Clinical Evaluation Reports for the GlinX InCoag Coagulation Testing System

1 Objectives

This report aims to evaluate the performance of the InCoag (Coagulation Operating System) and its companion test card in assessing a pet's coagulation function. The test is conducted by a professional veterinarian or nurse, who collects the animal's venous blood, tests it using the instrument, and compares the results, where possible, with those obtained from the pet's Sysmex automated coagulation analyzer and IDEXX coagulation system. This comparison allows for a thorough evaluation of the system's performance metrics.

2 Precautions

During the testing process, the following precautions should be observed:
2.1 Ensure the pet remains calm to avoid agitation, which could impact the quality of the blood sample.
2.2 Syringes, butterfly hoses, EP tubes used during testing, and test cards after use should be considered potential contaminants and must be disposed of in designated recycling containers according to the hospital's protocols.
2.3 When using syringes for blood collection, take care to protect yourself from needle injuries.
2.4 After completing the test, disinfect the pipette, laboratory bench, and instruments with 75% alcohol or alcohol disinfectant wipes.

3 Protocol

3.1 Test System Information

Table 1 InCoag Analyzer and Cartridge Information

Analyzer Mode	InCoag Coagulation Analyzer	**Cartridge Batch**	1072AK1212
SN	SN#15 #SN10	**Software**	V1.4.14
Remark： /			

3.2 Control System Information

Two control experiments were conducted for this report. Experiment 1 used the Sysmex CA1500 system as a control, while Experiment 2 used the IDEXX system as a control.

Table 2 Sysmex Control System Information

Control System	Sysmex CA1500	**Instrument Serial No.**	12397
Batch of PT reagent	2001491	**Batch of APTT reagent**	20102511
Batch of Fib reagent	565147	**Batch of TT reagent**	557663A
Batch of control level 1	564845A	**Batch of control level 2**	548516A
Remark：			

Experiment 1 in this report referenced Chapter 6 of the BSAVA Manual of Canine and Feline Clinical Pathology and utilized Sysmex coagulation test results as a reference. The key difference from the manual is that the reagents used in this report were not doubly diluted.

3.3 Sample Requirements

The samples selected for this test were sourced from pet hospitals and include:
1. Healthy pets;
2. Pets requiring blood clotting tests due to potential abnormalities in coagulation function.

For Experiment 1, non-anticoagulated venous whole blood was collected. The details for blood collection are as follows:

【Materials Prepared】

The necessary materials include syringes, butterfly tubes, alcohol swabs, and EP tubes containing 70 μL of anticoagulant. Depending on hospital practice, 1 mL or 2.5 mL syringes are used. Syringe needles should be 26G or 28G, and recommended butterfly tube sizes are 0.55, 0.60, or 0.70 mm (with colors medium purple, dark blue, or black), as shown in the figure below.

【Blood Collection Process】

1. Prepare the necessary materials for blood collection.
2. Clean the blood collection site with alcohol, and use a syringe and butterfly hose to collect at least 0.7 mL of venous blood.
3. After collection, withdraw the whole blood from the butterfly hose, discard the hose, and expel the air from the syringe's front end.
4. Add the blood to the fill hole of the test card that is in the fill state.
5. Click "Start Test."
6. Take a 650 μL sample of whole blood, add it to the EP tube containing anticoagulant, mix by inverting the tube five times, and store it for Sysmex testing.
7. Record the time taken for sample collection.

【Sample prepared process in laboratory】

In clinical tests, most pets are generally healthy, making it challenging to find animals with abnormal coagulation indexes within a short timeframe (2–3 days). To address this issue when using Sysmex as a reference system, the clinic collects additional anticoagulated blood samples from the pets and processes them in the laboratory to extend the clotting time of these samples. This procedure primarily targets PT (Prothrombin Time), APTT (Activated Partial Thromboplastin Time), and TT (Thrombin Time).

Treatment Method:

Addition of High Molecular Weight Heparin: High molecular weight heparin is added to the blood samples. Effect on Clotting Time: The heparin molecules work by inactivating coagulation factors, such as prothrombin, thereby prolonging the clotting time of the samples.

