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| Purpose | The purpose of this procedure is to provide instructions in performing Manual and Automated Body Fluid Cell Count (Red Blood Cells and Total Nucleated Cells) and Differential Count. |
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| Workplace Safety |  |

 | All laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, the safety of others and adhering to all departmental and medical center safety policies and procedures.* For standard precautions and safety practices in the laboratory; see **Safety Practices**, specifically, but not limited to, equipment safety, proper body mechanics, sharps exposure and proper use of personal protective equipment (PPE).
* For Universal Body Substance precautions, see **Universal Body Substance Precautions**, specifically, but not limited to, exposure to body fluids.
* For proper hand-washing, see **Hand washing Policy**, specifically, not limited to, proper hand-washing.
* For proper infection control, see **Infection Control**, specifically, but not limited to, proper use of gloves.
* For proper handling of regular and infectious waste, see **Handling of Regular and Infectious Waste**, specifically, but not limited to, proper disposal of regular and biohazardous waste.
* For proper cleaning of work area, see **Cleaning Work Areas**.
* For proper handling of chemicals and reagents, see the Chemical Hygiene Plan.
* For proper storage and disposal of chemical hazardous waste, see **Storage & Disposal of Chemical Hazardous Waste**. All laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, the safety of others and adhering to all departmental and medical center safety policies and procedures.
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| Specimen type | The following types of specimens are considered to be body fluids:* CSF
* Pleural fluid
* Peritoneal fluid
* Gastric fluid
* Synovial fluid
* Pericardial fluid
* Abdominal fluid
* Ascites fluid
* Thoracentesis fluid

Bronchial Lavage/Washing cell counts will follow the same procedure as body fluid cell counts. |

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| Specimen stability and storage | * STAT CBC and Body Fluids must be completed within 1 hour after received in the laboratory.
* Routine CSF and Body Fluids must be completed within 3 hours from time of collection.

Refrigerate samples after testing. |

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| Specimen collection | Fluid specimens for cell counts are collected in EDTA or sterile container.Note: CSF specimens are collected in sterile containers onlyNote: Observe specimen for fibrin clots and pellicle formation. If a small fibrin clots are detected, perform test as usual and include a comment in report stating that results may not be accurate due to fibrin clot formation.If specimen is completely clotted do not perform the cell count. Notify unit or provide that the specimen is clotted.Note: Always use sterile technique when handling body fluid specimens. |

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| Quality Control | See procedure **LAMC-PPP-0304 Fluid Quality Control Cell Count** |

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| Completing Manual cell count using Incyto C-chip | Follow the steps below to complete the manual cell count for RBCs and WBCs on body fluids. |

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| Step | Action |
| 1 | Mix specimen well. Use the Incyto C-chip disposable counting chamber.  Load both chambers of the hemacytometer, with undiluted body fluid specimen by dispensing 10uL of sample using a 10uL calibrated pipet per chamber. Note: On high counts a dilution may be necessary. Place hemacytometer in A covered Petri dish with a damp paper and two small applicator sticks. Let equilibrate for five minutes before counting. |
| 2 | Verify that QC is done before performing patient testing. If not perform QC in same manner as patient testing and document the results in the QC log. Refer to Fluid Quality Control Procedure (**LAMC-PPP-0304**) for detailed information about QC procedure. Verify that lines in all counting chambers or optical grids are bright and free from scratches, dirt, or debris. |
| 3 | * 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.
* Cell count will be performed in duplicate using 40x magnification. Typically, 9 large squares are counted on each side of the chamber. For very high counts, less squares may be counted, however, equal number of corresponding squares must be counted on both sides of the chamber. Follow the No cell counting border policy.
 |
| 4 | CLS will calculate per chamber using the following:chamber Large Square (W) = 1 mm3 Small Square (R) = 0.04 mm3 for one small square  = 0.2 mm3 for all 5 small squares Formula:**Total cells/ μl** = Total Average Count x 10 cells/μl  # of square mm counted OR**Large Square (W)** (TNCAVE\*DIL FACTOR)/(number of squares\*0.1) **Small Square (R)** (RBCAVE\*DIL FACTOR)\*10/(number of squares\*0.04)  |
| 5 | The calculated counts between the two chambers will be averaged as the final result but must agree either* within 20% difference **if averaged result is >10 cells/ul**
* within +/- 2 cells/ul from the average result **if average result is ≤10 cells/ul**

…or the count must be repeated. Formula for % difference:*% Difference is the difference between the 2 counts divided by the Average of 2 counts then multiplied by 100.* |
| 6 | After the count is completed, dispose disposable hemacytometer. |

