Introduction of Products

Outline of the Automated Hematology Analyzer

pocH-100*i*V Diff

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INTRODUCTION

In recent years, automated hematology analyzers have come into widespread use in veterinary clinics and hospitals. However, WBC differentiation still relies on visual observation with a microscope. Users have evaluated our Automated Hematology Analyzer pocH-100*i*V very favorably since its launch about two and a half years ago. Nevertheless, there has been a strong demand for the development of a hematology analyzer that could give differential WBC counts simultaneously with the 8 parameters of the Complete Blood Count (CBC). In response to this demand, we developed the Automated Hematology Analyzer pocH-100*i*V Diff, which is capable of analyzing the 8 CBC parameters as well as differential WBC counts, together with the hemolyzing reagent pocH-pack LVD meant exclusively for differential WBC analysis. We describe the functions and performance of this analyzer.

SPECIFICATIONS OF pocH-100*i*V Diff

The shape, dimensions and hardware components of pocH-100*i*V Diff (***Fig. 1***) are identical with those of pocH-100*i*V. In addition to the basic capabilities of pocH-100*i*V, the pocH-100*i*V Diff is capable of differentiating the WBCs of dogs and cats into 3 fractions, i.e., "lymphocytes", "eosinophils", and "other WBCs", and cattle WBCs into 2 fractions, i.e., "lymphocytes" and "other WBCs", with the help of the new hemolyzing reagent pocH-pack LVD. This is the major characteristic of the new analyzer. Apart from the measuring modes for dogs, cats, and cattle, for which the approval of the Ministry of Agriculture, Forestry and Fisheries of Japan has been obtained, it has an "OTHER" mode for setting measurement conditions for a maximum of 13 animal species, for research purposes. ***Table 1*** shows an outline of the specifications of pocH-100*i*V Diff (The parts where the specifications differ from those of pocH-100*i*V have been bolded).

Fig. 1 *pocH-100iV Diff*

Table 1 *Specifications of pocH-100iV Diff*

Conditions	Operating	Ambient temperature: +15°C to +30°C (+23°C would be ideal) Relative humidity: 30% to 85%
	Storage and Transportation*	Ambient temperature: -10°C to + 60°C Relative humidity: 95% or less (Non condensing/Keep dry)
Main Unit dimensions	Width: 185 mm (7.3 in) Depth: 460 mm (18.1 in) Height: 350 mm (13.8 in) Weight: approx. 14 kg (30.8 lbs)	
Power supply	100 to 240 VAC±10% (50/60 Hz)	
Power consumption	150 VA or less	
Paraments measured	WBC: Number of white blood cells, RBC: Number of red blood cells, HGB: Hemoglobin concentration, HCT: Hematocrit value: Red blood cell ratio of total blood volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Number of platelets	
Analysis parameters	**Dog mode**	**RDW-SD: Calculated distribution width of red blood cells, standard deviation** **RDW-CV: Calculated distribution width of red blood cells, coefficient of variation** **PDW: Calculated distribution width of platelets, standard deviation** **MPV: Mean platelet volume** **P-LCR: Ratio of large platelets (volume exceeding 12 fL) to the total number of platelets** **EO%: % of large white blood cells to total WBC** **They are assumed to be equivalent to eosinophils.** **OTHR%: % of middle white blood cells to total WBC** **They are assumed to be equivalent to neutrophils, monocytes, and basophils.** **LYM%: % of small white blood cells to total WBC** **They are assumed to be equivalent to lymphocytes.** **EO#: Absolute number of large white blood cells** **They are assumed to be equivalent to eosinophils.** **OTHR#: Absolute number of middle white blood cells** **They are assumed to be equivalent to neutrophils, monocytes, and basophils.** **LYM#: Absolute number of small white blood cells** **They are assumed to be equivalent to lymphocytes.**
	Cat mode	**RDW-SD: Calculated distribution width of red blood cells, standard deviation** **RDW-CV: Calculated distribution width of red blood cells, coefficient of variation** **EO%: % of large white blood cells to total** **WBC They are assumed to be equivalent to eosinophils.** **OTHR%: % of middle white blood cells to total WBC** **They are assumed to be equivalent to neutrophils, monocytes, and basophils.** **LYM%: % of small white blood cells to total WBC** **They are assumed to be equivalent to lymphocytes.** **EO#: Absolute number of large white blood cells** **They are assumed to be equivalent to eosinophils.** **OTHR#: Absolute number of middle white blood cells** **They are assumed to be equivalent to neutrophils, monocytes, and basophils.** **LYM#: Absolute number of small white blood cells** **They are assumed to be equivalent to lymphocytes.**
	Cattle mode	**RDW-SD: Calculated distribution width of red blood cells, standard deviation** **RDW-CV: Calculated distribution width of red blood cells, coefficient of variation** **PDW: Calculated distribution width of platelets, standard deviation** **MPV: Mean platelet volume** **P-LCR: Ratio of large platelets (volume exceeding 12 fL) to the total number of platelets** **OTHR%: % of middle white blood cells to total WBC** **They are assumed to be equivalent to WBC other than lymphocyte.** **LYM%: % of small white blood cells to total WBC They are assumed to be equivalent to lymphocytes.** **OTHR#: Absolute number of middle and large white blood cells** **They are assumed to be equivalent to WBC other than lymphocyte.** **LYM#: Absolute number of small white blood cells They are assumed to be equivalent to lymphocytes.**
	OTHER mode	**RDW-SD: Calculated distribution width of red blood cells, standard deviation** **RDW-CV: Calculated distribution width of red blood cells, coefficient of variation** **PDW: Calculated distribution width of platelets, standard deviation** **MPV: Mean platelet volume** **P-LCR: Ratio of large platelets (volume exceeding 12 fL) to the total number of platelets** **W-LCR: % of large white blood cells to total WBC (white blood cell-large cell ratio).** **W-MCR: % of middle white blood cells to total WBC (white blood cell-middle cell ratio)** **W-SCR: % of small white blood cells to total WBC (white blood cell-small cell ratio).** **W-LCC: Absolute number of large white blood cells (white blood cell-large cell count).** **W-MCC: Absolute number of middle white blood cells (white blood cell-middle cell count).** **W-SCC: Absolute number of small white blood cells (white blood cell-small cell count).**
Display range	**WBC 0 - 999.9 ($\times10^3$μL)** **RBC 0 - 99.99 ($\times10^6$μL)** **HGB 0 .0 - 999.9 (g/dL)** **PLT 0.0 - 9999 ($\times10^3$μL)**	
Background limits	WBC 0.3 ($\times10^3$μL) RBC 0.02 ($\times10^6$μL) HGB 0.1 (g/dL) PLT 10 ($\times10^3$μL)	
Analysis time	Approx. 125 seconds (After starting an analysis until displaying the analysis report)	

