

CAUTION

These anticoagulants are recommended by Beckman Coulter. Use of other anticoagulants may yield misleading results.

CAUTION

Follow the tube manufacturer's recommended procedure for the correct specimen collection.

For	the recommended anticoagulant is
Whole blood prediluted anti-coagulated blood Serous fluids	K ₂ or K ₃ - EDTA
Synovial fluids (pretreated with Hyaluronidase)	K ₂ or K ₃ - EDTA, or heparin

Aspiration

1.3.4. Veninio kraujo, kapiliarinio kraujo automatizuotam tyrimui atlikti reikalingas (jsiurbiamas) ėminio kiekis - 165 µL

DxH 800 aspiration volume is 165µL.

Pre-diluted blood CBC analysis requires a dilution of 50µL of blood and 200µL of diluent.

DxH 800 Sample Preparation

The DxH 800 will process specimens as indicated below.

Automated Cassette:	Whole Blood	Closed Vial
Single-tube Station:	Whole Blood	Closed Vial and Open Vial
	Body Fluid	Closed Vial and Open Vial
	Pre-dilute	Open Vial

Accuracy

Whole Blood - CBC

Accuracy for the CBC parameters is assessed by comparison of the results from the DxH 800 and a comparative method. The estimation of the difference is determined as described in CLSI EP09-A2 *Method Comparison and Bias Estimation Using Patient Samples*. When specimens covering the measuring range with no system messages are analyzed by both the DxH 800 and another automated hematology analyzer, the DxH 800 meets specification if the results are within the limits defined in the table below.

Table 1.1 Accuracy Specifications, Whole Blood - CBC

Parameter	Units	Measuring Range	Difference (whichever is greater)	
WBC	x10 ³ /μL	0.000–2.000	±0.1	±10%
		>2.000–100.000	±0.2	±3.0%
		>100.000–400.000	N/A	±5 %
RBC	x10 ⁶ /μL	0.000–8.500	±0.05	±2.0%
HGB	g/dL	0.00–25.50	±0.2	±3.0%
MCV	fL	50.00–150.00	N/A	±2%*
RDW	%	10.00–40.00	±0.5	±5.0%
RDW-SD	fL	15.00–220.00	±3.0	±10.0%
PLT	x10 ³ /μL	0.0–3000.0	±10.0	±7.0%
MPV	fL	5.00–25.00	N/A	±7.0%

*Due to the effect of temperature on red cell size, the specification applies to the temperature range of 70–80° F (21.1–26.7° C).

Differential

Accuracy for the Differential parameters is assessed by comparison of the results from the DxH 800 and a comparative method. Results may be compared to either an automated hematology analyzer or to a 400 cell manual differential prepared according to CLSI *H20-A2 Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods (H20)*.

The estimation of the bias/difference is described in CLSI *EP09-A2 Method Comparison and Bias Estimation Using Patient Samples*. When specimens covering the measuring range with no System messages are analyzed by the DxH 800, and by 400 cell manual differential, the DxH 800 meets specification if the results are within the Bias defined in [Table 1.2](#), below.

Table 1.2 Accuracy Specifications (DxH vs. Manual Diff), Whole Blood - Differential - CLSI *H20-A2*

Parameter	Units	Measuring Range	Bias (whichever is greater)	
NE	%	0.00–100.00	±2.0	±10%
LY	%	0.00–100.00	±3.0	±10%
MO	%	0.00–100.00	±3.0	±10%
EO	%	0.00–100.00	±1.0	±10%
BA	%	0.00–100.00	±1.0	±10%

When specimens covering the measuring range with no System messages are analyzed by the DxH 800 and a predicate device, the DxH 800 meets specification if the results are within the difference defined in [Table 1.3](#).

Table 1.3 Accuracy Specifications (DxH vs. Predicate), Whole Blood - Differential

Parameter	Units	Measuring Range	Difference (whichever is greater)	
NE	%	0.00–100.00	±2.0	±10%
LY	%	0.00–100.00	±1.5	±10%
MO	%	0.00–100.00	±1.0	±10%
EO	%	0.00–100.00	±0.5	±10%
BA	%	0.00–100.00	±0.5	±10%

Reticulocyte

Accuracy for the Reticulocyte parameters is assessed by comparison of results from the DxH 800 and a predicate instrument. Estimation of difference is determined using *CLSI EP09-A2*. The DxH 800 meets specification if results meet the limits defined in the table below.

Table 1.4 Accuracy Specification, Whole Blood - Reticulocytes

Parameter	Units	Measuring Range	Difference
RET	%	0.000–30.000	±0.5 or ±10% (whichever is greater)
IRF	—	0.000–1.000	±0.2
MRV	fL	50.00–190.00	±15.0

NRBC

Accuracy for the NRBC parameter is assessed by comparison of the results from the DxH 800 and a manual count. When specimens covering the measuring range without System messages are analyzed by both the DxH 800 and by manual methods as described by *CLSI H20-A2 Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods*, the DxH 800 meets specification if the results are within limits defined in the table below.

Table 1.5 Accuracy Specifications, Whole Blood -NRBC

Parameter	Units	Measuring Range	Correlation Coefficient
NRBC	per 100 WBC	0.00 to 600.00	$r \geq 0.90$

Body Fluids

Accuracy for body fluid parameters is assessed by comparison of the results from the DxH 800 and manual counts. The estimation of the bias is determined as described in *CLSI EP09-A2 Method Comparison and Bias Estimation Using Patient Samples*. When specimens covering the measuring range without System messages are analyzed by both the DxH 800 and by the manual method, the DxH 800 meets specification if the results are within the limits defined in the table below.

Table 1.6 Accuracy Specifications - Body Fluids

Parameter	Units	Measuring Range	Bias (whichever is greater)
TNC	cells/mm ³	20–89,000	±5 or ±10%
RBC	cells/mm ³	10,000–6,200,000	±500 or ±5.0%

Repeatability

Table 1.7 Repeatability - Whole Blood CBC, DIFF, Retic (N=10)

Parameter	Units	Range	Limit
WBC	x10 ³ /μL	0.500–2.000 5.000–10.000	≤5.0% CV ≤3.0% CV
RBC	x10 ⁶ /μL	4.5 to 5.5	≤1.5% CV
HGB	g/dL	14 to 16	≤1.5% CV
MCV	fL	80 to 90	≤1.0% CV
RDW	%	12 to 14	≤2.5% CV
RDW-SD	fL	33.00 to 48.00	≤2.5% CV
PLT	x10 ³ /μL	10.0 to 15.0 200 to 400	≤12.0% CV ≤3.5% CV
MPV	fL	8 to 10	≤2.5% CV
NE	%	50 to 60	≤3.5% CV
LY	%	25 to 35	≤5% CV
MO	%	5 to 10	≤10.0% CV
EO	%	2 to 5	SD ≤ 0.5 or ≤13.5% CV
BA	%	0.5 to 1.5	SD ≤ 0.5
NRBC	/100 WBC	1.00–2.00 >2.00–15.00 >15.00	SD ≤ 0.3 ≤20% CV ≤15% CV
RET	%	0.000 to 1.500 >1.500 to 4.000 >4.000 to 15.000	SD ≤ 0.25 SD ≤ 0.70 ≤7% CV
IRF	—	RBC ≥3.0 x 10 ⁶ μL and RETIC 1.0–4.0% and IRF ≥0.2	≤20% CV
MRV	fL	100.0–120.0	≤5% CV

