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InSight V5®R

Evaluation of InSight V5R for Dogs and Cats Compared to IDEXX ProCyte One and Manual Differential

Abstract

Background
Veterinary haematology analysers in a modern automated laboratory allow measurement of a variety of haematology parameters, which are used in the diagnosis, monitoring and treatment of disease. Therefore, it is important to verify the performance of the veterinary haematology analyser and to compare the performance of different instruments in measuring the same parameters.

Methods
In this study, the background, carry-over, repeatability and linearity of the InSight V5R was evaluated. The correlation of the complete blood count was evaluated between InSight V5R and IDEXX ProCyte One, as well as the comparison of the white blood cell parameters correlation between the two instruments and manual blood film.

Results
A good agreement between InSight V5R and ProCyte One under comparison by the correlation analysis. InSight V5R shows good white blood cell differentiation results compared with manual microscopy.

Conclusion
The performance of the InSight V5R Veterinary Haematology Analyser is good and can meet the requirements of the veterinary laboratory.

Keywords
Haematology analyser, blood cell count, comparison, performance evaluation.

1. Introduction

Complete blood cell count (CBC) plays a vital role in veterinary haematology evaluation. To deeply explain the physiological status, symptoms and disease process of animals, it is crucial to understand the abnormalities within the clinical parameters such as white blood cells (WBC) , red blood cells (RBC), platelets (PLT) and reticulocytes (RET) in peripheral blood.

Compared with traditional manual methods, the modern veterinary haematology analyser has higher objectivity and enables more accurate measurement of various haematological parameters (Rebar et al., 2001). These detailed haematological parameters not only provide a comprehensive understanding of the overall health status of the patients but also a basis for diagnosis and treatment. By monitoring the white blood cell count, the activity of the immune system can be assessed, thus helping to identify infection, inflammation or other underlying health problems. Red blood cell count and related parameters provide information about the oxygen transport function of the animals, which can help to detect anaemia or other blood diseases. Platelet count is a direct indicator of the ability to

haemostasis and is important for the assessment of risk of haemorrhage, and the diagnosis of coagulation disorders. Furthermore, the reticulocyte count can provide information about erythropoiesis in the bone marrow and is significant for assessing the type and degree of anaemia. The stable performance and accurate results of the InSight V5R is the basis of clinical veterinary diagnosis and treatment. To achieve this goal, the performance evaluation experiment is designed to verify the accuracy and reliability of the InSight V5R by measuring different haematology parameters, in order to ensure that the measurement of the instrument matches the established standards (Huang, 2004).

2. Materials and Methods

2.1 Materials

2.1.1 Samples
Fresh venous blood was collected using EDTA-K2 as the anticoagulant. All sample volume sizes were no less than 1.5ml. No abnormalities were observed in the samples, such as haemolysis and agglutination.

2.1.2 Instruments and Reagents
1. InSight V5R 5-Part Veterinary Haematology Analyser with Reticulocytes, haematology calibrators, haematology quality control (3 levels) and the corresponding supporting detection reagents.
2. IDEXX ProCyte One Haematology Analyser, haematology calibrators, haematology quality control (3 levels) and the corresponding supporting detection reagents.

2.2 Methods
Before the evaluation, it was confirmed that both InSight V5R and ProCyte One instruments were in good condition. The background, carry-over, repeatability and linearity of InSight V5R, and the correlation of the complete blood count were evaluated between InSight V5R and IDEXX ProCyte One, as well as the comparison of the white blood cells parameters correlation between the two instruments and manual microscope (Bollinger et al., 1987).

2.2.1 Background
After InSight V5R is turned on, select the test mode and test continuously on the analyser for 3 times using the diluent as the sample. The maximum value of the results of the 3 background results taken.

2.2.2 Carry-over
Perform continuous tests 3 times on the high and low concentration samples respectively (H1, H2, H3 and L1, L2, L3) to calculate the carry-over rate of the five main parameters: WBC, RBC, HGB, HCT, PLT. The carryover rate was calculated according to the following formula:

$$CR = \frac{|L1 - L3|}{|H3 - L3|} * 100\%$$

where:

CR – Carry-over rate
L1 – The first measurement result of low concentration sample
L3 – The third measurement result of low concentration clinical sample
H3 – The third measurement result of high concentration clinical samples

2.2.3   Repeatability

Select fresh EDTA venous blood samples from 5 cats and 5 dogs that meet the requirements of the detection range selected. Measure them 10 consecutive times under the counting interface and calculate the coefficient of variation (CV%), standard deviation (SD) or absolute deviation (d) of the 10 results for selected parameters (WBC, RBC, HGB, MCV, PLT, Neu%, Lym%, Mon%, Eos%, Bas%, RET#, RET%).

2.2.4   Linearity

Take EDTA whole blood samples and centrifuge them to remove plasma into concentrated blood cells. Then, the concentrated blood cells were gradient diluted with their own platelet-poor plasma/diluent to at least 5 concentrations of 20% from 100% to 0%, making the high concentration value close to the upper limit of the linear range and the low concentration value close to the lower limit of the linear range. Test each diluted sample using InSight V5R 3 times, calculate the average result of each sample. The regression equation and the correlation coefficient r are calculated using the following equation (1), using the dilution ratio as the independent variable (X) and the measured average result of each dilution as the dependent variable (Y). Use the regression equation to calculate the theoretical value corresponding to each dilution ratio, to ultimately calculate the absolute or relative deviation of the measured mean result and the theoretical value calculated. The linear deviation is calculated according to equation (2). Follow the instructions for the linearity test kit and calculate the linear deviation and correlation coefficient results.

