Evaluation of the ADVIA 120 for analysis of canine cerebrospinal fluid

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Background: Conventional techniques for canine cerebrospinal fluid (CSF) analysis require large sample volumes and are labor intensive and subject to operator variability.

Objective: The purpose of this study was to evaluate the ADVIA120 CSF assay for analysis of canine CSF samples.

Methods: CSF samples collected from 36 healthy control dogs and 17 dogs with neurologic disease were processed in parallel using the automated assay and established manual methods using a hemocytometer and cytocentrifugation. Results for WBC (total nucleated cell) count, RBC count, and differential nucleated cell percentages were compared using Spearman rank correlation coefficients and Bland–Altman bias plots.

Results: Correlation coefficients for WBC and RBC counts were 0.57 and 0.83 for controls, and 0.92 and 0.94 for ill dogs, respectively. Coefficients for the percentages of neutrophils, lymphocytes, and monocytes were 0.53, 0.26, and 0.12 for controls and 0.77, 0.92, and 0.70 for dogs with neurologic disease. When data were combined (n = 53), correlation coefficients were 0.86 and 0.91 for WBC and RBC counts, and 0.63, 0.43, and 0.30 for neutrophil, lymphocyte, and monocyte percentages. A 9.5% positive bias and 7.0% negative bias were obtained for the ADVIA 120 CSF assay for lymphocytes and macrophages in dogs with neurologic disease with Bland-Altman analysis. A 12.2% positive bias was found for lymphocyte percentage in dogs with neurologic disease.

Conclusions: Manual and automated CSF assays had moderate to excellent correlation for WBC and RBC concentrations, but results were more variable for differential cell percentages. The ADVIA assay may be more useful for assessment of canine CSF with adjustment of cell differentiation algorithms.

Introduction

Cerebrospinal fluid (CSF) functions to physically support and buoy the brain, to remove metabolic by-products from the nervous parenchyma, to provide a highly selective fluid composition around the nervous tissue, and to transport substances through unidirectional flow within the central nervous system (CNS).¹ Because of this intimate association with the CNS parenchyma, laboratory analysis of CSF may provide diagnostic information about conditions affecting the meninges and disease processes of the neuroparenchyma. Inflammatory seizure disorders, infectious meningitis, and neoplasia are among the conditions that may result in alterations in the cellular composition of CSF.²

The analysis of CSF in small animal medicine requires anesthetic immobilization of often critically ill patients to allow collection of fluid from the cerebello-medullary or lumbar cisterns. The volume of fluid that can be safely collected is dependent on the size of the patient, and rapid sample processing is necessary due to the low protein concentration and limited buffering capacity of CSF.² Without addition of stabilizing agents the integrity of cells in CSF is limited, and samples must be processed and analyzed within several hours.³ Hence, the collection and analysis of CSF samples has frequently been limited to veterinary referral facilities.

Conventional manual methods of CSF analysis have utilized hemocytometer chambers to enumerate total WBC (nucleated cells) and RBC numbers, while
cell type is differentiated by microscopic evaluation of concentrated CSF preparations. Such manual methods are relatively slow and subject to error from counting very few cells, interoperator variability, inadequate mixing of samples, pipetting, and subjectivity in the classification of leukocytes. Automated flow cytometric methods for enumerating and classifying cells from CSF have been investigated. Specifically, the ADVIA 120 CSF assay, which uses direct cytometry to enumerate RBCs and WBCs and to differentiate WBC types, has been evaluated for CSF samples from human patients. Using human samples, the ADVIA 120 CSF assay compared favorably with traditional manual methods with the added benefits of requiring a smaller CSF sample volume and less time for processing. Considering the successful use of the ADVIA 120 for blood sample analysis in many species including dogs, and the similar composition of CSF in dogs and humans, a prospective study was undertaken to evaluate the use of the ADVIA 120 CSF assay for canine samples.

Materials and Methods

CSF samples from 36 mixed-breed dogs of both sexes involved in terminal surgical procedures not affecting the CNS were aseptically collected from the cerebellomedullary cistern. The dogs appeared to be clinically healthy with no evidence of neurologic disease or any other abnormalities noted during preanesthetic evaluation. In addition, CSF samples were obtained from 17 dogs presented to the Veterinary Teaching Hospital neurology service, where CSF was analyzed as a component of the clinical evaluation. Animal-use protocols for dogs used in terminal surgeries and for use of samples from client-owned animals were approved by the institutional animal care review board.