Table 1.8 Repeatability - Prediluted Blood (N=10)

Parameter	Units	Range	Limit
WBC	$\times 10^3/\mu\text{L}$	5.000–10.000	$\leq 6.0\%$ CV
RBC	$\times 10^6/\mu\text{L}$	4.5 to 5.5	$\leq 3.0\%$ CV
HGB	g/dL	14 to 16	$\leq 3.0\%$ CV
PLT	$\times 10^3/\mu\text{L}$	200 to 400	$\leq 7.0\%$ CV

Table 1.9 Repeatability - CSF, Serous or Synovial Body Fluid Count (N=10)

Parameter	Unit	Repeatability	Limit
RBC	cells/mm ³	10,000 – 15, 000	$\leq 10.0\%$ CV
TNC	cells/mm ³	50 – 2000	$\leq 15.0\%$ CV

Measuring and Operating Ranges

Measuring Range

Measuring range is the range of values over which the acceptability criteria for the method are defined. Measuring range can be assessed using commercially available materials qualified for use on the DxH 800 System.

Operating Ranges

Operating range is the range over which the system, inclusive of the pre-dilute functionality, will report (display, print and/or transmit) results. Values that are between the Measuring range and Operating range are flagged.

Linearity

Linearity can be assessed by testing levels of an analyte that are known by formulation or by using commercially available materials qualified for use on the DxH 800 System.

Table 1.10 Whole Blood Measuring and Operating Ranges

Parameter	Units	Measuring Range	Operating Range	Linearity (r ²)
UWBC *	10 ³ /μL	0.000–400.000	0.000–999.999	N/A
WBC *	10 ³ /μL	0.000–400.000	0.000–999.999	>0.95
RBC	10 ⁶ /μL	0.000–8.500	0.000–10.000	>0.95
HGB	g/dL	0.00–25.50	0.00–30.00	>0.95
HCT	%	N/A	0.00–70.00	N/A
MCV	fL	50.00–150.00	40.00–200.00	N/A
MCH	pg	N/A	0.00–99.99	N/A
MCHC	g/dL	N/A	0.00–99.99	N/A
RDW	%	10.00–40.00	0.00–70.00	N/A
RDW-SD	fL	15.00–220.00	0.00–340.00	N/A
PLT	10 ³ /μL	0.0–3000.0	0.0–7000.0	>0.95
MPV	fL	5.00–25.00	0.00–25.00	N/A
NE	%	0.00–100.00	0.00–100.00	N/A
NE#	10 ³ /μL	0.000–400.000	0.000–600.000	N/A
LY	%	0.00–100.00	0.00–100.00	N/A
LY#	10 ³ /μL	0.000–400.000	0.000–600.000	N/A
MO	%	0.00–100.00	0.00–100.00	N/A
MO#	10 ³ /μL	0.000–400.000	0.000–600.000	N/A
EO	%	0.00–100.00	0.00–100.00	N/A
EO#	10 ³ /μL	0.000–400.000	0.000–600.000	N/A
BA	%	0.00–100.00	0.00–100.00	N/A
BA #	10 ³ /μL	0.000–400.000	0.000–600.000	N/A
NRBC	/100 WBC	0.00–600.00	0.00–600.00	N/A
NRBC#	10 ³ /μL	N/A	0.000–600.000	N/A
RET	%	0.000–30.000	0.000–50.000	N/A
RET#	10 ⁶ /μL	0.00000–1.00000	0.00000–2.50000	N/A
IRF	—	0.000–1.000	0.000–1.000	N/A
MRV	fL	50.00–190.00	0.00–500.00	N/A

* Operating range achieved when using predilute capability.

Table 1.11 Body Fluids (CSF, Serous, Synovial) Measuring and Operating Ranges

	Units	Measuring Range	Operating Range
RBC	cells/mm ³	10,000–6,200,000	0–10,000,000
TNC	cells/mm ³	20–89,000	0–600,000

Beckman Coulter recommends that a diluent be run as a Body Fluid sample prior to analysis of Body Fluid specimens. Backgrounds within specifications can influence the reported results on the samples with low abnormal or normal values. Beckman Coulter recommends that each laboratory establish criteria for evaluation of the impact on the background on the reported results.

Carryover

Carryover results should not exceed the following limits:

Table 1.12 High to Low Carryover

Parameter	Limit
WBC	≤0.5%
RBC	≤0.5%
HGB	≤1.0%
PLT	≤1.0%
NRBC	≤75 events
DIFF	≤200 events
RET	≤600 events

CBC High to Low Carryover is measured per ICSH guidelines⁴, and calculated as follows:

$$\text{Carryover} = [(1\text{st Diluent} - 3\text{rd Diluent}) / (3\text{rd Sample} - 3\text{rd Diluent})] \times 100.$$

For DIFF, Retic, and NRBC the counts for each diluent will be within limits as stated in [Table 1.12](#).

High to Low Carryover for Body Fluids is measured by analyzing a whole blood specimen followed by a diluent analyzed as a body fluid. The diluent sample should not exceed the Background limits as stated in [Table 1.14](#).

Background Counts

The following tables list the acceptable background limits for Daily Checks and Body Fluids.

Table 1.13 Background - Daily Checks

Parameter	Limit
WBC	≤0.05 x 10 ³ /μL
RBC	≤0.005 x 10 ⁶ /μL
HGB	≤0.1 g/dL
PLT	≤3 x 10 ³ /μL
NRBC Region	≤10 events
NRBC Total	≤60 events

Table 1.13 Background - Daily Checks

Parameter	Limit
DIFF	≤100 events
RET	≤600 events

Table 1.14 Background - Body Fluids*

Parameter	Limit
TNC	≤20 cells/mm ³
RBC	≤1000 cells/mm ³

* Analyzed by running a diluent using the Body Fluid Count cycle.

Throughput

The DxH 800 achieves the average throughput defined in the table below, when used in a routine laboratory environment with whole blood samples having hematology parameters with the values defined below. Throughput is achieved when cellular concentrations are in the following ranges: WBC = 7.00–10.0 x 10³/μL, RBC = 4.00–5.00 x 10⁶/μL, PLT = 250–400 x 10³/μL.

