Hematopathology // Evaluation of the Iris iQ200 Body Fluid Module With Manual Hemacytometer Count

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Key Words: Cerebrospinal fluid; CSF; Body fluids; Hemacytometer; iQ200; Cell count

DOI: 10.1309/AJCPSVG4POONQQ1R

Abstract

Manual cerebrospinal fluid (CSF) and body fluid cell counts done using a hemacytometer are labor-intensive and time-consuming. The automated Iris iQ200 with the Body Fluids Module (Iris Diagnostics, Chatsworth, CA) counts RBCs and nucleated cells in CSF and other body fluids using flow cell digital imaging. We compared the cell counts from the iQ200 and the hemacytometer. CSF and body fluid samples were first analyzed in duplicate using the Neubauer hemacytometer. We then counted the samples on the iQ200. CSF samples for RBC counts of 0 to 6.7 × 10^5/µL and nucleated cell counts of 0 to 1,707/µL resulted in r = 0.996 and r = 0.998, respectively. Body fluid samples for RBC counts of 0 to 2.7 × 10^5/µL and nucleated cell counts of 0 to 1.1 × 10^4/µL resulted in r = 0.988 and r = 0.987, respectively. Day-to-day precision with quality control yielded coefficients of variation of 12.5% and 13.3% for RBC counts and 9.4% and 12.3% for nucleated cell counts. Linearity studies yielded slopes of 0.99 and 1.010 for RBC and nucleated cell counts, respectively. The results from the iQ200 correlate well with the manual hemacytometer method.

The analysis of cerebrospinal fluid (CSF) and other body fluids provides useful diagnostic information to clinicians.1-4 Generally, CSF and body fluid cell counts are done manually using an improved Neubauer counting chamber.5 Manual cell counts are labor-intensive, time-consuming, technique-dependent, and prone to variability and low precision.6 Although electronic cell counters are available, traditionally they are not used for CSF and other body fluids with low cell counts owing in part to poor reproducibility and high background counts that may falsely elevate cell counts.5,6 The WBC count in normal CSF ranges from 0 to 5 cells/µL and up to 30 cells/µL in neonates. Body fluids aspirated from the pleural, pericardial, and peritoneal cavities are generally divided into transudates and exudates. WBC counts of less than 1,000 cells/µL are associated with transudates and WBC counts greater than 1,000 cells/µL with exudates; however, overlap can be seen.1-3

Newer instrumentation, such as the Iris iQ200 with Body Fluids Module (Iris Diagnostics, Chatsworth, CA), which uses flow cell digital imaging, has reduced the concerns of background interference and is linear to zero.5,7 Before analysis on the iQ200, 2 dilutions are made for the CSF or body fluid specimen being analyzed. One dilution is made using a lysing reagent that destroys the RBC membrane and leaves only nucleated cells intact. The other dilution is made using an isotonic diluent. A portion of each dilution is aspirated and sandwiched between enveloping layers of a suspending fluid. This fluid, or lamina, is positioned exactly within the depth of focus and field of view of the objective lens of the microscope that is coupled to a 1.3-megapixel CCD digital camera. The iQ Lamina (Iris Diagnostics) is used to position the cells in an orthoscopic
orientation that presents asymmetric particles with their largest profile facing the direction of view as the sample goes through the flow cell. The flow cell hydrodynamically orients particles within the focal plane of the microscope. The digital camera captures 500 frames per sample as each field is illuminated by the flash of a strobe lamp and magnified by the microscope. Individual particle images are digitized and sent to the instrument processor.7

The instrument processor autoclassifies the particle. Particles are classified as total cells and nucleated cells based on their size. Particle images derived from the sample treated with the lysing reagent are classified as nucleated cells, and images derived from the sample treated with diluent are classified as total cells.7

The number of RBCs is calculated as the difference between the number of user-edited total cells counted in the unlysed sample and the number of user-edited nucleated cells counted in the lysed sample. The nucleated cell count comes from the user-edited count in the nucleated cell screen classification. Bacteria and crystals are not quantified; rather, they are reported as observed or not observed by the user.7

The aim of this study was to evaluate the performance of the Iris iQ200 with Body Fluids Module as compared with the manual hemacytometer method. The study included comparison analysis, precision, linearity, and carryover.

Materials and Methods

Patient Samples

For the correlation, we used 66 CSF and 36 body fluid samples that were received in the laboratory for routine cell counts and differentials. The body fluid samples included pleural, pericardial, peritoneal, and drainage fluids. Synovial fluids and bronchoalveolar lavage fluids were excluded from this evaluation. We used CSF samples collected in sterile tubes and body fluid samples collected in sterile, EDTA, or heparinized tubes. Clotted, extremely viscous, and mucoid samples were not tested.

