

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K181599

B. Purpose for Submission:

To obtain a substantial equivalence determination for the UniceL DxH 800 Coulter Cellular Analysis System with Early Sepsis Indicator Application

C. Measurand:

Monocyte Distribution Width (MDW)

D. Type of Test:

Quantitative assay to measure Monocyte Distribution Width (MDW) parameter

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

UniceL DxH 800 Coulter Cellular Analysis System with Early Sepsis Indicator Application
Early Sepsis Indicator

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.3215

2. Classification:

Class II

3. Product codes:

QFS, GKZ

4. Panel:

83 – Microbiology

H. Intended Use:

1. Intended use(s):

Same as Indications for use

2. Indication(s) for use:

The Unicel DxH 800 Coulter Cellular Analysis System with Early Sepsis Indicator Application is the quantitative measurement of Monocyte Distribution Width (MDW). The Early Sepsis Indicator is intended for use with adult patients presenting to the emergency department, on whom a white cell differential test has been ordered.

MDW is measured from a (K2EDTA) whole-blood venous sample within 2 hours of collection. MDW values greater than 20.0 together with other laboratory findings and clinical information, aids in identifying patients with sepsis or at increased risk of developing sepsis within the first 12 hours of hospital admission.

MDW values greater than 20.0 should be interpreted in association with other clinical information and diagnostic testing, as a proportion of patients without sepsis may have an elevated MDW value at baseline.

MDW values less than or equal to 20.0 cannot rule out sepsis or the development of sepsis within 12 hours of hospital admission. The Early Sepsis Indicator should not be used as the sole basis to determine the absence of sepsis.

The predictive value of the Early Sepsis Indicator for identifying sepsis in patients with hematological abnormalities has not been established.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Unicel DxH 800 System

I. Device Description:

The Unicel DxH 800 Coulter Cellular Analysis System with Early Sepsis Indicator Application is the quantitative measurement of Monocyte Distribution Width (MDW).is a quantitative, automated hematology analyzer, and its reagents, controls and calibrators last cleared under K140911. The addition of the MDW parameter required software algorithm modifications. The system provides a Complete Blood Count (CBC), Leukocyte 5 Part Differential (Diff), Reticulocyte (Retic), Nucleated Red Blood Cell (NRBC) on whole blood, as well as, Total Nucleated Count (TNC), and Red Cell a quantitative, automated hematology analyzer Count (RBC) on Body Fluids (cerebrospinal, serous and synovial). The MDW parameter added in this submission measures the dispersion (i.e., standard deviation) around the mean of the monocyte volume population in whole blood. Throughput, as defined for CBC/DIFF (CD) or CBC/DIFF/Retic (CDR) panel panels, is not impacted with the addition of the MDW parameter to the differential results. The Early Sepsis Indicator Application is disabled by default at installation. When the Early Sepsis Indicator Application is activated, the MDW parameter is enabled for reporting.

The Unicel DxH 800 system is composed of two primary components, an analyzer which processes specimens and a workstation which processes results using a multidimensional clustering algorithm that identifies the WBC cell populations. The workstation implements the algorithm for calculating MDW results, which are generated by the analyzer from a scatterplot of light scatter correlated to the volume of the cells to identify the monocyte population. The monocyte population is characterized using Volume (V), Conductivity (C) and multiple angles of Light Scatter (Sn). Volume measurements correlate to cell size. Monocytes are identified in the Volume (V) versus Rotated Light Scatter (RLSn) dataplot. Once the monocyte population is identified, a histogram of monocyte volume distribution is generated from which the MDW value is calculated. The MDW is calculated as the Standard Deviation (SD) of a set of monocyte cell volume values.

J. Substantial Equivalence Information:

1. Predicate device name(s):

VIDAS BRAHMS PCT

2. Predicate 510(k) number(s):

K162827

3. Comparison with predicate:

Predicate Comparison		
Item	DxH 800 Coulter Cellular Analysis System with Early Sepsis Indicator Application (K181599)	VIDAS B·R·A·H·M·S PCT (PCT) (K162827)
Intended Use and Indications for Use	The Unicel DxH 800 Coulter Cellular Analysis System with Early Sepsis Indicator Application is the quantitative measurement of Monocyte Distribution Width (MDW). The Early Sepsis Indicator is intended for use with adult patients presenting to the emergency department, on whom a white cell differential test has been ordered. MDW is measured from a (K2EDTA) whole-blood venous sample within 2 hours of collection. MDW values greater than 20.0 together with other laboratory findings and clinical information, aids in identifying patients with sepsis or at increased risk of developing sepsis within the first 12 hours of hospital admission. MDW values greater than 20.0 should be interpreted in association with other clinical information and diagnostic testing, as a proportion of	VIDAS B·R·A·H·M·S PCT (PCT) is an automated test for use on the instruments of the VIDAS family for the determination of human procalcitonin in human serum or plasma (lithium heparinate) using the ELFA (Enzyme-Linked Fluorescent Assay) technique. Used in conjunction with other laboratory findings and clinical assessments, VIDAS BRAHMS PCT is intended for use as follows: • to aid in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock, • to aid in assessing the cumulative 28-day risk of all-cause mortality for patients diagnosed with severe sepsis or septic shock in the ICU or when obtained in the emergency department or other medical wards prior to ICU admission, using a change in PCT level over

Predicate Comparison		
Item	DxH 800 Coulter Cellular Analysis System with Early Sepsis Indicator Application (K181599)	VIDAS B·R·A·H·M·S PCT (PCT) (K162827)
	patients without sepsis may have an elevated MDW value at baseline. MDW values less than or equal to 20.0 cannot rule out sepsis or the development of sepsis within 12 hours of hospital admission. The Early Sepsis Indicator should not be used as the sole basis to determine the absence of sepsis. The predictive value of the Early Sepsis Indicator for identifying sepsis in patients with hematological abnormalities has not been established.	time • to aid in decision making on antibiotic therapy for patients with suspected or confirmed lower respiratory tract infections (LRTI) defined as community-acquired pneumonia (CAP), acute bronchitis, and acute exacerbation of chronic obstructive pulmonary disease (AECOPD) – in an inpatient setting or an emergency department, • to aid in decision making on antibiotic discontinuation for patients with suspected or confirmed sepsis.
Specimen	Venous Whole Blood	Human Serum or Plasma (lithium heparinate)
Analyte	MDW Parameter	Procalcitonin (PCT)
Automated	Automated Hematology Analyzer	Automated Assay
Assay Technique	Whole blood analysis and cellular differentiation on the DxH800	ELFA (Enzyme-Linked Fluorescent Assay) technique.
Assay Principle	Coulter Principle: Volume, Conductivity, Light Scatter Analysis (VCSn) Technology using: Aperture impedance (DC) Conductivity (RF) Laser Light Scatter (Multiple angles) Laser Light Absorbance	Immunoassay based on sandwich principle
Detection Method	VCSn	Fluorescence (ELFA) of 4-methyumbelliferyl measured at 450 nm
Assay/Parameter Duration	≥90 specimens per hour (40 sec/cycle) for CBC and Diff with NRBC cycle (approximately 40 seconds per sample)	Approximately 20 minutes
Combination Device	N/A	Instruments of the VIDAS family: VIDAS, miniVIDAS or VIDAS 3
Antibodies/Analysis Reagents	COULTER DxH Diluent COULTER DxH Diff Pack COULTER DxH Retic Pack COULTER DxH Cell Lyse	Conjugate: Alkaline phosphatase-labeled mouse monoclonal anti-human procalcitonin Solid phase: Mouse monoclonal anti-procalcitonin immunoglobulins coated on interior of the SPR Sample
Sample Volume	165 µL	200 µL

K. Standard/Guidance Document Referenced (if applicable):

- CLSI H26-A2: Validation, Verification and Quality Assurance of Automated Hematology Analyzers; Approved Standard – Second Edition - June 2010.
- CLSI C56-A: Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline - July 2012.
- CLSI EP07-A3: Interference Testing in Clinical Chemistry – Third Edition - April 2018.
- CLSI EP37-A: Supplemental Tables for Interference Testing in Clinical Chemistry, 1st Edition - April 2018.
- CLSI EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition
- CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
- CLSI C56-A: Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline – July 2012
- Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells; Final Guidance for Industry and FDA December 4, 2001 – Section 13: Specimens.
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests; Final Guidance for Industry and FDA Staff; March 2007
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, (10/2010)
- CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline. Second Edition guideline.
- CLSI EP24-A2: Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline – Second Edition. 2011.