All of the CSF samples were analyzed within a half-hour of collection by both the manual method and the ADVIA CSF assay (CSF Assay, Bayer Diagnostic, Tarrytown, NY, USA). Manual WBC and RBC counts were determined by placing 10-μL aliquots of undiluted CSF onto each side of a Neubauer hemocytometer chamber (Fisher Scientific, Fairlawn, NJ, USA). The cells were allowed to settle for 10 minutes in a humidified environment. WBC and RBC counts were determined by counting all cells in the 9 largest squares on each side of the chamber and then calculating the mean of both sides. Results were expressed as the number of cells x 10⁹/L. To identify individual leukocyte populations, 2 slides were prepared by applying 200 μL of CSF into cytocentrifugation chambers (Cytospin 4, Thermo Shandon, Pittsburgh, PA, USA) and centrifuging at 41 g for 6 minutes. The slides were stained with modified Wright–Giemsa in an automated stainer (Hematek Automated Stainer, Bayer Diagnostic) and a differential leukocyte count was performed on 200 cells or all cells if fewer than 200 cells were present on both slides. Leukocytes were classified as neutrophils, lymphocytes, monocytes, vacuolated macrophages, and eosinophils. Other cells such as plasma cells, ependymal cells, and mitotic figures were noted but not enumerated.

The ADVIA CSF assay is available on the ADVIA 120 hematology analyzers equipped with a specific CSF software key. Specific reagents and controls are required. Before analysis, 300 μL of CSF was incubated with 300 μL of CSF reagent (Bayer Diagnostic) for a minimum of 4 minutes at room temperature. Although human CSF samples are reported to remain stable for up to 4 hours following addition of the CSF reagent, all samples for this study were analyzed within 30 minutes of collection. Following the manufacturer’s guidelines and use of control reagents, acceptable background cell counts and control results were obtained before analyzing patient samples. Cells are differentiated and enumerated by the ADVIA 120 via 3 optical signals: 2 light scattering angles and an absorption measurement. After conversion to digital format, scatter and absorption signals are paired to generate CSF cytograms. Results for each sample included the total WBC and RBC counts, as well as percentages and absolute counts for neutrophils, eosinophils, lymphocytes, and monocytes. Macrophages are not identified as a separate category in the ADVIA 120 CSF assay, and it is unknown whether they fall within the monocyte gate; hence, macrophages were enumerated as a separate category only in manual differential counts. The CSF eosinophil count was considered reportable only for research purposes in human CSF samples at the time of this study. Although also reported by the analyzer, the subgroups polymorphonuclear cells (neutrophils and eosinophils) and mononuclear cells (lymphocytes and monocytes) were not used for the purposes of this study.

Statistical analysis

A Behrens–Fisher rank test was used to determine if a significant difference between automated and manual results was dependent on whether or not the sample was from a control dog or a dog with neurologic disease. A Wilcoxon signed rank test was used to determine whether within neurologic disease or control groups there was an effect of using the automated vs
the manual method. Results were considered significant at \( P < .05 \). The data were nonlinear in distribution; therefore, a Spearman rank correlation coefficient \( (r^2) \) was calculated to determine the degree of correlation between manual and automated methods for total WBC and RBC counts, and the percentages of neutrophils, eosinophils, lymphocytes, and monocytes. Correlation coefficients were determined for combined data and for neurologic disease and control groups separately. The correlation was considered excellent \((0.81–1.00)\), substantial \((0.61–0.80)\), moderate \((0.41–0.60)\), fair \((0.21–0.40)\), slight \((0.01–0.20)\), or poor \((0)\).

Agreement between the 2 methods for combined data and for neurologic disease and control groups separately was evaluated using Bland–Altman bias plots.

**Results**

CSF samples from control dogs had significantly lower WBC and RBC counts than samples from dogs with neurologic disease (Table 1). When data were combined, the correlation between manual and automated methods for WBC \( (r^2 = 0.86) \) and RBC \( (r^2 = 0.91) \) counts was excellent, while the correlation for the percentage of neutrophils \( (r^2 = 0.63) \) was substantial, for lymphocytes moderate \( (r^2 = 0.43) \), and for monocytes fair \( (r^2 = 0.30) \), Figure 1.

For control dogs alone, correlation for total RBC count was excellent \( (r^2 = 0.83) \), and for total WBC count was moderate \( (r^2 = 0.57) \). Correlations for leukocyte differential percentages were slight to moderate (Table 2, Figure 2). Correlation coefficients for samples from dogs with neurologic disease were substantial to excellent between manual and automated techniques for all parameters. However, in some samples it was obvious that not all cells were captured in the ADVIA 120 size window (Figure 3). The Behrens–Fisher rank test indicated a significant difference between manual and automated results for the percentages of neutrophils \( (P = .0127) \) and macrophages \( (P = .0004) \), depending on whether the sample was from a dog with neurologic disease or from a control dog.

