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Evaluation of the IDEXX Procyte Dx Analyzer for the Measurement of Total and Differential Cellular Counts of Bronchoalveolar Lavage Fluid in Healthy Beagles and Dogs with Lower Respiratory Tract Diseases

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ABSTRACT

Background: Bronchoalveolar lavage fluid (BALF) analysis is a key diagnostic tool in veterinary respiratory medicine. The IDEXX ProCyte Dx analyzer was developed for blood formulation and cellular counting in various fluids, but its applicability for BALF analysis remains unexplored.

Objectives: To assess the agreement between manual counting and automated counting using the IDEXX ProCyte Dx analyzer for total cell count (TCC) and differential cell count (DCC) in BALF samples from healthy and diseased dogs, under two pretreatment conditions: mucolytic treatment with dithiothreitol (DTT) or filtration.

Methods: BALF from 10 healthy beagles and 14 diseased dogs were analyzed to compare TCC and DCC between manual and automated methods across pretreatment conditions.

Results: In healthy dogs, Bland–Altman analysis on log-transformed data demonstrated a mean ratio close to 1 and acceptable 95% limits of agreement between manual and automated methods and between pretreatment conditions. In diseased dogs, good agreement was observed between manual and automated methods for filtered samples only; the other comparisons showed poor agreement. In both healthy and diseased dogs, Passing-Bablok regression analysis did not detect systematic or proportional bias between methods. Automated DCC was inconsistent compared with manual methods in both healthy and diseased dogs.

Conclusions: The IDEXX ProCyte Dx analyzer can be used for TCC measurement of pre-treated BALF samples in healthy dogs. In diseased dogs, filtration is preferred over DTT-treatment for automated counting, and results should be interpreted with caution due to the unpredictable influence of pretreatment on cellular counts. For DCC, automated methods are unreliable.

1 | Introduction

Bronchoalveolar lavage fluid (BALF) analysis is an essential diagnostic tool in veterinary respiratory medicine [1, 2], providing information on inflammatory bronchopneumopathy [3–5], infectious diseases, such as bacterial [6–8] or parasitic infections

[9], or even neoplastic pathologies of the lower airways in dogs and cats. Various analyses are routinely carried out on BALF samples to establish a diagnosis and guide treatment [1, 3].

Overall cellularity assessment, through either total cell count (TCC) or subjective cytological evaluation, combined with

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differential cell count (DCC) on BALF, is particularly relevant to characterize the type and severity of airway inflammation [1–6]. Manual counting is currently the method of choice to obtain these counts, using a hemocytometer chamber for TCC and cytologic evaluation for DCC [1, 2, 10]. These manual analyses are time-consuming, limited by intra- and inter-observer variability, and require specialized training and equipment [1, 2, 10].

Automated hematology analyzers, such as the IDEXX ProCyt Dx analyzer, offer promising advantages over manual counting, including speed, repeatability, and operator independence [11].

While initially developed for blood analysis [11], the IDEXX ProCyt Dx analyzer has also been evaluated for canine cerebrospinal fluid analysis, with acceptable sensitivity and specificity for TCC assessment and leukocyte differentiation [12]. Although it has been marketed for use on other fluids such as body effusions and synovial fluid, its applicability for BALF cellular counting has not yet been evaluated. Other hematology analyzers, such as the ADVIA 120 (Siemens, USA), have been assessed for automated total nucleated cell and leukocyte differential counts in pleural, peritoneal, and synovial fluid samples [13, 14]. The ADVIA 120 has also been used in several studies for TCC evaluation in BALF [4, 5, 15], but the diagnostic applicability of this automated technique has never been formally validated.

A specific challenge in BALF analysis is the high viscosity and mucus content of the fluid, which can interfere with cellular counting [16]. Although no formal validation study has evaluated the direct impact of BALF viscosity on automated hematology analyzers such as the IDEXX ProCyt Dx analyzer, reports from synovial fluid analysis have shown that high viscosity can lead to technical issues, including aspiration or mixing errors resulting in falsely low leukocyte counts, or the misclassification of noncellular material such as fat globules, crystals, joint cells, or debris as leukocytes [17, 18]. In human medicine, mucolytic agents such as dithiothreitol (DTT) are frequently used to reduce sample viscosity and improve cellular dispersion in sputum samples [19, 20], without affecting cell morphology [21] nor differential cell count [22, 23]. While DTT has been routinely used in human sputum processing, its effect on automated analyzers or its use in canine BALF analysis are not yet documented.