L. Test Principle:

The Coulter Principle of automated cell counting and sizing is used in the analysis of the whole blood and body fluid specimens; MDW measurements will be determined on whole blood specimens only. 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 two submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. While the number of pulses indicates particle count, the amplitude of the electrical pulse is proportional to the cell volume. These pulses are sent to the Signal Conditioner for analog to digital conversion. Pulse counts and digitized pulse measurements are sent to the System Manager for processing by the algorithms where the reported parameter values, flags and histograms are generated.

The COULTER VCSn technology is used to determine the white cell differential, nucleated red blood cell count, reticulocyte and monocyte parameters along with associated flags, messages, histograms and data plots.

The sample preparation and analysis use specific reagents and analytical processes for the WBC differential, NRBC and Retic analysis. The prepared sample is delivered to the flow cell for sample detection. As the cells pass through the sensing zone, a diode laser illuminates the particles causing light scatter and light absorption. Simultaneously to the light scatter measurements, cell volume and cell conductivity are also measured. Monocytes are identified in the volume versus Rotated Light Scatter (RLSn) dataplot. MDW is calculated as the standard deviation of the observed monocyte cell volumes in a whole blood specimen.

The data collected during each of the analytical processes is transferred to the System Manager where the digital raw values are processed by the algorithm using mathematical approaches designed for finding optimal separation between clusters of data. The identified clusters are used to calculate the frequency of cells within each population, generate parameter values, flags, histograms and data plots.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The system precision of the MDW parameter using COULTER 6C Plus Cell Controls on the DxH 800 system was assessed using CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition, October 2014. The measurement procedure precision using control materials evaluated with three (3) instruments at three (3) independent sites. A single lot of COULTER 6C Plus Cell Controls and at least three (3) lots of COULTER DxH Diluent and COULTER DxH Diff Pack reagents were used during the testing with each of the three (3) sites having a different set of reagent lots. The controls were run in triplicate, twice each day (morning and afternoon) for a minimum of five (5) days. A different vial of control was used for each test day. Repeatability and Reproducibility results are provided in Table 1 below.

Table 1: Repeatability of Controls

Repeatability						Between Runs		Between Days		Between Sites		Reproducibility		
Control Level	Parameter	N	Mean	SD	CV %	SD	CV %	SD	CV %	SD	CV %	Mean	SD	CV%
Level1	MDW	90	33.74	1.17	3.48	0.54	1.61	0.00	0.00	0.12	0.36	33.74	1.30	3.86
Level2	MDW	90	35.74	0.80	2.24	0.10	0.28	0.00	0.00	0.20	0.57	35.74	0.83	2.32
Level3	MDW	90	36.20	0.87	2.41	0.00	0.00	0.36	1.00	0.07	0.19	36.20	0.95	2.62

Repeatability was also assessed by replicate analysis of the same specimen (n=10). Repeatability was achieved with a %CV limit at or below 10.0. Specimens with system messages and/or suspect messages were not used for analysis.

The system precision performance for ESI on the DxH 800 hematology analyzer using whole blood specimens was assessed according to CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition, October 2014 and CLSI H26-A2: Validation Verification and Quality Assurance of Automated Hematology Analyzers, June 2010.

The precision profile was evaluated based on the CV% of ten replicates, from a total of 36 K2EDTA whole-blood samples. Analyzed samples recovered MDW values from 14 to 39. Whole blood repeatability was also assessed in conjunction with Monocyte% and Monocyte# for each individual sample. All CV% for individual specimens were <10% for MDW and similar to CV% for Monocyte %. Results with MDW flags and/or messages were excluded. The repeatability CV% was estimated at different MDW levels are shown in Table 2 below.

Table 2: Precision Performance for MDW at Multiple Cut-Off Values

Parameter			95% Confidence Limits	
Analyte	Level	CV %	Lower	Upper
MDW	19.00	5.28	4.55	6.01
	19.50	5.27	4.57	5.98
	20.00	5.27	4.59	5.94
	20.50	5.26	4.61	5.91

b. *Linearity/assay reportable range:*

The operating range for MDW is 0.00 - 255.50.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Sample Stability: To characterize the specimen age profile for the MDW parameter on the DxH 800 system stability was evaluated by testing 20 whole-blood samples in duplicate from zero to four hours. All samples were tested at each point in time. Samples were stored at 23.9°C (75°F). MDW must be tested from a (K₂EDTA) whole-blood venous sample within 2 hours of collection. The effect of time on MDW is summarized in Tables 3 - 5 below.

Table 3: Effect of Time on MDW

N=20	0 Hours	1 Hour	2 Hours	3 Hours	4 Hours
Mean	16.77	17.75	18.79	18.90	18.84

A second-degree polynomial was used to model the change of MDW over time. Drift at each time point was calculated as the difference between the predicted response from the regression model at each time point and the predicted response at time zero.

Standard error of the drift was based on the standard errors of the polynomial estimates and their covariance. The two-sided confidence limit of the drift was calculated based on the standard error and 95% confidence.

The estimates of regression parameters are shown in Table 4 and estimated drifts in Table 5.

Table 4: Regression Model – Parameter Estimate

Parameter	Estimate
Intercept (β_0)	16.79
Linear (β_1)	1.17
Quadratic (β_2)	-0.15

Table 5: Stability drift at different times as samples age

Time (hours)	Drift	Lower 95% confidence limit	Upper 95% confidence limit
1	1.03	0.77	1.28
2	1.75	1.35	2.14
3	2.16	1.72	2.60
4	2.27	1.85	2.68

Refrigerated samples cannot be used for MDW testing. Characterization studies have shown an increase in MDW values when samples are refrigerated.

COULTER Latron CP-X Control Lot to Lot Variability (long-term)

The repeatability and reproducibility specifications of the currently cleared Latron CP-X parameters as well as the D (V2) Sepsis parameter was verified per CLSI EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods. Three lots of Latron CP-X were evaluated. One new vial of each lot of Latron CP-X Control was sampled twice daily (AM and PM) with two aspirations being collected at each sampling for 20 days (20 x 2 x 2). The vials were discarded at the end of each day. Testing was conducted at ambient room temperature on one DxH 800. All currently cleared Latron CP-X parameters as well as the Sepsis D (V2) parameter met the Repeatability Specification of $\leq 3\%$ CV and all currently cleared Latron CP-X parameters met the Reproducibility specification of $\leq 5\%$ CV and the D (V2) met the Reproducibility Specification of $\leq 4\%$ CV.

Closed vial stability performance of three lots of Latron CP-X Control

The closed vial stability performance of three lots of Latron CP-X Control across the product's storage temperature range of 2-30°C was evaluated according to CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. Wayne, PA: Clinical and Laboratory Standards Institute; 2009. Test Methodology: Three lots of Latron CP-X Control were tested. All currently cleared parameters and the D(V2) parameter were evaluated. Two vials per lot of Latron CP-X were removed from each storage condition (2-8°C, 25°C and 30°C) and aspirated 6 times per vial. Lots were tested at approximately 0, 3, 6, 9, 12 and 13 months from date of fill. Closed vial stability is 365 days while open vial stability is up to 30 days.

Controls:

The system parameters and respective controls for the for Early Sepsis Indicator application are found in Table 6.

Table 6: System Parameters for Early Sepsis Indicator Application

PARAMETER	CONTROL(S)
MDW (Monocyte Distribution Width)	COULTER 6C Plus Cell Control and Patient Controls
LDW (Lymphocyte Distribution Width)	COULTER 6C Plus Cell Control
D (V2)	COULTER LATRON CP-X Control

COULTER 6C Plus Cell Control: The COULTER 6C Plus Cell Control included for use with the Early Sepsis Indicator Application is the current COULTER 6C Cell Control (K081822), assayed to include values for the MDW and LDW parameters. The COULTER 6C Plus Cell Control is an integrated control that enables monitoring of system performance for CBC, Diff, NRBC, MDW and LDW parameters. The two new parameters, MDW and LDW, are contained in each of the three levels of this cell control and represent dispersion values for different size populations of white cell subsets. The MDW parameter provides a larger dispersion value above the sepsis cutoff value. The LDW parameter provides a smaller dispersion value near or below the sepsis cutoff value. Both values indicate that the UniCel DxH 800 Coulter Cellular Analysis System is capable of calculating a range of clinical values for distribution width of cell populations.

NOTE: LDW is designed to enhance control of population dispersion calculations; it is not designed as part of patient result evaluation. LDW is only available in CBC/DIFF (CD) and CBC/DIFF/Retic (CDR) panels for cell controls when the Early Sepsis Indicator is activated.

COULTER LATRON CP-X: A suspension of stable polystyrene particles of uniform size with a diameter CV of $\leq 3.0\%$. Latron CP-X is used as part of the daily quality control procedure to monitor the stability of the electrical processing and the fluidic flow rate systems used to measure the volume (size), conductivity and light scattering characteristics of cells as they pass through the flow cell. It is also used by Beckman Coulter manufacturing and service engineers to calibrate the differential volume measurements. The MDW parameter cannot be reported if the D (V2) parameter fails for the COULTER LATRON CP-X Control.

