ICSH Guidelines: Verification and Performance of Automated Cell Counters for Body Fluids Counting

Barbara De la Salle
UK NEQAS Deputy Director and Organiser
haem@ukneqas.org.uk
ICSH guidelines for the verification and performance of automated cell counters for body fluids

G. BOURNER*, B. DE LA SALLE†, T. GEORGE‡, Y. TABE§•†, H. BAUM** N. CULP††, T. B. KENG‡‡, ON BEHALF OF THE INTERNATIONAL COMMITTEE FOR STANDARDIZATION IN HEMATOLOGY (ICSH)

SUMMARY

One of the many challenges facing laboratories is the verification of their automated Complete Blood Count cell counters for the enumeration of body fluids. These analyzers offer improved accuracy, precision, and efficiency in performing the enumeration of cells compared with manual methods. A patterns of practice survey was distributed to laboratories that participate in proficiency testing in Ontario, Canada, the United States, the United Kingdom, and Japan to determine the number of laboratories that are testing body fluids on automated analyzers and the performance specifications that were performed. Based on the results of this questionnaire, an International Working Group for the Verification and Performance of Automated Cell Counters for Body Fluids was formed by the International Council for Standardization in Hematology (ICSH) to prepare a set of guidelines to help laboratories plan and execute the verification of their automated cell counters to provide accurate and reliable results for automated body fluid counts. These guidelines were discussed at the ICSH General Assemblies and reviewed by an interna-
Body Fluid Counting

- **Types of fluids:**
  - Cerebrospinal fluid
  - Serous fluids (ascitic, pleural, pericardial)
  - Synovial fluid

- **Manual counting:**
  - Labour intensive
  - May be inaccurate and imprecise
  - Requires high level of expertise

- **Body fluid counting available on many fully automated cell counters**
Automated Counters: examples of functionality for Body Fluids

Patterns of Practice Questionnaire

<table>
<thead>
<tr>
<th>Analyzers</th>
<th>Fluids</th>
<th>Parameters reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Coulter LH 750/780</td>
<td>Serous, synovial, CSF</td>
<td>WBC, RBC</td>
</tr>
<tr>
<td>Beckman Coulter DxH 800</td>
<td>Serous, synovial, CSF</td>
<td>TNC, RBC</td>
</tr>
<tr>
<td>Sysmex XE 2100, XT 1800i/2000i</td>
<td>Serous, synovial, CSF</td>
<td>WBC, RBC</td>
</tr>
<tr>
<td>Sysmex XT-4000 and XE-5000</td>
<td>Serous, synovial, CSF</td>
<td>BF Mode : WBC-BF, TC-BF, RBC-BF, 2 part diff (mononuclear/polymorphonuclear)</td>
</tr>
<tr>
<td>Siemens Advia 2120, 2120i</td>
<td>Peritoneal, pleural, and peritoneal dialysate:</td>
<td>TNC, RBC</td>
</tr>
<tr>
<td>Iris iQ200</td>
<td>All fluids</td>
<td>Nucleated count, RBC</td>
</tr>
</tbody>
</table>
Body Fluid Counting

- Numerous articles on the use of automated body fluid counts
  - Verification may be incomplete
  - Performance specifications lacking
- Patterns of practice questionnaire
- ICSH WG for the preparation of guidelines
Patterns of Practice Questionnaire

Distributed to participants:

- QMP–LS (Ontario) 130
- CAP (United States) 1042
- UK NEQAS (H) 680
- JSLH (Japan) 273

Objectives:

- Whether laboratories used automated counters for CSF and other body fluid counts
- How the performance specifications had been determined
Numbers of respondents

![Bar Chart]

- **QMPLS**
- **CAP**
- **UK NEQAS (H)**
- **JSLH**

- **N**
- **Responses**
- **BFL counts**
- **CSF counts**
Numbers of CSF counts per month

![Bar Chart]

- QMPLS
- CAP
- UK NEQAS (H)
- JSLH

- Categories: 1-10, 11-20, 21-40, 41-80, 81-100, >100
Number of ‘other’ body fluid counts per month
Verification testing by laboratories

![Bar chart showing the percentage of laboratories for various criteria: Precision, Accuracy, Sensitivity, Specificity, and Report range. The criteria are compared across different categories: QMP-LS, CAP, UK-NEQAS, JSLH.](chart.png)
Procedures: Controls, Background Counts and Spurious Results

<table>
<thead>
<tr>
<th></th>
<th>QMP-LS</th>
<th>CAP</th>
<th>UK-NEQAS</th>
<th>JSLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>19</td>
<td>67</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Background each test</td>
<td>86</td>
<td>82</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Spurious results</td>
<td>79</td>
<td>82</td>
<td>40</td>
<td>54</td>
</tr>
</tbody>
</table>
Validation and verification of automated systems
  ◦ Manufacturer’s statement of intended use
  ◦ Specimen handling
  ◦ Performance specifications

Automated analysis of body fluids
  ◦ Procedures
  ◦ Units of measurement

Quality Control
Manufacturer’s statement of intended use

- Statement that indicates the type of body fluids for which the analyser has been validated
- Laboratory must verify the manufacturer’s claims
  - Full verification at one site
  - Transference verification at other analysers in the same network
- If the laboratory intends to use the instrument beyond the manufacturer’s scope, a full validation will be required
Specimen handling

- Pre-analytical variables
  - Sample container
  - Storage conditions
  - Transport conditions

- Sample stability
  - Cellular deterioration
  - Bacterial contamination
  - Correlation studies between methods should be within 2 hours of each other
Performance specification 1

- To provide evidence that the analyser produces reliable results
- Objectives are the responsibility of the laboratory
- Performance should be verified for each type of fluid to be counted
- Peripheral blood specimens should not be used
- Limited sample numbers may be a problem
  - Integrate into daily testing routine
  - Minimum of 40 recommended
Performance specification 2

- Accuracy
  - Split sample testing (40 samples recommended) OR
  - Recovery of expected values from reference materials or commercial controls

- Precision
  - 2 or more concentrations
  - 10 replicates (minimum 5)
  - May use a commercial control

- Patient correlation
  - 40 samples advised
Performance specification 3

- **Carryover**
- **Lower limits of detection**
  - Limit of blank (LOB)
  - Limit of detection (LOD)
  - Limit of quantitation (LOQ)
  \[ \text{LOB} < \text{LOD} \leq \text{LOQ} \]
- **Interfering substances**
  - Dependent on the patient population
- **Analytical Measurement Range, Linearity**
  - Defined by manufacturer, verified by laboratory
Laboratory procedures 1:

- Pre-analytical variables
  - Stability, transport, contamination
- Pre-treatment of samples,
  - e.g. Hyaluronidase treatment of synovial fluid
  - As stated in manufacturer’s statement
- Background counts
  - Equal to or less than lower limit of blank
Laboratory procedures 2:

- Spurious results
  - Debris, cell clumps
  - Irretrievable samples
  - Impact on accuracy of results

- Results outside the reportable range
  - Results that exceed upper or lower limits of the reporting range

- Units of measure
  - As for full blood count
    - To avoid confusion for requesting clinician
    - To avoid use of additional calculation steps
IQC and EQA

- Internal Quality Control
  - Commercial controls available
  - Use is advised if the body fluids are run in a different mode from whole blood
  - Differential count

- External Quality Assessment
  - EQA provider scheme if available
    - QMPLS (17%), CAP (94%), UK NEQAS (2%), JSLH (0%)
  - Blind testing
  - Interlaboratory exchange of samples
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