In this context, the objectives of this study were to evaluate the applicability of the IDEXX ProCyt Dx analyzer for canine BALF cellular counting in both healthy beagles and dogs affected with lower respiratory tract diseases in comparison with manual counting methods under two sample pretreatment strategies, specifically the addition of a mucolytic agent (DTT) and filtration.

2 | Materials and Methods

2.1 | Study Design and Population

This was a prospective observational study conducted on leftover BALF samples initially collected from 10 healthy purpose-bred beagles. Healthy dogs were recruited as part of another

unrelated experimental procedure and considered healthy based on the absence of respiratory clinical signs, normal physical examination, normal blood hematology and biochemistry, and normal thoracic radiographs. The number of dogs included was determined in accordance with the 3R principles and approved by the ethical committee of the University of Liège (approval number: 2299, approval date: 29/01/2021).

Subsequently, 14 leftover BALF samples collected from 14 dogs affected with various lower respiratory tract diseases were prospectively analyzed to validate the observations made in healthy dogs under clinical conditions.

2.2 | Sample Collection

For each included dog, a standardized BAL procedure was performed under general anesthesia as previously described [7]. All experimental beagle dogs were premedicated with butorphanol (0.2 mg/kg; Butomidor; Richter Pharma) in combination with medetomidine (5 µg/kg; Medetor; CP-Pharma) both IV, induced with propofol (2–4 mg/kg to effect; Propovet; Zoetis), IV, and maintained with isoflurane (Iso-Vet; Eurovet) in oxygen. Dogs were placed in sternal recumbency. BALF was collected using a flexible pediatric endoscope (FUJINON Pediatric Video-Bronchoscope EB-530S), cleaned and disinfected before each use.

In healthy dogs, three successive aliquots of 20 mL sterile saline solution (0.9% NaCl) were instilled separately: the first two into the right caudal lung lobe and the third into the left caudal lung lobe. This total volume of 60 mL per dog corresponded to a median of 3.5 mL/kg [3.2–3.9 mL/kg]. Each aliquot was gently aspirated immediately after instillation via the working channel of the endoscope and collected into a sterile specimen trap (Argyle Specimen Traps, Covidien, 40 mL capacity) before the next aliquot was administered. The recovered fluid from the three aliquots was then pooled for analysis.

In dogs with respiratory diseases, the anesthetic protocol was adapted individually to each patient's clinical status according to a board-certified anesthetist's expertise. Likewise, the volume of sterile saline solution employed was determined based on patient size. For the 10 dogs for which this information was available, the median volume used was 2.05 mL/kg [1.1–2.6 mL/kg]. For the remaining 4 dogs, the exact volume was not recorded; however, a volume of 2 mL/kg is typically used in such procedures. The pulmonary lobes into which sterile saline was instilled were selected based on the localization of radiographic or bronchoscopy lesions. Procedure for lavage and aspiration of BALF was identical to the one used on healthy beagles.

2.3 | Sample Processing

Three types of samples were prepared from the recovered leftover BALF: one volume of 1 mL was left naïve, a second volume of 1 mL was filtered through a 70 µm cell strainer (thereafter called filtered BALF), and a last volume of 0.5 mL was mixed with 0.5 mL of DTT 0.15% (Sigma-Aldrich, USA) (thereafter called DTT-treated BALF).

For naïve BALF samples, manual cellular counts were performed, but not automated measurements, as the high mucus content posed a risk of damaging the IDEXX ProCyt Dx analyzer. For filtered and DTT-treated BALF samples, both manual and automated counting were performed.

In healthy dogs, TCC of naïve, filtered, and DTT-treated BALF was assessed by two independent operators (one ECVIM-CA internal medicine resident [observer 1] and one veterinary laboratory technician experienced in cytology [observer 2]), with two repeated counts per operator, allowing evaluation of both inter- and intra-observer variability. TCC was determined manually using a Thoma hemocytometer (Marienfeld Superior, Germany). An equal volume of BALF and Türk's solution (1:1 ratio) was mixed to stain nucleated cells and lyse red blood cells. After gentle mixing, the sample was loaded into the counting chamber, and cells were counted in the 16 large squares (each subdivided into 25 small squares) of the central grid under $\times 400$ magnification. The total number of nucleated cells was then multiplied by 2 to correct for the 1:1 dilution and by 10 to express the result in cells per microliter.