Note: The COULTER LATRON CP-X Control is an existing product (initially cleared under (K885028) and has been modified with tighter expected ranges for control monitoring of the differential volume measurement.

Calibrator:

COULTER S-CAL Calibrator used with the DxH 800 is used to determine the calibration factors for directly measured CBC parameters; it is not required differential parameters. See K840794. Since the MDW and LDW parameters are derived from the monocyte and lymphocyte differential, the use of a calibrator is not required for the Early Sepsis Indicator Application.

Table 7 below summarizes the number of passed and failed quality control runs which were conducted during the course of the analytical and clinical studies.

Table 7: Cumulative Summary of Passed and Failed Quality Control Runs which were Conducted During the Course of the Analytical and Clinical Studies.

Control	Instrument	Control Level	Total # of Runs	# of Runs Passed*	# of Runs Failed
Latron	1	NA	29	28	1
	2		89	88	1
	3		67	64	3
	4		76	76	0
	5		49	49	0
6C Plus Control	1	Level 1	28	28	0
		Level 2	28	28	0
		Level 3	28	28	0
	2	Level 1	90	90	0
		Level 2	90	90	0
		Level 3	91	90	1
	3	Level 1	63	63	0
		Level 2	63	63	0
		Level 3	64	63	1
	4	Level 1	78	77	1
		Level 2	76	76	0
		Level 3	76	76	0
	5	Level 1	46	46	0
		Level 2	46	46	0
		Level 3	46	46	0

* Includes repeated runs due to QC failure

d. *Detection limit:*

See M.1.f Assay cut-off below.

e. *Analytical specificity:*

See Clinical Specificity

f. *Comparability Sampling Modes*

This study was performed to assess the impact on MDW parameter results due to sampling via the Closed Vial Cassette sampling mode and the Open Vial Single-Tube sampling mode on the DxH 800. A total of 60 whole blood specimens (20 normals and 40 abnormals) drawn into tubes containing K2EDTA anticoagulant, were randomly analyzed in duplicate on a single DxH 800, in closed vial (cassette) sampling mode and in the open vial (single-tube) sampling mode. Testing was performed at controlled room temperature. Testing of the MDW parameter in the Open Vial Single-Tube sampling mode was found to be equivalent to the Closed Vial Cassette sampling mode. Results are summarized in Tables 8 and 9 below.

Table 8:

Comparability Sampling Modes: Reference = Cassette Closed, Test = Single Open

N	Means		Difference	95% Confidence Limits	
	Reference	Test		Lower	Upper
60	23.180	23.124	-0.055	-0.441	0.330

Table 9: Comparability Sampling Modes -Regression Statistics and Correlation

Slope	95% Confidence Limits		Intercept	95% Confidence Limits		Correlation
	Lower	Upper		Lower	Upper	
1.005	0.910	1.099	-0.162	-2.312	1.987	0.947

g. *Comparability Tube Diameter*

To generate an accurate MDW requires that the aspirated sample is sampled from a homogeneous mixture. A nonhomogeneous sample resulting from poor quality sample mixing will result in changes in the monocyte volume distribution and consequently MDW. Effective sample mixing, i.e., creating a homogenous mixture, requires an air bubble and that the air bubble travel the length of the tube during inversion. As the sample tube diameter decreases, the quality of sample mixing can be adversely impacted because the effects of surface tension increase; which increases the difficulty for the air bubble to travel the length of the tube. The purpose of this study was to determine the effects of smaller diameter tubes on the quality of mixing as indicated by changes in the MDW parameter when compared to a standard sized 13 mm diameter tube. To limit the variability of the test to just tube diameter, tubes with the same anticoagulant from the same manufacturer were selected. This study demonstrated that K2EDTA tubes having a diameter of 8 mm and greater are acceptable for use on the DxH 800. The recommended

acceptable tube is based on the size which allows for proper mixing and is independent of a specific manufacturer. Results are summarized in Tables 10 and 11 below.

Table 10: Comparison of Reference MDW13MM vs Test=MDW8MM

MDW RANGE	N	MDW 8mm	MDW 13mm	Diff	Diff %
< 20	26	19.10	17.99	1.117	6.21
20 – 25	26	21.12	21.69	-0.561	-2.59
> 25	9	30.97	30.19	0.779	2.58

Table 11: Comparison of Reference of Reference=MDW13MM vs Test=MDW11MM

MDW RANGE	N	MDW 11mm	MDW 13mm	Diff	Diff %
< 20	26	18.37	17.99	0.384	2.13
20 - 25	26	20.86	21.69	-0.824	-3.80
> 25	9	30.20	30.19	0.002	0.01

h. Effect of Incomplete Lysing on MDW (NRBC Interference)

This study was performed to assess the potential interference on MDW's diagnostic ability in the presence of nucleated red blood cells (NRBC's). Within the differential analysis the NRBC's are spatially separated from the monocyte population; however, NRBC's have characteristics that place them in a region where other cellular material, such as red cell debris and giant platelets occurs, preventing the precise and accurate identification and enumeration within the differential analytical process. For these reasons the DxH 800 uses a separate VCS analytical process that uses different reagents to identify and enumerate the NRBC. This study demonstrated that the diagnostic ability of MDW is not affected by the presence of NRBC's above the clinical cut-off of $\geq 1.0\%$. For results, refer to Tables 12 and 13 below

Table 12: Number of Subjects and Percentage of Emergency Department Study Population for Combined Sites by NRBC's

Sepsis	NRBC<1.0%	NRBC $\geq 1.0\%$	Total	Percent (%)
No	1749	24	1773	1.4
Yes	373	12	385	3.1
Total	2122	36	2158	1.7
Percent (%)	17.6	33.3	17.8	

Table 13: Area Under the Curve for Specimens with NRBC <1.0% and NRBC $\geq 1.0\%$

NRBC	AUC	SE	95% Confidence Interval	
			Lower	Upper
NRBC $\geq 1.0\%$	0.778	0.097	0.588	0.968
NRBC <1.0%	0.789	0.014	0.762	0.816

i. Interfering Substances:

These studies were conducted to assess the effect of interfering substances (Lipemia, Hemolysis, Bilirubin and Hemoglobin) on the measurement of the MDW parameter on the DxH 800 system. Normal venous whole blood specimens collected in tubes containing K2EDTA anticoagulant and stored at controlled room temperature. The maximum concentration that does not interfere with

the MDW parameter is listed in Table 14 below:

Table 14: Interferent Results Summary

Interfering substance	Level of Interferent Added	Conclusion
Hemolysis	Up to 2.02 g/dL*	No MDW interference
Triglyceride	1500 mg/dL	No MDW interference
Conjugated Bilirubin	40 mg/dL	No MDW interference
Unconjugated Bilirubin	20 mg/dL	No MDW interference
Hemoglobin	Up to 1.87 g/dL	No MDW interference

*7.3% of hemolysate was used for the hemolysis study which corresponds to free hemoglobin levels up to 2.02 g/dL

See 3b below for interference which was assessed in vivo for clinical study patients data.

j. Assay cut-off:

MDW values >20.0 units should raise the level of suspicion that adult patients have or will develop sepsis within twelve hours of Emergency Department (ED) presentation.

Messages which will appear for MDW values >20 are summarized in Table 15 below.

Table 15– Lab Action Messages

Decision Rule: Adults/Unknown age in the ED with elevated MDW	
Adults	For adults in ED, MDW>20.0 may be associated with a higher risk of sepsis during the first 12 hrs of hospital admission
Unknown age	Confirm patient age is within intended use population (18 – 89 years) for MDW
Decision Rule: Adults/Unknown age in the ED with elevated MDW and suspect flag (without Left Shift flag)	
Adults	The predictive value of MDW for identifying sepsis in patients with hematological abnormalities has not been established
Unknown age	Confirm patient age is within intended use population (18 – 89 years) for MDW
Decision Rule: Adults/Unknown age in the ED with elevated MDW and Left Shift flag	
Adults	For adults in ED, MDW>20.0 may be associated with a higher risk of sepsis during the first 12 hrs of hospital admission
	The predictive value of MDW for identifying sepsis in patients with hematological abnormalities has not been established
Unknown age	Confirm patient age is within intended use population (18 – 89 years) for MDW
Adults/unknown age in the ED with normal MDW	
Adults/unknown age	For ED adult patients suspected of Sepsis, MDW<=20.0 does not rule out sepsis or risk of sepsis

When there is inadequate data, the algorithm will suppress the reporting of the MDW parameter and display this code (.....) for incomplete computation. Incomplete computation applies to

patient whole-blood results and patient controls:

- MDW results for patient samples will be shown as incomplete (.....).
- MDW results for patient samples processed as controls will be shown as incomplete (.....).