(1)
$$r = \frac{\sum (X - \overline{X})(Y - \overline{Y})}{\sqrt{\sum \left(X - \overline{X}\right)^2 \left(Y - \overline{Y}\right)^2}}$$

where:

r – Correlation efficient
$\overline{X}$ – Average value of X
$\overline{Y}$ - Average value of Y

(2)
$$\Delta x_i = \frac{X_i - X_{io}}{X_{io}} * 100\%$$

where:

ΔX_i – The linear deviation of X_i at dilution ratio i

X_i – Average measured result at dilution ratio i
X_{io} – Average theoretical value calculated by the regression equation at dilution ratio i

2.2.5   Correlation

1.   Internal Clinic Laboratory Correlation Evaluation
Test the correlation of 7 parameters (WBC, RBC, HGB, MCV, PLT, RET#, RET%) of the two instruments, InSight V5R and IDEXX ProCyte One, by internal clinical laboratory. Calibrate both the instruments according to their respective operator's manuals before the correlation evaluation. 42 cat samples and 45 dog samples are selected and measured on the InSight V5R and ProCyte One at room temperature. After judging the validity of the test results, the correlation analysis was performed. In the process of statistical results, it is required to eliminate the samples that can affect accuracy due to the limitation of principle, such as small erythroid cells, samples with nucleated red blood cells, etc.

Statistical Regression Equation: Y = aX + b and the correlation coefficient r is calculated.

2.   External Vet Clinic Correlation Evaluation
Test the correlation of 7 parameters (WBC, RBC, HGB, MCV, PLT, RET#, RET%) of the two instruments, InSight V5R and IDEXX ProCyte One, by an external vet clinic. Calibrate both the instruments according to their respective operator's manuals before the correlation evaluation. 15 dog samples are selected and measured on the InSight V5R and ProCyte One at room temperature. After judging the validity of the test results, the correlation analysis was performed.

Statistical Regression Equation: Y = aX + b and the correlation coefficient r is calculated.

3.   External Vet Clinic Correlation Evaluation for WBC Differentiation
Test the correlation of 5 parameters of WBC differentiation (Neu%, Mon%, Lym%, Eos%, Bas%) between InSight V5R, ProCyte One and manual microscopy by an external vet clinic. Calibrate both the instruments according to their respective operator's manuals before the correlation evaluation. A total of 17 dog samples are collected and each sample is divided into 3 samples, which are used for manual microscopic examination and two instrument tests. In the manual microscopic method, two blood smears are applied to each sample and 200 cells are analysed for a total of 400 cells. Two qualified examiners analyse one blood smear each, the results are averaged and recorded as percentages. After judging the validity of the test results, the correlation analysis was performed.

Statistical Regression Equation: Y = aX + b and the correlation coefficient r is calculated (Hyun et al., 1991).

3. Results

3.1 Background

Mode	WBC	RBC	HGB	HCT	PLT
Cat	0	0	0	0	0
Dog	0	0	0	0	0
Standard	≤0.20	≤0.20	≤1	≤0.5	≤10
Result	PASS	PASS	PASS	PASS	PASS

Table 1 Background Test Results

3.2 Carry-over

Mode	WBC	RBC	HGB	HCT	PLT
Cat	0.03	0.00	0.00	0.00	0.41
Dog	0.01	0.00	0.00	0.00	0.00
Standard	0.5%	0.5%	0.5%	0.5%	0.5%
Result	PASS	PASS	PASS	PASS	PASS

Table 2 Carry-over Test Results

3.3 Repeatability

Sample ID	WBC	Neu%	Lym%	Mon%	Eos%	Bas%
Cat 1	1.47%	0.06	0.05	0.20	0.10	0.29
Cat 2	2.63%	1.86	-1.24	0.91	0.74	-0.27
Cat 3	1.82%	-1.35	-1.32	-0.95	0.75	-0.23
Cat 4	1.33%	-1.32	1.12	-0.66	0.29	-0.13
Cat 5	1.03%	-1.27	-0.97	-0.42	0.6	-0.14
Dog 1	1.62%	0.72	0.7	-0.48	0.56	0
Dog 2	1.03%	0.96	-0.9	-1.02	0.55	-0.09
Dog 3	1.86%	-1.25	-0.64	0.59	0.38	-0.08
Dog 4	0.92%	1.14	0.94	0.26	-0.36	-0.08
Dog 5	2.90%	3.5	3.8	-1.88	-1.32	-0.94
Standard (≤CV/d/s)	3.0%	4.0	4.0	3.0	3.0	1.0
Result	PASS	PASS	PASS	PASS	PASS	PASS

Table 3 Repeatability Test Results (Part 1)

Sample ID	RBC	HGB	MCV	RET#	RET%	PLT
Cat 1	0.99%	0.70%	0.23%	11.64%	12.70%	4.29%
Cat 2	1.17%	0.91%	0.22%	9.40%	6.27%	3.79%
Cat 3	1.04%	0.54%	0.17%	13.6%	12.9%	2.88%
Cat 4	0.79%	0.58%	0.16%	12.84%	13.99%	5.31%
Cat 5	0.93%	0.68%	0.23%	12.77%	11.78%	2.80%
Dog 1	0.84%	0.74%	0.25%	13.32%	11.96%	2.47%
Dog 2	0.54%	0.54%	0.17%	7.5%	12.2%	2.02%
Dog 3	1.25%	0.91%	0.18%	10.56%	12.2%	3.42%
Dog 4	0.88%	0.90%	0.14%	13.94%	14.15%	2.92%
Dog 5	0.69%	0.83%	0.13%	4.1%	10.0%	2.98%
Standard (≤CV/d/s)	1.5%	1.5%	1.0%	15.0%	15.0%	6.0%
Result	PASS	PASS	PASS	PASS	PASS	PASS

Table 4 Repeatability Test Results (Part 2)