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| Performing cell count that needs dilution | In performing cell count using a hemacytometer, cells when checked on a microscope should be on a monolayer spread to have an accurate count. High counts that tend to make cells on top of each other should be diluted. Follow steps in performing cell count with dilution. |
| Step | Action |
| 1 | Diluent to be used for Cell count body fluid will be an aliquot from the Sysmex diluent Cellpack DCL ref. DCL-300A. |
| 2 | Perform diluent background check on diluent fluid by aliquoting diluent sample from diluent container and performing BF cell count mode(manual) using Sysmex. |
| 3 | Results should all be zero to make sure diluent can be used for cell count dilution. **Record diluent background result in the DCL Diluent Background Check Log.** |
| 4 | After passing the diluent background check, use the DCL diluent fluid aliquot to perform dilution analysis for Body Fluid Cell count. Use the lowest dilution factor possible to create a monolayer of cells on hemacytometer when checked on a microscope. |
| 5 | Mix diluted specimen well. Use either disposable counting chamber or reusable one. (Note: if using re-usable counting chamber cover slip the hemacytometer).  Load both chambers of the hemacytometer, with undiluted body fluid specimen by dispensing 10uL of sample using a 10uL calibrated pipet per chamber. Note: On high counts a dilution may be necessary. Place hemacytometer in A covered Petri dish with a damp paper and two small applicator sticks. Let equilibrate for five minutes before counting |
| 6 | Verify that QC is done before performing patient testing. If not perform QC in same manner as patient testing and document the results in the QC log. Refer to Fluid Quality Control Procedure (**LAMC-PPP-0304**) for detailed information about QC procedure. Verify that lines in all counting chambers or optical grids are bright and free from scratches, dirt, or debris. |
| 7 | Cell count will be performed in duplicate using 40x magnification. Typically 9 large squares are counted on each side of the chamber. For very high counts, less squares may be counted, however, equal number of corresponding squares must be counted on both sides of the chamber. Follow the No cell counting border policy. |
| 8 | CLS will calculate per chamber using the following:chamber Large Square (W) = 1 mm2 Small Square (R) = 0.04 mm2 for one small square  = 0.2 mm2 for all 5 small squares Formula:**Total cells/ μl** = Total Average Count x 10 cells/μl x **dilution factor**  # of square mm counted |
| 9 | The calculated counts between the two chambers will be averaged as the final result but must agree either* within 20% difference **if averaged result is >10 cells/ul**
* within +/- 2 cells/ul from the average result **if average result is ≤10 cells/ul**

…or the count must be repeated. Formula for % difference:*% Difference is the difference between the 2 counts divided by the Average of 2 counts then multiplied by 100.* |
| 10 | After the count is completed, dispose disposable hemacytometer. |

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| Verification of Cell count | Before resulting cell counts performed with unstained brightfield microscopy, verify results in correlation with the number and proportion of cells with the stained cytospin prepared slide used for differential count. Check box confirming that correlation was performed in the Body Fluid Patient Log form (Attachment A). For slide preparation see section ***preparing slide***. |

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| Preparing slide | Prepare a cytospin smear for differential count following Cytospin smear preparation using Thermo Scientific Cytospin 4 with cytocentrifuge rotor, procedure **LAMC-PPP-0310**. Dilute fluids with high cell counts with saline. Add a drop of albumin into the chamber. Once smear is ready let the smear air dry, fix and stain with Wright stain. |

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| Performing differential | Follow the steps below to complete the differential count on body fluids. |
| Step | Action |
| 1 | Count 100 WBCs , perform a 7 part differential * + Segs pct
	+ Lymphs pct
	+ Mono/Macro pct (include macrophage in the count)
	+ Eos pct
	+ Baso pct
	+ Mesothelial cells
	+ Oth nucleated cells(e.g. abnormal cells)
 |
| 2 | If less than 100 WBCs are present for differential count, calculate count to percent of the total number of WBCs differentiated. Note the **total number of cells differentiated** in the comment field. |
| 3 | Look for and note any abnormal cells and include the count under Oth nucleated cells field. |
| 4 | Slides will be saved for one week. |

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| Automated Cell count using Sysmex XN analyzer | **Fluid Types allowed to be performed in XN:**1. **Cerebrospinal Fluid (CSF)-** anticoagulant not required nor recommended
2. **Serous Fluids (Peritoneal and Pleural)-** collected in EDTA-K2
3. **Synovial/Joint fluid-** collected in EDTA-K2 with added hyaluronidase(a dab on applicator stick0 to break up mucous

**Note: Pericardial fluid, bronchoalveolar lavage, and amniotic fluid are not approved for analysis on the XN-series analyzers.**Required sample volume: 1.0 mL or more.Minimum sample volume:* Open tube: 300 uL
* Open microtainer tube: 160 uL

Aspirated sample volume: approximately 88 uLBefore you begin:* Inspect specimens for clots, ensuring specimens are properly mixed.
* Results may be compromised with improper mixing, cellular debris, or clotted specimens.
* Clotted and highly viscous specimens must not be run automated due to the mucous material that could clog up the instrument, causing erroneous or misleading results.

Follow the steps below to use the Sysmex XN analyzer for specimen analysis.