An incomplete computation is reported under the following conditions:

- When there are not enough (< 100) monocyte events for the reporting of results.
- When excessive debris is detected due to incomplete lysing.

In this case, the user will determine the cause for the incomplete computation by multiplying MO% x Displayed Count (located in the Additional Data screen - Diff tab):

- If the monocyte count is < 100 events, this is indicative of not enough events for the reporting of results.
- If the monocyte count is > 100 events in the presence of excessive debris, this is indicative of incomplete lysing.

Samples should be rerun if incomplete computation occurs. If a significant increase in the frequency of incompletes, the user will contact Beckman Coulter for assistance.

NOTE:

- Patients who receive an incomplete MDW result may not necessarily be excluded from the possibility of having sepsis
- The Patient Result screen displays MDW as part of the differential parameters. Flags, codes, and messages that apply to the differential parameter also apply to the MDW parameter. (Please refer to K081930, K120771, K140911).

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable

b. *Matrix comparison:*

Not applicable; K2EDTA venous whole blood only.

3. Clinical studies:

a. *Clinical Sensitivity:*

A blinded, prospective, observational, multicenter cohort study was conducted at three sites comprised of both academic and community hospital emergency departments in the United States. MDW performance was established using Sepsis-2 (ACCP/SCCM 2001 consensus criteria). The study enrolled a total of 2,158 consecutive adult emergency-department patients (≥ 18 and ≤ 89 years of age) meeting inclusion criteria for:

- Having a CBC-DIFF performed upon presentation and
- Subjects remaining in the hospital (emergency department or in-patient) for at least 12 hours

The prevalence of sepsis as defined by the Sepsis-2 (ACCP/SCCM 2001 consensus criteria) was

17.8%. All subjects were adjudicated by two qualified physicians following the ACCP/SCCM 2001 consensus definition for Sepsis-2 and the recent Sepsis-3 definition. Discordant results were arbitrated by a 3rd physician. No cases were excluded due to inability to arbitrate. Clinical diagnosis was based upon medical information obtained within 12 hours of emergency-department presentation. The presence of infection was determined based upon retrospective chart review of clinical symptoms and tests performed within 12 hours of ED presentation. Test results were obtained from the medical records 5-10 days later, including cultures, molecular tests, tissue pathology, etc. Additional blood cultures obtained after 12 hours of presentation were not considered in the determination of sepsis. For subjects to be considered for infection or sepsis diagnosis, evidence of a protocol defined infection work-up was required in the data extracted from the medical charts. If infection work-up was not performed within 12 hours or if the adjudicator believed that the infection work-up showed no evidence of infection then, the patient was classified as “Control” or “SIRS”. In order to categorize a patient as “Sepsis”, SIRS criteria and an infection-work-up had to be fulfilled within 12 hours of presentation. The subject categories included:

- Non-SIRS - case controls, subjects having 0 to 1 SIRS (Systemic Inflammatory Response Syndrome) criteria and no infection
- SIRS - subjects having ≥ 2 SIRS criteria
- Infection -subjects having suspected or confirmed infection with 0 – 1 SIRS criteria
- Sepsis - subjects having infection plus ≥ 2 SIRS
- Severe sepsis - subjects having sepsis with one or more organ failure
- Septic shock -subjects having sepsis with severe hypotension

All sites were required to extract the same information to enable adjudication based on the case report form, however the diagnostic evaluation for infection was not prescribed by the study but rather followed the standard of care at the institution. Some subjects lacked cultures (87% had cultures drawn, 46% were positive), and empiric therapy was considered to be part of the infectious disease work-up. Some subjects (<1%) may not have had any additional evaluation for sepsis besides the administration of antimicrobials but may have had an infectious workup conducted with a previous admission or physical exam findings suggestive of infection. Differences in diagnostic evaluations for infection added to the uncertainty in the clinical trial results.

The emergency-department population demographics based upon presenting clinical status are summarized in Table 16, subject demographics are summarized in Table 17, and the results of microbiological testing for subjects diagnosed with sepsis are summarized in Table 18 below.

Table 16: Emergency Department Population Demographics Based on Presenting Diagnosis

Site	Category per Sepsis-2 Criteria				
	Case Control	SIRS	Infection	Sepsis*	Total
1	320	111	95	139	665
2	440	222	60	115	837
3	328	108	89	131	656
Total	1088	441	244	385	2158

Table 17: Summary Subject Demographics -Multi-Center Blinded Prospective Clinical Trial

	Summary Demographics by Group			
	Control	SIRS	Infection	Sepsis
Total Subjects	1088	441	244	385
Subject Age - Mean (Min - Max)	60 (18 - 89)	59 (18 - 89)	63 (21 - 89)	61 (18 - 89)
Male gender, no. (%)	529 (49%)	202 (46%)	107 (44%)	195 (51%)
Race:				
White	731	318	181	260
Black or African American	247	90	40	82
American Indian or Alaska Native	1	0	1	2
Native Hawaiian or other Pacific Islander	2	0	0	0
Asian	28	9	5	11
Not Provided (includes Others)	79	24	17	30
Pre-Existing Conditions, no. (%)				
Immune-Suppression / Immune Stimulant	129 (12%)	80 (18%)	36 (15%)	88 (23%)
Malignancy	132 (12%)	87 (20%)	41 (17%)	77 (20%)
Antibiotics	69 (6%)	33 (7%)	55 (23%)	75 (19%)
Alcoholism	58 (5%)	29 (7%)	5 (2%)	8 (2%)
Smoking	202 (19%)	91 (21%)	35 (14%)	70 (18%)

* Sepsis includes sepsis, severe sepsis, and septic shock.

Table 18: Microbiological Testing (Sepsis Positive N=385)*

Test(s)	Performed	% Performed	Confirmed	Percent Confirmed
All Microbial Testing	335	87%	155	46%
Bacterial Tests (All Culture Sources)	330	86%	146	44%
Bacterial Blood Cultures **	275	71%	45	16%
Viral Tests	27	7%	11	41%
Fungal Tests	11	3%	4	36%
Serological Tests	8	2%	0	0%
Rapid Tests	11	3%	1	9%
<i>Not Performed</i>	<i>50</i>	<i>13%</i>		
Confirmed Cases	Gram +	Gram -	Mixed Gram Stain	Gram + & Gram - from different cultures
Bacterial Culture (All Sources)/subject	43	59	34	10
Bacterial Culture (Blood Only)	24	19	2	0

* Numbers do not add to 385 as there were many cases of more than one microbial testing performed

**Blood cultures are subset of bacterial culture total

Monocyte Volume Width Distribution Testing

Whole-blood venous samples collected in K2 EDTA, stored at room temperature, and analyzed on the UniCel DxH 800 Coulter Cellular Analysis System within two hours of venipuncture. MDW results were compared to the clinical adjudication of sepsis per Sepsis-2 Criteria (defined as a documented or suspected infection together with two or more SIRS criteria).

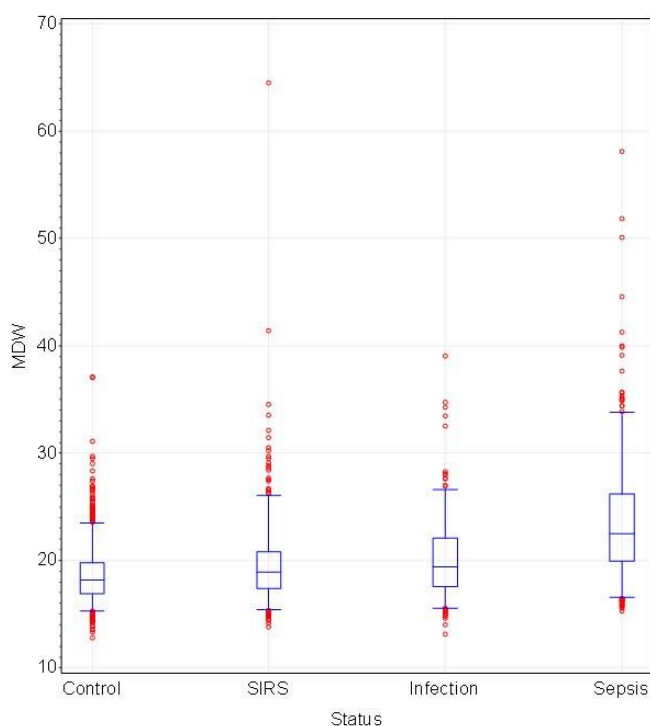
Of the 2,158 subjects enrolled in the prospective clinical study, MDW trended upwards with sepsis and MDW values >20 were highly prevalent in the sepsis group. However, a proportion of subjects without sepsis also had elevated MDW values (Table 19).

