



# KAISER PERMANENTE®

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<b>AUTHOR:</b>	<b>PREVIOUS NUMBER:</b>
<b>OWNER:</b>	<b>CHANGE NUMBER:</b>

## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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<b>Purpose</b>	This procedure describes how to do a body fluid cell count using the SYSMEX XN hematology analyzer and WAM 5.0 middleware.
<b>Scope</b>	This procedure is intended for the use of Clinical Laboratory Scientist (CLS) that will use the SYSMEX XN analyzer to perform an automated body fluid cell count and other laboratory personnel who may need to review the assay as part of quality management.
<b>Principle</b>	Fluorescent flow cytometry using side scattered light and side fluorescent are used to determine WBC counts. The direct current detection method is used for the RBC counts.
<b>Specimen sources</b>	Acceptable Body Fluid Types are: <ol style="list-style-type: none"><li>1. Cerebrospinal Fluid (CSF) – The use of anticoagulant is not required nor recommended.</li><li>2. Serous Fluids (Peritoneal and Pleural) – Collected in EDTA-K<sub>2</sub> anticoagulant.</li><li>3. Synovial/Joint Fluid – Collected in EDTA-K<sub>2</sub> anticoagulant with added hyaluronidase (a dab on applicator stick) to break up mucous.</li></ol> <p>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 µL.</p>
<b>Specimen stability</b>	Body fluid specimens should be analyzed as soon as possible. The longer the delay, the more likely are elements to lyse and deteriorate.
<b>Equipment</b>	Sysmex® XN™ Analyzer

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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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**Reagents** Sysmex XN Reagents  
Sysmex® DCL CELLPACK

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**Materials and supplies** The following contains the list of materials and supplies required.

12x75 mm Tubes  
Microtainers  
Calibrated Pipettes for dilutions  
Hyaluronidase lyophilized powder, 400-1000 units/mg, 100 mg pack

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**Safety Precautions** Refer to the safety manual for general safety requirements.

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**Quality Control** XN CHECK BF – Automated Body Fluid Controls

- XN CHECK BF control levels: **All levels** will be run at least once daily on each XN instrument in the Manual BF mode.
- Results must be recorded and reviewed for acceptability prior to testing patient specimens.
- Follow local facility protocols if any.

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**Before 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.

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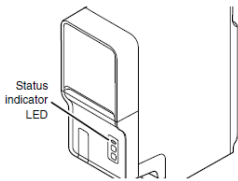
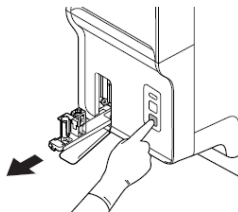
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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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### Procedure – Analysis Preparation

Follow the steps below to prepare the analyzer for body fluid specimen analysis.

