Automated Body Fluid Analysis

Matthew Riley, MT (ASCP)
Technical Integration Specialist, Sysmex America Inc.

Objectives

- Discuss the advantages of the automated cell count over a manual count
- Review the meaning of analytical reference range and clinical reportable range
- Identify features of benign and malignant cells
Why Do We Fear Body Fluids?

Icky Specimens  Scary Cells

Manual Cell Counting

The Formula

cells/µL = \frac{\text{cells counted}}{\text{area} \times \text{depth} \times \text{dilution}}
Automated Cell Counting

Automated Counts Are Better

• Better Precision
• Easier to Perform
• Safer!!!
Rules to Follow

- Treat Synovial Fluids with Hyaluronidase
- Original Reference – Add 5mg to a 0.5 – 1ml aliquot.

SIGMA #H-3757
TYPE VIII BOVINE
STERILE
FILTERED

Rules to Follow

- It’s a Total Nucleated Count – NOT just a WBC count
  - Mesothelial Cells, Macrophages
- If there are clots or fibrin……… Take them out and report as approximate
  - You can still make a smear
Automated Cell Counts

- What’s Important?
  - Background counts
  - Scattergrams
  - AMR – Analytical Measurement Range
  - CCR – Clinical Reportable Range

Scattergrams

SYSMEX XE2100
Know When it is Good

And When it is Not
Things to Consider

• AMR
  – How low or high can the instrument measure

• CRR
  – How low or high can you report results

Terminology

• Linearity = Analytical Measurement Range (AMR)
  – Range of analyte values that a method can directly measure on the specimen without any dilution, or other pretreatment not part of the usual assay process
### AMR = LINEARITY

#### XE5000 LINEARITY - SYNOVIAL FLUID

<table>
<thead>
<tr>
<th>DILUTION</th>
<th>nucleated cells</th>
<th>XE5000</th>
<th>EXPECTED</th>
<th>XE5000</th>
<th>EXPECTED</th>
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<tbody>
<tr>
<td>0</td>
<td>5901</td>
<td>5901</td>
<td>1445000</td>
<td>1445000</td>
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<tr>
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<td>3015</td>
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<td>749000</td>
<td>722500</td>
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<td>4</td>
<td>1631</td>
<td>1507.5</td>
<td>384000</td>
<td>374500</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>809</td>
<td>815.5</td>
<td>191000</td>
<td>192000</td>
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</tr>
<tr>
<td>16</td>
<td>411</td>
<td>404.5</td>
<td>97000</td>
<td>95500</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>213</td>
<td>205.5</td>
<td>50000</td>
<td>48500</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>107</td>
<td>106.5</td>
<td>27000</td>
<td>25000</td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>56</td>
<td>53.5</td>
<td>14000</td>
<td>13500</td>
<td></td>
</tr>
<tr>
<td>256</td>
<td>29</td>
<td>28</td>
<td>7000</td>
<td>7000</td>
<td></td>
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<tr>
<td>512</td>
<td>11</td>
<td>14.5</td>
<td>4000</td>
<td>3500</td>
<td></td>
</tr>
<tr>
<td>1024</td>
<td>6</td>
<td>5.5</td>
<td>2000</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>2048</td>
<td>3</td>
<td>3</td>
<td>1000</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>4096</td>
<td>1</td>
<td>1.5</td>
<td>1000</td>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>

| CORR     | 1.0            |        | 1.0      |
| SLOPE    | 1.0            |        | 1.0      |

#### AMR - Linearity

![XE5000 LINEARITY - WBC](image)

$R^2 = 0.9996$
BUT!!

There are limits ……….

That MUST Be Taken Into Consideration

Things to Consider

• Does the method have limitations based on:
  – Linearity Limits
  – Precision or Reproducibility
  – Decimal Place limits
  – Carryover/Background
  – Clinical Decision Points
    YES
    YES
    YES
    YES
    POSSIBLY
Clinical Reportable Range (CRR)

• Clinical Reportable Range (CRR) - Analyte values that a method can report as a quantitative result, allowing for specimen dilution, concentration or other pre-treatment used to extend the AMR

• Establishment of the CRR is a medical judgment made by the Laboratory Medical Director, and is based in part on the assay technology.