Table 19: Distribution of Elevated MDW Values in Prospective Clinical Trial

Clinical Population	Subjects with MDW values greater than 20.0 (n (%))
Non-SIRS (Case Controls)	241/1088 (22.2%)
SIRS	151/441 (34.2%)
Infection	104/244 (42.6%)
Sepsis	285/385 (74.0%)

The distribution of MDW for the various sub-populations represented in the clinical study is illustrated by the box plot in Figure 1.1.

Figure 1.1: Distribution of MDW Values for Various Sub-populations



A cutoff of 20.0 units was validated to differentiate between sepsis and other conditions in emergency-department patients. The MDW parameter upon emergency-department presentation is modestly predictive of the development of sepsis within the first 12 hours of emergency-department presentation. Receiver Operator Characteristic Curve (ROC) analysis yielded an area under the curve (AUC) of 0.789 (95% CI 0.762 to 0.815). See Figure 1.2, Capacity of MDW to Differentiate between Sepsis (ROC Curve) and All Other Conditions.

Table 20 titled “Performance of MDW for Sepsis”, summarizes the sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios with their respective 95% confidence intervals based on the established optimal cutoff of 20.0 units.

MDW values between 19.0 and 19.5 units have a higher sensitivity for predicting sepsis, but lower specificity (for example, more false-positive results). The impact on sensitivity and specificity for early detection of sepsis at different MDW cutoffs is provided in Table 21.

Figure 1.2: Capacity of MDW to Differentiate between Sepsis (ROC Curve) and All Other Conditions

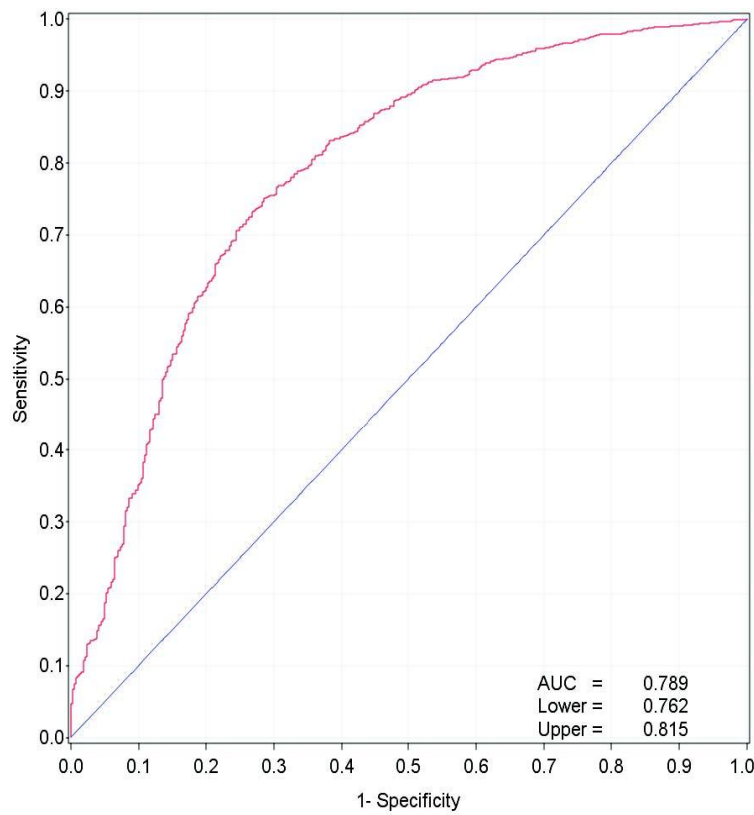


Table 20: Performance of MDW as a Predictive Marker of Sepsis

MDW Cutoff at 20						
	Sensitivity	Specificity	Predictive Values		Likelihood Ratios	
			Positive	Negative	Positive	Negative
Estimate	0.740	0.720	0.365	0.927	2.646	0.361
Lower 95% Confidence Interval	0.694	0.699	0.332	0.912	2.406	0.304
Upper 95% Confidence Interval	0.782	0.741	0.399	0.940	2.911	0.428

Table 21: Sensitivity and Specificity at Various MDW Cutoffs

Cutoff	Sensitivity	95% Confidence Interval		Specificity	95% Confidence Interval	
		Lower	Upper		Lower	Upper
19.0	0.821	0.779	0.856	0.594	0.571	0.617
19.5	0.784	0.741	0.823	0.661	0.639	0.683
20.0	0.740	0.694	0.782	0.720	0.699	0.741
20.5	0.683	0.635	0.728	0.771	0.751	0.790

Added Value Analyses

In the following analyses, the current standard of care is guided by WBC which is a component of the SIRS criteria. Based on the data from the clinical trial, AUCs of the ROC curves for the detection of sepsis are calculated for WBC as a single parameter and WBC+MDW combination. Results are graphically presented in Figure 1.3, ROC Curves Comparison while AUC estimates along with their 95% confidence intervals and are summarized in Table 22, AUC for WBC and Combined WBC+MDW. These results apply to this multi-center observational clinical trial and may not be generalizable to other patients in different institutions.

Figure 1.3: ROC Curves Comparison

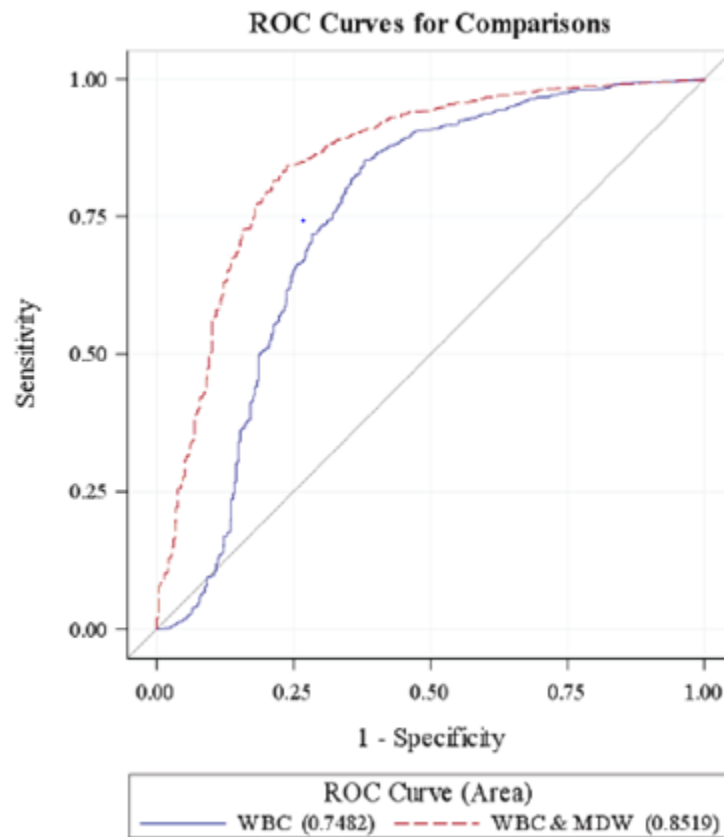


Table 22: AUC for WBC and Combined WBC+MDW

Parameter	AUC	SE*	95% Confidence Limits	
WBC	0.75	0.02	0.72	0.78
WBC+MDW	0.85	0.01	0.83	0.88
Difference	0.1	0.01	0.08	0.13

*Standard Error

The AUC for WBC+MDW is greater than the AUC for WBC alone. The difference between the two models was shown to be statistically significant.

The added value of MDW combined with WBC was integrated into an assessment for the probability of the disease. Pre-test and post-test probabilities for different combinations of tests are shown in Table 23, Pre-Test and Post-Test Probabilities for Different Combinations of Tests. This table summarizes the positive likelihood ratios and post-test probabilities for WBC only or when used in conjunction with MDW. The following decision rules are used for considering a result as abnormal:

- $\text{WBC} > 12 \times 10^3/\mu\text{L}$
- $\text{WBC} < 4 \times 10^3/\mu\text{L}$
- $\text{MDW} > 20.0$
- $(\text{WBC} > 12 \times 10^3/\mu\text{L} \text{ or } \text{WBC} < 4 \times 10^3/\mu\text{L}) \text{ and } \text{MDW} > 20.0$

An abnormal WBC result increases the probability of a patient having or developing sepsis to 44.7% when the pre-test probability is 17.8%, based on the incidence of sepsis in the clinical study. The post-test probability increases to 63.5% when WBC is abnormal and $\text{MDW} > 20.0$. This represents an overall increase of 46% in the probability of being septic when both tests being abnormal. This analysis includes patients based on request for CBC-DIFF therefore, results may not be generalizable. Normal WBC test results will reduce the probability of sepsis. The post-test probability of a normal WBC test is 7.9%, but this value goes down to 2.9% if both WBC and MDW are normal. Thus, the probability of sepsis in this clinical cohort, based on a sepsis incidence of 17.8%, is reduced when both tests are normal.