Table 1.15 Throughput per Test Panel Mode

Test Panel	Specimens per Hour
CBC	≥100
CBC and Differential	≥100
CBC and Differential with NRBC	≥90
Any Retic cycle	≥45
Mixed Samples	
80% CBC/Diff with NRBC	≥72
20% CBC/Diff/Retic with NRBC	

1.3.1 Vieno analizatoriaus našumas tiriant veninį kraują su diferenciacija (CBC+DIFF) – ne mažiau kaip 100 tyrimų per valandą

Reference Range Studies

A Normal Range study was conducted to assess the Reference Ranges for the DxH 800. Whole-blood samples were collected from approximately 240 donors (males and females). The selection of donors was consistent with guidelines stated in CLSI, C28-A2. These ranges are used as the System Manager default normal range flags. your patient population ranges may be different.

Table 1.16 Whole Blood Reference Ranges Overall

Parameter	Units	Overall		
		Mean	95% Confidence Low Limit	95% Confidence High Limit
WBC	$\times 10^3/\mu\text{l}$	6.3	3.6	11.2
RBC	$\times 10^6/\mu\text{l}$	4.52	3.73	5.50
HGB	g/dl	13.4	11.4	15.9
HCT	%	39.0	33.3	45.7
MCV	fL	86.4	73.7	95.5
MCH	pg	29.6	24.3	33.2
MCHC	g/dl	34.2	32.5	35.8
RDW	%	13.8	12.3	17.0
RDW-SD	fL	41.4	37.1	47.8
PLT	$\times 10^3/\mu\text{l}$	257	159	386
MPV	fL	9.2	7.5	11.2
NE	%	58.5	43.3	76.6
LY	%	29.6	16.0	43.5
MO	%	8.3	4.5	12.5
EO	%	2.8	0.6	7.9
BA	%	0.7	0.2	1.4
NE#	$\times 10^3/\mu\text{l}$	3.7	1.8	7.8
LY#	$\times 10^3/\mu\text{l}$	1.8	1.0	3.0
MO#	$\times 10^3/\mu\text{l}$	0.5	0.3	1.0
EO#	$\times 10^3/\mu\text{l}$	0.2	0.0	0.5
BA#	$\times 10^3/\mu\text{l}$	0.0	0.0	0.1
NRBC	/100 WBC	0.1	0.0	0.4
NRBC#	$\times 10^3/\mu\text{l}$	0.01	0.00	0.02
RET	%	1.10	0.50	2.17
RET#	$\times 10^6/\mu\text{l}$	0.0498	0.0221	0.0963
MRV	fL	108.8	97.4	120.2
IRF	—	0.40	0.29	0.53

Table 1.17 Whole Blood Reference Ranges Male

Parameter	Units	Male		
		Mean	95% Confidence Low Limit	95% Confidence High Limit
WBC	$\times 10^3/\mu\text{l}$	5.9	3.6	10.2
RBC	$\times 10^6/\mu\text{l}$	4.81	4.06	5.63
HGB	g/dl	14.2	12.5	16.3
HCT	%	41.3	36.7	47.1
MCV	fL	86.1	73.0	96.2
MCH	pg	29.6	23.8	33.4
MCHC	g/dl	34.4	32.5	36.3
RDW	%	13.6	12.1	16.2
RDW-SD	fL	40.8	36.5	45.9
PLT	$\times 10^3/\mu\text{l}$	234	152	348
MPV	fL	9.2	7.4	11.4
NE	%	57.3	43.5	73.5
LY	%	29.8	15.2	43.3
MO	%	9.0	5.5	13.7
EO	%	3.2	0.8	8.1
BA	%	0.7	0.2	1.5
NE#	$\times 10^3/\mu\text{l}$	3.4	1.7	7.6
LY#	$\times 10^3/\mu\text{l}$	1.7	1.0	3.2
MO#	$\times 10^3/\mu\text{l}$	0.5	0.3	1.1
EO#	$\times 10^3/\mu\text{l}$	0.2	0.0	0.5
BA#	$\times 10^3/\mu\text{l}$	0.0	0.0	0.1
NRBC	/100 WBC	0.2	0.0	0.6
NRBC#	$\times 10^3/\mu\text{l}$	0.01	0.00	0.02
RET	%	1.09	0.42	2.23
RET#	$\times 10^6/\mu\text{l}$	0.0523	0.0188	0.1086
MRV	fL	109.5	97.5	122.7
IRF	—	0.41	0.30	0.54

Table 1.18 Whole Blood Reference Ranges Female

Parameter	Units	Female		
		Mean	95% Confidence Low Limit	95% Confidence High Limit
WBC	$\times 10^3/\mu\text{l}$	6.7	3.8	11.8
RBC	$\times 10^6/\mu\text{l}$	4.26	3.63	4.92
HGB	g/dl	12.6	10.9	14.3
HCT	%	36.9	31.2	41.9
MCV	fL	86.8	75.5	95.3
MCH	pg	29.6	24.7	32.8
MCHC	g/dl	34.1	32.3	35.6
RDW	%	14.0	12.3	17.7
RDW-SD	fL	42.0	37.6	50.3
PLT	$\times 10^3/\mu\text{l}$	278	179	408
MPV	fL	9.2	7.9	10.8
NE	%	59.7	42.7	76.8
LY	%	29.4	16.0	45.9
MO	%	7.6	4.3	10.9
EO	%	2.4	0.5	7.0
BA	%	0.7	0.2	1.3
NE#	$\times 10^3/\mu\text{l}$	4.1	1.9	8.2
LY#	$\times 10^3/\mu\text{l}$	1.9	1.1	3.1
MO#	$\times 10^3/\mu\text{l}$	0.5	0.2	0.9
EO#	$\times 10^3/\mu\text{l}$	0.2	0.0	0.5
BA#	$\times 10^3/\mu\text{l}$	0.0	0.0	0.1
NRBC	/100 WBC	0.1	0.0	0.3
NRBC#	$\times 10^3/\mu\text{l}$	0.01	0.00	0.02
RET	%	1.11	0.51	2.17
RET#	$\times 10^6/\mu\text{l}$	0.0474	0.0230	0.0935
MRV	fL	108.1	96.4	118.0
IRF	—	0.40	0.26	0.52

Body Fluids Reference Ranges

Reportable body fluid results obtained from the DxH 800 System may exceed commonly accepted normal reference ranges for all body fluids. Results should always be interpreted in light of the total clinical presentation of the patient, including clinical history, data from additional tests, and other appropriate information.

Cerebrospinal Fluid

The inability to collect cerebrospinal fluid specimen in the normal, non-diseased population limits the ability to determine reference ranges. Literature¹ suggests the following normal reference ranges.