Additionally, filtered and DTT-treated BALF samples were analyzed using the IDEXX ProCyt Dx analyzer in two different setting modes: one using the standard hematology mode, and one using the analyzer's "other fluid" mode.

Following TCC evaluation, a manual DCC was performed for each sample on cytospin preparation (centrifugation at 221, for 4 min at 20°C, Thermo Shandon Cytospin4) [7]. Two smears per sample were prepared using Diff-Quik staining (Hemacolor Rapid staining of blood smear, Sigma-Aldrich, Germany) and independently reviewed by the same two operators as previously described, who performed a differential count based on 200 nucleated cells at high power field. These results were then compared with the automated differential leukocyte classification provided by the IDEXX ProCyt Dx analyzer.

In dogs affected with respiratory diseases, processing of BALF leftover samples was identical to that used for healthy dogs, with preparation of naïve, filtered, and DTT-treated BALF samples. Based on the results obtained in healthy dogs, TCC and DCC were calculated manually by one single observer (two readings) and by the machine (using the two different setting modes).

2.4 | Statistical Analysis

Results were reported as median and interquartile ranges for continuous variables and as numbers and percentages for qualitative variables. Normality of continuous variables was assessed using the Shapiro-Wilk test.

For TCC measurements, the effects of observer (intra- and inter-observer variation) on manual counts, as well as the effects of repeated readings from two different setting modes on automated counts, for each pretreatment condition, were evaluated using intraclass-coefficient (ICC) with 95% confidence (IC95%) and coefficient of variation (CV) expressed in %. An ICC value above 0.9 was considered excellent, 0.75–0.9 as good, 0.5–0.75 as moderate, and below 0.5 as poor. CV values less than 15%, between

15% and 30%, and more than 30% were considered indicative of low, moderate, and high variability, respectively. For further analysis, TCC mean values were calculated from all observer readings for the manual count and from both setting-mode readings for the automated count. Agreement between methods (manual vs. automated) and pretreatment techniques (naïve vs. filtrated vs. DTT-treated) was primarily assessed using Bland–Altman analysis on log-transformed data, as TCC values were not normally distributed in the diseased group due to some extreme values. Bland–Altman results on log-transformed data were expressed as mean ratio and 95% limits of agreement. A mean ratio close to 1 indicated good agreement between methods. The 95% limits of agreement defined the range within which most proportional differences between paired measurements were expected to lie. In addition, the linear association between paired measurements was evaluated using the Pearson correlation coefficient to quantify the strength of the association between methods (considered strong between 0.7 and 0.9 and very strong > 0.9), and Passing-Bablok regression analysis was used to detect potential systematic or proportional bias between methods. Systematic bias was considered present if the IC 95% of the intercept did not include 0, and proportional bias when the IC 95% of the slope did not include 1.

Regarding DCC measurements, due to major discrepancies in cell population identification between manual and automated DCC methods, statistical comparisons were not applicable. Results were therefore described qualitatively based on observed differences.

A retrospective power analysis using a Student's *t*-test was conducted on data collected from diseased dogs only, as the large TCC difference between healthy and diseased populations made healthy dogs unsuitable for this purpose. The analysis showed that a sample size of 12 diseased dogs provides 80% power to detect a difference of 1000 cells/ μ L between methods, with a standard deviation of 1500 cells/ μ L. Fourteen diseased samples were included (1.4 times the number of healthy samples), which was considered sufficient to provide preliminary method-comparison data for this exploratory study.

For all statistical analysis, a *p*-value < 0.05 was considered significant. All statistical analyses were performed using SAS software (v9.4) and figures were done in R version 4.3.1.

3 | Results

3.1 | Study Population

Healthy dogs were purpose-bred Beagles aged from 2 to 15 years with a median age of 5.6 years. Median body weight was 17.2 kg [15.8–18.2 kg].