* You have to perform Reagent Draining in prior to the storage or transportation.

Table 1 *Specifications of pocH-100iV Diff*

Analysis principle	WBC: DC detection method RBC/PLT: Hydrodynamic Focusing DC detection method HGB:Non-cyanide HGB method		
Required temperature compensation	Approx. 512 BTU/h (Approx. 130 kcal/h)		
Class of electric shock protection measures	Class I Equipment		
EMC characteristics	Conforms with IEC 61326-1 (Class B, Group 1, Industrial environment)		
Safety	Conforms with IEC 61010-1 (Overvoltage Category II, Pollution degree 2, Portable equipment)		
Reproducibility (With 95% reliability limit)	**WBC (≥4.0×10^{3}μL)** **RBC (≥3.00×10^{6}μL)** **HGB** **HCT** **MCV** **MCH** **MCHC** **PLT (≥100×10^{3}μL) (for dog and cattle blood)** **PLT (≥100×10^{3}μL) (for cat blood)** **LYM#** **OTHR#** **EO# (≥0.2×10^{3}μL)** **LYM% (≥LYM 15%)** **OTHR%** **EO% (≥EO 2%)** **RDW-SD** **RDW-CV** **PDW(for dog and cattle blood)** **MPV (for dog and cattle blood)** **P-LCR (for dog and cattle blood)**	**3.5% or less** **2.0% or less** **2.0% or less** **2.0% or less** **2.0% or less** **2.0% or less** **2.0% or less** **10.0% or less** **30.0% or less** **30.0% or less** **15.0% or less** **50.0% or less** **30.0% or less** **15.0% or less** **50.0% or less** **4.0% or less** **6.0% or less** **12.0% or less** **5.0% or less** **20.0% or less**	
Linearity	**WBC (RBC<7.00×10^{6}μL) (for dog and cattle blood)** **WBC (RBC<7.00×10^{6}μL) (for cat blood)** **RBC** **HGB** **HCT** **PLT (RBC<7.00×10^{6}μL)**	**1.0 - 99.9 (×10^{3}μL)** **1.0 - 75.0 (×10^{3}μL)** **0.3 - 13.00 (×10^{6}μL)** **0.1 - 25.0 (g/dL)** **10.0 - 60.0 (HCT%)** **10 - 1200 (×10^{3}μL)**	**±0.3 (×10^{3}μL) or less, or ±5% or less** **±0.3 (×10^{3}μL) or less, or ±5% or less** **±0.03 (×10^{6}μL) or less, or ±5% or less** **±0.2 (g/dL) or less, or ±5% or less** **±1 (HCT%) or less, or ±5% or less** **±10 (×10^{3}μL) or less, or ±10% or less**
Carry-over	WBC 3% or less RBC 1.5% or less HGB 1.5% or less HCT 1.5% or less PLT 5% or less		
Consumables	Reagents: pocH-pack D, **pocH-pack LVD** Detergent: CELLCLEAN Control material: EIGHTCHECK-3WP		
Aspirated sample volume	approx. 15μL		
Number of analyses that can be performed with 1 reagent bottle	pocH-pack D: Approximately 30 pocH-pack LVD: Approximately 235 (These figures assume 10 measurements per day, and include the background check, shutdown, and other processes.)		

NEW FEATURES OF pocH-100*i*V Diff

The pocH-100*i*V Diff has the following new features that are not in pocH-100*i*V.