Samples were first analyzed and results completely determined by laboratory personnel following standard operating procedures. After all requested tests were performed and results determined, the samples were used for the correlation, provided there was a sufficient amount of residual sample. For specimens with low cell counts, minimum volumes of 500 μL of CSF and 200 μL of the other body fluids were required for analysis on the iQ200. Samples were analyzed on the iQ200 within 1 to 12 hours of being analyzed by the manual method. Any discrepant results were reanalyzed by the hemacytometer method first and then by the iQ200, if necessary, to account for method variability or cell loss owing to delays between testing methods. If there was not a sufficient amount of sample to double-check discrepant results, the specimen was excluded from the evaluation.

Manual Method

Neubauer counting chambers were used to perform manual RBC and nucleated cell counts in duplicate. Clear and colorless samples were counted undiluted, provided there was clear separation of the cells when viewed under the microscope. Dilutions were prepared on samples as needed using 0.9% saline for RBC counts and Turk solution, containing acetic acid and dye, for nucleated cell counts.

Both sides of the Neubauer chamber were filled. For undiluted samples, the RBCs and nucleated cells in all 9 large squares on each side of the counting chamber were counted. For samples that required dilution, the dilutions were prepared in duplicate, one dilution for each side of the chamber. The RBC counts for those specimens were determined by counting the cells in the standard 5 small squares in the center square on each side of the chamber, and the nucleated cells were counted in the 4 large corner squares on each side of the chamber. The counts were averaged, provided they agreed within ±10% of each other. The standard Neubauer calculation formula was used to determine the number of cells per cubic millimeter.5

Iris iQ200 With Body Fluids Module

Before analyzing specimens on the iQ200, the maintenance, calibration, quality control (QC), and background check were confirmed as having been done in accordance with the manufacturer’s recommendations. Each sample analyzed on the iQ200 required 2 dilutions. Pairs of iQ Body Fluids Conical Bottom tubes (Iris Diagnostics) were prepared in advance each day of correlation testing with equal amounts of iQ Body Fluids Lysing Reagent (Iris Diagnostics) in one tube and Iris Diluent (Iris Diagnostics) in the other. The appropriate dilution for each sample was determined based on the sample type and gross appearance in accordance with the manufacturer’s recommendation.

The analyzer aspirated approximated 1.0 mL of each dilution. The aspirate was then surrounded by iQ200 Lamina, which stabilized the sample through the flow cell. The flow cell hydrodynamically oriented the particles in the sample within the focal plane of a 20× objective of a microscope. The magnified particles were illuminated by a strobe light, and 500 images were captured by a 1.3-megapixel CCD digital camera as the sample flowed through the flow cell. The digitized images of the particles were then sent to the instrument processor where they were categorized as total cells, nucleated cells, or artifact based on their size.7

Selective lysis occurred in the tube diluted with iQ Body Fluids Lysing Reagent. Selective lysis is a chemical process that destroys the RBC membranes, leaving only nucleated
cells intact. The aliquot diluted with iQ Body Fluids Lysing Reagent (lysed) was reported as nucleated cells and the aliquot diluted with Iris Diluent (unlysed) as total cells. The number of RBCs was calculated as the difference between the number of total cells counted in the unlysed sample and the number of nucleated cells counted in the lysed sample. The results were automatically calculated and corrected for the dilution factor.

Based on the dilution determined, the appropriate amount of patient sample was added to the 2 tubes containing the appropriate amount of lyse and diluent. Patient identification labels were affixed to each tube, and secondary preprinted body fluid/dilution-specific labels were affixed to the bottom of each tube. The secondary label, which was provided by the manufacturer, identified the specimen type, dilution factor, and diluent. Both tubes were mixed by tapping the bottoms and then tested on the analyzer.

Users reviewed each particle image that the analyzer had classified and then reclassified any images that they believed had been misclassified. All particle images that users believed were not total cells (RBCs and/or nucleated cells) or nucleated cells were reclassified as artifact and were not included in the final count. After all images were reviewed and reclassified, if necessary, the analyzer automatically adjusted the results and generated a final report of RBCs and nucleated cells.

**Precision**

Day-to-day precision was done by analyzing both levels of QC material for several days; 25 data points were collected. The QC materials manufactured by Iris Diagnostics consist of “stabilized human red blood cells and simulated white blood cells in a preserved medium.” The within-run precision was done by using 2 CSF samples and 2 peritoneal fluid samples. Each CSF sample was analyzed 10 consecutive times, and each peritoneal fluid sample was analyzed 12 consecutive times on the iQ200. EP Evaluator Software (David Rhodes Associates, Kennett Square, PA) was used to analyze the data.