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| Step | Action |
| 1 | Check the status of the analyzer. Check the Status indicator LED on theanalyzer to confirm analyzer is in **READY**state. |
| 2 | Press the mode switch to eject the tubeholder.  |
| 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 thediluent 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 103 / μL or lessRBC-BF 0.003 x 106 / μL or less |

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| 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 thetube all the way in, so that the bottom of the tube contactsthe 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, Continue**Lower Limit of Detection by Sysmex XN** |

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| 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 WAM  |
| 13 | * Manually input TNC, RNC result in Cerner under TNC Auto or RNC Auto respectively.
* For fluid types like **peritoneal, abdominal, and ascites** fluid without the Auto field, include with a result 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.  |

**Perform the Cell count manually whenever:**TC-BF RESULT OF ≤ 0.003 x 103 μL, and/orRBC-BF result of < 0.002 x 106 μL(< 2000 μL) |
| Instrument Ranges | Display range is the range over which the analyzer will report, display, print and transmit results.Body Fluids maybe diluted using Cellpack DCL.

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| Parameter | Analytical Measurement Range | Display Range | Units |
| WBC-BF | 0.003 to 10.000 | 0.000 TO 999.999 | x 103 μL |
| RBC-BF | 0.002 to 5.000 | 0.000 TO 999.999 | x 106 μL |
| TC-BF# | 0.003 to 10.000 | 0.000 TO 999.999 | x 103 μL |

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| Resulting count | Follow the steps below to complete the reporting of body fluid counts.

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| Step | Action |
| 1 | Use the Body Fluid Results Log to show raw counts and calculations. See Attachment A(Body Fluid Results Log) |
| 2 | Results to be entered are:* Tot vol.(CSF only):
* Xanthochromia (CSF only): Yes or No
	+ Xanthochromia is a yellow, orange, or pink discoloration of cerebrospinal fluid (CSF). It's caused by the breakdown of hemoglobin in red blood cells, which releases bilirubin into the CSF
	+ For bloody CSF, confirm xanthochromia by spinning the sample at 3000rpm for 5 min. after the cell count has been completed.
* Color (CSF only): Colorless, Yellow, Pink, Red,
* Appearance: Bloody, Cloudy, Clear, Clotted, Hazy, Slightly Hazy
* RBC(cells/mm3) count Manual
* TNC(cells/mm3) count Manual
* Differential
	+ Segs pct
	+ Lymph pct
	+ Mono/Macro pct (include macrophage in the count)
	+ Eos pct
	+ Baso pct
	+ Mesothelial cells
	+ Oth 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. See **Performing differential section above.**
 |
| 3 | Final Results are manually entered in Cerner through Accession Result Entry. Click perform and review results entered then verify results. |

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Accession result entry



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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/mm3. *A corrected PMN count greater than 250 cells/mm3 is diagnostic of Spontaneous Bacterial Peritonitis.*Follow these steps in Cerner for **peritoneal, ascites and abdominal fluids**:

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| If | Then |
| RBC count <=10,000 cells/mm3 | Report the neutrophil absolute count. |
|  | Follow the numbered steps below for resulting in Cerner.  |

A screenshot of a computer  AI-generated content may be incorrect.

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| If | Then |
| RBC count >10,000 cells/mm3 | 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) |

A screenshot of a computer  AI-generated content may be incorrect.

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| If | Then |
| RBC count >10,000 cells/mm3 and the corrected neutrophil absolute count exceeds 250 cells/mm3 | Critical flagging is triggered in Cerner |
|  | See example below |

A screenshot of a computer  AI-generated content may be incorrect.

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| If | Then |
| RBC count <=10,000 cells/mm3 and the corrected neutrophil absolute count exceeds 250 cells/mm3 | Critical flagging is triggered in Cerner |
|  | See example below |

A screenshot of a computer  AI-generated content may be incorrect. |

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| Calculations for peritoneal, ascites and abdominal fluid PMN correction | * RBC count <=10,000 cells/mm3

Neutrophil Abs Man = TNC Man Count \* (Neutrophils pct/100)* RBC count >10,000 cells/mm3

Corrected Neut Abs Count = TNC \* (Neut pct /100) – (RBC Count / 250) |

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| Alert Values | Corrected neutrophil absolute count exceeds 250 cells/mm3 |

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| Controlled Documents |

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| --- | --- |
| **Document Number** | **Document Name** |
| LAMC-PPP-0123 | Safety Practices |
| LAMC-PPP-0127 | Infection Control |
| LAMC-PPP-0128 | Universal Body Substance Precaution |
| LAMC-PPP-0129 | Handling of Regular and Infectious Waste |
| LAMC-PPP-0130 | Cleaning Work Areas |
| LAMC-PPP-0132 | Hand-washing Policy |
| LAMC-PPP-0134 | Storage and Disposal of Chemical Hazardous Waste |
| LAMC-PPP-0310 | Cytospin smear preparation using Thermo Scientific Cytospin 4 |
| LAMC-PPP-0304 | Fluid Quality Control Procedure |
| Attachment A | Body Fluid Log Form |
| Attachment R1 | Pathologist Review for Suspicious cells Log |
|  | Diluent Background Check procedure |

See below the list of controlled documents |
| Author(s) | Alvin Castillo |

**ATTCHMENT A**



**ATTACHMENT R1**

