents

The following controlled documents support this procedure.

SCPMG-PPP-0452 Body Fluid Cell Count Using Sysmex XN and WAM Middleware.

Non-Controlled Documents

The following non-controlled document support this procedure.

CLSI. Body Fluid Analysis for Cellular Compositions; Approved Guideline. CLSI document H56-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.

Author(s)

Hematology-Urinalysis Working Group