Step	Action						
1	<p>Check the status of the analyzer.                      Check the Status indicator LED on the analyzer to confirm analyzer is in <b>READY</b> state.</p> 						
2	<p>Press the mode switch to eject the tube holder.</p> 						
3	Select the Change Analysis Mode button on the control menu.						
4	Select analysis mode [ <b>BODY FLUID</b> ], then select [ <b>OK</b> ]						
5	<p>Analyzer automatically performs a Background Check on the diluent fluid and lysing agent to check for contamination that will affect cell counts.</p> <p><b>Note:</b></p> <ul style="list-style-type: none"> <li>The analyzer will automatically perform a background check up to three times (3X) to achieve an acceptable background check value.</li> <li>When performing a specimen dilution, first run the DCL (diluent) as a sample to verify that there are no contaminants in the DCL. Enter the name of the aspirate sample as “SALINE”. Print the result and attach to the specimen result printout.</li> </ul>						
6	<p>Ensure <b>Background Check</b> passes, then proceed to sample or QC analysis.</p> <p>Acceptable Background Limits are as follows:</p> <table border="1"> <thead> <tr> <th>Checked Parameter</th> <th>Acceptable Value</th> </tr> </thead> <tbody> <tr> <td>WBC-BF</td> <td>0.001 x 10<sup>3</sup> / µL or less</td> </tr> <tr> <td>RBC-BF</td> <td>0.003 x 10<sup>6</sup> / µL or less</td> </tr> </tbody> </table>	Checked Parameter	Acceptable Value	WBC-BF	0.001 x 10 <sup>3</sup> / µL or less	RBC-BF	0.003 x 10 <sup>6</sup> / µL or less
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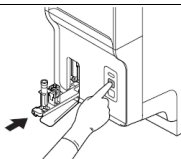
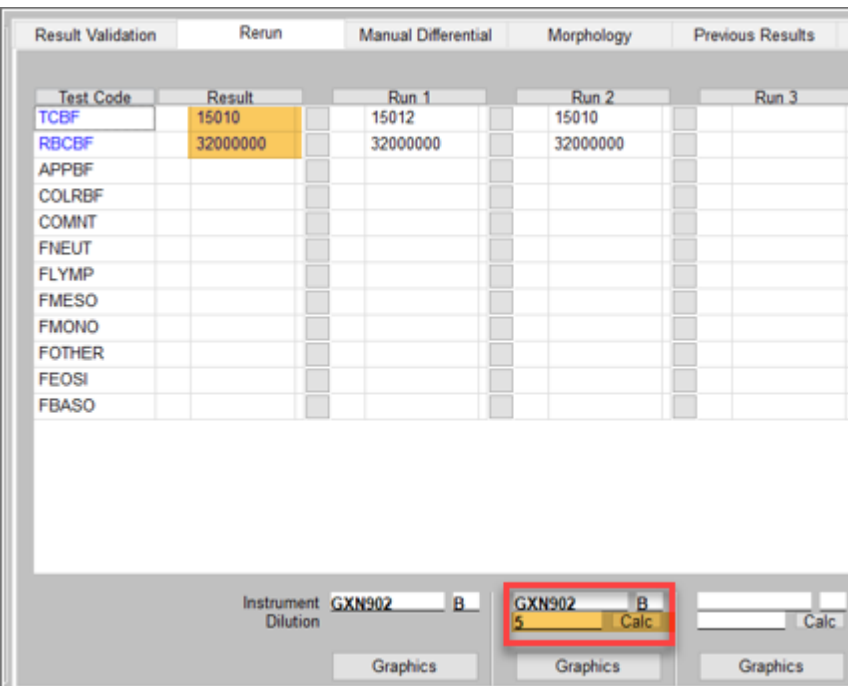
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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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### Procedure – Body Fluid Specimen Analysis

Follow the steps below to perform cell count using the Sysmex XN analyzer.


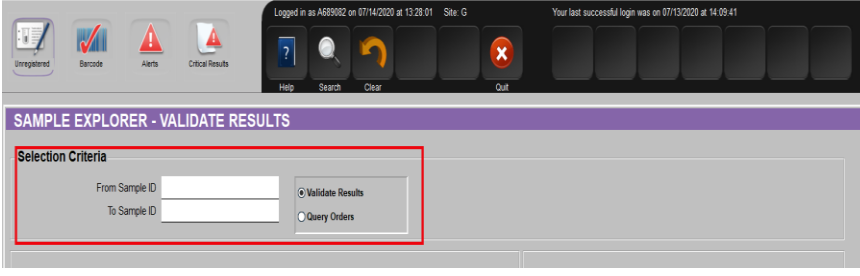

Step	Action
1	Place a well-mixed patient body fluid in a vial with the correct sample barcode for analysis in the sample tube holder.
2	Click the <b>[Manual Analysis]</b> button in the analyzer area.
3	Input the Sample ID or select <b>[READ ID]</b> to read the barcode.
4	If sample tube is uncapped, check the <b>[CAP OPEN]</b> box. If sample tube is capped ensure the <b>[CAP OPEN]</b> box is unchecked.
5	Click <b>[OK]</b> and press the start switch (Blue Button).  <b>Perform AUTORINSE between sample runs.</b> 
6	Verify that the body fluid result is acceptable and there are no flagging present such as “@”, *, etc. <ul style="list-style-type: none"> <li>• If linearity flag “@” is present, perform an offline dilution, and rerun the sample.</li> <li>• The dilution factor can be applied in WAM under run #2 in the rerun tab after the assay is done by the instrument.</li> </ul> 
7	Report TCBF and RCBF, Appearance, Color and Differential in WAM Middleware.