1. Differentiation of WBCs

In order to obtain the WBC count, the intermixed RBCs are destroyed (lysed) with a hemolyzing reagent before counting the WBCs. The lysing reagent not only destroys the RBC membranes, but also acts on the WBCs and shrinks them. The extent of this shrinking is believed to differ, depending on the properties of the membrane, shape and size of the nucleus, and the size and density of granules within the cells. Therefore, by suitably adjusting the composition of the lysing reagent, the WBCs can be differentiated according to their size. The pocH-pack LVD is a new lysing reagent developed exclusively for the pocH-100*i*V Diff, and its main component is a quaternary ammonium salt. ***Fig. 2*** shows basic blood cell size distribution curves of a dog, cat, and cow.

2. Changeover to a different animal species

With pocH-100*i*V, if the measurements are made after setting the analyzer for the wrong animal species, a fresh measurement has to be made after making the correct selection. Such re-measurement is not necessary with pocH-100*i*V Diff. Re-calculation can be done by simply selecting the mode of the correct animal species. The printout produced after such a switch of the species will have a "Manual Ana. [S]" printed on it (***Fig. 3***).

3. Manual reanalysis function

For automatic differentiation of blood cells, each cell type should have a clear peak and trough. If the cell size distribution is unimodal, and the peak and trough are not clear, as shown in ***Fig. 4***, differentiation is not possible, and then "– – –. –" is printed. If differentiation is not possible because the discrimination value was not set automatically or if the automatic setting of discrimination value is not appropriate, we can carry out reanalysis after changing the discrimination value appropriately (***Fig. 5***). The printout after changing the discrimination value has "Manual Ana." and the parameter, i.e., "W", "R", or "P", printed on it (***Fig. 5***).

4. The "OTHER" mode for research purposes

When analyzing the blood of animals other than dogs, cats, and cattle, for research, the operator can enter measurement conditions for a maximum of any 13 animal species using the "OTHER" mode. After making measurement for the first time in the "OTHER" mode, the discrimination values for WBC size distribution are to be reset manually to make 2 fractions, depending on the type of animal (***Fig. 6***), because they have been initially set for 3 fractions. When the measurement is done in the "OTHER" mode, "-RESEARCH-" will be printed in the output (***Fig. 6***).