Diseased dogs included different breeds, with two mixed-breed dogs and one of each of the following breed: Italian Greyhound, American Staffordshire Terrier, Australian Shepherd, Pyrenean Mountain Dog, Dachshund, Jack Russell Terrier, Bichon Frise, English Bulldog, French Bulldog, Siberian Husky, West Highland White Terrier, and Dutch Shepherd. Median age was 5.9 years [1.8–9.2 years]. There were 12 males (7 intact, 5

neutered) and 2 females (one intact, one neutered). Median body weight was 16.25 kg [9.3–27.8 kg]. Final diagnosis included chronic bronchitis ($n=5$), infectious bronchopneumonia ($n=4$), eosinophilic bronchopneumopathy ($n=2$), neoplasia ($n=1$), foreign body ($n=1$) and pulmonary thromboembolism ($n=1$).

3.2 | Total Cell Counts (TCC)

Raw TCC data for all included BALF samples are provided in Supporting Information 1.

ICC analysis showed that, for both manual and automated measurements across pretreatment conditions, there were respectively small intra- or inter-observer effects and influence of the setting mode on the TCC measurements with good to excellent agreement and acceptable CV (Supporting Information 2 and 3). Consequently, mean TCC values for each BALF sample were calculated from the repeated readings of each observer for manual counting and from repeated readings obtained under two setting modes for automated counting. These mean values were used to generate boxplots for each BALF type, namely naïve BALF (manual count), DTT-treated BALF (manual and automated count), and filtered BALF (manual and automated count) from healthy and diseased dogs (Figures 1 and 2, respectively).

In healthy dogs, comparisons of manual TCC across pretreatment conditions, as well as between manual and automated TCC within each pretreatment condition, demonstrated good to excellent agreement according to ICC values with acceptable CV (Table 1). In diseased dogs, ICC between pretreatment conditions for manual counts and between manual and automated TCC also remained good, except for DTT-treated samples, where it dropped to moderate levels. Variability was markedly higher in the diseased group, particularly in the DTT-treated sample (Table 1 and Figure 2).

In healthy dogs, Bland–Altman analysis performed on log-transformed data demonstrated mean ratios close to 1 between pretreatment conditions and between manual and automated methods, with acceptable 95% limits of agreement (Figure 3) ranging from approximately half to twice the measured value. The distribution of points was homogeneous around the mean ratio, and most points lay within the agreement limits (Table 2 and Figure 3).

In diseased dogs, Bland–Altman analysis revealed that pretreatment with DTT or filtration resulted in poor agreement when compared with manual count on naïve samples (Table 2 and Figure 4). The manual naïve TCC count was on average 40% lower than the DTT-treated count and 15% lower than the filtered count, with wide 95% limits of agreement, consistent with the high CV observed.

Agreement between automated and manual methods in diseased dogs was good for filtrated samples, with a mean ratio close to 1 and acceptable 95% limits of agreement. For DTT-treated samples, agreement was moderate to poor, as manual counts were,

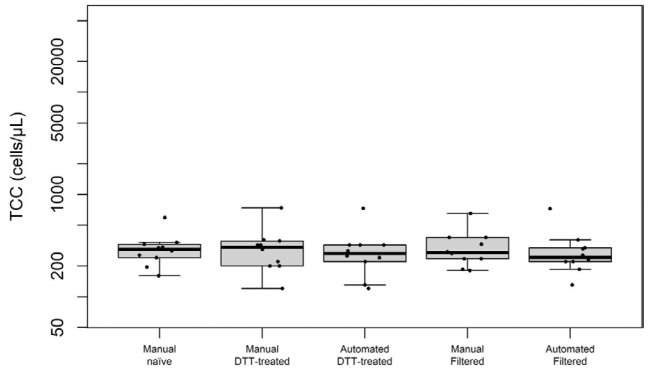


FIGURE 1 | Boxplots of total cell count (TCC) according to pretreatment condition in healthy dogs.