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# Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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## WAM Middleware Reporting

Step	Action
<b>Automated Cell Count</b>	
1	From the Sysmex WAM Main Menu screen, select the <b>Sample Explorer</b> icon. 
2	In the <b>Selection Criteria</b> screen, enter the Sample ID in the <b>“From Sample ID”</b> field to retrieve the Sample ID that require manual validation. 
3	Click <b>[SEARCH]</b> icon to retrieve the Sample ID 
4	The <b>Result Validation</b> screen will display the searched Sample ID and review of results.

## Result Validation Screen

**RESULT VALIDATION**

<b>Selection Criteria</b>	<b>Patient Demographics</b>	<b>OP Alerts</b>
	Sample ID: 220114000026A MRN: ZZ000007325 Name: MDIASSAY,HBM DOB/Age/Sex: 06/02/2000 20 Years Male Collection d/t: 04/23/2020 08:23:00 Receipt d/t: 04/23/2020 08:23:26 Sample Loc: ...	Run Description No Operator Alerts
	Diagnosis 1: _____ Diagnosis 2: _____ Req. Location: NP,HBM LAB Req. Phys.: _____ Req. Name: DUMMY,TEST Req. Phone: _____ Care Unit: NP,HBM LAB Room Number: _____	

<b>Samples</b>	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th colspan="2">Instrument</th> <th colspan="2">Manual</th> </tr> <tr> <th>Instr</th> <th>Result</th> <th>Count 1</th> <th>Count 2</th> </tr> </thead> <tbody> <tr> <td><input type="checkbox"/> RBCBF</td> <td></td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> TNCFBM</td> <td></td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> BFVOL</td> <td></td> <td></td> <td>2</td> </tr> <tr> <td><input type="checkbox"/> APPBF</td> <td></td> <td></td> <td>#CLR</td> </tr> <tr> <td><input type="checkbox"/> COLRBF</td> <td></td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> XANTHR</td> <td></td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> TNCAVE</td> <td></td> <td></td> <td>1.5</td> </tr> </tbody> </table>	Instrument		Manual		Instr	Result	Count 1	Count 2	<input type="checkbox"/> RBCBF				<input type="checkbox"/> TNCFBM				<input type="checkbox"/> BFVOL			2	<input type="checkbox"/> APPBF			#CLR	<input type="checkbox"/> COLRBF				<input type="checkbox"/> XANTHR				<input type="checkbox"/> TNCAVE			1.5	<b>Flags</b> No Flags to report for any Run  Images for Run 1
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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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### WAM Middleware Reporting

Test Code	Interpretation
<b>TCBF</b>	<b>TOTAL NUCLEATED BODY FLUID</b> Total Nucleated Count result from automated BF analysis
<b>RBCBF</b>	<b>RED BLOOD CELL BODY FLUID</b> Total RBC Count result from automated BF analysis
<b>BFVOL</b>	<b>BODY FLUID TOTAL VOLUME</b> Manually enter BF total volume in mL, if applicable
<b>APPBF</b>	<b>BODY FLUID APPEARANCE</b> Manually enter BF appearance, double click on the field for choices. <ul style="list-style-type: none"> <li>➤ BLOODY</li> <li>➤ CLEAR</li> <li>➤ CLOUDY</li> <li>➤ CLOTTED</li> <li>➤ HAZY</li> <li>➤ SLIGHT HAZY</li> </ul>
<b>COLBF</b>	<b>BODY FLUID COLOR</b> Manually enter BF color, double click on the field for choices <ul style="list-style-type: none"> <li>➤ COLORLESS</li> <li>➤ YELLOW</li> <li>➤ PINK</li> <li>➤ RED</li> </ul>
<b>XANTHR</b>	<b>XANTHOCHROMIA (If indicated)</b> Manually enter Xanthochromia response <ul style="list-style-type: none"> <li>➤ YES</li> <li>➤ NO</li> </ul>