- WBC 0–5 cells/mm³ in adults
- WBC 0–30 cells/mm³ in children 1 to 4 years of age
- WBC 0–20 cells/mm³ in children 5 years of age to puberty
- RBC none to few

Serous Fluids

The accumulation of fluid in a serous cavity is an indication of a disease state. The normal, non-diseased population has no fluid accumulation. Therefore, there are no normal reference ranges for serous fluids. However, the number of cells present in a serous fluid are used to aid in the classification, diagnosis and treatment of disease.¹

Synovial Fluid

The inability to collect synovial fluid specimens in the normal, non-diseased population limits the ability to determine reference ranges. Literature suggests the following normal reference ranges.

- WBC 0–150 cells/mm³
- RBC none

Sample Stability and Storage

IMPORTANT Refer to *CLSI H18-A3, Procedures for the Handling and Processing of Blood Specimens for guidelines*.

Overview

The following types of sample can be analyzed on the SPM.

- Anti-coagulated human whole blood in K₂ or K₃ EDTA
- Prediluted anti-coagulated human blood in K₂ or K₃ EDTA
- Human Cerebrospinal fluid (CSF)
- Human Synovial fluid in K₂ or K₃ EDTA or Heparin (pretreated with Hyaluronidase)
- Human Serous fluids in K₂ or K₃ EDTA

Sample stability is measured by the ability of results to be within the stated specifications for a given period of time and storage condition. A minimum of ten (10) samples are analyzed in duplicate at time zero and the room temperature defined in [Table 1.19](#). The mean of those results is compared to the mean of the same samples analyzed at the times and storage conditions noted in those tables. The difference in mean results will be within the stability ranges defined in [Table 1.19](#) and [1.20](#).

Beckman Coulter recommends analyzing all non-refrigerated whole blood samples within 24 hours.

Whole Blood

Long term stability is determined by comparing results from the initial analysis (within two hours of collection) to results from samples stored at controlled room temperature for 24 hours and refrigerated temperature for 48 hours. Upon removal from refrigerated storage, samples were hand mixed by inversion 20 times, allowed to warm at room temperature for a minimum of 30 minutes and then hand mixed by inversion 20 times prior to analysis.

Table 1.19 Sample Stability (Whole Blood)

Parameter	Stability Range	At Controlled Room Temperature (18 to 26°C or 64 to 79°F) Time	At Refrigerated Temperature (2 to 8°C or 35.6 to 46.4°F) Time
WBC ($\times 10^3/\mu\text{L}$)	≤ 0.5	24 hours	48 hours
RBC ($\times 10^6/\mu\text{L}$)	≤ 0.10	24 hours	48 hours
HGB (g/dL)	≤ 0.2	24 hours	48 hours
MCV fL	≤ 3.0	24 hours	48 hours
RDW (%)	≤ 1.0	24 hours	48 hours
RDW-SD	≤ 5.0	24 hours	48 hours
PLT ($\times 10^3/\mu\text{L}$)	≤ 30	24 hours	48 hours
MPV fL	≤ 1.0	24 hours	48 hours
NE (%)	≤ 5.0	24 hours	48 hours
LY (%)	≤ 4.0	24 hours	48 hours
MO (%)	≤ 3.0	24 hours	48 hours
EO (%)	≤ 1.5	24 hours	48 hours
BA (%)	≤ 1.5	24 hours	48 hours
NRBC (%)	≤ 0.5	24 hours	24 hours
RET (%)	≤ 0.30	24 hours	72 hours
MRV fL	≤ 4.0	24 hours	72 hours
IRF	≤ 0.30	24 hours	72 hours

Predilute

Results from prediluted samples analyzed between 5 minutes and 1 hour after preparation and compared to those same samples from whole blood analyses should agree within the limits in [Table 1.20](#).

Table 1.20 Sample Stability (Pre-diluted Whole Blood)

Parameter	Difference (whichever is greater)
WBC $\times 10^3/\mu\text{L}$	± 0.4 or $\pm 10\%$
RBC $\times 10^6/\mu\text{L}$	± 0.1 or $\pm 4\%$

Table 1.20 Sample Stability (Pre-diluted Whole Blood)

Parameter	Difference (whichever is greater)
HGB g/dL	±0.4 or ±6%
PLT x 10 ³ µL	±10.0 or ±15%

Body Fluids

Per established literature, Body Fluid samples should be stored at room temperature and analyzed within 1 hour of collection.

Clinical Sensitivity and Specificity Performance Characteristics

Clinical sensitivity and specificity of WBC differential flagging performance can be influenced by a number of factors relating to instrument technology, cellular frequency, uncertainty in the reference determination of a “positive,” and the sample population evaluated. The DxH 800 provides the ability to set the levels and sensitivities of a variety of Flags and Messages to meet individual laboratory requirements.

Beckman Coulter, Inc. recommends completion of sensitivity and specificity studies using your sample population to establish these settings.

Specimen Tubes

The DxH 800 is capable of processing a wide variety of specimen tubes. Please refer to the tube list at www.beckmancoulter.com for additional information.

1.3.2 Tiriami éminiai: veninis kraujas, kapiliarinis kraujas

Venous and Capillary Sample Performance Characteristics

Twenty-nine specimens from normal Beckman Coulter in-house donors were collected as whole blood venous and whole blood capillary specimens. When analyzed on the DxH 800, a number of specimens did not provide parameter results and were excluded from the analysis. The results of the study are shown below.

Table 1.21 Venous and Capillary Sample Performance Characteristics

Parameter	n	Correlation	Intercept	Slope	Mean		Units
					Venous	Capillary	
WBC	29	0.963	0.573	0.978	6.28	6.71	x10 ³ /µL
RBC	29	0.940	0.324	0.972	4.75	4.94	x10 ⁶ /µL
HGB	29	0.917	1.849	0.908	14.26	14.80	g/dL
MCV	29	0.994	-0.852	0.995	87.43	86.15	fL
PLT	29	0.936	-34.627	1.047	247.37	224.48	x10 ³ /µL
MPV	29	0.944	1.478	0.891	8.59	9.13	fL

Table 1.21 Venous and Capillary Sample Performance Characteristics

Parameter	n	Correlation	Intercept	Slope	Mean		Units
					Venous	Capillary	
RDW	29	0.960	-0.169	1.010	13.27	13.23	CV%
RDW-SD	29	0.964	-5.252	1.113	40.72	40.05	fL
NE%	25	0.978	0.995	0.966	56.65	55.58	%
LY%	25	0.974	1.488	0.968	30.75	31.46	%
MO%	25	0.949	1.573	0.864	9.11	9.38	%
EO%	25	0.979	0.039	0.992	2.78	2.81	%
BA%	25	0.333	0.551	0.309	0.70	0.77	%
NRBC%	27	0.567	0.045	0.30	0.10	0.09	%
RET%	25	0.954	0.078	0.856	1.44	1.34	%
MRV	28	0.898	24.299	0.784	107.13	108.29	fL
IRF	28	0.790	0.078	0.831	0.34	0.36	—

Closed and Open Vial Performance Characteristics

Twenty-five specimens from normal Beckman Coulter in-house donors were collected and analyzed as closed vial and open vial specimens. The results of the study are shown below.