3.4 Testing Protocol

Exp.1

3.4.1 Testing Method

In this experiment, each sample was simultaneously tested on two InCoag blood clotting analyzers, while anticoagulated blood samples were analyzed using the Sysmex system. Following the tests, all results were compiled and analyzed.

3.4.2 Testing Procedure

1. Prepare the Instrument:
    - Remove the cartridge from the foil pouch and insert it into the instrument’s slot.

- Input the relevant sample information, select the testing item, and initiate the test. The instrument will automatically proceed to the sample addition interface after completing the heating process.

2. Collect the Sample.
3. Start the Test:
    - Click the Start button to begin the testing process.
4. Process the Sample:
    - Centrifuge the collected anticoagulated blood sample to obtain plasma.
5. Conduct Testing**:**
    - Perform PT, APTT, Fib, and TT tests simultaneously on the Sysmex instrument to obtain reference values.

Exp.2

3.4.3 Method

Each sample was tested simultaneously on both IDEXX and InCoag analyzers. After completing the tests, all results were compiled and analyzed.

3.4.4 Procedure

1. Prepare the Instrument:
    - Remove the cartridge from the foil pouch and insert it into the slot of the instrument.
    - Input the relevant sample information, select the testing item, and click the button. The instrument will automatically proceed to the sample addition interface once heating is completed.
2. Collect the Sample.
3. Start the Test:
    - Click the Start button to initiate the test.
4. Analyze the Sample:
    - Perform the test on the IDEXX coagulation analyzer.

3.5 Data and Analysis

This report aims to evaluate the performance of the InCoag Coagulation Operating System and its companion test card when assessing a pet's coagulation function. Testing is conducted by a professional veterinarian or nurse who collects the animal's venous blood, tests it on the instrument, and compares the results with those obtained from the pet's Sysmex automated coagulation analyzer and IDEXX coagulation system. This comparison helps assess the performance metrics of the InCoag system.

Data Acceptable Criteria

The following conditions render the data invalid for this test and are excluded from statistical analysis:

1. Collection of whole blood by syringe exceeds 30 seconds.
2. More than 3 punctures at the same site during blood collection.
3. Presence of blood clots after collection.
4. Obvious hemolysis in the blood intended for system testing.

Method for Data Analysis

Correlation between InCoag and reference systems, including Sysmex and IDEXX, is analyzed using the least squares method. Correlation coefficients are calculated to assess the relationship between the systems.

4 APTT Data Analysis

4.1 Species Distribution

The species distribution for Exp.1 is as follows:

Table 5 Species Distribution in Exp.1

Species	Quantity of case
Beagle	7
Labrador Retriever	2
Toy Poodle	12
French Bulldog	2
Golden Retriever	2
Pug	1
German Shepherd	6
Shiba Inu	3
Bichon Frise	2
Schnauzer	1
Yorkshire Terrier	1
Miniature Doberman Pinscher	2
Pomeranian	1
Springer Spaniel	1
China Canines	2
Jack Russell Terrier	3
Corgi	1
Border Collie	1
Samples with UFH	24
Total	74