Lower Limit of  
 Detection by  
 Sysmex XN

**Perform the Cell Count manually whenever:**

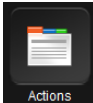
TC-BF result of  $\leq 0.003 \times 10^3 \mu\text{L}$  ( $\leq 3 \mu\text{L}$ ), AND/OR  
 RBC-BF result of  $< 0.002 \times 10^6 \mu\text{L}$  ( $< 2000 \mu\text{L}$ )

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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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### WAM Middleware Reporting


<b>Manual Cell Count</b>									
5	<p>Perform manual cell count, if necessary, otherwise proceed to step 7.</p> <ul style="list-style-type: none"> <li>➤ Click on the [Action]  icon</li> <li>➤ Select by clicking on the selection box                             <table border="1" style="margin-left: 20px;"> <tr> <td style="text-align: center;"><b>TNCBFM</b></td> <td>For manual total nucleated count (hemocytometer count)</td> </tr> <tr> <td style="text-align: center;"><b>RBCBFM</b></td> <td>For manual total red blood cell count (hemocytometer count)</td> </tr> </table> </li> <li>➤ Select the [ADD] button. This will add additional result field in the <b>Result Validation</b> screen.</li> </ul>	<b>TNCBFM</b>	For manual total nucleated count (hemocytometer count)	<b>RBCBFM</b>	For manual total red blood cell count (hemocytometer count)				
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6	<p>Manually enter the hemocytometer result in the following result field.</p> <table border="1" style="width: 100%;"> <thead> <tr> <th style="text-align: center;">Test Code</th> <th style="text-align: center;">Interpretation</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;"><b>TNCCALC/ RBCCALC</b></td> <td> <b>TOTAL NUCLEATED / RED BLOOD HEMOCYTOMETER CHAMBER SQUARES COUNTED</b>                       Select which square counted on the hemocytometer                     <ul style="list-style-type: none"> <li>➤ Large</li> <li>➤ Small</li> </ul> </td> </tr> <tr> <td style="text-align: center;"><b>TNCSD1/ RBCSD1</b></td> <td> <b>RAW COUNT ON ONE CHAMBER OF THE HEMOCYTOMER</b>                       Manually enter the TNC or RBC raw count of one chamber of the hemocytometer.                 </td> </tr> <tr> <td style="text-align: center;"><b>TNCSD2/ RBCSD2</b></td> <td> <b>RAW COUNT ON THE OTHER CHAMBER OF THE HEMOCYTOMER</b>                       Manually enter the TNC or RBC raw count of the other chamber of the hemocytometer.   <b>NOTE:</b>                      CLS must verify that the counts from each chamber must agree within 10% or the count must be repeated. Multiply 10% against the bigger count value obtained.                       The difference between the two chamber counts must be less than the product obtained.                 </td> </tr> </tbody> </table>	Test Code	Interpretation	<b>TNCCALC/ RBCCALC</b>	<b>TOTAL NUCLEATED / RED BLOOD HEMOCYTOMETER CHAMBER SQUARES COUNTED</b>  Select which square counted on the hemocytometer <ul style="list-style-type: none"> <li>➤ Large</li> <li>➤ Small</li> </ul>	<b>TNCSD1/ RBCSD1</b>	<b>RAW COUNT ON ONE CHAMBER OF THE HEMOCYTOMER</b>  Manually enter the TNC or RBC raw count of one chamber of the hemocytometer.	<b>TNCSD2/ RBCSD2</b>	<b>RAW COUNT ON THE OTHER CHAMBER OF THE HEMOCYTOMER</b>  Manually enter the TNC or RBC raw count of the other chamber of the hemocytometer.  <b>NOTE:</b> CLS must verify that the counts from each chamber must agree within 10% or the count must be repeated. Multiply 10% against the bigger count value obtained.  The difference between the two chamber counts must be less than the product obtained.
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<b>TNCSD2/ RBCSD2</b>	<b>RAW COUNT ON THE OTHER CHAMBER OF THE HEMOCYTOMER</b>  Manually enter the TNC or RBC raw count of the other chamber of the hemocytometer.  <b>NOTE:</b> CLS must verify that the counts from each chamber must agree within 10% or the count must be repeated. Multiply 10% against the bigger count value obtained.  The difference between the two chamber counts must be less than the product obtained.								