Table 1.22 Closed and Open Vial Performance Characteristics

Parameter	n	Correlation	Intercept	Slope	Mean		Units
					Closed Vial	Open Vial	
WBC	25	0.9982	0.135	0.974	6.435	6.404	$\times 10^3/\mu\text{L}$
RBC	25	0.9936	0.136	0.973	4.83	4.84	$\times 10^6/\mu\text{L}$
HGB	25	0.9912	1.213	0.985	13.72	13.73	g/dL
MCV	25	0.9927	2.152	0.974	85.29	85.22	fL
PLT	25	0.9906	-0.288	1.011	265.03	267.81	$\times 10^3/\mu\text{L}$
MPV	25	0.9913	0.671	0.919	8.53	8.51	fL
RDW	25	0.9344	1.568	0.879	13.82	13.72	CV%
RDW-SD	25	0.8766	10.782	0.731	41.58	41.16	fL
NE%	25	0.9877	-0.837	1.019	59.27	59.53	%
LY%	25	0.9809	-1.013	1.031	29.54	29.44	%
MO%	25	0.8794	0.530	0.923	8.02	7.93	%
EO%	25	0.9756	-0.073	1.022	2.49	2.48	%

Table 1.22 Closed and Open Vial Performance Characteristics

Parameter	n	Correlation	Intercept	Slope	Mean		Units
					Closed Vial	Open Vial	
BA%	25	0.7909	0.080	0.796	0.68	0.62	%
NRBC%	25	0.8899	0.017	0.752	0.15	0.13	%
RET%	25	0.9112	0.187	0.849	1.35	1.33	%
MRV	25	0.9584	-0.467	1.009	104.32	104.75	fL
IRF	25	0.8569	0.069	0.801	0.36	0.36	—

Whole Blood and Pre-Dilute Performance Characteristics

Fifty-seven specimens were analyzed as whole blood and pre-dilute. The results of the study are shown below.

Table 1.23 Whole Blood and Pre-Dilute Performance Characteristics

Parameter	n	Correlation	Intercept	Slope	Mean		Units
					Whole Blood	Pre-Dilute	
WBC	57	0.999	-0.341	1.079	14.856	15.690	$\times 10^3/\mu\text{L}$
RBC	57	0.999	-0.010	1.043	3.67	3.82	$\times 10^6/\mu\text{L}$
HGB	57	0.999	0.165	1.041	10.95	11.57	g/dL
MCV	57	0.996	-2.686	1.004	90.42	88.11	fL
PLT	57	0.998	3.127	1.003	281.69	285.70	$\times 10^3/\mu\text{L}$
MPV	57	0.940	0.349	0.924	8.47	8.18	fL
RDW	57	0.995	-0.625	1.005	16.86	16.31	CV%
RDW-SD	57	0.991	-1.054	0.964	52.38	49.44	fL

Limitations

All Specimens	<p>Misleading results can occur if the specimen is not properly collected, stored or transported. Beckman Coulter, Inc. recommends that you follow CLSI or equivalent procedures to ensure proper specimen collection, storage and transport. Always follow manufacturer's recommendations when using microcollection devices for capillary specimen collection.</p> <p>Misleading results can occur if specimens contain clots. Always use good laboratory practices for inspecting specimens for clots and verifying results.</p> <p>Misleading results can occur if the specimen is not properly mixed. Always use good laboratory practices to ensure specimens are appropriately mixed. Do not bypass or circumvent the automated mixing process used on the DxH 800.</p> <p>NOTE When running a test panel, with NRBC analysis enabled, the information from the NRBC analysis is used to supplement interference detection, flagging and correction.</p>
WBC and TNC	<p>NRBCs, giant platelets, platelet clumps, malarial parasites, precipitated elevated proteins, cryoglobulin, microlymphoblasts, very small lymphocytes, fragmented white cells, agglutinated white cells, lyse resistant red cells, unlysed particles > 35 fL in size.</p> <p>Elevated WBC counts may have a carryover effect on subsequent leukopenic specimens, within the limits specified in the Carryover section.</p>
RBC	<p>Very high WBC count, high concentration of very large platelets, auto-agglutination.</p> <p>If hemolysis is occurring in vivo, the instrument RBC may be flagged as low, reflecting the true circulating cells. If, however, the hemolysis is in vitro, the specimen may give falsely low RBC results. Cell counts due to in vitro hemolysis do not represent the number of circulating red blood cells.</p>
HGB	Severe lipemia, heparin, certain unusual RBC abnormalities that resist lysing.
MCV	Very high WBC count, high concentration of very large platelets, auto-agglutination.
RDW, RDW-SD	Very high WBC count, high concentration of very large platelets, auto-agglutination
PLT	Giant Platelets, platelet clumps, white cell fragments, electronic noise, very small red cells, red cell fragments.
HCT	Known interferences related to RBC and MCV.
MCH	Known interferences related to HGB and RBC.
MCHC	Known interferences related to HGB, RBC, and MCV.
NRBC	<p>Known interferences may be related to the following:</p> <ul style="list-style-type: none"> • lyse resistant red cells • malarial parasites • very small or multi-population lymphocytes • precipitated elevated proteins
Differential	<p>Hypogranular granulocytes, agranular granulocytes, lyse resistant red cells, very small or multi-population lymphocytes, elevated triglycerides, precipitated elevated proteins.</p> <p>A transient basophilia may be observed in samples that have been exposed to high temperatures (~ 90°F or ~ 32°C). The temporary basophilia should resolve after stabilization at room temperature (~ 72°F or ~ 22°C).</p>

Reticulocytes	Erythrocyte inclusions stained by New Methylene Blue, if sufficiently numerous within a sample, and some hemoglobinopathies (SS, SC) might affect the accuracy of the reticulocyte enumeration ² .
Body Fluids	<ul style="list-style-type: none">• Clotted specimens may lead to misleading or erroneous results. Follow standard operating procedure for inspecting specimens for clots.• Improperly mixed specimens may lead to misleading or erroneous results.• Cellular debris may lead to misleading or erroneous results.• Results should be interpreted in light of the total clinical presentation of the patient, including clinical history, data from additional tests, smear review, and other appropriate information.
Cerebrospinal Fluid	<ul style="list-style-type: none">• The low levels of albumin and lipids in cerebrospinal fluid may accelerate cell lysis, leading to decreased manual counts and an apparent lack of correlation.³• Delays in processing may lead to misleading or erroneous results.
Synovial Fluid	<ul style="list-style-type: none">• Fat globules may lead to misleading or erroneous results.• Crystals may lead to misleading or erroneous results.• Highly viscous synovial fluids may trap cells leading to misleading or erroneous results.