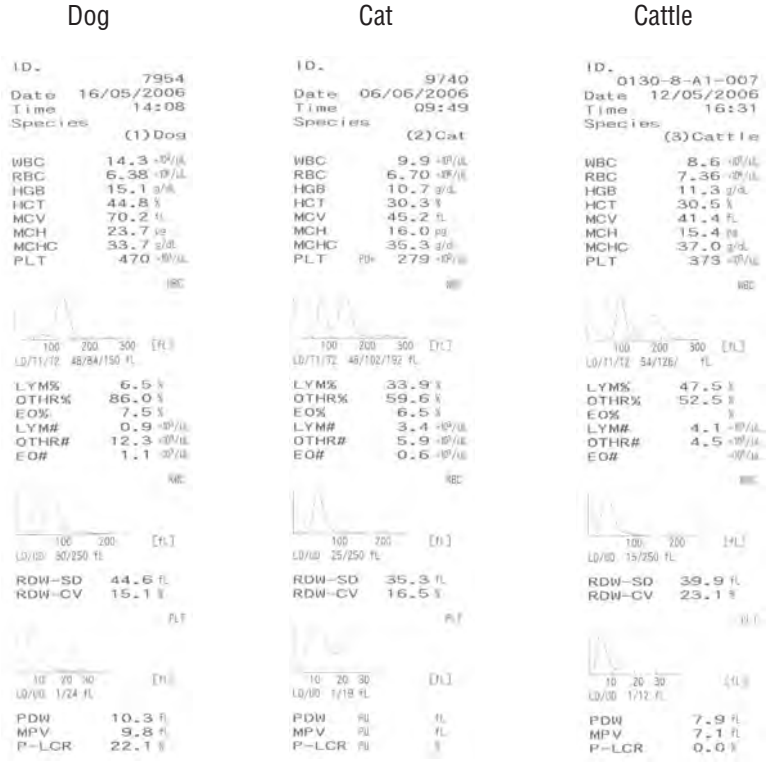

Fig. 2 *The basic cell size distribution*

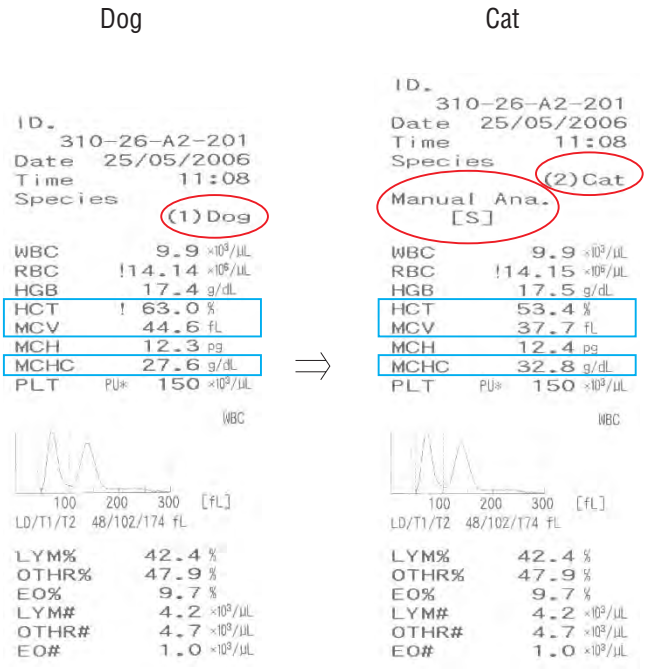

Fig. 3 *Changeover of animal species*

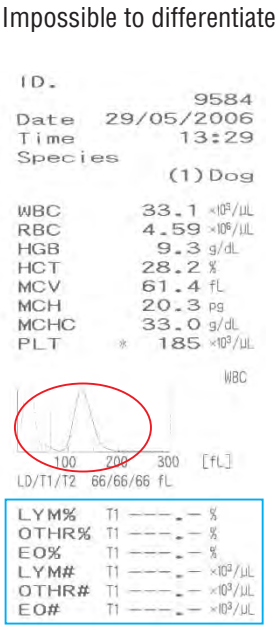

Fig. 4 *A sample for which differentiation was possible*

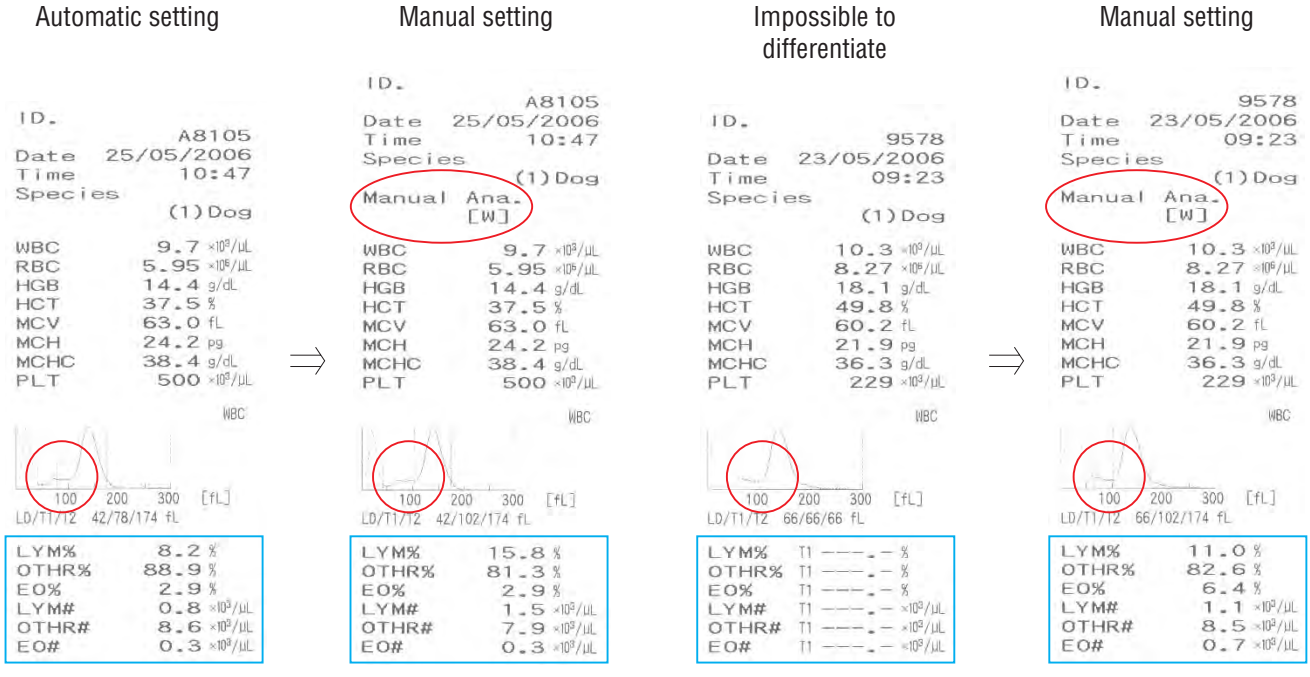

Fig. 5 *Manual setting*

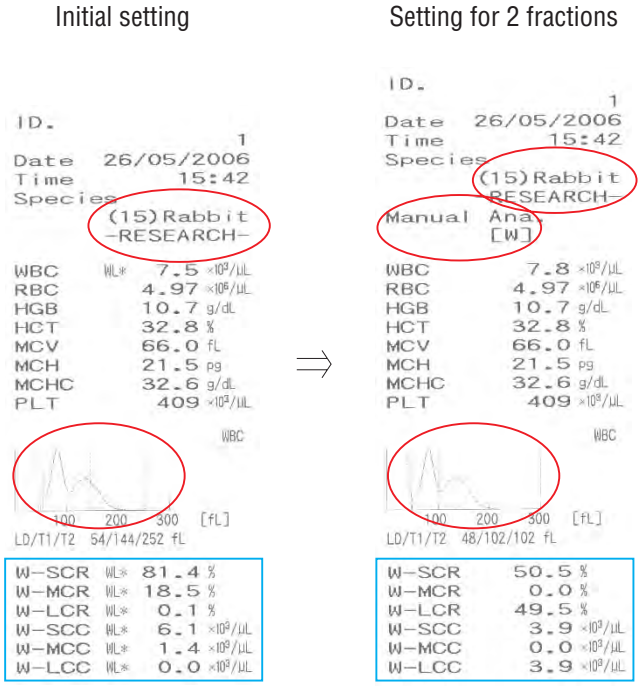

Fig. 6 *OTHER mode for research*

5. Abnormal measured values and cell size distribution abnormality flags

With pocH-100*i*V and pocH-100*i*V Diff, one can obtain various kinds of information about the analyzed sample, not only from the measured values but also from the cell size distribution of WBC, RBC, and platelets. Therefore, a thorough knowledge of the cell size distribution pattern is useful for properly evaluating the results of measurement and for using the results in diagnosis. It is important to develop the habit of checking the cell size distribution, which is the key to full utilization of the potential of pocH-100*i*V Diff. ***Table 2*** lists the various flags and their meanings.

WBC DIFFERENTIATION PERFORMANCE

1. Reproducibility

The cell size distribution shows subtle changes with each measurement, which affect the reproducibility of the differentiation. ***Table 3*** shows the reproducibility of differentiation in successive measurements on 3 samples each of dog, cat, and cow blood. The highest coefficients of variation were 12.8% in dog and 10.9% in cat. Platelet aggregation sometimes significantly affects WBC cell size distribution, lowering reproducibility. Therefore, sufficient care must be taken at the time of blood collection. The reproducibility in the cattle was very good, with a maximum coefficient of variation of 4.0%.

2. Time Course

Fig. 7 shows changes in differential WBC percentages with time in the blood of a dog, cat, and cattle, collected with K_2EDTA as the anticoagulant, and stored at room temperature for 24h. The lymphocyte fraction of the dog and cat showed a slight decreasing trend, and the eosinophil fraction of the dog showed an increasing trend. The cattle blood showed almost no change.

Table 2 *Information on cell distribution*

Flag	Probable sample cause	Correction
WL	Incomplete lysing of red blood cells, presence of nucleated red blood cells, increase of large platelets, platelet aggregation or agglutination, precipitation of fibrin, presence of proteins or lipids.	Check smear. Warm sample and repeat analysis. If incomplete lysing is suspected, perform a 1:5 dilution of the sample (50µL of whole blood added to 200µL of diluent) and re-analyze. Adjust the results for the dilution factor.