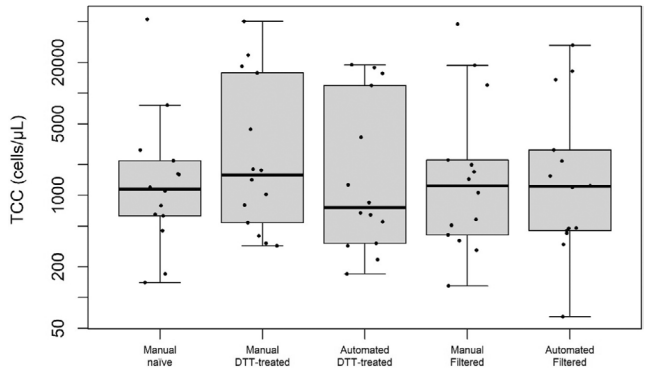


FIGURE 2 | Boxplots of total cell count (TCC) according to pretreatment condition in diseased dogs.

TABLE 1 | Agreement between manual and automated total cell count in healthy and diseased dog across pretreatment conditions.

	ICC (IC 95%)		CV (%)	
	Healthy	Diseased	Healthy	Diseased
Manual naïve compared with				
Manual DTT-treated	0.78 (0.43–0.94)	0.85 (0.67–0.95)	22	78.7
Manual filtered	0.94 (0.84–0.98)	0.91 (0.80–0.97)	10.5	70.6
Manual DTT-treated compared with automated DTT-treated	0.86 (0.65–0.96)	0.68 (0.34–0.88)	20.6	94.7
Manual filtered compared with automated filtered	0.83 (0.57–0.96)	0.90 (0.79–0.97)	20.5	60.7