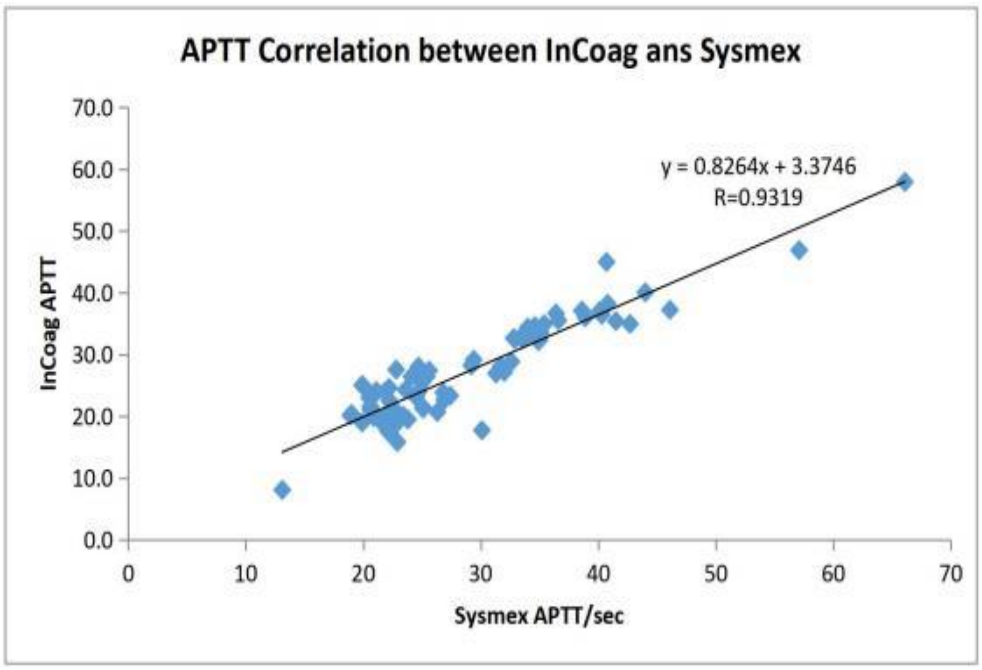
Species Distribution According to Sysmex APTT Results

Sysmex APTT/sec	<15	15~25	25~35	35~50	>50	Total
Quantity	1	31	25	15	2	74

4.2 APTT correlation between InCoag and Sysmex

The correlation of APTT results between InCoag and Sysmex is illustrated in Figure 1.

Figure 1 APTT correlation between InCoag and Sysmex



As shown in the correlation figure above, the linear correlation coefficient (R) between InCoag APTT and Sysmex APTT is 0.9283. This indicates a strong correlation between the two systems, suggesting that the InCoag system is highly effective in distinguishing between samples with coagulation disorders.

5 PT Data Analysis

5.1 Species distribution according Sysmex PT results

In Exp.1, the species distribution for the PT samples was similar to that of the APTT samples. A total of 22 samples were prepared.

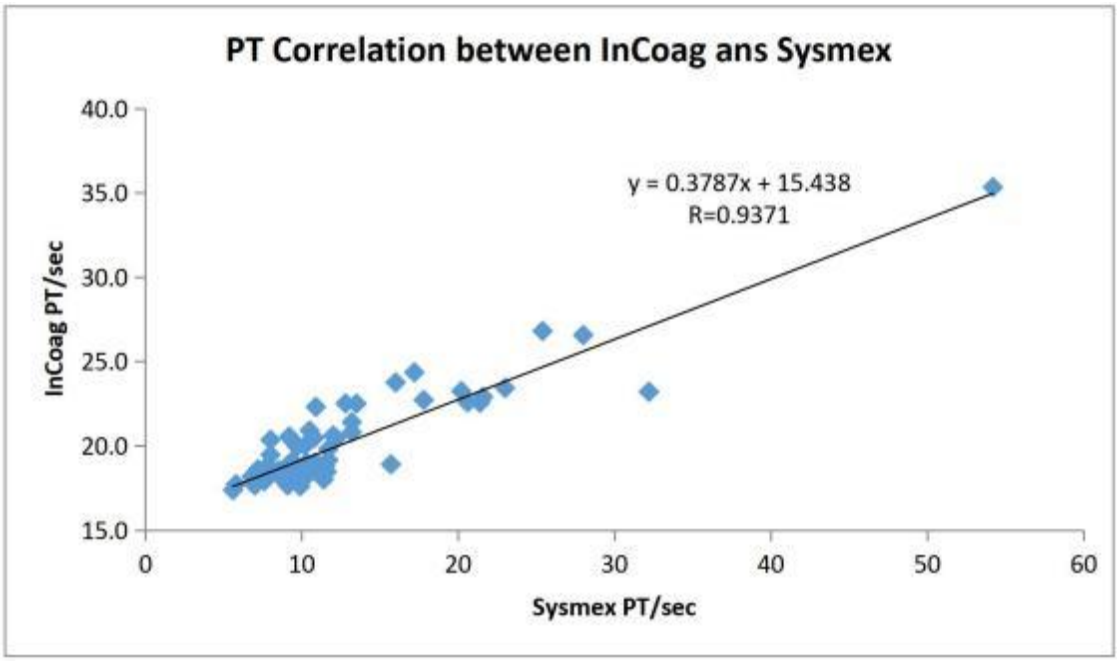
The species distribution according to Sysmex PT results is as follows:

Sysmex PT/sec	<7	7~15	15~25	25~40	>40	total
Quantity	3	56	9	3	1	72

5.2 PT Correlation Between InCoag and Sysmex

The correlation of PT results between InCoag and Sysmex is illustrated in Figure 2.

Figure 2: PT Correlation Between InCoag and Sysmex



As illustrated in the correlation figure above, the linear correlation coefficient (R) between InCoagPT and SysmexPT is 0.9371, indicating a strong relationship between the two systems. Compared to Sysmex, InCoag's results were relatively higher at lower values and relatively lower at higher values.

6 Fib Data Analysis

6.1 Species Distribution According to Sysmex Fib Results

In Experiment 1, the distribution of sample species was consistent with that of APTT.

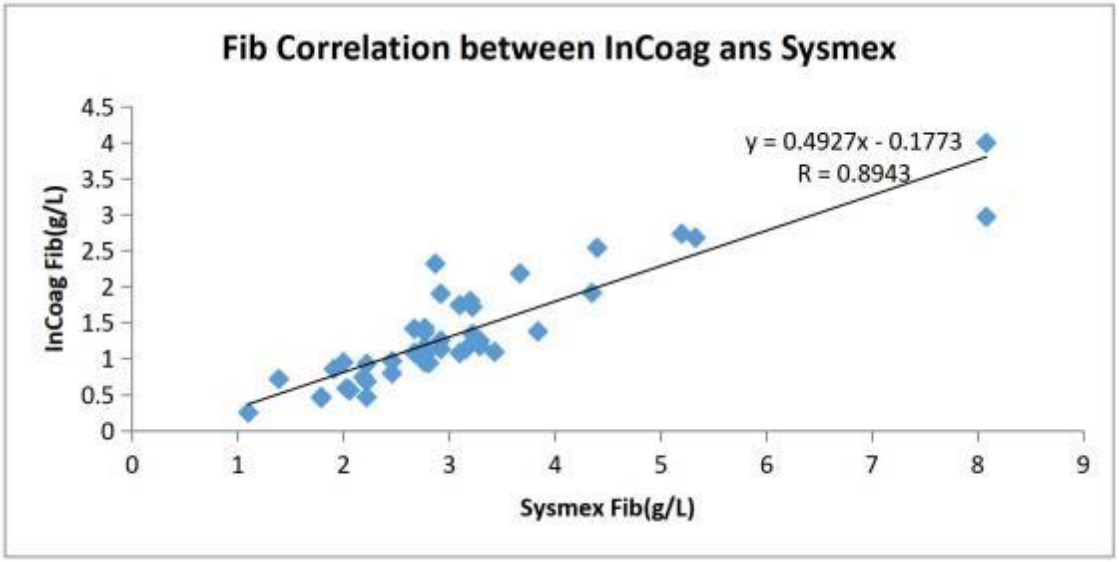
The species distribution based on Sysmex Fib results is as follows:

Sysmex Fib (g/L)	<1	1~2	2~4	4~6	>6	total
Quantity	0	5	32	4	2	43

6.2 Fib Correlation Between InCoag and Sysmex

The correlation of Fib between InCoag and Sysmex is illustrated in the figure below:

Figure 3 Fib Correlation



As shown in the correlation figure above, the linear correlation coefficient (R) between InCoag Fib and Sysmex Fib is 0.8943, indicating a relatively strong correlation between the two systems. The correlation equation has a slope of 0.4927, suggesting that InCoag's results are generally about 50% lower than those of Sysmex.

7 TT Data Processing

7.1 Species Distribution According to Sysmex TT Results

In Experiment 1, the distribution of sample species was consistent with that of APTT, and a total of 23 samples were prepared.

