WHITE PAPER

anvajo

Digital holographic microscopy for **cerebrospinal fluid (CSF) analysis in veterinary diagnostics**

Accurate quantification of red blood cells (RBCs) and white blood cells (WBCs) in cerebrospinal fluid (CSF) is critical in veterinary medicine for diagnosing neurological conditions such as meningitis, hemorrhage, trauma, and neoplasia. This study evaluates the analytical performance of DHM compared to the ADVIA 120 hematology analyzer, a widely accepted laboratory reference standard. Here, DHM demonstrates strong agreement for both RBC and WBC counts across 30 measurements, with high linearity (R^2 ≈ 0.96) and excellent correlation (Pearson r ≈ 0.98) providing a reliable, rapid, and potentially field-deployable alternative for CSF cytology in veterinary settings.

In veterinary diagnostics, quantification of white blood cells (WBCs) and red blood cells (RBCs) in cerebrospinal fluid (CSF) is a fundamental component of neurologic assessment, providing critical insight into inflammatory, infectious, neoplastic, and hemorhagic conditions affecting the central nervous system. Elevated WBC counts (pleocytosis) are indicative of underlying inflammatory or infectious processes, with differential patterns offering further diagnostic refinement, while the presence and magnitude of RBCs help distinguish true hemorhage from iatrogenic blood contamination during sample collection. However, accurate measurement of these parameters remains technically challenging due to the typically low cellularity and limited sample volumes characteristic of CSF in veterinary patients. Despite the clinical importance, there is a notable lack of automated hematology analyzers specifically optimized for CSF analysis in veterinary medicine. Most laboratories rely either on manual hemocytometer-based counting—subject to significant operator variability and limited precision—or on blood-optimized automated analyzers, which may suffer from reduced sensitivity, misclassification of debris, and suboptimal performance at low cell concentrations. This gap underscores the need for dedicated or adapted

technologies capable of delivering reliable, reproducible CSF cell counts to support timely and accurate clinical decision-making in veterinary neurology.

Based on availability, the analyzed sample volume span from 3.6 µl (triplicates) to 6µl (quintuplicates) enabling precise analytical results.

The WBC concentrations measured in this veterinary CSF sample set covered a clinically relevant range for companion-animal and large-animal neurological diagnostics. Reference concentrations ranged from 0–492 WBC/µL. In dogs, commonly cited CSF reference intervals are approximately 0.5–5 cells/µL depending on the sampling site, while feline CSF is reported around 2–8 cells/µL, with stricter reference limits of 0–2 cells/µL also described for clinically normal dogs and cats in some studies; therefore, values above approximately 5 WBC/µl may be considered compatible with pleocytosis in many veterinary contexts, although interpretation remains species-age-, laboratory-, and sampling-site dependent. [veteriankey.com],

Using veterinary CSF interpretation categories, the study population included samples within the normal or near-normal range as well as samples representing mild, moderate, and marked pleocytosis. Veterinary references commonly describe pleocytosis as mild when cell counts are above the reference interval but <25 cells/µL; moderate at approximately 26–100 cells/µL; and marked at >100 cells/µL. In the present dataset, the reference method therefore covered the normal/near-normal range, low-grade inflammatory changes, moderate pleocytosis, and marked pleocytosis up to 492 WBC/µL. DHM reproduced this distribution well, with mean sensor values spanning 126 to 514,708 WBC/mL and maintaining strong agreement with the reference method.

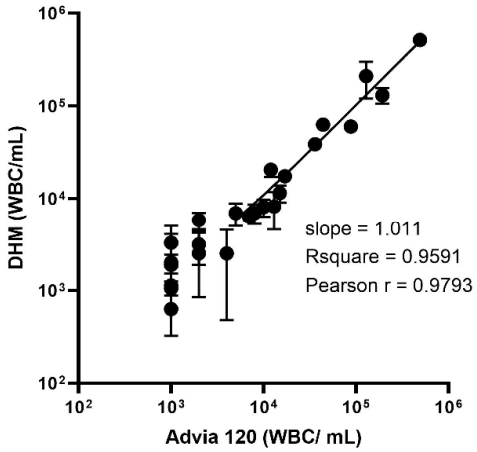

Figure 1. Correlation of WBC counts per mL between DHM and Advia 120

When applying a threshold of approximately 5 WBC/µL to distinguish normal/near-normal CSF from pleocytosis, DHM correctly identified all reference-positive samples and showed only one borderline discordant result.