3.4 Linearity
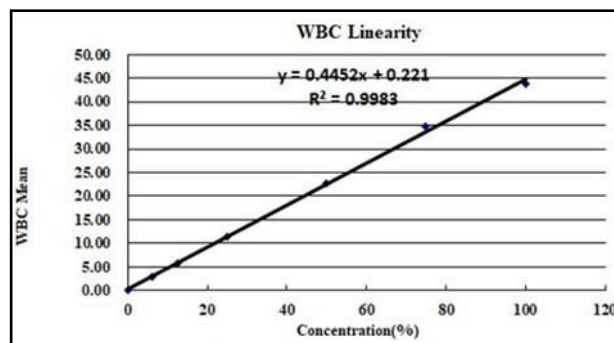
Figure 1 WBC Linearity Test Results 1 (0 – 43.67*10^9/L)
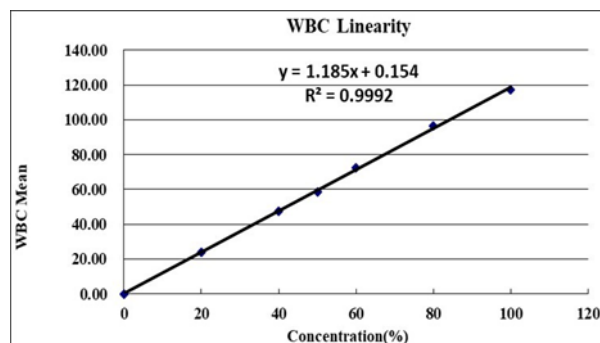
Figure 2 WBC Linearity Test Results 2 (23.73 – 117.09*10^9/L)
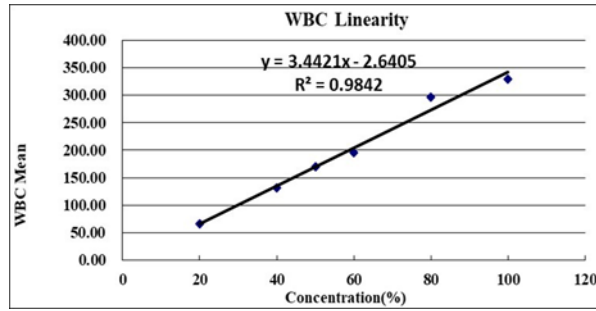
Figure 3 WBC Linearity Test Results 3 (66.84 – 329.11*10^9/L)
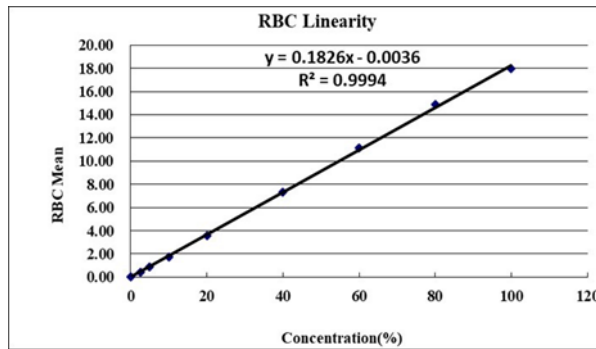
Figure 4 RBC Linearity Test Results (0 – 17.95*10^{1}2/L)
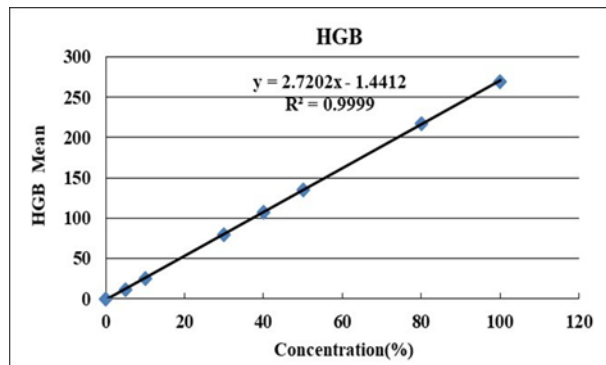
Figure 5 HGB Linearity Test Results (0 – 269.33 g/L)
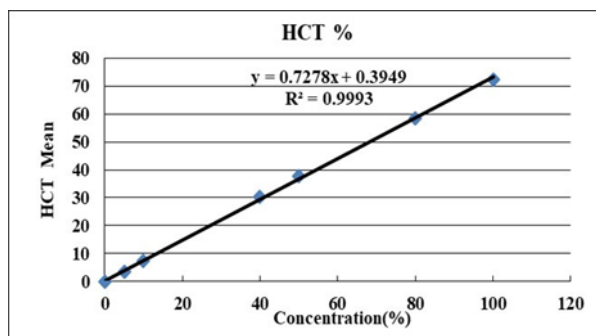
Figure 6 HCT Linearity Test Results (0 – 72.4)
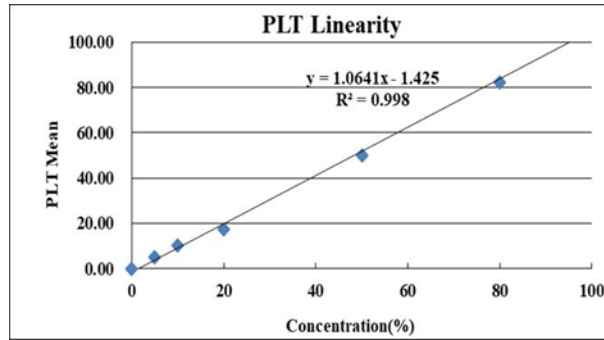
Figure 7 PLT Linearity Test Results 1 (0 – 114*10^9/L)
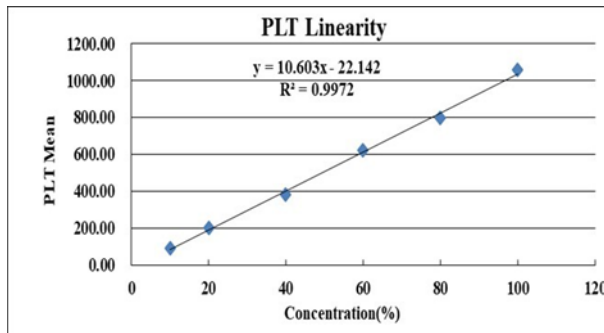
Figure 8 PLT Linearity Test Results 2 (89 – 1060*10^9/L)
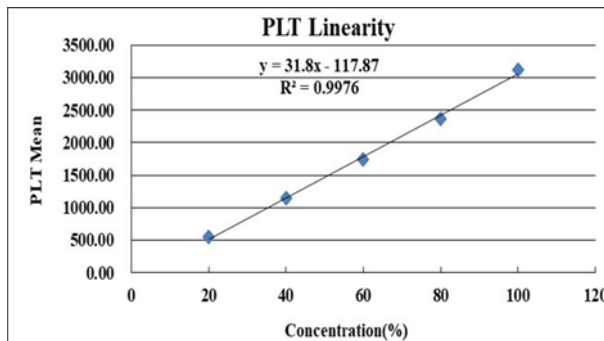
Figure 9 PLT Linearity Test Results 3 (556 – 3124*10^9/L)