• Values outside the CRR are reported as < or > a numeric value.

WBC-BF & TC-BF are Linear to Zero

- Expected: 0.001 x 10^3/µl
- Recovered: 0.000 x 10^3/µl

Not a Clinical Decision Point

0 cells/µl

1 cells/µl
**RBC-BF is Linear to Zero**

- **EXPECTED**: 0.000 x 10^6/µl
- Or
- 0 cells / µl

- **RECOVERED**: 0.001 x 10^6/µl
- Or
- 1000 cells/µl

Is This a Clinical Decision Point?

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**Clinically Reportable Range**

Example: RBC-BF

- **AMR** is 0.000 - 5.000 x 10^6/µl (method specification).
- **CRR** is 0.001 - 8.000 x 10^6/µl (clinical usefulness).
- Results of 0.000 are reported as “< 0.001 x 10^6/µl ”.
- Results >5.000 are diluted and rerun to obtain quantitative values up to 8.000 x 10^6/µl.
- Results >8.000 are reported as “>8.000 x 10^6/µl ”.

**NOTE:** CRR limits are the Judgment of the Lab Director and may be different for different labs.
### Clinical Significance of Body Fluids

<table>
<thead>
<tr>
<th>Body Fluid Type</th>
<th>WBC</th>
<th>RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>Adults: 0-5 mononuclear cells/μL. Children: 8-30 mononuclear cells/μL.*</td>
<td>Limited diagnostic value</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>&gt;500/mL</td>
<td>NA</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>WBC have limited value</td>
<td>&gt;10,000/μL</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>Up to 200/μL. Gp 1: Noninflammatory 200-3000/μL. Gp 2: Inflammatory 3000-75,000/μL. Gp 3: Infectious 50,000-200,000/μL. Gp 4: Crystal associated 500-200,000/μL.</td>
<td>NA</td>
</tr>
<tr>
<td>Lavage (Peritoneal)</td>
<td>&gt;500/mL</td>
<td>&gt;10,000/μL</td>
</tr>
</tbody>
</table>

*Children have intermediate leukocyte values, less than 20/μL the first year of life and less than 10/μL until adolescence.

Reasons for knowing RBC values in CSF

• Subarachnoid or intracranial bleeding
  – **RBC Count** unchanged between first and third tubes
  – Blood does not clot
  – Xanthochromia suggests bleeding
    • See CSF Color
    • CSF supernatant xanthochromic on centrifugation
    • Requires at least 2 to 4 hours from onset of bleed

• Traumatic tap
  – **RBC Count** decreases between first and third tubes
  – CSF becomes clear on centrifugation
  – Consider repeating tap at higher interspace

• References
  • Kooiker in Roberts (1998) Procedures in ER, p. 1067-75
  • Ravel (1995) Lab Medicine, Mosby, p. 294-9
  • Tunkel in Mandell (2000) Infectious Disease, p. 974-8
  • Seehusen (2003) Am Fam Physician 68:1103

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Sysmex Body Fluid Comparison Chart

<table>
<thead>
<tr>
<th></th>
<th>Sysmex XE-Series &amp; XT-Series</th>
<th>Sysmex XE-5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC-BF</td>
<td>N/A</td>
<td>0.000 – 10.000 x 10^3/µL*</td>
</tr>
<tr>
<td>TC-BF</td>
<td>&gt;= 0.050 x 10^3/µL</td>
<td>0.000 – 10.000 x 10^3/µL*</td>
</tr>
<tr>
<td>RBC</td>
<td>&gt;= 0.01 x 10^6/µL</td>
<td>0.000 – 5.000 x 10^6/µL**</td>
</tr>
</tbody>
</table>

* +/- 10 µl (0.000 – 0.050 x 10^3/µl) ; +/- 20% (0.050 – 10.000x 10^3/µl
** +/- 2% (0.000 – 5.000 x 10^6/µl
What’s Really Important