Table 23: Pre-Test and Post-Test Probabilities for Different Combinations of Tests

Test	Likelihood Ratio	Lower 95% CI	Upper 95% CI	Probability
Pre-Test	N/A	N/A	N/A	N/A
Post-Test: Positive Test Results				
WBC Abnormal* & MDW Normal	1.48	1.15	1.90	24.3%
WBC Abnormal*	3.72	3.30	4.20	44.7%
WBC Abnormal* & MDW Abnormal	8.01	6.52	9.84	63.5%
Post-Test: Negative Test Results				
WBC Normal* & MDW Normal	0.14	0.10	0.19	2.9%
WBC Normal*	0.39	0.34	0.46	7.9%
WBC Normal* & MDW Abnormal	1.10	0.90	1.34	19.3%

*Abnormal White Blood Cells Range $>12 \times 10^3/\mu\text{l}$ or $<4 \times 10^3/\mu\text{l}$

The clinical study data was also analyzed using an alternative definition of sepsis to define a comparator method positive. MDW performance is lower when applying Sepsis-3 criteria as the comparator method when compared with Sepsis-2. This is expected as MDW is intended to identify patients early in the disease progression, prior to organ dysfunction. The performance of MDW when using Sepsis-3 criteria to determine a positive comparator method result is summarized in Tables 24 and 25.

Table 24: Performance for MDW based on the established optimal cutoff of 20.0 units per Sepsis-3 Criteria

Parameter	Sensitivity	Specificity	Predictive Values		Likelihood Ratios	
			Positive	Negative	Positive	Negative
Estimate	0.679	0.678	0.211	0.943	2.111	0.473
Lower 95% Confidence Interval	0.618	0.657	0.184	0.930	1.894	0.393
Upper 95% Confidence Interval	0.735	0.699	0.241	0.954	2.352	0.570

Table 25 Performance of MDW at Varying Cut-offs: Cut-offs, Sensitivity, Specificity, Lower and Upper 95% Confidence Interval for Combined Sites vs. Final Diagnosis per Sepsis-3 Criteria

		95% Confidence Intervals					95% Confidence Intervals	
Cut-off	Sensitivity	Lower	Upper	Specificity	Lower	Upper	Lower	Upper
19.0	0.774	0.717	0.822	0.557	0.535	0.579		
19.5	0.724	0.665	0.777	0.620	0.598	0.642		
20.0	0.679	0.618	0.735	0.678	0.657	0.699		
20.5	0.630	0.567	0.688	0.731	0.710	0.750		

Clinical Performance differentiating between SIRS and Sepsis

An assessment was done to establish the performance of the MDW parameter to differentiate between adult patients with inflammation (≥ 2 SIRS) and infection (sepsis) from adult patients with inflammation only (≥ 2 SIRS). A total of 441 patients had ≥ 2 SIRS which represents 20% of the emergency-department population.

The performance of MDW to differentiated between SIRS and sepsis in emergency-department patients at a cutoff of 20.0 units was reported. Receiver Operator Characteristics Curve (ROC) analysis yielded an area under the curve (AUC) of 0.756 (95% CI 0.723 to 0.789). See Figure 1.4, Capacity of MDW to Differentiate between SIRS and Sepsis (ROC Curve).

A summary of the sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios with their respective 95% confidence intervals is shown in table 26, Performance of MDW to Differentiate between SIRS and Sepsis. The impact on sensitivity and specificity for early detection of sepsis from SIRS at different MDW cutoffs is provided in Table 27, Sensitivity and Specificity at Various MDW Cutoffs to Differentiate between SIRS and Sepsis.

Figure 1.4 Capacity of MDW to Differentiate between SIRS and Sepsis (ROC Curve)

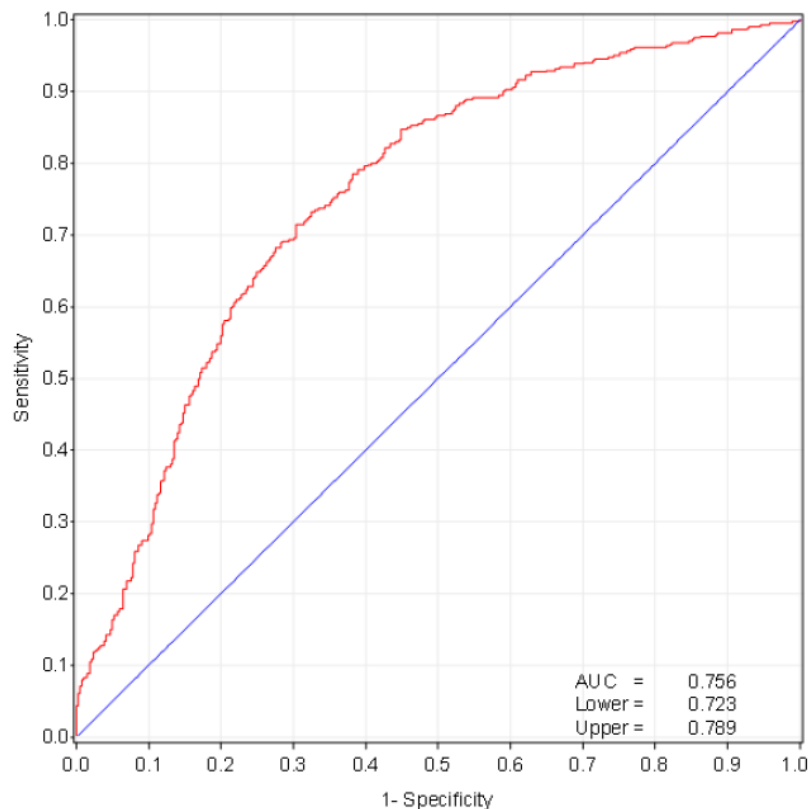


Table 26: Performance of MDW to Differentiate between SIRS and Sepsis

Parameter	MDW Cutoff at 20.0					
	Sensitivity	Specificity	PPV	NPV	LR+	LR
Performance	0.740	0.658	0.654	0.744	2.162	0.395
Lower 95% Confidence Interval	0.694	0.612	0.608	0.698	1.875	0.329
Upper 95% Confidence Interval	0.782	0.700	0.697	0.784	2.492	0.474

Table 27: Sensitivity and Specificity at Various MDW Cutoffs to Differentiate between SIRS and Sepsis

Cut-off	Sensitivity	95% Confidence Intervals		Specificity	95% Confidence Intervals	
		Lower	Upper		Lower	Upper
19.0	0.821	0.779	0.856	0.517	0.470	0.563
19.5	0.784	0.741	0.823	0.601	0.555	0.646
20.0	0.740	0.694	0.782	0.658	0.612	0.700
20.5	0.683	0.635	0.728	0.719	0.675	0.759

b. Clinical specificity:*Clinical Population Analyzed by White Blood Cell Interferences and MDW Cut-off*

The pivotal clinical data was evaluated for the number of samples and relative percent of total samples found with and without White Blood Cell flags at the MDW cut-off values and by the MDW's diagnostic ability in the presence of other abnormal cell types as indicated by the presence of suspect messages (blasts, immature granulocytes and variant lymphocytes) that occur in the peripheral blood in the presence of a clinical condition or disease. Differential suspect messages are summarized in Table 28. While there does not appear to be significant interference with the MDW assay, the number of patients with sepsis was too small to make any conclusions on the potential impact upon the MDW parameter. A labeling limitation has been included to indicate the clinical performance of the MDW has not been established in patients with hematological abnormalities, such as blast cells. Results are found in Tables 29-41 below.

Table 28: Differential suspect messages

Diff Suspect Messages	Description
Imm Grans	Pattern characteristic of specimen containing: a) metamyelocytes and myelocytes and/or promyelocytes, or b) myelocytes and/or promyelocytes without metamyelocytes.
Left Shift	Pattern is characteristic of specimen containing metamyelocytes, but without myelocytes, promyelocytes, or blasts.
LY Blast	Blasts in the Lymphocyte region of the data plot
MO Blast	Blasts in the Monocyte region of the dataplot
NE Blast	Blasts in the Neutrophil region of the dataplot
Variant LY	Pattern characteristic of specimen with variant lymphs, including mature lymphocytes such as those observed in viral infections, as well as immature and/or abnormal lymphocytes.