Parameter	Product Specification Requirements	Linear Results
WBC	$0 - 300.00 \times 10^9$/L	$0 - 43.67 \times 10^9$/L
		$23.73 - 117.09 \times 10^9$/L
		$66.84 - 329.11 \times 10^9$/L
RBC	$0 - 17 \times 10^12$/L	$0 - 17.95 \times 10^12$/L
HGB	$0 - 250$ g/L	$0 - 269.33$ g/L
HCT	$0 - 67\%$	$0 - 72.4\%$
PLT	$0 - 3000 \times 10^9$/L	$0 - 114 \times 10^9$/L
		$89 - 1060 \times 10^9$/L
		$556 - 3124 \times 10^9$/L

Table 5 Linearity Test Results

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3.5 Correlation

Internal and external correlation evaluation between InSight V5R and ProCyte One results are shown in the following figure, where 'V' stands for InSight V5R and 'I' stands for IDEXX ProCyte One.

1. **Internal Clinical Laboratory Test Results (Dog)**
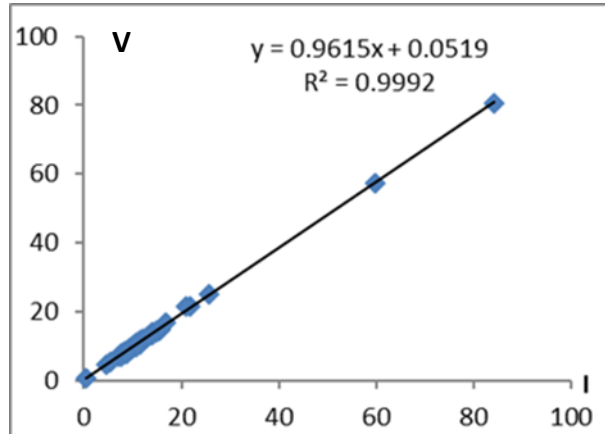
Figure 10 WBC Correlation Result

$y = 0.9615x + 0.0519$
$R^2 = 0.9992$
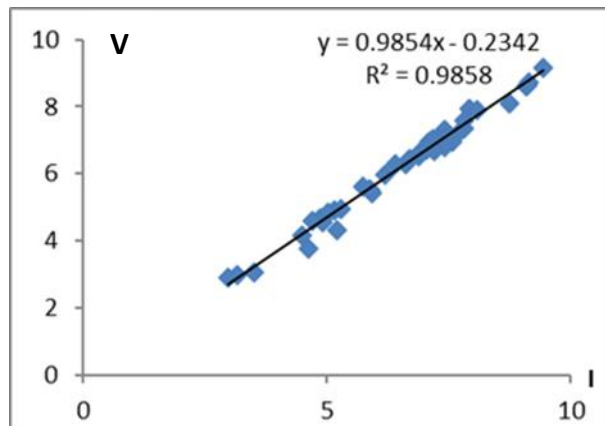
Figure 11 RBC Correlation Result

$y = 0.9854x - 0.2342$
$R^2 = 0.9858$

$y = 0.9984x - 2.3391$
$R^2 = 0.9897$
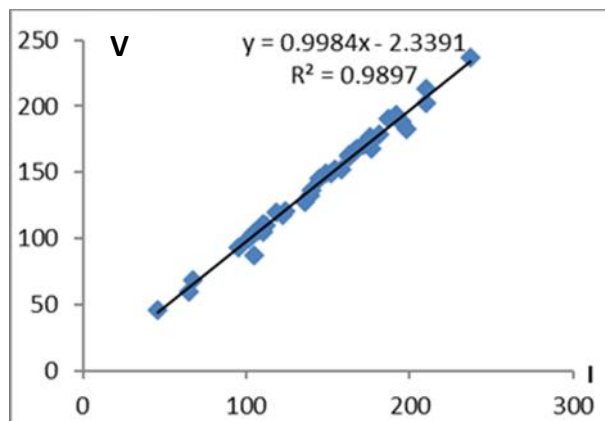
Figure 12 HGB Correlation Result

$$y = 0.966x + 1.1773$$
$$R^2 = 0.9833$$
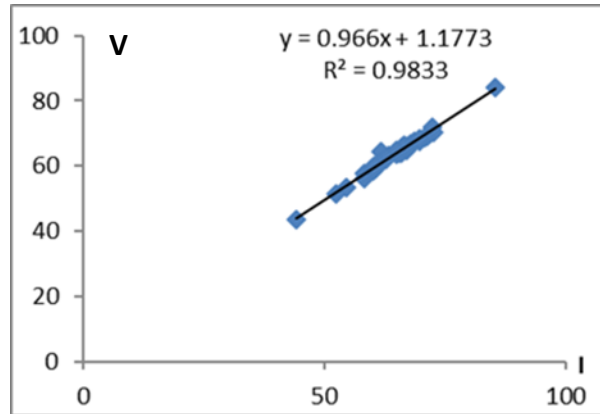
Figure 13 MCV Correlation Result

$$y = 0.9709x + 4.4064$$
$$R^2 = 0.9778$$
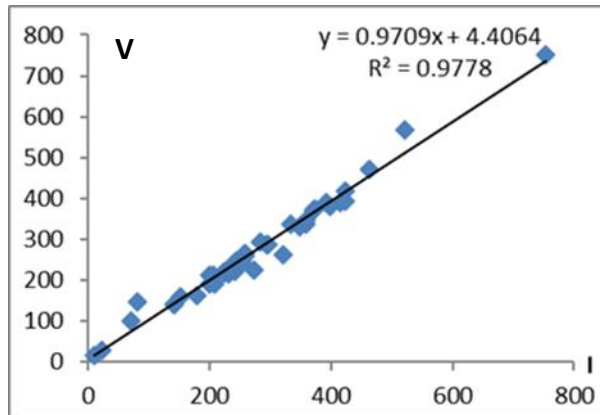
Figure 14 PLT Correlation Result

