510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K152776

B. Purpose for Submission:

Clearance of new device

C. Measurand:

Total nucleated cells and erythrocytes in cerebrospinal fluid

D. Type of Test:

Quantitative determination of fluorescence labeled total nucleated cells and erythrocytes in cerebrospinal fluid

E. Applicant:

Advanced Instruments, Inc.

F. Proprietary and Established Names:

GloCyte® Automated Cell Counter System
GloCyte® Low and High Level Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.5200, Automated cell counter
   21 CFR 864.8625, Hematology quality control mixture

2. Classification:

   Class II (instrument, assay, and controls)

3. Product code:

   GKL, counter, cell, automated (particle counter)
   JPK, mixture, hematology quality control

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   The GloCyte® Automated Cell Counter System is intended for use by trained healthcare professionals in clinical laboratories to provide quantitative determination of fluorescence labeled total nucleated cells and erythrocytes in cerebrospinal fluid collected from adult and pediatric patients.

   The GloCyte® Low and High Level Controls are assayed hematology controls designed to monitor the performance of the GloCyte® Automated Cell Counter System. Assayed parameters include total nucleated cells and erythrocytes.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   GloCyte Instrument

I. Device Description:

The GloCyte® Automated Cell Counter System is an automated cell counter that concentrates and enumerates total nucleated cells (TNCs) and red blood cells (RBCs) using fluorescent microscopy and digital image analysis principles. The test method uses one of two reagents to stain TNCs (propidium iodide with detergent) or RBCs (fluorochrome labeled anti-human RBC antibody in buffer with stabilizers), and a digital imaging system to count the cells. The image is captured by a digital CCD camera, and the fluorescent stained cells are counted via digital image processing.

The GloCyte® Automated Cell Counter System includes the GloCyte instrument, computer (hardware and software), Vacuum Station, Sample Preparation Tray, barcode reader, pipettes (10 and 30 μL), test cartridge, TNC and RBC reagents, Low and High Level Controls.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Sysmex XN-10

2. Predicate 510(k) number(s):
   K112605

3. Comparison with predicate:

Similarities		
Item	Device GloCyte® Automated Cell Counter System	Predicate Sysmex XN-10
Sample Type	Cerebrospinal fluid (CSF)	CSF (and other body fluids)
Hardware	Semiconductor laser with optical components	Flow system, semiconductor laser with optical components
Parameter(s)	TNC, RBC	WBC-BF#, RBC-BF#

Differences		
Item	Device GloCyte® Automated Cell Counter System	Predicate Sysmex XN-10
Intended Use	The GloCyte® Automated Cell Counter System is intended for use by trained healthcare professionals in clinical laboratories to provide quantitative determination of fluorescence labeled total nucleated cells and erythrocytes in cerebrospinal fluid collected from adult and pediatric patients.	The XN-Series modules (XN-10, XN-20) are quantitative multi-parameter automated hematology analyzers intended for in vitro diagnostic use in screening patient populations found in clinical laboratories. The XN-Series modules classify and enumerate the following parameters in whole blood: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, NEUT%/#, LYMPH%/#, MONO%/#, EO%/#, BASO%/#, IG%/#, RDW-CV, RDW-SD, MPV, NRBC#/%, RET%/#, IPF, IRF, RET-He and has a Body Fluid mode for body fluids. The Body Fluid mode enumerates the WBC-BF, RBC-BF, MN%/#, PMN%/#, and TC-BF parameters in cerebrospinal fluid (CSF), serous fluids (peritoneal, pleural) and synovial fluids. Whole blood should be collected in K_2 or K_3EDTA anticoagulant and, Serous and Synovial fluids in K_2EDTA anticoagulant to prevent clotting of fluid. The use of anticoagulants with CSF specimens is neither required nor recommended.