RL	Presence of fragmented red blood cells, increase of large platelets, platelet aggregation or agglutination, presence of micro-red blood cells.	Check smear. Warm sample and repeat analysis. Manual count.
PL	Effects of cryoglobulins, fragmented red blood cells, or cellular fragments of white blood cells.	Check smear. Warm sample and repeat analysis. Manual count.
WU	Incomplete lysing of red blood cells, presence of immature white blood cells, white blood cell aggregation, platelet satellite phenomenon, etc.	Check smear. Dilute sample and repeat analysis. If incomplete lysing is suspected, perform a 1:5 dilution of the sample (50µL of whole blood added to 200µL of diluent) and re-analyze. Adjust the results for the dilution factor.
RU	Effects of cold agglutinin, inclusion of white blood cells.	Check smear. Warm sample and repeat analysis.
PU	Increase of large platelets, inclusion of fragmented red blood cells, effects of cryoglobulins, platelet aggregation or agglutinative, presence of micro-red blood cells.	Warm sample and repeat analysis. Manual count. Take an another blood sample.
DW (RBC)	Significant anisocytosis, etc.	Check smear.
DW (PLT)	Inclusion of fragmented red blood cells, nonuniformity in size of platelets, effects of cryoglobulins, etc.	Check smear. If cyroglobulins are suspected, first warm the sample and repeat analysis. If error message persists, perform a plasma replacement (remove plasma and replace with equal volume of diluent) and repeat analysis.
MP (RBC)	Effects of anemia treatment or blood transfusion causing the presence of cells of multiple sizes.	Check smear.
MP (PLT)	Platelet aggregation, low platelet count.	Check smear.
T1	Presence of immature white blood cells, incomplete lysing of red blood cells, etc., causing the first two WBC populations in the WBC-Histogram not to be separated.	Check smear. If incomplete lysing is suspected, perform a 1:5 dilution of the sample (50µL of whole blood added to 200µL of diluent) and re-analyze. Adjust the results for the dilution factor.
T2	Presence of immature white blood cells, incomplete lysing of red blood cells, etc., causing the first two WBC populations in the WBC-Histogram not to be separated.	Check smear. If incomplete lysing is suspected, perform a 1:5 dilution of the sample (50µL of whole blood added to 200µL of diluent) and re-analyze. Adjust the results for the dilution factor.
F1, F2, F3	Presence of immature white blood cells, incomplete lysing of red blood cells, etc., causing the first two WBC populations in the WBC-Histogram not to be separated.	Check smear. If incomplete lysing is suspected, perform a 1:5 dilution of the sample (50µL of whole blood added to 200µL of diluent) and re-analyze. Adjust the results for the dilution factor.