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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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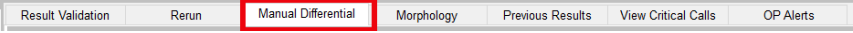


<p><b>TNCAVE/ RBCAVE</b></p>	<p><b>AVERAGE COUNT OF THE TWO CHAMBERS OF THE HEMOCYTOMETER</b></p> <p>Calculated TNC/RBC average for both raw counts.</p> <p><b>WAM will automatically calculate after CLS enters responses on the:</b></p> <ul style="list-style-type: none"> <li>- TNCSD1 &amp; TNCSD2 for TNCAVE;</li> <li>- RBCSD1 &amp; RBCSD2 for RBCAVE.</li> </ul>
<p><b>TNCDIL/ RBCDIL</b></p>	<p><b>DILUTION FACTOR</b></p> <p>Manually enter the dilution factor.</p> <p>Enter 1 if no dilution was performed.</p>
<p><b>TNCSQ/ RBCSQ</b></p>	<p><b>NUMBER OF SQUARES COUNTED IN EACH CHAMBER OF THE HEMOCYTOMETER</b></p> <p>Manually enter the number of squares counted.</p>
<p><b>TNCBFM/ RBCBFM</b></p>	<p><b>TOTAL NUCLEATED / RED BLOOD CELL MANUAL COUNT</b></p> <p>Calculated field for TNC/RBC.</p> <p><b>WAM will automatically calculate after CLS enters responses on the following:</b></p> <ul style="list-style-type: none"> <li>-TNCCALC, TNCSD1, TNCSD2 and TNCDIL for TNCBFM;</li> <li>-RBCCALC, RBCSD1, RBCSD2 and RBCDIL for RBCBFM.</li> </ul> <p>WAM Calculation Formula:</p> <ul style="list-style-type: none"> <li>A. If large squares were counted:  <math>(RBCAVE * RBCDIL) / (RBCSQ * 0.1)</math> for large squares; replace for TNC as applicable.</li> <li>B. If small squares were counted:  <math>(RBCAVE * RBCDIL) * 10 / (RBCSQ * 0.04)</math> for small squares; replace TNC as applicable.</li> </ul>
<p>Select the <b>[SAVE]</b> icon  to calculate all the automated calculations by WAM.</p>	

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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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### WAM Middleware Reporting

7	<p>Select the <b>[MANUAL DIFFERENTIAL]</b> tab to perform the BF differential.</p>  <p>In the Manual Differential screen, change the default <b>[MDIFF]</b> and select <b>[FDIFF]</b> in the drop-down menu from <b>[Select Interpretation]</b></p> 																
8	<p>Using the counter key, perform the Body Fluid Differential. Counting will automatically stop at 100 cell count.</p> <table border="1" data-bbox="781 785 1245 1150"> <thead> <tr> <th>TEST</th> <th>COUNTER KEY</th> </tr> </thead> <tbody> <tr> <td>FNEUT</td> <td><b>+</b></td> </tr> <tr> <td>FLYMPH</td> <td><b>6</b></td> </tr> <tr> <td>FMONO</td> <td><b>5</b></td> </tr> <tr> <td>FMESO</td> <td><b>4</b></td> </tr> <tr> <td>FEOSI</td> <td><b>7</b></td> </tr> <tr> <td>FBASO</td> <td><b>8</b></td> </tr> <tr> <td>FOTHER</td> <td><b>1</b></td> </tr> </tbody> </table>	TEST	COUNTER KEY	FNEUT	<b>+</b>	FLYMPH	<b>6</b>	FMONO	<b>5</b>	FMESO	<b>4</b>	FEOSI	<b>7</b>	FBASO	<b>8</b>	FOTHER	<b>1</b>
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FOTHER	<b>1</b>																
9	<p>Select the <b>[SAVE]</b> icon to save all the changes made.</p>																
10	<p>Go back to the result validation tab. All fields must be answered, including the "CMNT" field, for all results to transmit to Cerner LIS. Select <b>[Val All]</b> icon  to validate the responses.</p> <p><b>Note:</b> Only the manual diff count must be present; otherwise, if automated count is present, this will cross to Cerner LIS.</p>																