The ADVIA 120 assay had a positive bias of 9.5% \( (P < .0001) \) for lymphocyte percentage and a negative bias of \(-0.5\%\) for macrophages \( (P = .0313) \) in samples from dogs with neurologic disease. Furthermore, the ADVIA assay had a negative bias of 7% for the percentage of macrophages \( (P < .0001) \) in samples from control dogs. Bland–Altman analysis indicated a negative bias ranging from \(-0.0003\) to \(-0.1507 \times 10^9/L\) for total WBC counts determined by the ADVIA 120 for all data combined and for neurologic disease and control groups separately (Table 3, Figure 4). Only lymphocyte percentage had a significant positive bias, of 12.2% \( (P = .0014) \).

**Discussion**

Although several studies have been conducted to assess the performance of the ADVIA 120 CSF assay for human samples, to our knowledge this is the first to evaluate its performance for canine samples. The dogs in this study were grouped according to clinical history and physical examination findings into groups with and without neurologic disease. The control dogs had a limited clinical history and therefore, underlying neurologic disease could not be entirely ruled out; however, the objective of the study was to evaluate the automated CSF assay in animals with a range of normal and abnormal CSF results. The slightly increased \((>0.003 \times 10^9/L)\) manual WBC count for control dogs could have been the result of prolonged anesthesia.

Excellent correlation was found between automated and manual methods for total WBC and RBC concentrations when data were combined and for dogs with neurologic disease. However, total WBC counts from the clinically normal dogs were only moderately correlated between the 2 methods. In contrast, previous research on human CSF samples found that the ADVIA total WBC count was accurate to 5 cells/\( \mu L \) over the range of 0–50 cells/\( \mu L \) and accurate to 10% over the range of 50–5000 cells/\( \mu L \). The relatively poor correlation for canine samples appeared to be attributable to a single incongruent result, whereby the total WBC count was \(0.004 \times 10^9/L\) by the manual method and \(0.009 \times 10^9/L\) by the ADVIA assay. The cause of this discrepancy was not further investigated.

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**Table 1.** Cell concentrations in canine cerebrospinal fluid samples measured by the manual and automated assays.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Manual assay</th>
<th>ADVIA assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>WBC count ( (\times 10^9/L) )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All samples</td>
<td>0.152</td>
<td>0.003</td>
</tr>
<tr>
<td>Clinically healthy</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>Neurologic disease</td>
<td>0.469</td>
<td>0.370</td>
</tr>
<tr>
<td>RBC count ( (\times 10^9/L) )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All samples</td>
<td>0.760</td>
<td>0.004</td>
</tr>
<tr>
<td>Clinically healthy</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>Neurologic disease</td>
<td>2.173</td>
<td>0.280</td>
</tr>
</tbody>
</table>
due to insufficient remaining sample volume; however, in the ADVIA cytogram it appeared that separation of RBCs from leukocytes was incomplete (Figure 2). Removal of this outlier resulted in a correlation coefficient of 0.85 (95% confidence interval, 0.72–0.92) for WBC counts from control dogs, similar to the accuracy obtained with human samples. Also, Bland–Altman analysis indicated excellent agreement between manual and automated methods for total WBC and RBC counts. Therefore, except in rare instances, the ADVIA CSF assay appears to provide reliable results for total WBC and RBC numbers over a broad range of values, including those in clinically normal dogs. As such, the assay could be relied upon to provide rapid total WBC counts for canine CSF samples without the need for cumbersome manual cell counting.

CSF samples from dogs with clinical evidence of neurologic disease tended to have higher WBC counts and better correlation between methods for leukocyte differential percentages compared with samples from clinically healthy dogs. Other researchers also noted

Table 2. Correlation coefficients and 95% confidence intervals (CI) for cell concentrations and cell types in canine cerebrospinal fluid by the manual and automated assays.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Clinically healthy dogs (n = 36)</th>
<th>Dogs with neurologic disease (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (× 10^9/L)</td>
<td>r^2 0.57* 0.30–0.76*</td>
<td>r^2 0.92 0.79–0.97</td>
</tr>
<tr>
<td>RBC (× 10^9/L)</td>
<td>0.83 0.69–0.91</td>
<td>0.94 0.84–0.98</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>0.53 0.25–0.73</td>
<td>0.77 0.45–0.91</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>0.26 – 0.08–0.54</td>
<td>0.92 0.79–0.97</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.12 – 0.21–0.44</td>
<td>0.70 0.34–0.88</td>
</tr>
</tbody>
</table>

*r^2 = 0.85 (95% CI = 0.72–0.92) following removal of a single outlier.
better correlation in differential cell percentages between automated and manual methods at cell counts ≥0.1 × 10⁹/L.⁶ The manufacturer of the ADVIA suggests that differentials from CSF samples with a total WBC count < 0.020 × 10⁹/L should be confirmed by an alternate method.⁹