Operation Principles

History

Coulter Principle

W.H. Coulter (1956) describes the Coulter Principle:⁵

A suspension of blood cells is passed thru [sic] a small orifice simultaneously with an electric current. The individual blood cells passing through the orifice introduce an impedance change in the orifice determined by the size of the cell. The system counts the individual cells and provides cell size distribution. The number of cells counted per sample is approximately 100 times greater than the usual microscope count to reduce the statistical error by a factor of approximately 10 times.

This substantial improvement in precision over previous methods helped to establish the erythrocyte count as a sensitive index of erythropoietic dyscrasia, particularly when considered together with Hct and Hgb measurements.⁶

The COULTER COUNTER Model S analyzer was the first instrument that automated simultaneous multiparameter measurements on blood. Brittin et al., Gottmann, and Hamilton and Davidson, reviewed the performance and clinical value of the Model S.^{7,8,9}

Refinements of the COULTER COUNTER analyzer to provide accurate size (volume) distribution data led to a reawakening of interest in pathological erythrocyte size distribution, first sparked by Price-Jones.^{10,11}

Among the advantages offered by the Coulter method of counting and sizing was the ability to derive an accurate Hct measurement by summing the electronic volume of erythrocytes. England et al. speculated that electronic Hct measurements did not contain the trapped plasma error of centrifugal Hct measurements.¹²

Bull et al. described the use of a COULTER COUNTER analyzer for counting thrombocytes.¹³ This method, useful as it was, depended on preparing thrombocyte-rich plasma to avoid counting erythrocytes as thrombocytes. Mundschenk et al. and Schulz and Thom discussed the possibility of counting thrombocytes in the presence of erythrocytes and classifying them by size.^{14,15} Electronic refinements in the Model S-PLUS enhanced the accuracy of the hydrodynamic method. Von Behrens and Paulus have also cited the feasibility of counting thrombocytes by the Coulter method.^{16,17}

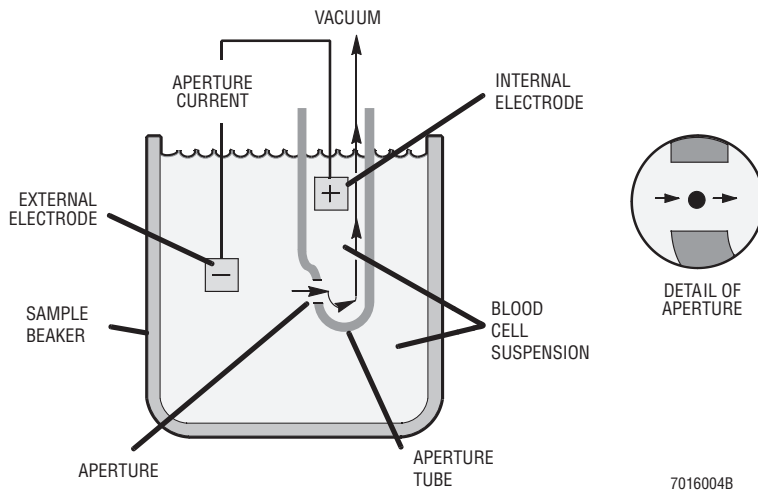
1.3.2. 1) Elektrinės
varžos pokyčio matavimo
metodas

Coulter Method

1.3.2. 1) Elektrinės varžos pokyčio matavimo metodas

The Coulter Method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. See Figure 2.1, Coulter Method.

Figure 2.1 Coulter Method



Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume.^{18,19,20,21}

While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

VCS Technology

The COULTER VCS established WBC differential technology using three measurements: individual cell volume, high-frequency conductivity and laser-light scatter.

The combination of low-frequency current, high-frequency current and light-scattering technology provided abundant cell-by-cell information that is translated by the SPM into dataplots.

Volume Analysis

Electronic Leukocyte Volume Analysis using low-frequency current, has been used since 1967.²² It has been evaluated as a possible adjunct to the differential white cell count.^{23, 24, 25, 26}

Conductivity Analysis

Cell walls act as conductors to high frequency current. The current, while passing through the cell walls and through each cell interior, detects differences in the insulating properties of the cell components. The current characterizes the nuclear and granular constituents and the chemical composition of the cell interior.^{27, 28, 29}

1.3.2. 2) Šviesos sklaidos (priekinės ir šoninės) matavimo metodas (tėkmės citometrija)

Light Scatter Analysis

Coulter's experience in flow cytometry dates back decades to Fulwyler's pioneering use of light scatter for cell analysis. Loken et al. and Jovin et al. discuss the relationship of particle size and refractivity to the angle of light scattered from a laser beam.^{30, 31, 32}

TTM

Historically, Beckman Coulter analyzers housed a flow cell in a Triple Transducer Module (TTM), first introduced commercially in the 1980's. The TTM flow cell was the location for detection of the processed samples. The TTM produced three measurement signals – volume, conductivity and light scatter.

The DxH 800 replaces the TTM with the Multi-transducer Module (MTM), which measures additional multiple angles of light scatter, a major improvement over the single light scatter measured by the TTM.

Reticulocyte Analysis

Reticulocytes are immature, non-nucleated erythrocytes retaining a small network of basophilic organelles, consisting of RNA and protoporphyrin. The enumeration of reticulocytes provides a simple, effective means to determine red cell production and regeneration.^{33, 34, 35, 36}

The most common means of measuring reticulocytes is to use supravital dyes, such as New Methylene Blue or Brilliant Cresyl Blue. These dyes precipitate and aggregate the basophilic substances within the reticulocyte, resulting in a granular, staining pattern easily seen with light microscopy.³⁷

Reticulocyte immaturity is related to cell volume and light scatter. Since more immature reticulocytes are larger, contain more RNA and cause increased light scatter, the cell volume and light scatter will increase with immaturity of the cell.

DxH 800 Operation Principles

CBC Analysis

In hematology, the complete blood count, the CBC, is the fundamental analytical test that evaluates the three main cellular components: white blood cells, red blood cells and platelets. The DxH 800 CBC analysis is based on the Coulter Principle. Show me CBC analysis.

The sample preparation and data collection occurs in the SAM and CBC modules and analysis is handled by the System Manager.