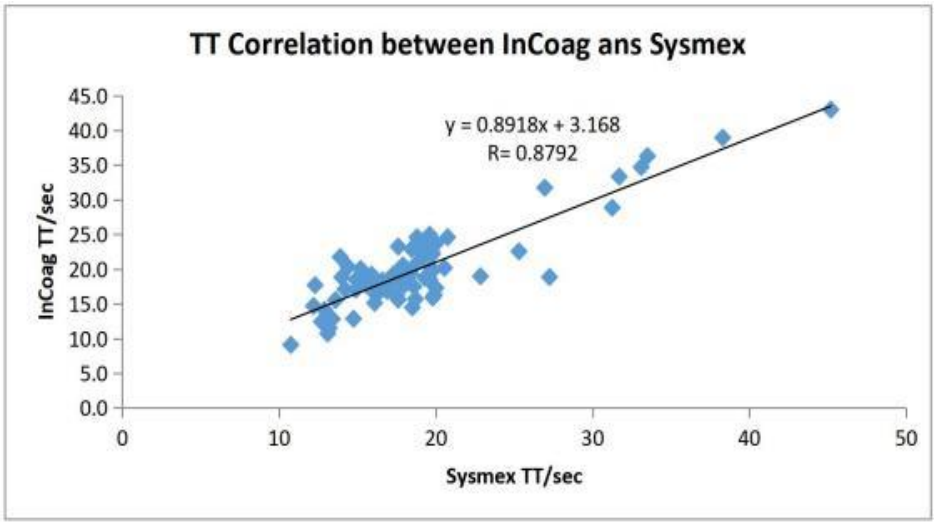
The species distribution based on Sysmex TT results is as follows:

Sysmex TT/sec	<12	12~25	25~35	>35	Total
Quantity	1	63	6	1	71

7.2 TT Correlation Between InCoag and Sysmex

The correlation of TT between InCoag and Sysmex is shown in the figure below:

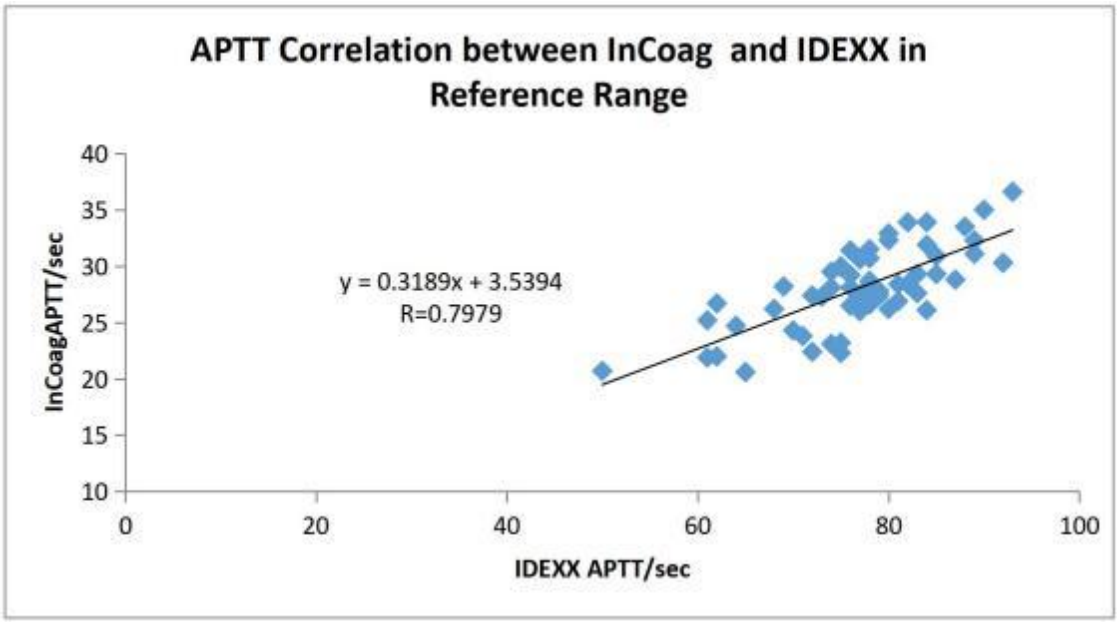
Figure 4 TT Correlation



As shown in the correlation figure above, the linear correlation coefficient (R) between InCoag TT and Sysmex TT is 0.8792, indicating a relatively strong correlation between the two systems.