Table 3 *Reproducibility of WBC differentiation*

Dog

	LYM(%)			OTHR(%)			EO(%)		
	Sample A	Sample B	Sample C	Sample A	Sample B	Sample C	Sample A	Sample B	Sample C
1	29.1	19.3	16.7	66.8	62.9	70.0	4.1	17.8	13.3
2	28.2	20.3	17.4	67.8	65.2	69.4	4.0	14.5	13.2
3	31.0	19.1	15.0	65.3	65.6	72.3	3.7	15.3	12.7
4	28.7	18.4	15.1	67.3	65.5	71.9	4.0	16.1	13.0
5	31.1	22.9	17.2	64.9	58.5	72.2	4.0	18.6	10.6
6	30.3	18.8	18.1	66.6	61.9	68.8	3.1	19.3	13.1
7	29.0	19.4	20.7	67.0	64.3	68.7	4.0	16.3	10.6
8	31.0	22.0	18.6	64.7	61.3	71.7	4.3	16.7	9.7
9	29.9	26.9	18.8	66.8	53.7	70.4	3.3	19.4	10.8
10	29.4	23.1	18.6	67.0	59.2	71.0	3.6	17.7	10.4
Mean	29.8	21.0	17.6	66.4	61.8	70.6	3.8	17.2	11.7
Standard diviation	1.0	2.7	1.7	1.1	3.8	1.4	0.4	1.7	1.4
Coefficient of variation	3.5	12.8	9.9	1.6	6.2	2.0	9.9	9.7	12.2

Cat

	LYM(%)			OTHR(%)			EO(%)		
	Sample A	Sample B	Sample C	Sample A	Sample B	Sample C	Sample A	Sample B	Sample C
1	42.3	13.5	33.8	53.0	66.4	57.6	4.7	20.1	8.6
2	44.7	14.2	34.7	50.9	66.7	56.7	4.4	19.1	8.6
3	44.8	11.8	32.9	50.9	69.6	58.0	4.3	18.6	9.1
4	41.4	15.5	33.7	53.7	66.2	56.9	4.9	18.3	9.4
5	44.4	16.2	34.5	51.1	64.0	56.1	4.5	19.8	9.4
6	45.6	15.4	33.2	50.2	65.3	57.9	4.2	19.3	8.9
7	45.0	16.8	34.3	50.8	65.0	57.2	4.2	18.2	8.5
8	42.9	15.5	32.5	51.5	65.9	59.8	5.6	18.6	7.7
9	44.2	14.8	33.9	51.5	67.5	56.9	4.3	17.7	9.2
10	45.0	13.2	33.3	51.2	67.5	58.1	3.8	19.3	8.6
Mean	44.0	14.7	33.7	51.5	66.4	57.5	4.5	18.9	8.8
Standard diviation	1.4	1.5	0.7	1.1	1.6	1.0	0.5	0.8	0.5
Coefficient of variation	3.1	10.3	2.1	2.1	2.4	1.8	10.9	4.0	5.9

Cattle

	LYM(%)			OTHR(%)		
	Sample A	Sample B	Sample C	Sample A	Sample B	Sample C
1	40.9	31.4	48.8	59.1	68.6	51.2
2	40.4	29.5	48.0	59.6	70.5	52.0
3	39.4	30.2	51.0	60.6	69.8	49.0
4	42.4	28.3	50.7	57.6	71.7	49.3
5	40.4	29.9	48.3	59.6	70.1	51.7
6	42.9	32.1	51.1	57.1	67.9	48.9
7	44.0	31.0	49.8	56.0	69.0	50.2
8	44.5	30.9	48.7	55.5	69.1	51.3
9	43.1	32.0	50.7	56.9	68.0	49.3
10	41.6	31.7	47.9	58.4	68.3	52.1
Mean	42.0	30.7	49.5	58.0	69.3	50.5
Standard diviation	1.7	1.2	1.3	1.7	1.2	1.3
Coefficient of variation	4.0	4.0	2.6	2.9	1.8	2.6