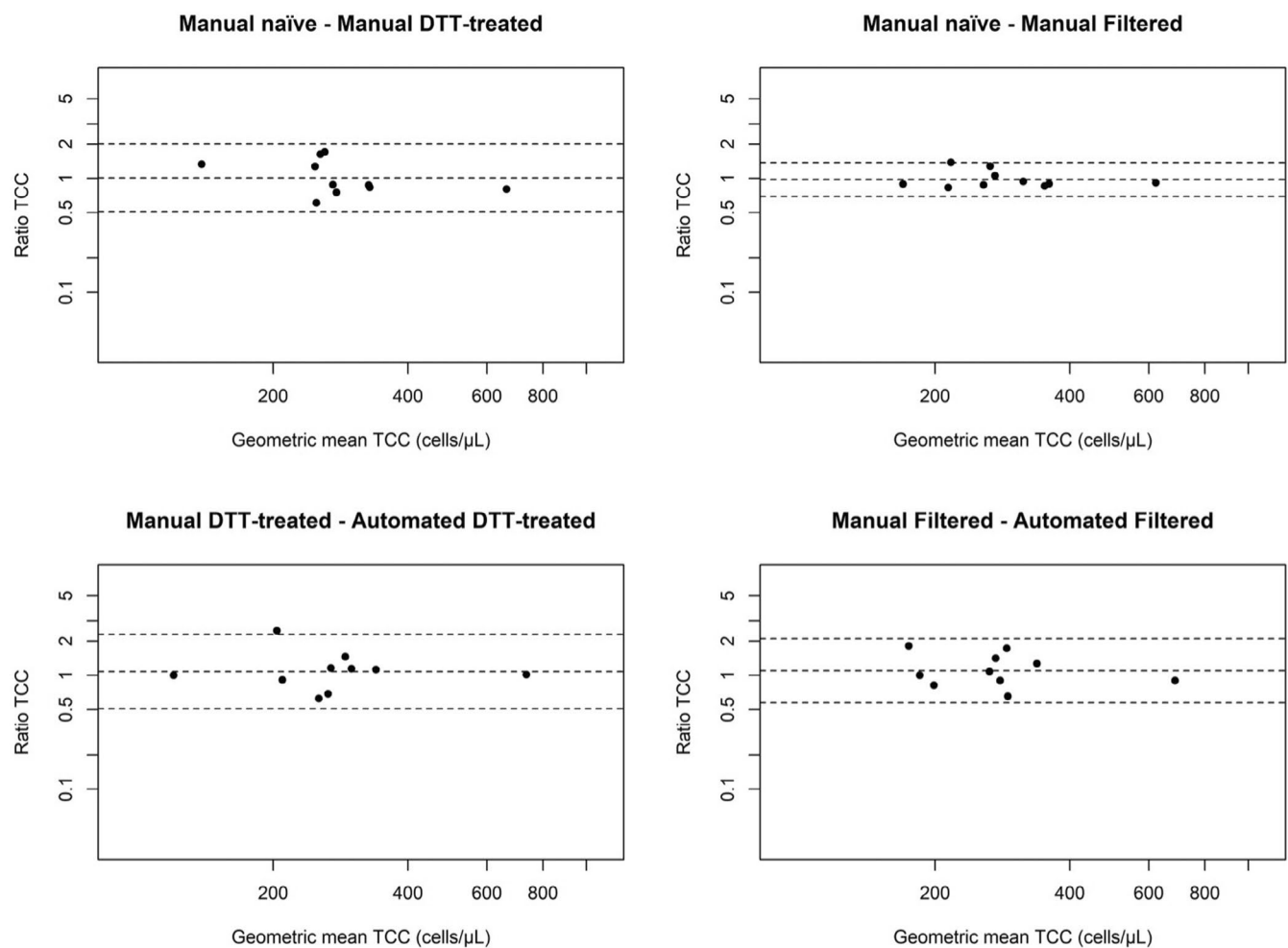


FIGURE 3 | Bland–Altman plot comparing pretreatment methods on manual count and comparing manual and automated counts in healthy samples ($n = 10$).

TABLE 2 | Bland–Altman analysis results between manual and automated total cell count in healthy and diseased dogs across pre-treatment conditions.

	Mean ratio		95% limits of agreement	
	Healthy	Diseased	Healthy	Diseased
Manual naïve compared with				
Manual DTT-treated	1.010	0.548	0.509–2.004	0.064–4.677
Manual filtered	0.976	0.871	0.693–1.376	0.099–7.664
Manual DTT-treated compared with automated DTT-treated	1.078	1.560	0.509–2.285	0.794–3.063
Manual filtered compared with automated filtered	1.100	1.063	0.575–2.104	0.601–1.883

on average, 51% higher than automated counts, with wide 95% limits of agreement.

Finally, Pearson correlation coefficient indicated strong positive linear associations between pretreatments and between manual and automated methods in both healthy and diseased dogs (Table 3) and Passing-Bablok regression analysis did not reveal significant systematic or proportional bias for any comparison in either group (Supporting Information 4).

3.3 | Differential Cell Counts (DCC)

When comparing DCC results obtained using manual counting and the IDEXX ProCyt Dx analyzer, significant inconsistencies in the detection of certain cell types were identified (raw data available on Supporting Information 5). Basophils and monocytes were wrongly reported present by the automated analyzer, while they were not identified during manual cytologic evaluation. Conversely, macrophages and mast