Table 29: Clinical Accuracy by White Blood Cell Interferences by MDW Cut-off-19.00

MDW	WBC Flagged		WBC Not Flagged		Totals
	Sepsis [#/%]	Non-Sepsis [#/%]	Sepsis [#/%]	Non-Sepsis [#/%]	
≤ 19.00	2 / 4	24 / 32	67 / 20	1029 / 61	1122
> 19.00	50 / 96	51 / 68	266 / 80	669 / 39	1036
Total	52	75	333	1698	2158

Table 30: Clinical Accuracy by White Blood Cell Interferences by MDW Cut-off-19.50

MDW	WBC Flagged		WBC Not Flagged		Totals
	Sepsis [#/%]	Non-Sepsis [#/%]	Sepsis [#/%]	Non-Sepsis [#/%]	
≤ 19.50	3 / 6	28 / 37	81 / 24	1145 / 67	1257
> 19.50	49 / 94	47 / 63	252 / 76	553 / 33	901
Total	52	75	333	1698	2158

Table 31: Clinical Accuracy by White Blood Cell Interferences by MDW Cut-off-20.00

MDW	WBC Flagged		WBC Not Flagged		Totals
	Sepsis [#/%]	Non-Sepsis [#/%]	Sepsis [#/%]	Non-Sepsis [#/%]	
≤ 20.00	7 / 13	30 / 40	94 / 28	1248 / 73	1379
> 20.00	45 / 87	45 / 60	239 / 72	450 / 27	779
Total	52	75	333	1698	2158

Table 32: Number of Subjects and Percentage of Emergency Department Study Population for Combined Sites by Suspect Messages

Suspect Message Flag	N	Percent (%)
All	127	6%
Left Shift / Immature Granulocyte	100	5%
Variant Lymphocyte	22	1%
Blasts	30	1%

Table 33: Total Numbers of Subjects With and Without Left Shift/Immature Granulocytes Suspect Messages by Diagnosis

Sepsis	Non Flagged	Flagged	Total	Percent (%)
No	1715	58	1773	3.3
Yes	343	42	385	10.9
Total	2058	100	2158	4.6
Percent (%)	16.7	42.0	17.8	.

Table 34: Total Numbers by Suspect Message and Final Diagnosis for Specimens with Left Shift/Immature Granulocytes Suspect Messages

Left Shift / Immature Granulocyte Suspect Messages				
Suspect Message(s)	All Subjects	Non-sepsis	Sepsis	Total
LS	37	14	23	37
LS+IG	24	18	6	24
IG	20	16	4	20
LS+IG+NeBlt	6	6	0	6
LS+IG+MoBlt+LyBlt+VarLy	2	0	2	2
LS+IG+MoBlt+NeBlt	2	1	1	2
LS+IG+MoBlt	2	0	2	2
LS+IG+MoBlt+VarLy	1	0	1	1
LS+IG+LyBlt	1	0	1	1
LS+IG+VarLy	1	1	0	1
IG+MoBlt	1	1	0	1
IG+LyBlt+VarLy	1	0	1	1
LS+MoBlt	1	1	0	1
LS+NeBlt	1	0	1	1
Total	100	58	42	100

Table 35: Area Under the Curve (AUC) for Specimens with Left Shift/Immature Granulocytes Suspect Messages

				95% Confidence Interval	
LS / IG	N	AUC	SE	Lower	Upper
Flagged Only	100	0.754	0.049	0.657	0.850
Non Flagged	2058	0.782	0.015	0.753	0.810
All Samples	2158	0.789	0.014	0.762	0.815

Table 36: Total Numbers of Subjects With and Without Variant Lymphocyte Suspect Messages by Diagnosis

Sepsis	Non Flagged	Flagged	Total	Percent (%)
No	1763	10	1773	0.6
Yes	373	12	385	3.1
Total	2136	22	2158	1.00
Percent (%)	17.5	54.5	17.8	

Table 37: Total Numbers by Suspect Message and Final Diagnosis for Specimens with Variant Lymphocyte Suspect Messages

Variant Lymphocyte Messages				
Suspect Message(s)	All Subjects	Non-sepsis	Sepsis	Total
VarLy	15*	8	7	15
VarLy+MoBlt+LyBlt+LS+IG	2**	0	2	2
VarLy+MoBlt+LS+IG	1	0	1	1
VarLy+LS+IG	1**	1	0	1
VarLy+LyBlt+IG	1**	0	1	1
VarLy+MoBlt+LyBlt	1**	0	1	1
VarLy+LyBlt	1	1	0	1
Total	22	10	12	22

* 11 out of 15 Absolute lymphocytosis

** 5 out of 7 Absolute monocytosis

Table 38: Area Under the Curve (AUC) for Specimens with Variant Lymphocyte Suspect Messages

				95% Confidence Interval	
Variant Lymph	N	AUC	SE	Lower	Upper
Flagged Only	22	0.833	0.088	0.662	1.000
Non Flagged	2136	0.786	0.014	0.758	0.813
All Samples	2158	0.789	0.014	0.762	0.815

Table 39: Total Numbers of Subjects With and Without Blast Suspect Messages by Diagnosis

Sepsis	Non Flagged	Flagged	Total	Percent (%)
No	1755	18	1773	1.0
Yes	373	12	385	3.1
Total	2128	30	2158	1.40
Percent (%)	17.5	40.0	17.8	.

Table 40: Total Numbers by Suspect Message and Final Diagnosis for Specimens with Blast Suspect Messages

Blast Suspect Messages				
Suspect Message(s)	All Subjects	Non-sepsis	Sepsis	Total
MoBlt	7	6	1	7
NeBlt+LS+IG	6	6	0	6
MoBlt+NeBlt+LS+IG	2	1	1	2
MoBlt+LyBlt+LS+IG+VarLy	2	0	2	2
MoBlt+LS+IG	2	0	2	2
NeBlt	2	2	0	2
MoBlt+LyBlt+VarLy	1	0	1	1
MoBlt+LS+IG	1	1	0	1
MoBlt+VarLy+LS+IG	1	0	1	1
MoBlt+IG	1	1	0	1
NeBlt+LS	1	0	1	1
LyBlt+VarLy+IG	1	0	1	1
LyBlt+LS+IG	1	0	1	1
LyBlt+VarLy	1	1	0	1
LyBlt	1	0	1	1
Total	30	18	12	30

Table 41: Area Under the Curve (AUC) for Specimens with Blast Suspect Messages

				95% Confidence Interval	
Blast	N	AUC	SE	Lower	Upper
Flagged	30	0.870	0.065	0.743	0.997
Non Flagged	2128	0.787	0.014	0.760	0.814
All Samples	2158	0.789	0.014	0.762	0.815

Pre-existing Clinical Conditions

Assay performance was assessed with respect to the potential differences in monocyte response to sepsis in the presence of pre-existing clinical conditions. The following five (5) categories of pre-existing conditions (summarized below) were evaluated based on their potential impact to the measurement of MDW: Immune Suppression, Immune Stimulants, Malignancy, Antibiotic Treatment, and Lifestyle/Behavior. There does not appear to be statistically significant interference with the measurement of MDW for the pre-existing conditions tested but the number of patients is small. The assay labeling includes a limitation indicating that clinical performance in patients receiving immune stimulants or those with alcoholism is unknown.

Category 1: Immune Suppression

- Subjects with neutropenia (defined as absolute neutrophil count $<1,500/\mu\text{L}$)
- Subjects with suspected immune suppression due to HIV, organ or bone marrow transplant.
- Subjects with suspected immune suppression due to chemotherapy. The chemotherapeutic agent was recorded as well as the time from last chemotherapeutic treatment [<1 month; >1 month].
- Subjects on chronic treatments (≥ 2 weeks) with potent immune suppressants, including high-dose corticosteroids (prednisone ≥ 20 mg/day), anti-TNF α molecules, methotrexate (≥ 15 mg/week), azathioprine (≥ 75 mg/day), cyclophosphamide (any dose) or mycophenylate (≥ 1000 mg/day) or any combination of these treatments (at any dose) .
- Subjects currently or recently (within one week) treated with Hydroxyurea.

Category 2: Immune Stimulant Treatments

- Subject on treatment for neutropenia, including Neupogen, Filgrastim, Leukine, Granix or Sargramostim.

Category 3: Malignancy

- Subjects with malignancy, hematologic (lymphomas, multiple myeloma, acute and chronic leukemias, myelodysplastic syndromes, aplastic anemia). The malignancy was recorded.

Category 4: Antibiotics

- Subjects treated with antibiotics within the past 7 days for active infection prior to ED. The antibiotic was recorded.
- Subjects treated with antibiotics for primary prophylaxis prior to ED (HIV, PSCT, etc.). The prophylactic antibiotic was recorded.