$$y = 0.8751x + 0.0039$$
$$R^2 = 0.8994$$
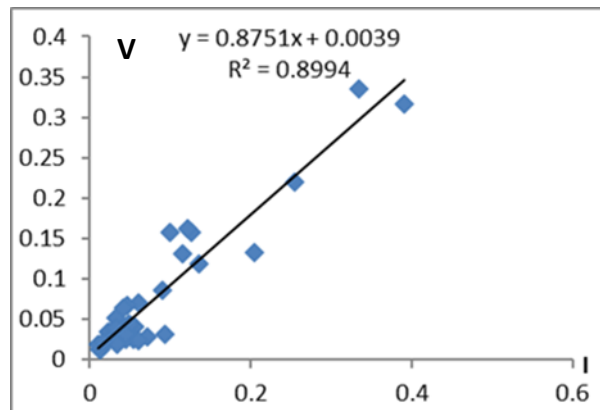
Figure 15 RET# Correlation Result
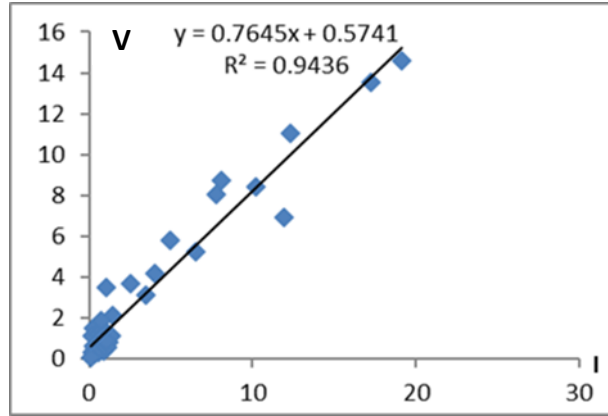
Figure 16 RET% Correlation Result

2. **Internal Clinical Laboratory Test Results (Cat)**
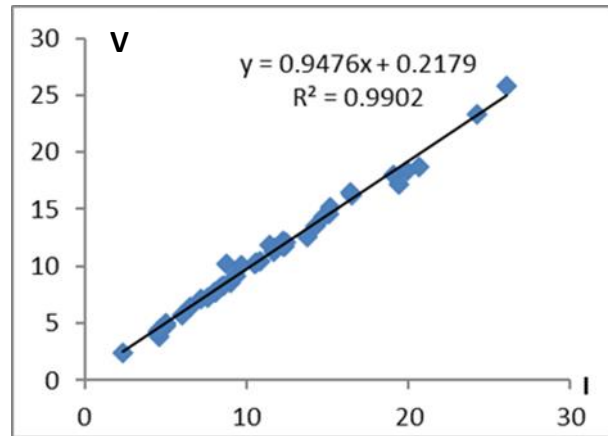
Figure 17 WBC Correlation Result
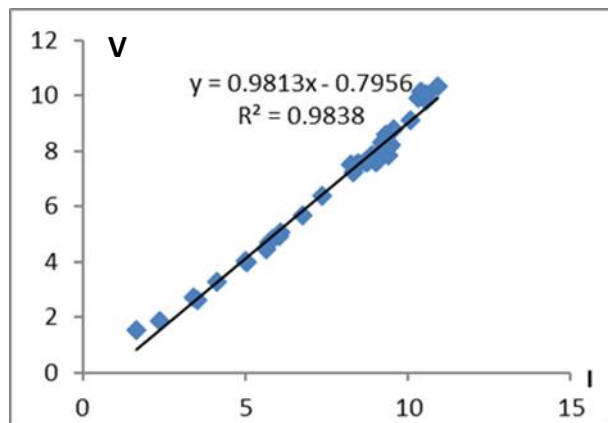
Figure 18 RBC Correlation Result
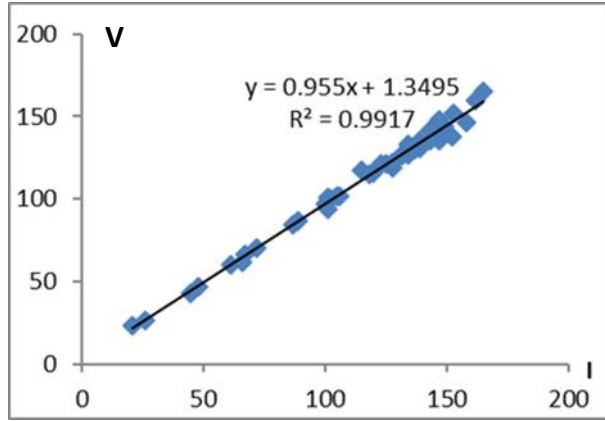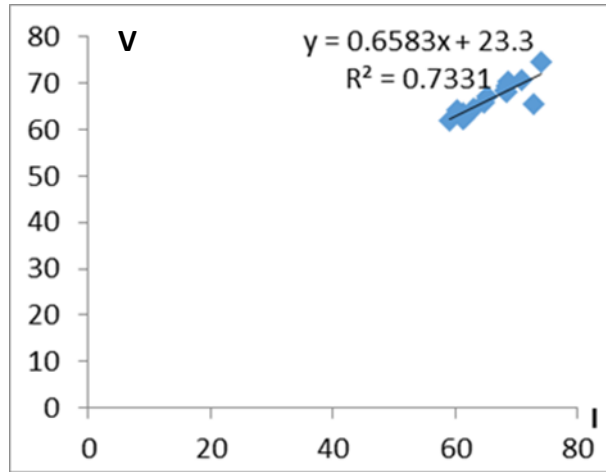
Figure 19 HGB Correlation Result
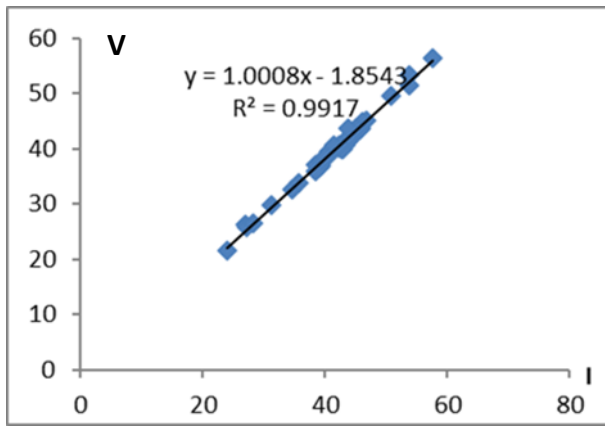
Figure 20 MCV Correlation Result
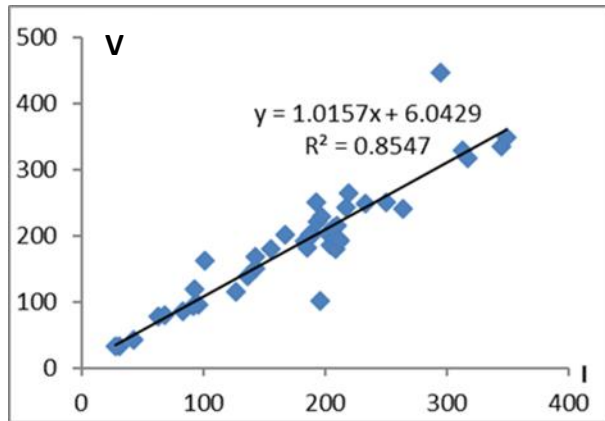
Figure 21 PLT Correlation Result
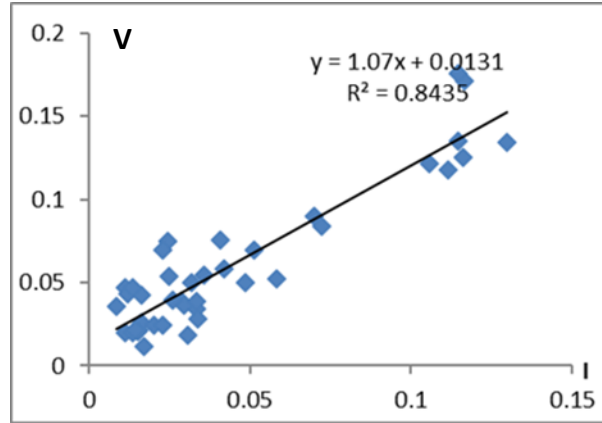
Figure 22 RET# Correlation Result
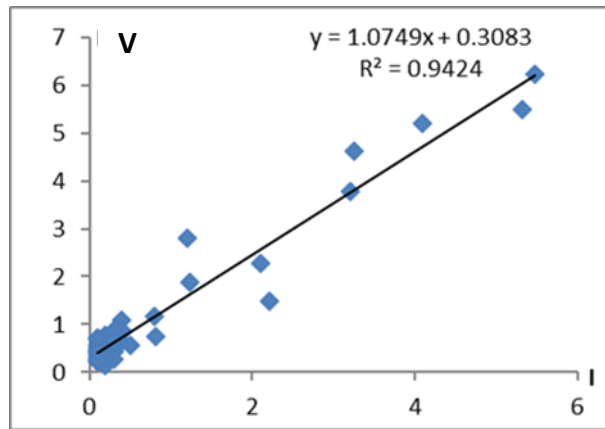
Figure 23 RET% Correlation Result