This corresponds to an apparent 100% sensitivity, 93.8% specificity for detecting pleocytosis in this sample set.

However, because the highest reference concentration in this study was 492 cells/µL, additional samples with very high pleocytosis would be useful to establish performance across the full range of severe infectious or suppurative CNS disease encountered in veterinary patients.

RBC concentrations in the same CSF samples spanned a similarly broad range. Reference RBC concentrations ranged from 0–19.500 RBC/µL, while mean DHM-derived RBC concentrations ranged from 0 to 19.600 RBC/µL showing strong agreement with the reference method across this range, with Pearson r = 0.9862 and Spearman ρ = 0.94.

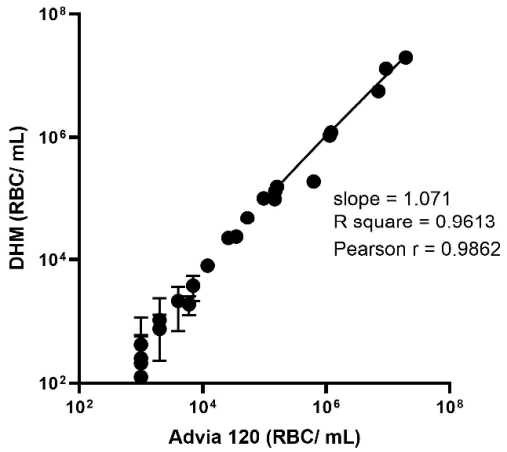

Figure 2. Correlation of RBC counts per mL between DHM and Advia 120

RBCs are relevant because normal CSF should contain few or no erythrocytes; their presence most commonly reflects iatrogenic blood contamination during CSF collection, for example from needle trauma or puncture of small vessels, although true CNS or subarachnoid hemorrhage can also introduce RBCs into CSF. Blood contamination may affect CSF interpretation because peripheral blood can increase the apparent nucleated cell count and correction formulas are not always reliable.[1]

Thus, the RBC detection also performed well for identifying clinically relevant blood contamination levels. At a threshold of ≥5 RBC/µL, the sensor achieved 87.5% sensitivity and 100% specificity.

To assess whether RBC contamination interfered with WBC performance, WBC classification was evaluated across increasing RBC burdens. Despite substantial RBC concentrations in several samples, WBC category agreement remained high. Among samples with ≥5 RBC/µL, WBC category agreement was 16/16; among samples with ≥100 RBC/µL, agreement was 9/9; and among samples with ≥1,000 RBC/µL, agreement was 5/5. Thus, RBC contamination did not alter the clinical WBC classification in this dataset. However, higher RBC

concentrations were associated with greater absolute WBC scattering between replicates, as indicated by a moderate correlation between RBC burden and absolute WBC error. This suggests that RBC-rich or visibly blood-contaminated CSF may increase variability but did not compromise the sensor's ability to identify clinically relevant pleocytosis in the tested samples.

Taken together, these results indicate that DHM provides robust performance for both WBC and RBC enumeration in veterinary CSF samples. The WBC measurements covered the normal-to-markedly pleocytic range relevant for veterinary neurological diagnostics, while the RBC measurements captured a broad range of blood contamination. Importantly, even in samples with substantial RBC contamination, WBC classification remained stable, supporting the suitability of the sensor for veterinary CSF screening while highlighting the need to consider RBC contamination during quantitative interpretation.

[1]Martina Klinkon and Jožica Ježek (2012). Values of Blood Variables in Calves, A Bird's-Eye View of Veterinary Medicine, Dr. Carlos C. Perez-Marin (Ed.), ISBN: 978-953-51-0031-7