The Type of Cell Present

Necessary Tools

- CytoCentrifuge
Body Fluid Fears

• Problem Cells
  – Mesothelial Cells
  – Malignant Cells

How To Tell Them Apart

**Mesothelial**
• Individual
• Uniform
• Flat clusters
• N/C ratio low

**Malignant**
• Cannibalism
• Bizarre
• Ball-like clusters
• N/C ratio high
Mesothelial Cells

Malignant Cells
### How To Tell Them Apart

<table>
<thead>
<tr>
<th>Mesothelial</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Smooth chromatin</td>
<td>- Uneven chromatin</td>
</tr>
<tr>
<td>- Smooth nuclear membrane</td>
<td>- Irregular nuclear membrane</td>
</tr>
<tr>
<td>- Round/oval nuclei</td>
<td>- Nuclear clefting/molding</td>
</tr>
<tr>
<td>- Multinucleated/uniform</td>
<td>- Multinucleated /non-uniform</td>
</tr>
<tr>
<td>- Minimal vacuolization</td>
<td>- Dramatic vacuolization</td>
</tr>
</tbody>
</table>
Malignant Cells

Mesothelial Cells
Case Study 1

• 67 YEAR OLD FEMALE
  – MEDICAL HISTORY UNREMARKABLE
  – SEEN IN ER WITH COMPLAINTS OF HEADACHE AND DIZZINESS

• CSF
  – RBC  NONE SEEN
  – WBC  135 / mcL
Case Study 1

- Ugly cells
- Cell size
- Irregular chromatin and large vacuoles

Malignant cells from metastasized breast cancer
Mamograms are important!!
• Very big cells or clusters of cells will appear here because they have a lot of fluorescence.

Case Study 2

• 24 YEAR OLD FEMALE
  – SEEN IN ER FOR HEADACHE AND NAUSEA
  – PREVIOUS HISTORY OF MENINGITIS

• CSF
  – RBC 17 / mcL
  – WBC 754 /mcL
Viral Meningitis

More specifically a recurrence of herpes meningitis

She is positive for HSV- Type 2. Recurrent meningitis is known as ‘Mollaret’s Meningitis’.

Herpes meningitis occurs in 10% of cases of primary genital HSV-2. Women are at higher risk for herpes meningitis than men.

Surprisingly, herpes meningitis resolves without complications in 7 – 10 days but can recur in some patients.
Case Study 3

- 67 YEAR OLD MALE
  - 5.5 YRS POST HEART TRANSPLANT

- PLEURAL FLUID
  - NUCLEATED CELLS 5,998 /mcL
Burkitt’s lymphoma
Monomorphic post transplant lymphoproliferative disorder

Case Study 4

Synovial Fluid
Uric Acid Crystals – Polarized Light

Red Compensator Filter
The diff channel on the XE showed interference from the uric acid crystals as did the WBC/BASO channel. A manual count would need to be performed but........

HOW ARE YOU GOING TO FIND THE CELLS?

AND DOES IT MATTER?
Background count after running the fluid

Case Study 5

- **47 YEAR OLD FEMALE**
  - ER PATIENT
  - ALTERED VISION ??

- **CSF**
  - NUCLEATED CELLS 4 / mcL
  - RBC 3 / mcL
SOMETHING TO REMEMBER....

CYTOCENTRIFUGE CONCENTRATION

~ 20 FOLD

...IF YOU DO IT RIGHT!
Case Study 5

• 47 YEAR OLD FEMALE
  – ER PATIENT
  – ALTERED VISION ??

• CSF
  – NUCLEATED CELLS 4 / mcL
  – RBC 3 / mcL
  – REPEAT COUNT -NUCLEATED CELLS 29 / mcL

Case Study 6

Should we dilute?
References

The Best Body Fluid References:

Body Fluids, 3rd edition (Kjeldsberg) - ASCP Press
Color Atlas of Body Fluids - CAP
Questions