3. External Vet Clinic Test Results (Dog)

$y = 1.1259x - 1.396$
$R^2 = 0.9988$
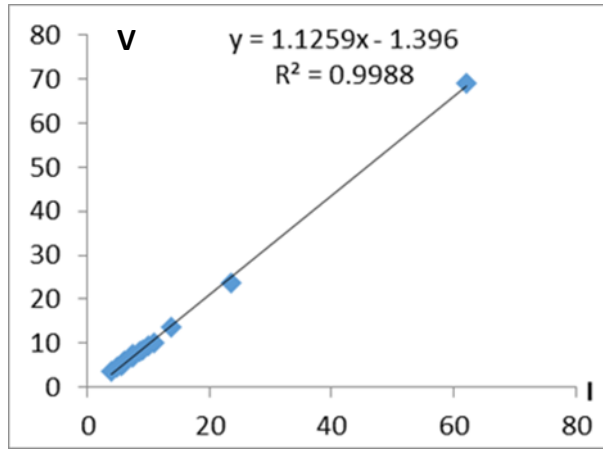
Figure 24 WBC Correlation Result

$y = 0.938x + 0.6562$
$R^2 = 0.9965$
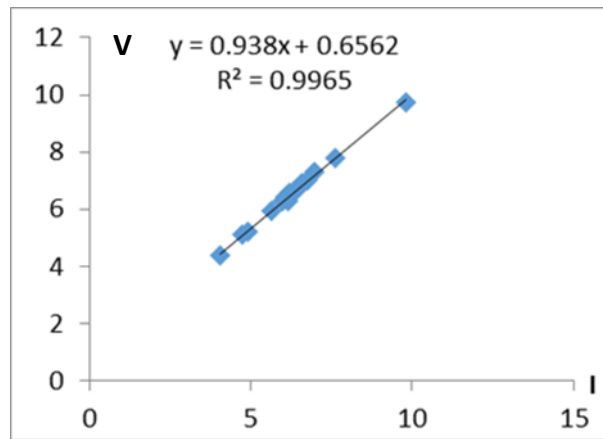
Figure 25 RBC Correlation Result

$y = 1.0469x - 0.1456$
$R^2 = 0.9936$
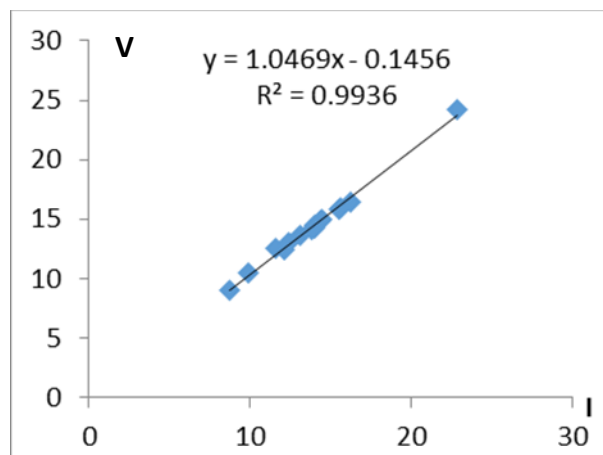
Figure 26 HGB Correlation Result