Differences		
Item	Device GloCyte® Automated Cell Counter System	Predicate Sysmex XN-10
Test Principles	Detection of fluorescence from stained TNCs and RBCs using a semiconductor laser and optical system to capture fluorescent cell images and calculate and display cell counts.	Performs hematology analyses according to the Hydro Dynamic Focusing (DC Detection), flow cytometry method (using a semiconductor laser), and SLS-hemoglobin method.
Reagents	TNC Reagent (hemolyzes RBCs and stains nucleated cells). RBC Reagent (anti-human glycophorin A/B antibody fluorescent stain).	LYSERCELL WDF (Lyse) FLUOROCELL WDF (Stain) CELLPACK DCL (Diluent) CELLPACK™ DFL (Diluent)
Calibrators	No external calibrator	XN-10 Calibrator (XN CAL)
Sample/Fluidic Pathway	No fluidic pathway	Single fluidic pathway
Throughput	6–15 samples/hour	40 samples/hour maximum
Sample Volume	30µL for TNC test 30µL for RBC test	88µL
Controls	GloCyte® Low and High Level Controls – 2 Levels	XN Check BF – 2 Levels

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

IEC61010-1:01 Safety requirements for electrical equipment for measurement, control and laboratory use - Part 1: General requirements.

IEC61010- 2-101:02 Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.

IEC60825-1 Safety of laser products - Part 1: Equipment classification and requirements.

IEC 62304: 2006 Medical device software - Software life cycle processes.

ISO 14971: 2007 Medical devices - Application of risk management to medical devices.

CLSI Document H26-A2 Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard - Second Edition. 2010.

CLSI Document EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline. 2014.

CLSI Document EP09-A3 Method Comparison and Bias Estimation Using Patient Samples. 2013.

CLSI Document EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. 2009.

CLSI Document EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline- Second Edition. 2012.

CLSI Document EP06-A Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. 2003.

CLSI Document H56-A Body Fluid Analysis for Cellular Composition; Approved Guideline. 2006.

L. Test Principle:

Detection of fluorescence from stained TNCs and RBCs using a semiconductor laser and optical system to capture fluorescent cell images and calculate and display cell counts.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Repeatability

Repeatability was accessed in-house using two test cartridge lots, two reagent lots (one TNC and one RBC) and GloCyte® Low and High Level Controls. Fourteen manipulated human CSF samples (7 TNC samples and 7 RBC samples) with concentrations evenly distributed throughout the reportable range of the GloCyte® Automated Cell Counter System were tested. Ten replicates were performed on the GloCyte System per cell concentration level for a total of 70 tests each for the RBC counts and TNC counts. The SD and %CV were calculated for each study sample. The results of the repeatability met the performance specifications (%CV) for the GloCyte® Automated Cell Counter System.

Summary of Repeatability Results: In-house TNC

Assay	Test Level	N	Dilution Performed	Mean	Median	Standard Deviation	%CV
TNC	TNC 1*	10	1:1,000	10,313	10,500	874.4	8.5
	TNC 2*	10	1:1,000	8,707	8,767	907.6	10.4
	TNC 3	10	1:1,000	6,807	7,000	492.3	7.2
	TNC 4	10	1:100	5,225	5,190	223.2	4.3
	TNC 5	10	1:100	2,846	2,873	176.6	6.2
	TNC 6	10	1:100	1,898	1,890	138.5	7.3
	TNC 7	10	1:10	426	427	13.6	3.2

*indicates TNC concentration above the GloCyte System reportable range

Summary of Repeatability Results: In-house RBC

Assay	Test Level	N	Dilution Performed	Mean	Median	Standard Deviation	%CV
RBC	RBC 1*	10	1:10,000	727,800	736,000	35,383	4.9
	RBC 2*	10	1:10,000	647,800	650,000	45,200.30	7.0
	RBC 3	10	1:10,000	446,000	441,333	25,066.80	5.6
	RBC 4	10	1:10,000	263,800	261,667	14,429.30	5.5
	RBC 5	10	1:10,000	98,400	99,334	6,403.70	6.5
	RBC 6	10	1:1,000	49,467	49,567	1,751.10	3.5
	RBC 7	10	1:100	10,546	10,680	584.60	5.5