**Linearity**

The iQ200 was shown to be linear through zero as established by a linearity analysis done during the linearity validation of the iQ200 urinalysis validation protocol. The urinalysis linearity assessment validated linearity from 0 to 1,000 cells/µL. An extended linearity study was performed to validate linearity at the higher range.

The extended linearity was assessed by following the iQ200 Body Fluids Module Field Validation Protocol. Several dilutions were made using the Iris Level II Body Fluid Control (Iris Diagnostics) and Iris Diluent. Each dilution pool was run in duplicate. The suggested acceptable limits are a slope of 0.9 to 1.1 and an $r^2$ of 0.9 or more.

**Carryover**

The carryover was assessed by analyzing a serous fluid with elevated RBC and nucleated cell counts (H1, H2, and H3) 3 consecutive times immediately followed by a CSF sample with low RBC and nucleated cell counts (L1, L2, and L3) analyzed 3 consecutive times. The carryover was calculated using the following formula: Carryover % = (L1 – L3)/(H3 – L3) × 100.

**Regression Analysis Summary for the Y Method (iQ200) and the X Method (Hemacytometer)**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No. of Samples (Y Method/X Method)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation (R)</th>
<th>Range (/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs in CSF</td>
<td>65/65</td>
<td>0.811</td>
<td>104.8</td>
<td>0.9956</td>
<td>0-6.7 × 10^5</td>
</tr>
<tr>
<td>RBCs in body fluid*</td>
<td>29/31</td>
<td>1.030</td>
<td>-1,217.6</td>
<td>0.9883</td>
<td>0-2.7 × 10^5</td>
</tr>
<tr>
<td>Nucleated cells in CSF</td>
<td>66/66</td>
<td>1.101</td>
<td>-1.0</td>
<td>0.9982</td>
<td>0-1,707</td>
</tr>
<tr>
<td>Nucleated cells in body fluid*</td>
<td>34/36*</td>
<td>0.882</td>
<td>3.1</td>
<td>0.9871</td>
<td>0-1.1 × 10^4</td>
</tr>
</tbody>
</table>

CSF, cerebrospinal fluid.

*Body fluid types include pleural, peritoneal, pericardial, and drainage fluids.

† Body fluids that showed clumping on the iQ200 digital images were excluded from the calculations.
iQ200 images. Neither of the specimen hemacytometer results correlated with the iQ200 results. The iQ200 results were significantly lower than the hemacytometer results for both specimens. Because the iQ200 analyzer is not able to isolate individual nucleated cells when they occur in clumps, the nucleated cell counts from such specimens are falsely decreased. These 2 counts were excluded from the regression analysis but listed in the experimental data results.

**Precision**

We used 2 levels of QC material to collect 25 data points to assess the day-to-day precision. The iQ Body Fluids Control Level I resulted in coefficients of variation (CVs) of 12.5% and 9.4% for the RBC and nucleated cell counts, respectively. The mean RBC count was 22,492/µL with an SD of 2,805.7. The mean nucleated cell count was 1,444/µL with an SD of 135.9.

The iQ Body Fluids Control Level II resulted in CVs of 13.3% and 12.3% for the RBC and nucleated cell counts, respectively. The mean RBC count was 39,269/µL with an SD of 5,217.8. The mean nucleated cell count was 2,539/µL with an SD of 311.4. Table 2 shows the day-to-day precision statistical data.

The within-run precision was assessed by using 2 CSF samples and 1 body fluid sample run 10 consecutive times. The CSF samples had mean RBC counts of 342/µL and 4/µL with CVs of 18.0% and 55.3%, respectively, and mean nucleated cell counts of 44/µL and 0.2/µL with CVs of 21.7% and 300.0%, respectively. The peritoneal body fluid sample had a mean RBC count of 188/µL with a CV of 16.6% and a mean nucleated cell count of 63/µL with a CV of 30.4% (Table 3).

**Linearity**

The body fluids extended linearity results showed that the iQ200 was accurate up to 43,829/µL for the RBC count with a slope of 0.99 and an average agreement of 97.9% (Figure 1). The nucleated cell count was accurate up to 2,558/µL with a slope of 1.010 and an average agreement of 98.9% (Figure 2). The linearity was performed using a 1:5 dilution of the various pools; therefore, higher cell counts are accommodated by making higher dilutions (ie, 1:10, 1:20, 1:50, and 1:100) of the original sample. The iQ200 can accommodate cell counts up to $4.4 \times 10^6/µL$ and $2.6 \times 10^5/µL$ for RBC and nucleated cell counts, respectively.