8 Clinical Data Compared to IDEXX System

8.1 APTT Data



In this experiment, a total of 68 healthy pets were tested, as confirmed by a doctor. The error results were as follows, with a similar error proportion for both InCoag and IDEXX systems, each showing 5 errors out of 68 tests, which corresponds to 7%.

Error Results:

Total test cases	InCoag	IDEXX
68	5	5

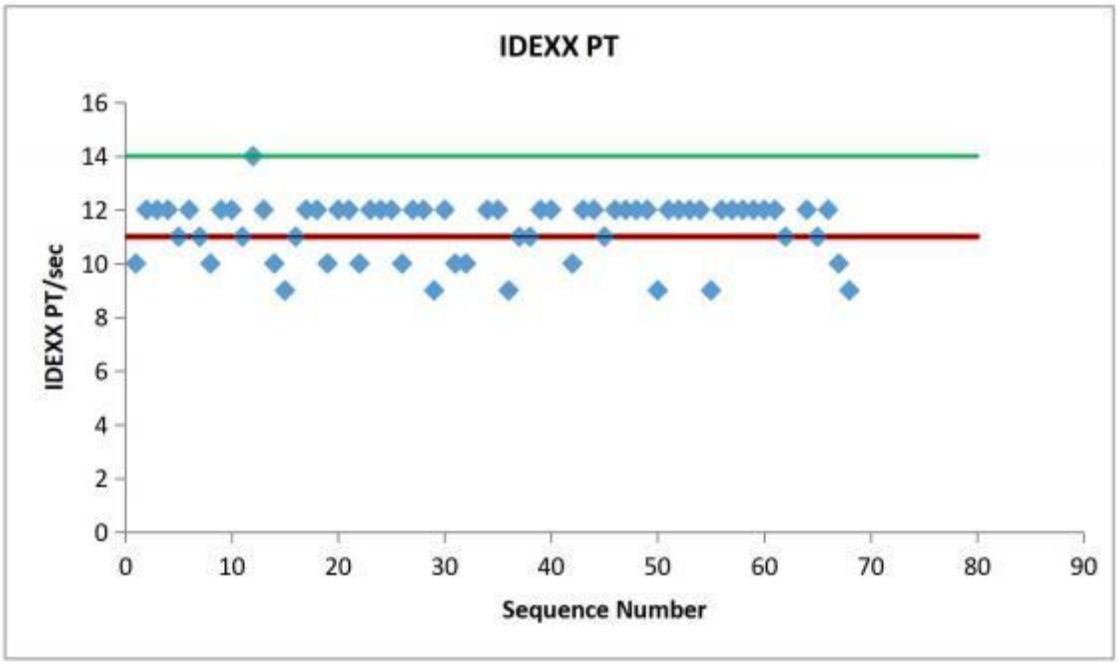
The distribution of test results inconsistent with clinical expectations is as follows:

Total test cases	InCoag	IDEXX
68	1	2

The inconsistent results for the InCoag system showed an APTT of 8.6 seconds, while the inconsistencies for IDEXX were 34 seconds and 23 seconds, respectively.

After removing the abnormal data, the correlation between InCoag and IDEXX within the reference range is strong, with a correlation coefficient of approximately 0.8.