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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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### LIS Calculation

- WBC Cnt-BF is calculated by Cerner LIS, using the following equation:

- $$\text{WBC Cnt Auto} = \text{TNC Auto} - [\text{TNC Auto} \times (\text{Meso} + \text{Other Nucleated Cells})/100]$$

- If values from manual count were entered in WAM:

$$\text{WBC Cnt Man} = \text{TNC Manual} - [\text{TNC Manual} \times (\text{Meso} + \text{Other Nucleated Cells})/100]$$

### Instrument Ranges

- Display range is the range over which the analyzer will report, display, print and transmit results.
- Body fluids may be diluted offline using Cellpack DCL. See step #6 above of Body Fluid specimen analysis by Sysmex XN section of this procedure.

Parameter	Analytical Measurement Range	Display Range	Units
WBC-BF	0.003 to 10.000	0.000 to 999.999	x 10 <sup>3</sup> /uL
RBC-BF	0.002 to 5.000	0.000 to 999.999	x 10 <sup>6</sup> /uL
TC-BF#	0.003 to 10.000	0.000 to 999.999	x 10 <sup>3</sup> /uL

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## Body Fluid Cell Count Using Sysmex XN and WAM Middleware

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**Reference  
 Range**

• CSF

Color	Colorless
Appearance	Clear
TNC Count	0-5 cells/mm <sup>3</sup>
WBC Count	0-5 cells/mm <sup>3</sup>
RBC Count	0 cells/mm <sup>3</sup>

• Synovial Fluid

TNC Count	0-200 cells/mm <sup>3</sup>
WBC Count	0-200 cells/mm <sup>3</sup>
RBC Count	0-500 cells/mm <sup>3</sup>

• Pleural Fluid

Appearance	Clear
TNC Count	0-9 cells/mm <sup>3</sup>
WBC Count	0-9 cells/mm <sup>3</sup>
RBC Count	0-500 cells/mm <sup>3</sup>

• Other Body Fluid

Appearance	Clear
TNC Count	0-9 cells/mm <sup>3</sup>
WBC Count	0-9 cells/mm <sup>3</sup>
RBC Count	0-500 cells/mm <sup>3</sup>

**Non-Controlled  
 Document/s**

The following non-controlled document/s support this policy.

Sysmex XN-9000 Instructions for Use (North American Edition), Sysmex Corporation, Kobe, Japan.
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**Author(s)**

SCPMG Hematology Working Group  
 Marlon Esguerra

## Signature Manifest

**Document Number:** LAMC-PPP-1388

**Revision:** 01

**Title:** Body Fluid Cell Count Using Sysmex XN and WAM Middleware

**Effective Date:** 17 Jan 2022

All dates and times are in Pacific Standard Time.

### Hematology Regional Documents

#### Initial Approval

Name/Signature	Title	Date	Meaning/Reason
Jocelyn Javier (T684676)	Assist. ADA	07 Dec 2021, 11:02:25 AM	Approved

#### Operations Approval

Name/Signature	Title	Date	Meaning/Reason
Julie Toti (K084521)	DIR AREA LAB	05 Jan 2022, 06:32:15 PM	Approved

#### Final Approval

Name/Signature	Title	Date	Meaning/Reason
Hedyeh Shafi (I086749)	Pathologist	14 Jan 2022, 09:59:26 AM	Approved