Bland–Altman analysis indicated broad 95% limits of agreement for differential leukocyte counts. Based on examination of scatter/scatter and scatter/absorption cytograms, different leukocyte populations, particularly the neutrophil population, did not fall cleanly within preset regions. Adjustment of these regions or the method of light scatter detection to canine leukocytes would likely result in improved differential results. Significant differences were also found between manual and automated results for the percentages of neutrophils and macrophages depending upon whether the sample was from a control dog or a dog with neurologic disease. This finding was not surprising when considering that dogs with neurologic disease are more likely to have increased numbers of neutrophils and/or macrophages in CSF,²,¹⁵ and that the ADVIA 120 CSF assay does not purport to be able to separate macrophages from other mononuclear cells.⁹ Although it has been suggested that macrophages with vacuolation may be a result of the cytocentrifuge technique, others consider them to be activated monocyted cells and indicative of CNS inflammation, irritation, or degeneration; the presence of erythrophagia and leukophagia in some cells argues against them being simply an artifact.³,¹⁵ Because many of the CSF samples in this study contained substantial numbers of macrophages with abundant foamy cytoplasm, it was considered important to quantify their numbers. The negative bias of the ADVIA 120 for macrophages was not unexpected because the automated assay does not claim to differentiate these cells from monocytes. The positive bias of the ADVIA 120 for lymphocytes suggested underestimation of these cells by microscopic evaluation. A flow cytometric study using specific markers for B and T lymphocytes found that a large percentage of mononuclear cells within the CSF were lymphoid in origin; some of these cells may be misclassified as monocytes in microscopic examination.¹⁶ As all of the CSF samples in this study were

### Table 3. Bland–Altman analysis of manual versus automated assays for determining cell concentrations and cell types in canine cerebrospinal fluid.

<table>
<thead>
<tr>
<th>analyte</th>
<th>all dogs (n = 53)</th>
<th>clinically healthy dogs (n = 36)</th>
<th>dogs with neurologic disease (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bias</td>
<td>95% limits of agreement</td>
<td>bias</td>
</tr>
<tr>
<td>WBC (× 10⁹/L)</td>
<td>-0.0485</td>
<td>-0.0939 to -0.0029</td>
<td>-0.0003</td>
</tr>
<tr>
<td>RBC (× 10⁹/L)</td>
<td>-0.1661</td>
<td>-2.4005 to -2.0684</td>
<td>0.0004</td>
</tr>
<tr>
<td>neutrophils (%)</td>
<td>0.8</td>
<td>33.6 to 35.1</td>
<td>4.2</td>
</tr>
<tr>
<td>lymphocytes (%)</td>
<td>3.0</td>
<td>56.5 to 62.6</td>
<td>1.3</td>
</tr>
<tr>
<td>monocytes (%)</td>
<td>3.7</td>
<td>49.7 to 57.2</td>
<td>6.0</td>
</tr>
</tbody>
</table>

*Significantly different between methods (P < .05).
processed within 30 minutes of collection, it is unlikely that sample deterioration resulting in altered cellular morphology that might have impacted significantly the leukocyte differentials.

Reported limitations of the ADVIA 120 CSF assay include its inability to detect atypical cells and the recommendation by the manufacturer to use eosinophil results for research purposes only. No CSF samples in this study had a high percentage of eosinophils; hence, the reliability of the automated assay with respect to this cell type could not be assessed. Similarly, no CSF samples had neoplastic cells. Several CSF samples had low numbers of plasma cells and rare mitotic figures, which would not have been noted if only the automated differential was applied. Although it may be argued that rare cells are not likely of clinical significance, their presence is not detected by the ADVIA CSF assay. This, along with marked discrepancies in CSF differential results in rare cases, illustrates the continued need for trained professionals to microscopically examine concentrated slide preparations of CSF.

Technically, the ADVIA 120 CSF assay was easily adapted for use in the laboratory. Time and reagent savings were maximized when CSF samples were batched; this allowed multisample analysis following single wash and initialization steps of approximately 6 to 10 minutes in duration. Considerable disruption to workflow on the ADVIA analyzer occurred when a single CSF sample required analysis, especially if multiple wash steps due to high background cell counts were required. In our laboratory, the sporadic submission of individual CSF samples resulted in inefficient use of costly reagents as the ADVIA 120 CSF reagent and controls have shelf-lives of 30 and 10 days, respectively, once opened. As such, the ADVIA 120 CSF assay appears technically best suited to a laboratory where large numbers of CSF samples are analyzed daily.

Acknowledgments

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References


Figure 4. Bland–Altman plots for total WBC counts using the manual method and automated assays. (Top) All data combined (n = 53), (middle) clinically healthy dogs (n = 36), and (bottom) dogs with neurologic disease (n = 17). The line of identity (x = 0), 95% limits of agreement (hatched line), and bias (thick line) are indicated.