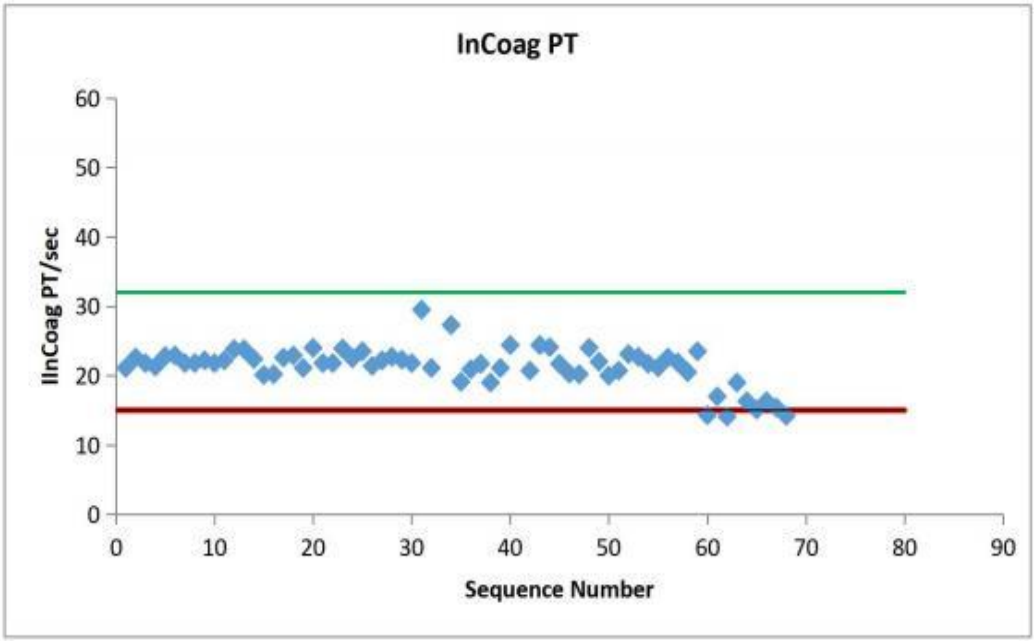
8.2 PT Data



Note: The horizontal axis represents the serial number, and the vertical axis represents the IDEXX test result. The red line indicates the lower limit of the IDEXX reference range, while the green line represents the upper limit. The IDEXX reference range for PT is 11 to 14 seconds.

According to the figures, the IDEXX reference range appears significantly higher, with 16 results falling below the given reference limit. This suggests that the IDEXX reference range may be inappropriate for this experiment.

The PT distribution for InCoag is shown in the following figure:



Note: The horizontal axis represents the serial number, and the vertical axis represents the test results from InCoag. The red line marks the lower limit of the InCoag reference range, while the green line indicates the upper limit. The reference range for InCoag is PT 15–32 seconds.

Analysis of the graph shows that the reference range of InCoag is wider compared to IDEXX, and the test results are generally within this reference range. This suggests that the InCoag reference range is more compatible with clinical testing and aligns well with clinical expectations.

The reported errors for PT testing from both IDEXX and InCoag are as follows, with the proportion of reported errors being comparable between the two systems:

Total test cases	InCoag errors	IDEXX errors
68	1	1

9 Reporting Results

The correlation between InCoag and Sysmex is as follows:

Item	Correlation Equation	Correlation Coefficient R
PT	y = 0.8264x + 3.3746	0.9319
APTT	y = 0.3787x + 15.438	0.9371
Fib	y = 0.4927x - 0.1773	0.8943
TT	y = 0.8918x + 3.168	0.8792

Although the results of the two systems, InCoag and Sysmex CS1500, are not identical, the correlations are sufficiently strong, ranging from 0.8 to 0.95. This indicates that

InCoag effectively distinguishes samples with coagulation disorders and is suitable for clinical use.

The error ratios for InCoag and IDEXX are similar: InCoag had a 5% error rate for APTT and a 1% error rate for PT. Within the reference range, the correlation between InCoag and IDEXX's APTT is 0.8. Notably, the narrow distribution of APTT in Experiment 1 relative to the detection range may contribute to a lower correlation coefficient, but an R value of 0.7979 is still considered strong. This suggests that InCoag's APTT test results are reliable.

The InCoag test results for PT were generally within the given reference range, whereas 24% of IDEXX's results fell below the reference range. From this perspective, InCoag's results are more aligned with clinical expectations.

In summary, the clinical data indicates that the InCoag system is reliable for veterinary blood coagulation testing.