Figure 27 MCV Correlation Result
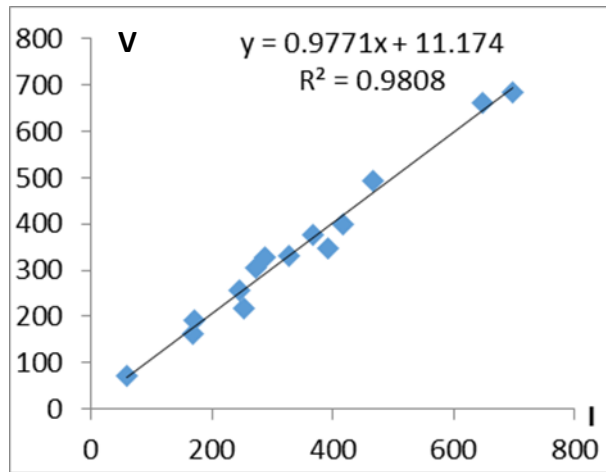
Figure 28 PLT Correlation Result
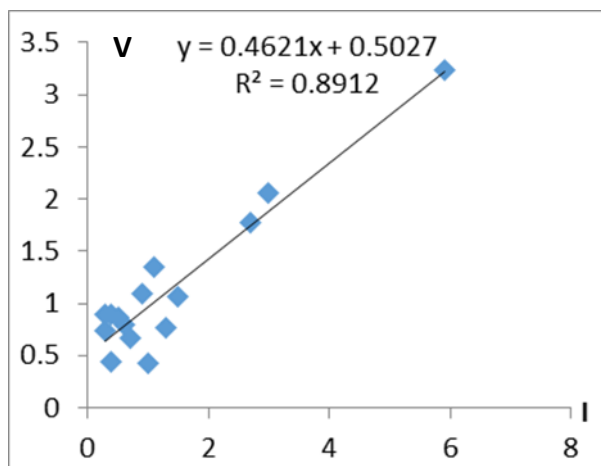
Figure 29 RET# Correlation Result
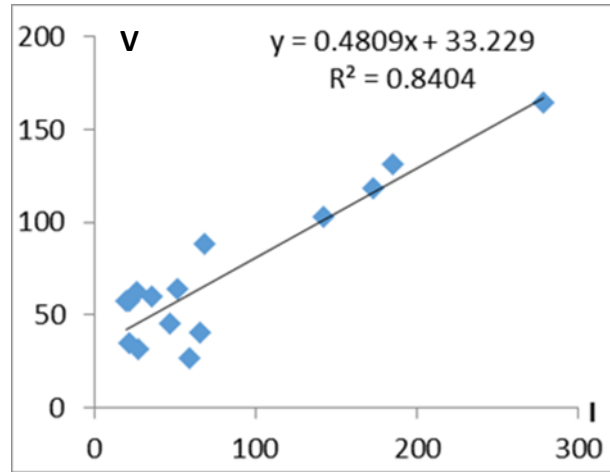
Figure 29 RET% Correlation Result

r	WBC	RBC	HGB	MCV	PLT	RET#	RET%
Internal Clinical Laboratory (Dog)	1.000	0.993	0.995	0.992	0.989	0.948	0.972
Internal Clinical Laboratory (Cat)	0.995	0.992	0.996	0.996	0.925	0.918	0.971
External Vet Clinic (Dog)	0.999	0.998	0.997	0.856	0.990	0.944	0.917

Table 6 Correlation Test Results (Between Both Instruments)

External Vet Clinic Correlation Evaluation for WBC Differentiation between Analysers and Manual Microscopic Method
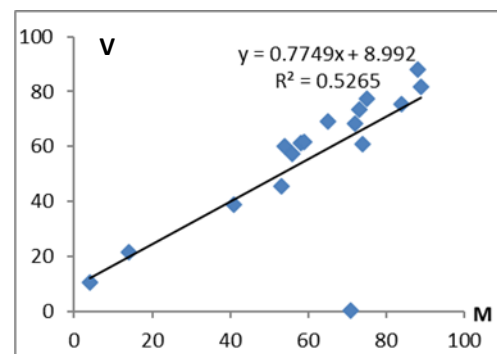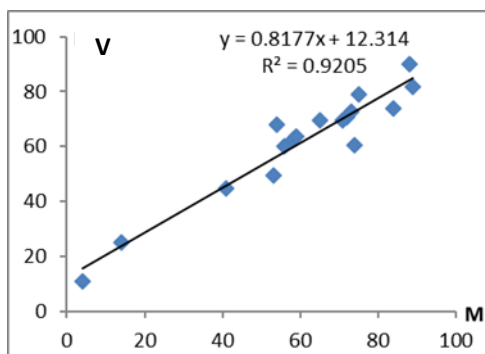

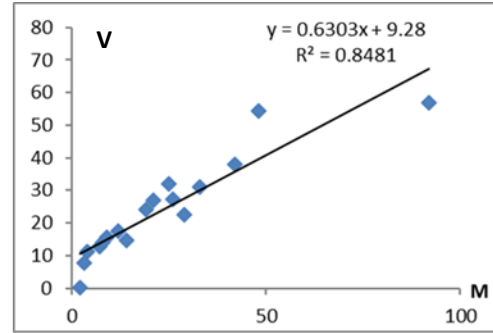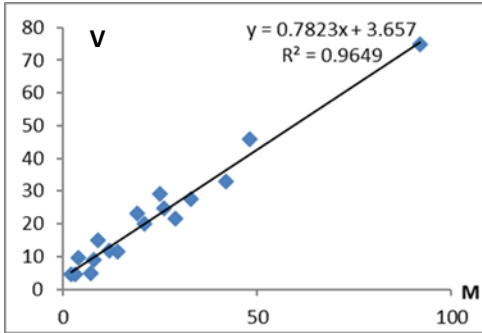**Figure 31 Comparative Evaluation of Neu% Correlation**