*indicates RBC concentration above the GloCyte System reportable range

An additional repeatability was conducted at three intended use sites using 13 TNC and 16 RBC clinical patient clinical CSF samples, nine test cartridges lots, six reagent lots (3 TNC and 3 RBC), and two lots of the GloCyte® Low and High Level Controls. Low Level Control samples (approximately 12 cells/µL of TNC and RBC) were tested using the 30 µL test volume; High Level Control samples (approximately 130 cells/µL of TNC and RBC) were tested using the 10 µL test volume. Each sample and control was run 10 times by a single operator for RBC and/or TNC counts. A total of 100 tests each for RBC counts and TNC counts were run at each site. The results of the repeatability met the performance specifications (%CV) for the GloCyte® Automated Cell Counter System.

Repeatability Performance

Site 1		N	Mean	Median	Standard Deviation	%CV
Cell Type	Sample ID					
TNC	303	10	17.9	18.0	1.1	6.2
	304	10	71.6	71.0	3.8	5.3
	306	10	4.9	5.0	0.6	11.6
	H1103	10	123.2	123.5	3.1	2.5
	L1103	10	10.6	11.0	0.8	8.0
RBC	301	10	17.1	17.0	1.0	5.8
	302	10	75.7	74.5	4.5	5.9
	305	10	148.3	149.0	7.9	5.3
	307	10	101.3	101.0	6.5	6.4
	H1103	10	131.1	130.5	8.7	6.6
	L1103	10	11.7	11.5	1.1	9.1

Site 2		N	Mean	Median	Standard Deviation	%CV
Cell Type	**Sample ID**					
TNC	300	10	4.1	4.0	0.7	18.0
	304	10	147.8	152.0	10.5	7.1
	305	10	78.5	79.0	2.2	2.8
	308	10	1.2	1.0	0.4	35.1*
	310	10	11.7	12.0	0.9	8.1
	313	10	177.6	171.0	29.4	16.5
	H1105	10	118.6	119.5	4.7	4.0
	L1105	10	10.3	10.5	0.9	9.2
RBC	303	10	77.5	78.0	6.0	7.8
	306	10	138.5	138.5	4.2	3.0
	307	10	6.3	6.0	0.7	10.7
	309	10	60.3	59.5	6.7	11.1
	311	10	62.9	61.5	5.7	9.1
	314	10	838.2	844.0	73.5	8.8
	H1105	10	134.2	134.0	3.8	2.9
	L1105	10	11.4	11.5	1.0	8.5

*Sample diluted 1:10. The mean of this sample (1.2 cells/µL) is below the LoQ of the GloCyte System, and hence is not within the measuring range.

Site 4		N	Mean	Median	Standard Deviation	%CV
Cell Type	**Sample ID**					
TNC	125	10	2.1	2.0	0.3	15.1
	128	10	7.8	8.0	0.9	11.8
	129	10	5.5	5.5	0.9	15.5
	198	10	65.9	65.0	4.3	6.5
	199	10	7.9	8.0	1.0	12.6
	200	10	183.3	182.0	7.4	4.0
	H1103	10	128.6	127.0	4.4	3.4
	L1103	10	9.8	10.0	0.9	9.4
RBC	125	10	34.3	34.5	3.0	8.8
	126	10	5.0	5.0	0.8	16.3
	127	10	209.1	211.0	6.3	3.0
	195	10	7.1	7.0	1.0	14.0
	196	10	66.7	66.5	5.6	8.5
	197	10	131.0	131.0	3.6	2.7
	H1103	10	125.3	127.5	6.8	5.4
	L1103	10	10.0	10.0	1.1	10.5

Reproducibility

Reproducibility performance was conducted at three intended use sites over 20 operating days utilizing 15 test cartridges lots, six reagent lots (3 TNC and 3 RBC), and one lot of Low and High Level Controls for both the RBC and TNC assays. Testing was done twice daily using the same set of controls, for 20 days. Each control set was run, in duplicate, independently, by two operators at each site. Standard deviation and %CV were calculated for each measurand and the results obtained were within specifications.