**Carryover**

A CSF sample with low RBC and nucleated cell counts that was analyzed after a serous fluid sample with an approximate RBC count of $3.6 \times 10^4/µL$ and a nucleated cell count of 665/µL demonstrated no carryover (Table 4).

**Discussion**

CSF and body fluid cell count analysis typically done manually using a hemacytometer can be labor-intensive and time-consuming and exhibit low reproducibility. Electronic cell counters are usually ineffective for CSF and other cytopenic body fluids owing in part to elevated background counts and other interference that can falsely elevate low cell counts. The iQ200 digital imaging technology virtually eliminates some of the problems encountered with manual counting and electronic cell counting analyzers.

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**Table 2**

**Day-to-Day Precision Summary for the iQ200 Instrument**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample</th>
<th>No. of Samples</th>
<th>Mean (/µL)</th>
<th>SD</th>
<th>CV (%)</th>
<th>2 SD Range (/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs</td>
<td>Level I QC</td>
<td>25</td>
<td>22,492</td>
<td>2,805.7</td>
<td>12.5</td>
<td>21,334-23,650</td>
</tr>
<tr>
<td>RBCs</td>
<td>Level II QC</td>
<td>25</td>
<td>39,269</td>
<td>5,217.8</td>
<td>13.3</td>
<td>38,833-41,423</td>
</tr>
<tr>
<td>Nucleated cells</td>
<td>Level I QC</td>
<td>25</td>
<td>1,444</td>
<td>135.9</td>
<td>9.4</td>
<td>1,172-1,716</td>
</tr>
<tr>
<td>Nucleated cells</td>
<td>Level II QC</td>
<td>25</td>
<td>2,539</td>
<td>311.4</td>
<td>12.3</td>
<td>1,916-3,162</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; QC, quality control.

**Table 3**

**Within-Run Precision Summary for the iQ200 Instrument**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample</th>
<th>No. of Samples</th>
<th>Mean (/µL)</th>
<th>SD</th>
<th>95% Confidence Interval (/µL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs</td>
<td>CSF</td>
<td>10</td>
<td>342</td>
<td>61.4</td>
<td>297.6-385.4</td>
<td>18.0</td>
</tr>
<tr>
<td>Nucleated cells</td>
<td>CSF</td>
<td>10</td>
<td>44</td>
<td>9.5</td>
<td>36.8-50.4</td>
<td>21.7</td>
</tr>
<tr>
<td>RBCs</td>
<td>CSF</td>
<td>9</td>
<td>4</td>
<td>2.3</td>
<td>2.4-6.0</td>
<td>55.3</td>
</tr>
<tr>
<td>Nucleated cells</td>
<td>CSF</td>
<td>9</td>
<td>0.2</td>
<td>0.7</td>
<td>–0.3 to 0.7</td>
<td>300.0</td>
</tr>
<tr>
<td>RBCs</td>
<td>Peritoneal</td>
<td>12</td>
<td>188</td>
<td>31.3</td>
<td>169-208</td>
<td>16.6</td>
</tr>
<tr>
<td>Nucleated cells</td>
<td>Peritoneal</td>
<td>12</td>
<td>63</td>
<td>19.3</td>
<td>51-76</td>
<td>30.4</td>
</tr>
</tbody>
</table>

CV, coefficient of variation.
Although the iQ200 requires 2 dilutions for each sample, the dilutions can be prepared ahead of time. Once the sample arrives, the appropriate amount of sample can be pipetted into the prepared dilution tubes. After sample analysis is complete, laboratory personnel only need to review the images to see if they were correctly classified by the analyzer and reclassify any misclassified images. The analyzer automatically calculates the RBC and nucleated cell counts, which eliminates the time, labor, and possible errors associated with manual cell counting and calculations.