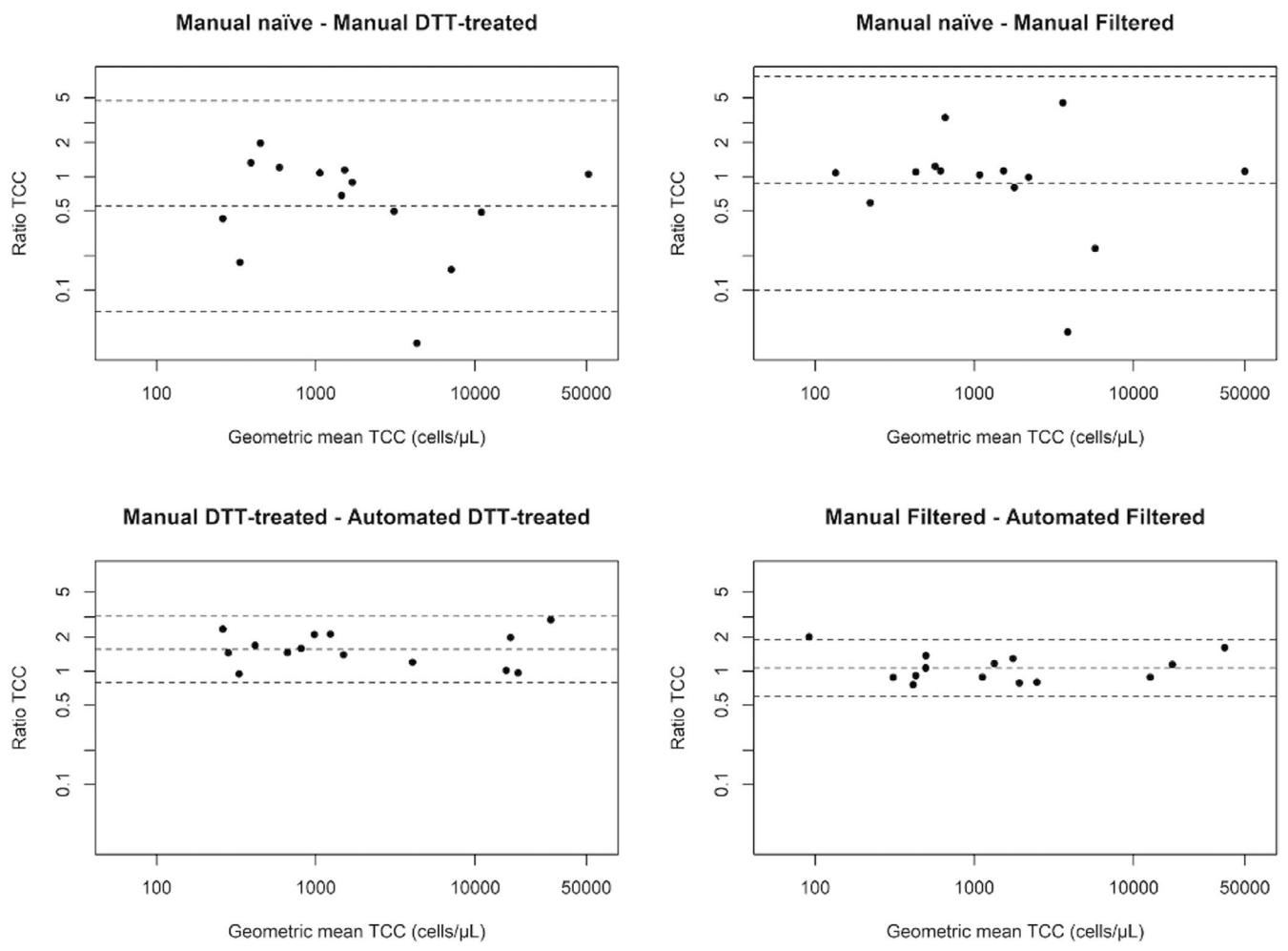


FIGURE 4 | Bland–Altman plot comparing pretreatment methods on manual count and comparing manual and automated counts in diseased samples ($n = 14$).

TABLE 3 | Pearson correlation coefficients between methods in healthy and diseased dogs.

	Healthy	Diseased
Manual naïve compared with		
Manual DTT-treated	0.819	0.869
Manual Filtered	0.947	0.904
Manual DTT-treated compared with automated DTT-treated	0.857	0.852
Manual filtered compared with automated filtered	0.839	0.977

cells were not counted by the IDEXX ProCyte Dx analyzer, even though they were present in large numbers on the manual cytologic examination. The scatter plots provided by the IDEXX ProCyte Dx analyzer were also analyzed (available in Supporting Information 6) and did not provide reliable information for any of the cell types.

As a result, the percentages of cell types reported by both techniques were not directly comparable.

4 | Discussion

The IDEXX ProCyte Dx analyzer provided reliable TCC measurements in healthy BALF samples regardless of the pretreatment method. In diseased samples, greater variability among pretreatment methods was observed, especially for DTT-treated samples. Good agreement between manual and automated counts was observed only in filtrated samples, supporting filtration as the preferred pretreatment for diseased BALF, although a non-negligible risk of deviation from the naïve count remains. Automated DCC was inconsistent and could not be considered reliable.

In healthy dogs, the IDEXX ProCyte Dx results were in good agreement with manual counting across pretreatment methods for TCC measurements. On the contrary, DCC obtained with the IDEXX ProCyte Dx analyzer was not consistent with manual counts. The analyzer failed to detect typical cells found in BALF, such as macrophages and mast cells, while incorrectly identifying other cell types, such as monocytes and basophils. This misclassification came from the fact that BALF cellular populations differ significantly from those in peripheral blood, both in composition and morphology [1, 2]. As a result, the relative proportions of cell types reported by the analyzer did not match those obtained manually, and

direct comparison was not meaningful. Knowing this, automated differential count cannot replace manual cytologic interpretation, especially in inflammatory conditions where subtle variations in cell types have diagnostic and therapeutic implications [3–6]. In order to compensate for this inability of the IDEXX Procyte Dx analyzer to establish a DCC corresponding to the manual count, certain new techniques, including those based on artificial intelligence, offer promising results [24, 25].