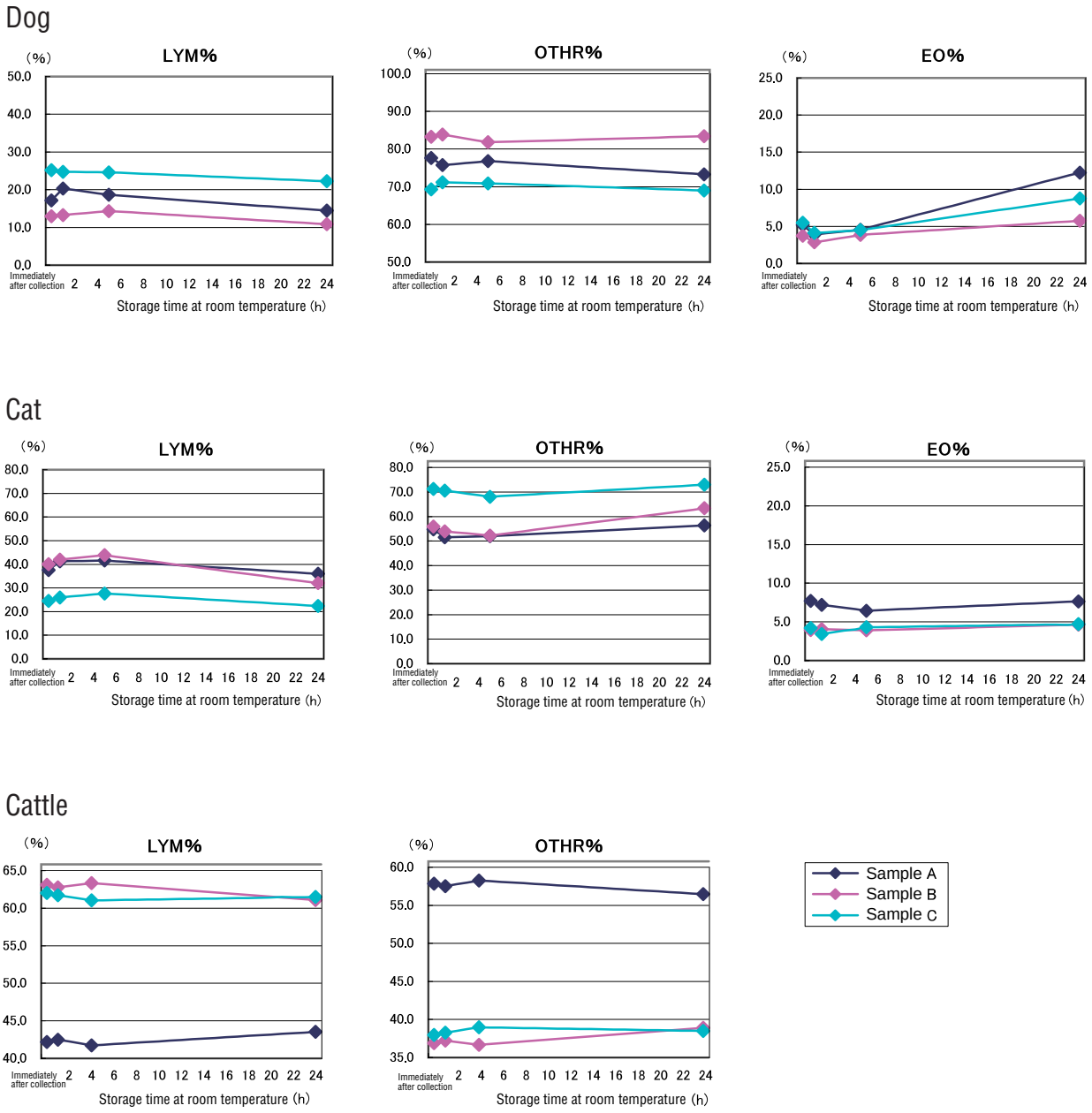

Fig. 7 *Time course of the differential WBC percentages*

3. Correlations

Fig. 8 shows correlations between visual differential WBC count percentages and those measured by pocH-100*i*V Diff in dogs, cats, and cattle. The numerator "n" is the number of samples where automatic differentiation could be done, and the denominator is the total number of samples analyzed. The lymphocyte percent of dogs, cats, and cattle were well correlated with the visual count values. However, the eosinophil percent of both dogs and cats had poorer correlation than the lymphocyte percent, partly because they had smaller absolute numbers of eosinophils. ***Fig.9*** "a" to "f" shows the cell size distribution curves of the discrepant cat blood samples. We can see that the LD trough (O) was abnormally high compared to the basic cell size distribution of ***Fig. 2***. This strongly suggests platelet aggregation. It appeared that comparatively small aggregated platelet masses increased the lymphocyte fraction and decreased the other WBC fractions, and larger aggregated platelet masses increased the eosinophil fraction (O). Compared to cats, the eosinophils of dogs do not show a distinct peak and trough in the cell size distributions curve. Because of this, some samples gave a higher value of the eosinophil fraction when measured by pocH-100*i*V Diff in the range where the visual count value was 5% or less.

