COMPARISON OF A NOVEL BLOOD FILM ESTIMATION AND A FLOW CYTOMETRIC METHOD

PLATELET ENUMERATION TO THE MANUAL HEMACYTOMETER AND AUTOMATED HEMATOLOGY ANALYZER PLATELET COUNTS

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Abstract:
Differences in platelet counts have been documented regarding the counting method or analyzer used. In this study, two unconventional and multiple hematology analyzer platelet counting methods were used to investigate these differences. Three manual hemacytometer platelet counts were used as the reference method and automated counts were performed on the Advia120 (Siemens), Sysmex XT-V (Sysmex, Inc.), Cell-Dyn 3500 (Abbott Laboratories) and ProCyte Dx (IDEXX Laboratories, Inc.). With each blood film (BF) estimate, ten 1000x monolayer fields of view digital images were captured; all erythrocytes (range=2.543-3.682) and platelets (range=93-172) were enumerated and the platelet/erythrocyte ratio (P/E) was calculated. Flow cytometric (FC) counts of erythrocytes (range=33.963-91.266) and CD61 labeled platelets (range=1.191-3.288) were counted by flow-based fluorescence cytometry to obtain the P/E. Platelet counts were calculated using the P/E and the known erythrocyte counts from the different analyzers. Eight canine EDTA anticoagulated blood samples (normal HCT) were used; an average of three automated platelet count replications was recorded. Manual counts ranged from 236-270 k/µL. Compared to the manual platelet counts, average percent recovery by the ProCyte, XT-V, Cell-Dyn and Advia120 were 95.51%, 102.67%, 114.32% and 121.20%, respectively. Estimate percent recoveries of platelet counts using erythrocyte counts from the ProCyte, XT-V, Cell-Dyn and Advia120 for the two methods (BF/FC) were 106.42%/95.16%, 105.03%/93.90%, 104.31%/93.26% and 102.61%/91.70%, respectively. In summary, Advia120 and Cell-Dyn consistently over-recovered platelet numbers and XT-V and ProCyte counts were more similar to the manual counts. In addition, the BF and FC methods (within the 10-20% precision limits of the manual count) consistently recovered slightly more and less platelets, respectively, and proved effective in estimating platelet counts.

Introduction:
Differential counting of platelet counts (canine, feline and equine) are observed from various hematology analyzers (Advia, ProCyte and Sysmex XT-V). Concerns arise when a user expects platelet count (PLT) results to fall within a certain range based on previous or matched studies with more than one of these analyzers. Additionally, knowledge of what is real for platelet counts is important for proper hematology analyzer development and improvement. Previous studies have shown an approximate 20% difference in reported PLT between Advia and ProCyte. This study is to introduce a novel blood film estimation and a flow cytometric method of platelet enumeration to compare manual hemacytometry and automated hematology analyzer platelet counts. From this study, we are expecting to determine which analyzers respond most closely to the manual platelet counting.

Objectives:
The goal of this study is to identify the analyzers closely respond to the manual hemacytometry platelet counts, to understand why the discrepancy is observed from the analyzers, and to define a true reference method for counting platelet in standard hematology samples if possible.

Materials and Methods:

Instruments

Accutest (BD Biosciences), Advia120 (Siemens), Sysmex XT-V (Sysmex, Inc.), Cell-Dyn 3500 (Abbott Laboratories) and ProCyte Dx (IDEXX Laboratories, Inc.), Olympus microscope system (Olympus).

Materials

Daily fresh or day old canine EDTA anticoagulated whole blood samples (Information on canine age, gender and health status was not included in the data analysis), anti-platelet-CD61 (BD Biosciences) conjugated with AF647 (Invitrogen), ProCyte CD61 1% FBS buffer (Celloglo), Fetal bovine serum (FBS) (SaltSci, Inc.), 1X PBS (pH 7.4) EDTA-1% FBS buffer, Tyrode (5 mM) EDTA-1% FBS buffer, BMP LeukoChek, hemacytometry (InCyte)

Methods

Accutest flow-based fluorescence cell counting: Pipette 20 µL whole blood into 2 mL 1X PBS (5 mM) EDTA-1% FBS buffer using a 12 x75 mm tube. Invert the tube 5 times. Pipette 30 µL of the mixture into 2 mL 1X PBS (5 mM) EDTA-1% FBS buffer using a 12 x75 mm tube. Pipette 400 µL of the mixture into a 12 x75 mm tube and add 3 µL anti-CD61-AF647, incubate this mixture for 20 minutes and keep in dark; then run Accutest to determine PLT/RBC ratio (Figure 1). Estimate percent recoveries of platelet counts using erythrocyte counts from the ProCyte, XT-V, Cell-Dyn and Advia120.

Platelet counts: Follow the LeukoChek protocol to obtain the platelet mixture from canine EDTA anticoagulated whole blood samples. Load this mixture to an InCyte hemacytometer, wait 10 minutes and count platelet in 50 squares (25x on each chamber). Average the platelets counts from two chambers.

Blood film cell counts: Make blood films for blood samples, count platelets and red cells from 10 photomicrographs collected from the monolayer region of the blood film. 10 fields of view were evaluated to determine the PLT/RBC ratio (Figure 2). Estimate percent recoveries of platelet counts using erythrocyte counts from the ProCyte, XT-V, Cell-Dyn and Advia120.

Results:

Eight canine EDTA anticoagulated blood samples (normal HCT, 4 day old and 4 day fresh) were used; an average of three automated platelet count replications was recorded. Compared to manual platelet counts, average percent recovery by the ProCyte, XT-V, Cell-Dyn and Advia120 was shown in Table 1. Additional 8 normal and 29 patient EDTA anticoagulated blood samples (daily fresh, 4 of the patient samples were observed mild platelet clumping on their blood films) were used for further investigation; three automated platelet count replications were recorded for the 8 samples and one automated platelet count was recorded for patient samples. The platelet count difference between Advia and ProCyte was shown in Figure 3 for all samples. Compared to manual platelet count, Advia120 and Cell-Dyn cell counts over-recovered platelet counts; XT-V and ProCyte performed similarly to the manual count (Figure 4) for all samples. Accutest CD61 platelet count compares to manual platelet count, Advia120, Cell-Dyn, XT-V and ProCyte performed similarly to the manual count (Figure 5) for all samples. Blood film platelet count compares to manual platelet count, Advia120, Cell-Dyn, XT-V and ProCyte perform similarly to the manual count (Figure 6) for all samples.

Average Percent Recovery (ADV + TIDE)

Comparison

XTV Cell-Dyn Advia ProCyte

PLT manual 102.67 ± 5.6% 114.82 ± 5.5% 121.2 ± 10.4% 95.16 ± 6.9%

Platelet blood film 105.03 ± 9.78% 104.31 ± 9.48% 102.61 ± 9.85% 106.42 ± 5.97% flow cytometry 93.9 ± 6.21% 92.26 ± 5.89% 91.27 ± 6.65% 95.16 ± 6.04%

Conclusions:

The platelet count of ProCyte and XT-V performed similarly to the manual platelet count; in contrast, Advia120 and Cell-Dyn over-recovered platelet counts. The platelet counts from all analyzers using the blood film platelet estimation and flow cytometric method were consistently recovered more and less platelets, respectively, and proved effective in estimating platelet counts.

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