Category 5: Lifestyle/Behavior

- Subjects with a current diagnosis of “alcoholism” or current history of heavy alcohol consumption, as defined by the CDC (≥ 15 drinks/week for men; ≥ 8 drinks/week for women) or a blood alcohol level on admission to the ED.
- Subjects that smoke.

Tables 42-54 below summarizes the total number of specimens and percentages for each pre-existing category as well as all pre-existing conditions for combined sites.

Table 42: Number of Subjects and Percentage of Study Population by Pre-Existing Conditions

Pre-Existing Condition Group	N	%
Immunosuppression	330	15%
Immune-Stimulant	15	1%
Malignancy	337	16%
Antibiotics	232	11%
Lifestyle/Behavioral [Alcohol]	100	5%
Lifestyle/Behavioral [Smoking]	398	18%
All Pre-Existing Conditions	661	31%

Table 43: Category 1 - Subjects With and Without Immune Suppression by Diagnosis

Sepsis	No Immune Suppression	Immune Suppression
No	1530	243
Yes	298	87
Total	1828	330
Percent (%) Sepsis	16.3	26.4

Table 44: Category 1 - Area Under the Curve (AUC) for Immune Suppression

Category 1	AUC	SE	Lower	Upper
Immune Suppression Only	0.763	0.032	0.701	0.825
No Immune Suppression	0.790	0.015	0.760	0.820
All Samples	0.789	0.014	0.762	0.815

Table 45: Category 2 - Number of Subjects With and Without Immune Stimulant Pre-Existing Conditions by Diagnosis

Sepsis	No Immune Stimulants	Immune Stimulants	Total	Percent (%)
No	1762	11	1773	0.6
Yes	381	4	385	1.0
Total	2143	15	2158	0.7
Percent (%)	17.8	26.7	17.8	

Table 46: Category 2 - Area Under the Curve (AUC) for Immune Stimulant

Category 2	AUC	SE	Lower	Upper
Immune Stimulants Only	0.795	0.126	0.549	1.000
No Immune Stimulants	0.788	0.014	0.761	0.815
All Samples	0.789	0.014	0.762	0.815

Table 47: Category 3 - Number of Subjects With and Without Malignancy by Diagnosis

Sepsis	No Malignancy	Malignancy
No	1513	260
Yes	308	77
Total	1821	337
Percent (%) Sepsis	16.9	22.8

Table 48: Category 3: Area Under the Curve (AUC) for Malignancy

Category 3	AUC	SE	Lower	Upper
Malignancy Only	0.772	0.031	0.711	0.832
No Malignancy	0.789	0.015	0.759	0.819
All Samples	0.789	0.014	0.762	0.815

Table 49: Category 4 – Number of Subjects With and Without Antibiotics Prior to Presentation by Diagnosis

Sepsis	No Antibiotics	Antibiotics
No	1616	157
Yes	310	75
Total	1926	232
Percent (%) Sepsis	16.1	32.3

**Table 50: Category 4 –
Area Under the Curve (AUC) for Antibiotics Prior to Presentation**

Category 4	AUC	SE	Lower	Upper
Antibiotics Only	0.740	0.038	0.666	0.815
No Antibiotics	0.793	0.015	0.765	0.822
All Samples	0.789	0.014	0.762	0.815

Table 51: Category 5 - Number of Subjects With and Without Lifestyle/Behavioral – Alcoholism

Sepsis	No Alcoholism	Alcoholism
No	1681	92
Yes	377	8
Total	2058	100
Percent (%) Sepsis	18.3	8.0

Table 52: Category 5 - Area Under the Curve (AUC) for Alcoholism

Category 5	AUC	SE	Lower	Upper
Alcoholism Only	0.842	0.055	0.735	0.949
No Alcoholism	0.788	0.014	0.761	0.815
All Samples	0.789	0.014	0.762	0.815

Table 53: Category 5 - Number of Subjects With and Without Lifestyle/Behavioral – Smoking

Sepsis	Non Smoker	Smoker
No	1445	328
Yes	315	70
Total	1760	398
Percent (%) Sepsis	17.9	17.6

Table 54: Category 5 - Area Under the Curve (AUC) for Smoking

Category 5	AUC	SE	Lower	Upper
Smoker Only	0.781	0.032	0.719	0.844
Non Smoker	0.791	0.015	0.761	0.820
All Samples	0.789	0.014	0.762	0.815

c. *Clinical supportive data (when a. and b. are not applicable):*

Not applicable.

4. Clinical cut-off:

The clinical MDW cut-off of 20.0 was established through a blinded prospective multi-center pilot study; other cut-offs are provided as informational purposes only

5. Expected values/Reference range:

To assess the normal reference interval of the MDW parameter for apparently healthy adults a total of 146 specimens (70 females and 76 males) were collected and tested within two (2) hours of collection at the sites and evaluated per CLSI EP28-A3c: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory. Approved Guideline (10/2010). Subjects were enrolled from health and wellness screening programs and had normal WBCs and monocyte differentials. The 146 subjects were enrolled from three locations and were representative of the geographic and demographic diversity of the United States. A non-parametric approach was used to calculate the lower and upper limits of the reference interval using 95% confidence. The 95% reference interval for MDW on the DxH 800 hematology analyzer across all participating sites is 13.98 - 21.28. The 90% confidence intervals for the lower and upper bounds of the reference limits were also calculated. Tables 55-58 summarize the characteristics of the expected values and the demographics of the study subjects.

Table 55: Normal Reference Interval of MDW for Apparently Healthy Adults

Name	N	Mean	SD	MDW RANGE		95% Confidence Reference Interval			
				Min	Max	Lower Limit		Upper Limit	
MDW	146	16.96	1.88	13.37	22.56	13.98		21.28	
						90% Confidence Interval for the Lower bound		90% Confidence Interval for the Upper bound	
						Lower Limit	Upper Limit	Lower Limit	Upper Limit
						13.37	14.57	20.38	22.56

Table 56: Table of Gender for Normal Reference Interval Study

AGE	Female	Male	Not Provided	Total
Adolescent 18-21 Years	1	0	0	1
Adult 22-65 Years	69	76	0	145
Geriatric 66-89 Years	0	0	0	0
Total	70	76	0	146
Minimum (years)	19	22		19
Maximum (years)	62	64		64
Mean	45	46		45

Table 57: Table of Ethnicity for Normal Reference Interval Study

AGE	Hispanic or Latino	Not Hispanic or Latino	Not Provided	Total
Adolescent 18-21 Years	0	1	0	1
Adult 22-65 Years	41	65	39	145
Geriatric 66-89 Years	0	0	0	0
Total				146

Table 58: Table of Race for Normal Reference Interval Study

Age	White	Black or African American	Asian	American Indian or Alaska Native	Native Hawaiian or other Pacific Islander	Not Provided	Total
Adolescent 18-21 Years	0	1	0	0	0	0	1
Adult 22-65 Years	109	31	3	1	0	1	145
Geriatric 66-89 Years	0	0	0	0	0	0	0
Total							146

N. Instrument Name:

UniCel DxH 800 COULTER Cellular Analysis System

O. System Descriptions:**P.****1. Modes of Operation:**

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes X or No

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes or No X

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes X or No

3. Specimen Identification:

Specimen identification is automated or by manual sample identification with the use of a hand held barcode scanner.

4. Specimen Sampling and Handling:

DxH 800 specimen analysis can occur via several sampling methods on the analyzer (see modes of operation above).

5. Calibration:

The DxH 800 COULTER S-CAL Calibrator is used to determine the calibration factors for directly measured CBC parameters; it is not required for differential parameters. See K840794. Since the MDW and LDW parameters are derived from the monocyte and lymphocyte differential, the use of a calibrator is not required for the Early Sepsis Indicator Application.

6. Quality Control:

COULTER 6C Cell Control (K081822) enables monitoring of system performance for all directly measured and calculated CBC, Diff and NRBC parameters.

COULTER LATRON CP-X Control (K885028) determines calibration factors to ensure accurate measurements of directly measured CBC parameters. Assigned assay values are traceable to reference methods. The MDW parameter cannot be reported if the D (V2) parameter fails for the COULTER LATRON CP-X Control.

Q. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

Not Applicable

R. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

S. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

The potential value of MDW reporting to help aid in the diagnosis sepsis should be established by the individual testing site in consideration of current testing practices. MDW is not validated for clinical use in patient populations outside adults treated in the emergency department.

Local education concerning reporting and interpretation guidelines should be established and facilitated by joint decisions between various clinical care groups (Laboratory, Infectious Diseases, Intensivists, antibiotic stewardship committee, etc.)

T. Patient Perspectives: This submission did not include specific information on patient perspectives for this device.