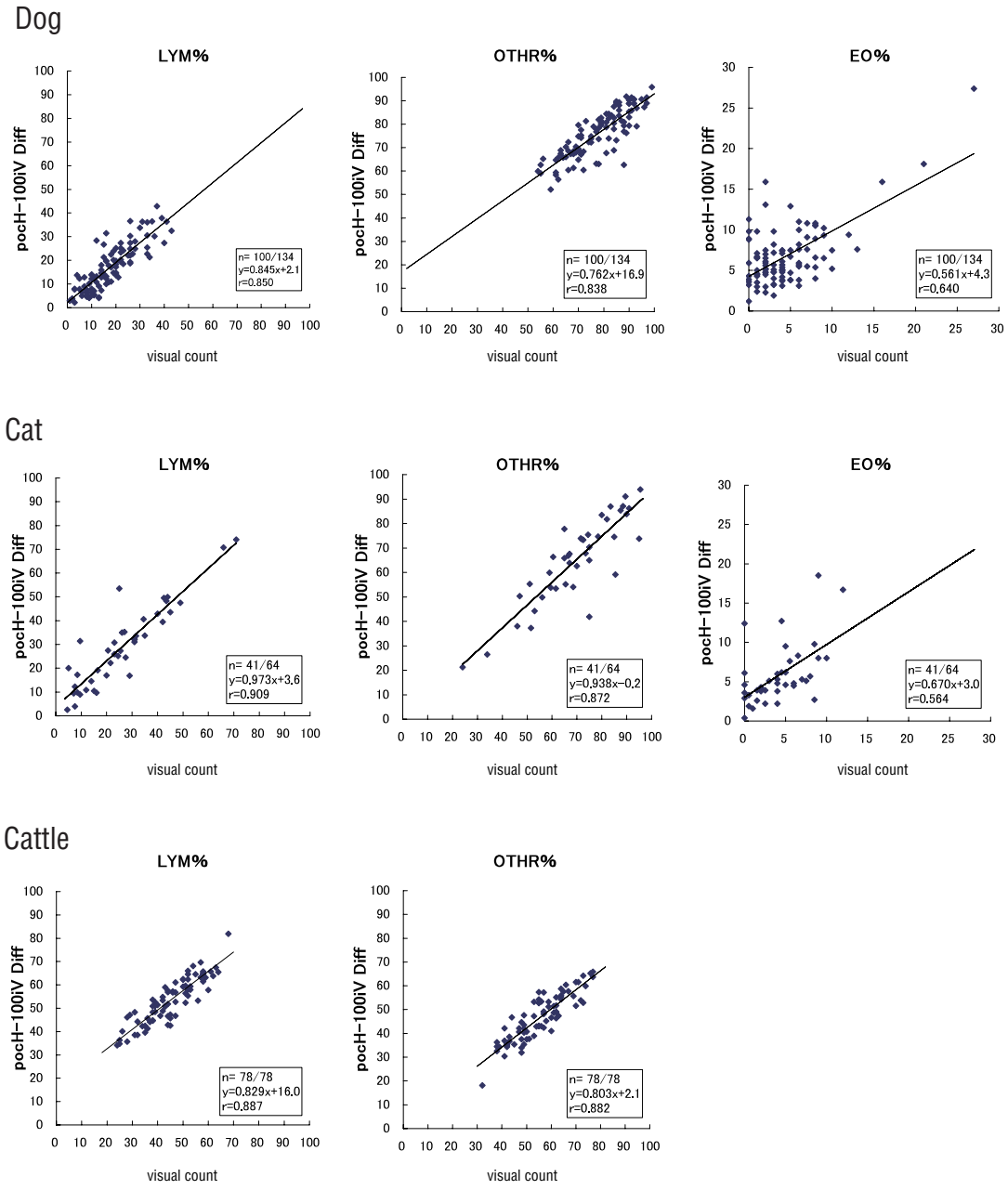

Fig. 8 *Correlation diagrams for differential WBC percentages*

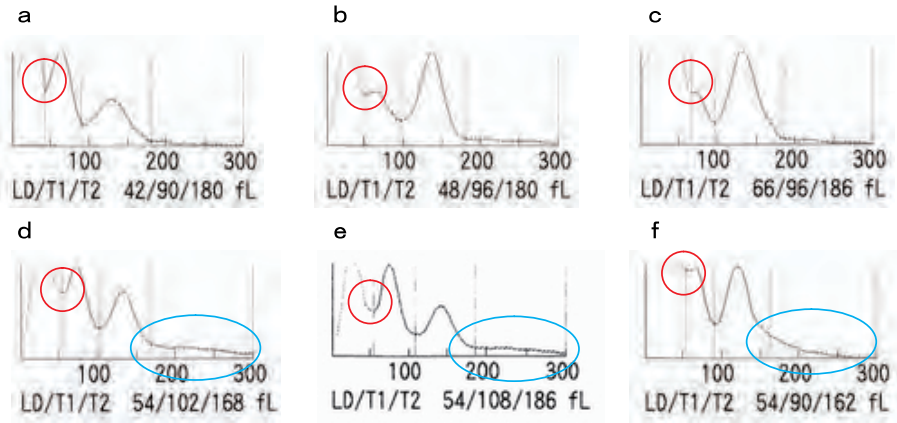

Fig. 9 *Deviant samples from cats*

CONCLUSION

We have now developed the pocH-100*i*V Diff, which can be used for differentiation of WBCs, apart from measuring blood cell counts, and can be of assistance in saving the time of veterinary doctors and veterinary technicians. Here, we have outlined the specifications and functions, and described the WBC differentiation performance of this analyzer. We believe that the pocH-100*i* Diff is accurate enough for use as a screening tool for WBC differential, but observation of smear samples is still the standard method. It is important to verify with smear samples when the cell size distribution pattern is found to be abnormal. In future we plan to prepare better algorithms so that fewer samples would be impossible to differentiate.

Reference

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