Specimen Preparation

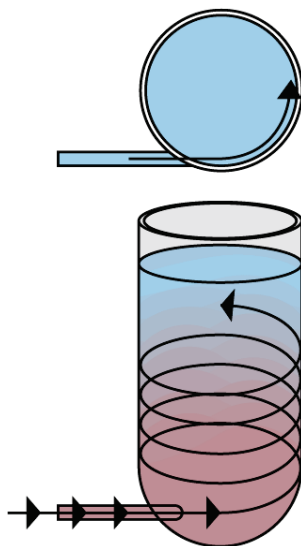
The aspiration pump activates and aspirates 165 μL of sample. After the probe is removed from the specimen tube a second pull of the aspiration pump draws the blood through the BSV pathway, verifying a proper aspiration at the blood detectors.

With each cycle, the BSV directs the delivery of sample and DxH Diluent to the WBC and RBC triple aperture baths.

The RBC diluent and WBC diluent/Lyse dilutions enter through a port in the bath that is located at the bottom and tangential to a sloping surface for bubble free delivery and mixing. Show me tangential mixing.

In the WBC bath, ~6.0 mL of DxH diluent and ~28 μL of sample are combined with ~1.08 mL of DxH Cell Lyse for a final dilution of 1:251. In the RBC bath, ~10 mL of DxH diluent and ~1.6 μL of sample are combined for a final dilution of 1:6250.

Figure 2.2 Tangential Mixing



Detection/Sensing

After the mixing and incubation of sample and reagents, 6 inches of vacuum and aperture current are applied to the apertures simultaneously for the measurements of cell count and cell volume. The RBC and PLT count includes the application of sweep flow to prevent the recirculation of cells behind the aperture. All pulses generated by the apertures are collected and sent to the Signal Conditioner Analyzer Card for analog to digital conversion. The process provides the following raw counts and digital measurements to the System Manager:

- Time
- Volume (pulse peak amplitude)
- Count rate
- Wait time
- Pulse width

The System Manager processes the measurements. The process includes:

- Coincidence correction
- Voting
- The generation of 256 channel histograms for WBC, RBC, and PLT and their voting pattern analysis
- Interference correction

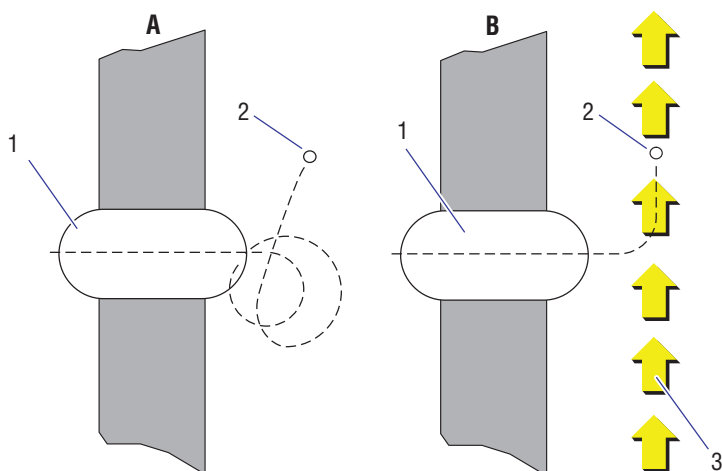
Pulse Editing

When cells pass through the aperture near the edge or at an angle rather than at the center, they create atypical pulses. These atypical pulses are excluded from analysis because they distort the true size of the cell. This prevents the atypical pulses from influencing size measurement.

Sweep Flow

The sweep flow is a steady stream of diluent that flows behind the RBC aperture during the sensing period. This prevents cells from re-entering the sensing zone and being counted as platelets. See [Figure 2.3](#).

Figure 2.3 Sweep Flow



Example A: No Sweep Flow
Show me No Sweep Flow.

Example B: Sweep Flow Added
Show me Sweep Flow.

1. Sensing Zone
2. Cell Recirculates

1. Sensing Zone
2. Cell Carried to Waste
3. Sweep Flow

Counting/Sizing

The RBC and WBC baths each have three discrete apertures that function as independent systems, utilizing the [Coulter Method](#) to accurately count and size cells.

Coincidence Correction

Occasionally, more than one cell passes through the aperture at one time. When cells coincide, the SPM counts only one pulse. As the frequency of coincidence is proportional to the actual count, the system automatically corrects results for coincidence.

Scaling

Scaling adjusts for calibration and reportable format.

Voting and Averaging

To prevent data errors due to statistical outliers or obstructions that may block an aperture, the SPM votes on the data from all of the apertures, and rejects any questionable data. For the WBC, RBC, MCV, RDW, Plt, and MPV, the SPM compares the data from the three apertures. It verifies that at least two apertures have produced data within an established statistical range of each other.

When a parameter totally votes out, the system does not give any results for the affected parameter or for any parameters that are derived from it. See [Flags](#) and [Codes](#) in the Data Review chapter of this manual for codes and messages that appear in these circumstances.

Hemoglobinometry

The lytic reagent used for the WBC prepares the blood so the system can count leukocytes and measure the amount of hemoglobin. The lytic reagent rapidly and simultaneously destroys the erythrocytes and converts a substantial proportion of the hemoglobin to a stable pigment while it leaves leukocyte nuclei intact. The absorbance of the pigment is directly proportional to the hemoglobin concentration of the sample.

The accuracy of this method equals that of the hemoglobincyanide method, the reference method of choice for hemoglobinometry recommended by the International Committee for Standardization in Hematology.³⁸

After the WBC are counted, the lysed WBC dilution drains into the hemoglobin cuvette for Hgb measurement. Hgb is measured photometrically at 525 nm using the sample from the WBC analysis. A blank is introduced into the cuvette during each operating cycle. The Hgb blank provides a reference to which the sample signal is compared.

Generation of Histograms

The digital information from each aperture is stored according to volume in 256-channel, size distribution histograms.

To ensure that the size-distribution curves accurately reflect the true cell population, the sensing may be extended whenever the data accumulations are below a predetermined value.

VCSn

All Diff, NRBC, and Retic analysis occurs in the VCSn module. The VCSn module is responsible for controlled sample preparation and delivery of the prepared sample to the flow cell for analysis of the WBC differential, reticulocytes and NRBC. The VCSn module includes the Air Mix and

Temperature Control (AMTC) Module and the Multi-transducer Module (MTM).
Show me Diff analysis. Show me NRBC analysis. Show me Retic analysis.

Sample Preparation

The sample preparation for Diff, NRBC, and Retic analysis occurs in the mix chambers in the AMTC module of the VCSn module. The blood samples used for analysis are delivered by the SAM and dispensed directly to the appropriate mix chamber. Next, the temperature-controlled reagents are delivered and the sample and reagents are mixed using a focused jet of air regulated to 4 psi. Show me air mixing. The mix chambers, reagents and air are all temperature controlled.