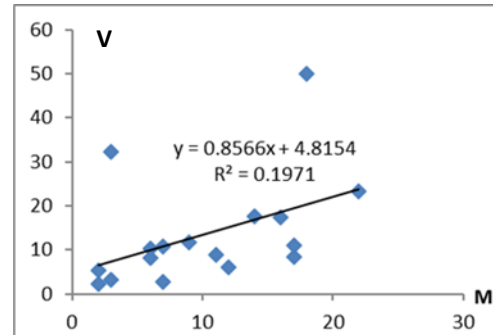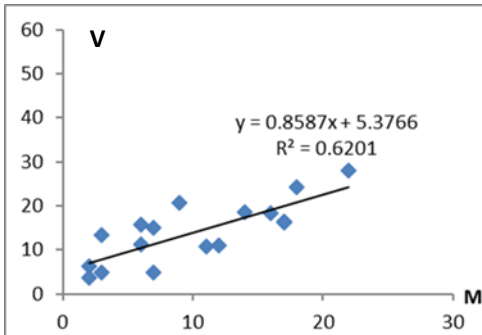**Figure 32 Comparative Evaluation of Lym% Correlation**

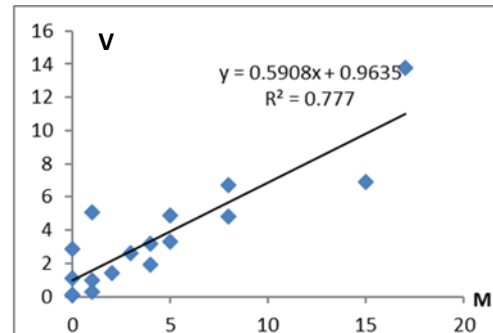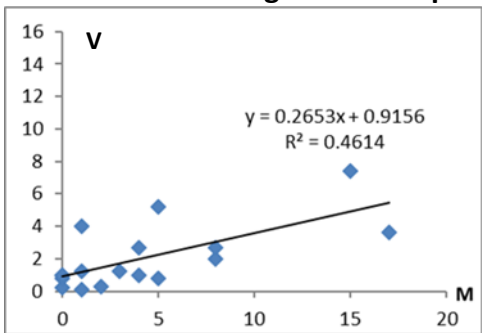**Figure 33 Comparative Evaluation of Mon% Correlation**

Figure 34 Comparative Evaluation of Eos% Correlation
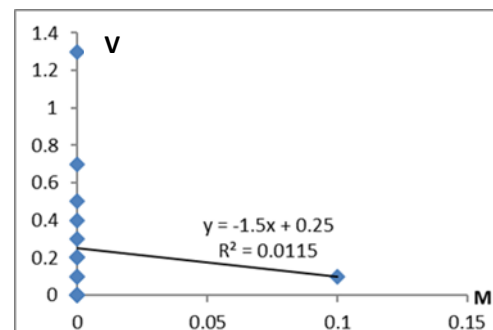
Figure 35 Comparative Evaluation of Baso% Correlation
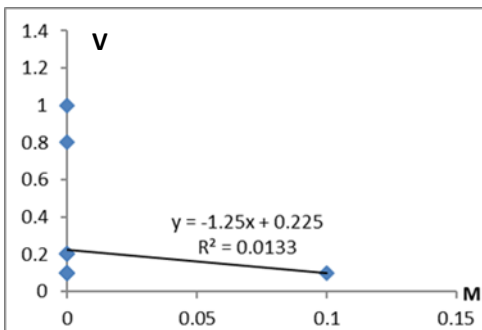
r	Neu%	Lym%	Mon%	Eos%	Baso%
InSight V5R	0.959	0.982	0.787	0.679	-0.115
ProCyte One	0.726	0.921	0.444	0.881	-0.107

Table 7 Correlation Test Results (Between Instruments and Manual Microscopic Method)

4. Conclusion

The evaluation results of InSight V5R are as follows:

- The background counting results of five parameters of WBC, RBC, HGB, HCT and PLT are satisfactory and meet the technical standards set by the manufacturer.
- The carry-over rate is within the acceptable range, meeting the technical standards set by the manufacturer.
- In terms of repeatability, the 12 parameters of the instrument show the precision level in line with the current technical standards, indicating that the instrument is precise and reliable and meets the standards set by the manufacturer.
- In the linear range evaluation, the correlation coefficient (r) of WBC, RBC, HGB, HCT and PLT were all greater than 0.99. This result shows a significant positive correlation between the measured values and the theoretical values, namely, the detected concentration is linearly correlated with the dilution ratio. It is worth noting that the linear range of InSight V5R in WBC, RBC, HGB, HCT and PLT is wider than the IDEXX ProCyte One instrument, which means that InSight V5R provides reliable measurements in a much wider range of concentrations, enhancing its flexibility and applicability in laboratory applications. This extension of the linear range is important for responding to different sample types and experimental requirements, and can provide a more comprehensive support for the accuracy and reliability of the experimental data (Rebar et al., 2001).
- When comparing the correlation between InSight V5R and IDEXX ProCyte One, the correlation between WBC, RBC, HGB and MCV in dogs and cats (correlation coefficient were all greater than 0.99) and the correlation in the two RET parameters also met the manufacturer's technical standards. In the external vet clinic evaluation, the two instruments showed good correlation on the 6 parameters of WBC, RBC, HGB, PLT, RET# and RET%. The internal and external data showed little difference in terms of correlation, which can be used to evaluate the correlation results. In addition, in the results of the correlation between instrument and manual microscopy, InSight V5R showed a better correlation in Neu% and Lym% (r>0.95). While Mon%, Eos% and Bas% are limited by the sample state (the result of these parameters in the samples were low) but still in the acceptable range. Overall, the five parameters of InSight V5R leucocyte differentiation were comparable with manual microscopy. Combining all the correlation results, it is showed that InSight V5R and IDEXX ProCyte One are comparable in performance.

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