Another problem encountered with CSF analysis is limited sample volume. To accommodate the dilutions that must be made for a sample to be analyzed on the iQ200, clear and colorless CSF requires a minimum of 500 µL of sample. Other body fluids analyzed on the iQ200 can require a minimum of 200 µL depending on the gross analysis (Table 1). Smaller CSF and body fluid samples are needed for bloody or cloudy samples, which typically have higher RBC and/or nucleated cell counts and require a higher dilution for analysis on the iQ200. Samples that do not have the minimum required volume cannot be analyzed on the iQ200. This study did not analyze the number of specimens that are rejected for insufficient quantity. The hemacytometer method typically analyzes 1 µL of sample.6

The data analyzed in this study showed that the iQ200 demonstrated excellent correlation with the manual hemacytometer method. The $r$ values yielded greater than 0.980 for RBC and nucleated cell counts in CSF and body fluids. The iQ200 even demonstrated excellent correlation in samples with low nucleated cell counts, less than 10/µL. However, samples that exhibit nucleated cell clumping did not correlate well with the manual method. The iQ200 nucleated cell counts were falsely decreased in such samples. The results from this study indicate that whenever iQ200 images show excessive nucleated cell clumping, the manual method is the method of choice to achieve an accurate cell count. The human eye is better able to discern and count individual cells when they appear in clumps.

The precision analysis indicated that the iQ200 demonstrated good day-to-day precision for RBC and nucleated cell counts. The IQ Body Fluids Control Levels I and II were used to assess the day-to-day precision. Both levels of control for RBC and nucleated cell counts resulted in CVs less than 14.0%.

Because the QC WBCs are not human-derived, the iQ200 images of the QC nucleated cells do not look the same as the
nucleated cells in a patient sample. However, the simulated WBCs in the control material function the same as a patient’s nucleated cells in that they are resistant to cell lysis when diluted with the Iris Lysing Reagent. Although the iQ200 images of the QC nucleated cells do not look like a patient’s nucleated cell images, they have a specific and distinct appearance that would enable laboratory personnel to distinguish them from RBCs or artifact.

The within-run precision yielded higher CVs, especially in samples with low cell counts. The highest CV was 300.0% for a CSF sample with a mean nucleated cell count of 0.2/µL. The sample was run 9 consecutive times and resulted in a nucleated cell count of 0/µL 8 times and 2/µL 1 time. The low cell count and the small sample would account for the high CV but do not indicate analyzer imprecision. The same sample generated a CV of 55.3% for the RBC count with a mean value of 4/µL. This study did not compare the precision of the iQ200 with that of the hemacytometer. However, other studies indicate that CSF samples with hemacytometer nucleated counts in the reference range of 0 to 5/µL yielded CVs between 18% and 56%.9,10 The other CSF sample with a higher RBC count (mean, 342/µL) resulted in lower CVs of 18.0% and 21.7%, respectively. The same is true for the body fluid sample assayed 12 times with a mean RBC count of 188/µL and nucleated cell count of 63/µL and CVs of 16.6% and 30.4%, respectively. Analysis of these data indicates that the differences in cell counts between runs is not clinically significant and does not indicate that the iQ200 is imprecise.

The extended linearity analysis revealed that the iQ200 is linear up to 43,829/µL for RBC counts and 2,558/µL for nucleated cell counts at a 1:5 sample dilution. The linearity can be extended with higher dilutions (ie, 1:10, 1:20, 1:50, and 1:100) to 4.4 × 10^5/µL and 2.6 × 10^5 for RBC and nucleated cell counts, respectively. Because the instrument is linear to a nucleated count of 0/µL 8 times and 2/µL 1 time. The low cell count and the small sample would account for the high CV but do not indicate analyzer imprecision. The same sample generated a CV of 55.3% for the RBC count with a mean value of 4/µL. This study did not compare the precision of the iQ200 with that of the hemacytometer. However, other studies indicate that CSF samples with hemacytometer nucleated counts in the reference range of 0 to 5/µL yielded CVs between 18% and 56%.9,10 The other CSF sample with a higher RBC count (mean, 342/µL) resulted in lower CVs of 18.0% and 21.7%, respectively. The same is true for the body fluid sample assayed 12 times with a mean RBC count of 188/µL and nucleated cell count of 63/µL and CVs of 16.6% and 30.4%, respectively. Analysis of these data indicates that the differences in cell counts between runs is not clinically significant and does not indicate that the iQ200 is imprecise.

This study shows that the iQ200 results correlate well with the manual method. The iQ200 is capable of analyzing samples with low RBC and nucleated cell counts, along with samples with cell counts as high as in whole blood. The iQ200, however, requires a significantly higher sample volume than the manual method. The hemacytometer count is also the method of choice when the iQ200 images show nucleated cell clumping. Compared with the manual method, the iQ200 requires less labor and less time. The within-laboratory cost savings of this approach relate to the reduction in technical time involved. This may vary from institution to institution and depends on test volumes. Therefore, any savings would be dependent on those factors, plus pricing of the instrument. However, the results of this study indicate that the iQ200 is a viable alternative to hemacytometer cell counts.

References

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