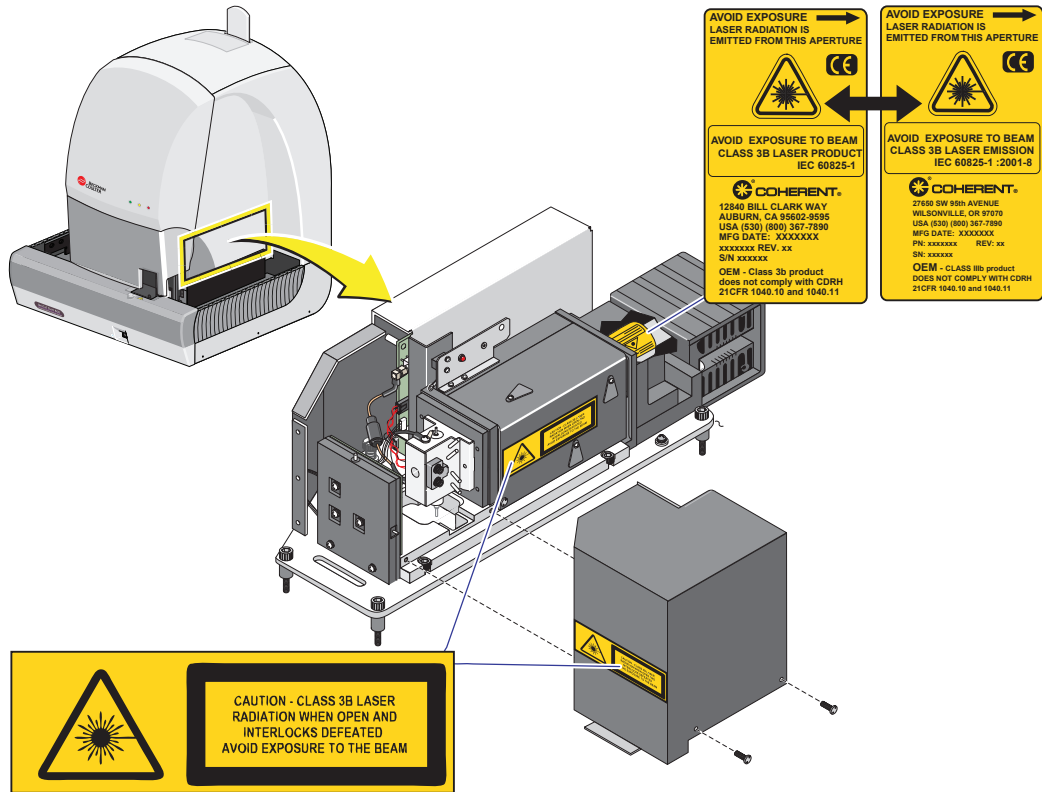
Detection/Sensing

Once the sample is prepared, the sample is delivered via the Distribution Valve (DV) to the MTM for sample detection.

MTM

The MTM measures particle light scatter by utilizing a flow cell to pass particles through a sensing zone one cell at a time. [Figure 2.4](#) shows the MTM without its protective housing to display the laser and flow cell and label locations. As the particles pass through the sensing zone, a diode laser illuminates the particles. The MTM flow cell measures volume, conductivity, multiple angles of light scatter and axial light loss.

Figure 2.4 Multi-Transducer Module with Protective Housing Cut Away



Volume and Conductivity

In the flow cell, low-frequency, direct current measures volume, while high-frequency (RF) current senses cellular internal content through measuring changes in conductivity.

Measuring Light Scatter and Axial Light Loss

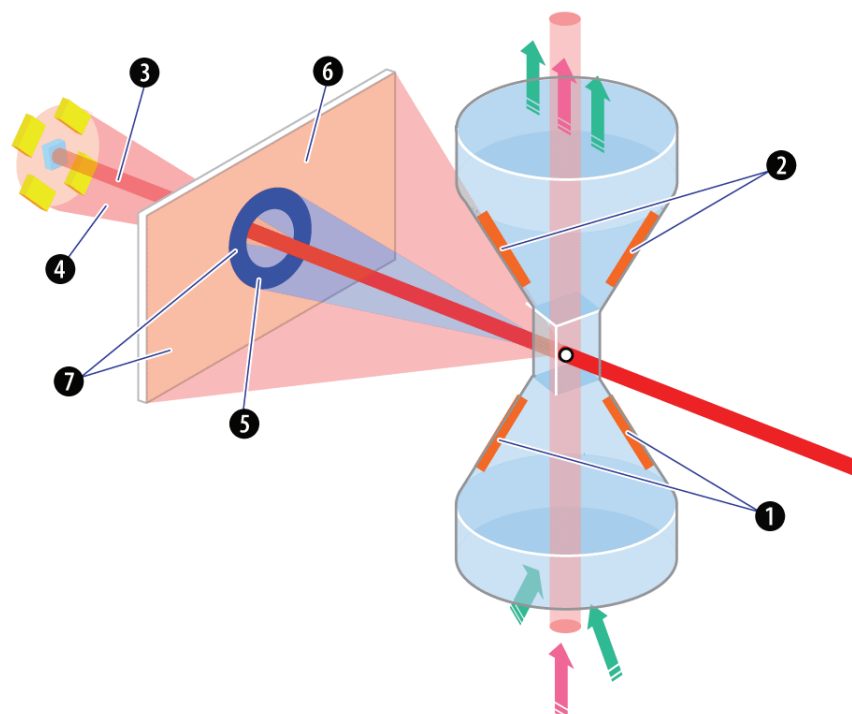
The MTM utilizes a flow cell to pass particles through a sensing zone one particle at a time and a diode laser to illuminate the particles. The illuminated particles both scatter and absorb a portion of the incident light.

Sensors strategically placed around the flow cell collect the scattered light of interest.

An additional sensor placed in the laser path measures the amount of light removed due to light scatter and absorption. This measurement is called Axial Light Loss.

1.3.2. 2) Šviesos sklaidos (priekinės ir šoninės) matavimo metodas (tėkmės citometrija)

Figure 2.5 Light Scatter on the DxH 800



1. Lower Electrode (DC and RF)
2. Upper Electrode (DC and RF)
3. Axial Light Loss (ALL) 0°
4. Low Angle Light Scatter (LALS) 5.1°
5. Lower Median Angle Light Scatter (LMALS) 10°–20°
6. Upper Median Angle Light Scatter (UMALS) 20°–42°
7. The fifth light scatter channel is the sum of the UMALS and the LMALS regions (called MALS).

Dataplot Development

The System Manager performs a series of operations on the stored digital raw values received from the flow cell to identify populations and calculate the frequency of cells within each population. The system produces the Dataplot displays for visual representation of the Differential, NRBC and Reticulocyte membership and density.

The DxH 800 System algorithm uses tools designed for finding optimal separation between overlapping clusters of data. The algorithm can

- adapt to unusual population shifts and overlaps
- define highly irregular separation
- make subsequent analysis of the identified regions
- correct deficiencies in separation