In dogs affected with lower respiratory tract diseases, ICC values remained moderate to good across pretreatment conditions and between manual and automated methods. However, compared with healthy samples, an increased variability was observed in diseased samples, reflected by deviant mean ratio and by wide 95% limits of agreement in the Bland–Altman analyses. The highest variability was found for the DTT-treated samples. A plausible explanation is that DTT, while reducing viscosity, may release cell fragments or debris that can be mistaken for nucleated cells, leading to artificially elevated counts in DTT-treated samples when compared with naïve samples [17]. Conversely, excessive cell fragmentation may decrease the number of intact cells detected by the IDEXX Procyte Dx machine, thereby reducing cellular counts when automated counts were compared with manual counts. Filtration accounted for less but not negligible variation. By trapping mucus within the filter, some cells embedded in the mucus may also be retained, which can result in an underestimation of total counts, as previously described [16]. With regard to DCC, the same inconsistencies observed in healthy dogs were also present in diseased dogs, affecting both cell numeration and cell visualization on the scatter plots.

Pearson correlation coefficients were strong in both healthy and diseased dogs across pretreatment condition and across methods, suggesting that the linear association between methods was preserved even in the presence of greater dispersion. Passing-Bablok regression did not identify significant systematic or proportional bias in any comparison, as confidence intervals for the intercepts included 0 and those for the slopes included 1. This suggests that the observed discrepancies in diseased samples, particularly in DTT-treated samples, were primarily driven by increased variability rather than by a constant analytical bias between methods.

Several limitations must be acknowledged. First, the monocentric design of the study may limit the generalizability of the results. However, conducting all analyses at a single institution ensured consistent sample handling, standardized procedures, and the use of a single analyzer, thereby minimizing pre-analytical variability. Manual DCC was performed on 200 cells. Although analysis of a larger number of cells could have improved data precision, this was not undertaken to reflect the typical clinical practice of the institution. Secondly, the relatively small number of dogs within each group may have limited the robustness of the observed findings. However, the sample size was determined in accordance with the 3R principle, and although the sample size was small, retrospective power analysis confirmed that it was sufficient to generate preliminary exploratory data in diseased dogs. Additionally, the diversity of respiratory conditions represented further reflects the clinical

spectrum commonly encountered in practice, supporting the relevance of the findings [3, 6–9]. Thirdly, manual counts in diseased dogs were performed by a single operator, preventing assessment of inter-observer variability in this group. However, results from healthy dogs demonstrated high reproducibility and low inter-observer variability, making this limitation less likely to have influenced the results. Naïve BALF samples were not analyzed with the IDEXX ProCyte Dx due to concerns about potential mucus-related interferences. Future studies are needed to determine whether unprocessed samples can be analyzed directly, as this could further improve the speed of the analysis. Additionally, only two pretreatment strategies were evaluated. Exploring alternative mucolytic agents or varying DTT concentrations might help reduce variability and optimize BALF preparation, although this was beyond the scope of the present study.

Finally, the study was conducted exclusively in dogs. While similar applicability is expected in cats, independent validation is required in this species.

In conclusion, this study demonstrated that the IDEXX ProCyte Dx analyzer can be reliably used to assess TCC in BALF from healthy dogs after pretreatment to reduce fluid viscosity. In dogs affected with lower respiratory tract diseases, it appears that filtration should be preferred over DTT pretreatment as it accounted for less variation in TCC results and showed good agreement between automated and manual counts, at least until the impact of DTT on sample representativeness is better characterized or alternative mucolytic agents are evaluated. However, because agreement between manual counts of naïve and filtered samples was poor in diseased dogs, automated TCC results should be interpreted cautiously, as they may deviate from the true value.

These findings open the possibility of integrating automated BALF analysis into clinical practice, allowing faster and more reproducible TCC assessment, thereby improving diagnostic efficiency, reducing delays, and avoiding inter-operator variability. At present, DCC should still be performed manually to ensure accuracy and reliability.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the [Supporting Information](#) of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** vcp70132-sup-0001-DataS1.xlsx.