Reproducibility Performance

Combined Sites				Within Run		Between Run		Between Day		Between Site		Between-Operator		Total	
Cell Type	**Control**	**N**	**Mean**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
TNC	Low	480	10.6	1.1	10.1	0.0	0.0	0.2	1.6	0.4	3.9	0.3	2.4	1.2	11.2
	High	480	122.9	7.3	5.9	0.0	0.0	0.0	0.0	3.8	3.1	1.8	1.5	8.4	6.9
RBC	Low	480	11.3	1.0	9.2	0.0	0.0	0.2	1.9	0.4	3.8	0.3	2.7	1.2	10.5
	High	480	130.0	6.8	5.3	0.0	0.0	0.9	0.7	2.2	1.7	1.3	1.0	7.4	5.7

Site 1				Within-Run		Between-Run		Between-Day		Between-Operator		Total	
Cell Type	**Control**	**N**	**Mean**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
TNC	Low	160	11.0	1.0	9.4	0.0	0.0	0.0	0.0	0.3	3.0	1.1	9.9
	High	160	124.4	6.0	4.8	0.0	0.0	0.4	0.3	5.3	4.3	8.0	6.5
RBC	Low	160	11.7	1.0	9.2	0.1	1.2	0.0	0.0	0.4	3.7	1.1	9.2
	High	160	129.7	5.6	4.3	0.0	0.0	0.0	0.0	2.6	2.0	6.2	4.8

Site 2				Within-Run		Between-Run		Between-Day		Between-Operator		Total	
Cell Type	**Control**	**N**	**Mean**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
TNC	Low	160	10.6	1.0	9.8	0.1	1.0	0.1	1.0	0.0	0.0	1.1	9.1
	High	160	125.7	7.4	5.9	0.0	0.0	0.2	0.1	0.0	0.0	7.4	5.9
RBC	Low	160	10.8	1.0	9.2	0.2	1.9	0.2	1.4	0.3	2.7	1.1	9.9
	High	160	132.4	6.9	5.2	1.3	1.0	0.6	0.4	3.6	2.7	7.9	6.0

Site 4				Within-Run		Between-Run		Between-Day		Between-Operator		Total	
Cell Type	Control	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
TNC	Low	160	10.1	1.0	10.1	0.0	0.0	0.1	1.4	0.4	4.0	1.1	11.0
	High	160	118.5	7.5	6.3	0.8	0.7	0.4	0.3	0.0	0.0	7.5	6.4
RBC	Low	160	11.2	0.9	8.4	0.0	0.0	0.1	1.3	0.2	1.6	1.0	8.6
	High	160	127.9	7.4	5.8	0.0	0.0	0.0	0.0	2.0	1.6	7.7	6.0

A reproducibility study was also performed in-house over 25 days using two GloCyte instruments, 19 test cartridge lots, and 10 reagent lots (5 TNC and 5 RBC), one lot of GloCyte® Low and High Level Controls as samples as well as a Mid-Level Control sample that was made from a combination of the Low and High Level Control, and two operators. Three cell concentration levels were tested in duplicate for both TNCs and RBCs, twice daily (2 runs) by each operator on each GloCyte instrument. The Low Level Control targeted (approximately12 cells/µL), the Mid-Level targeted (approximately 40–50 cells/µL), and the High Level targeted (approximately120 cells/µL) each targeted cell concentration for each control level were for TNCs and RBCs. The Low Level Control was tested with the 30 µL test volume and the High Level Control was tested with the 10 µL test volume. The mid-level control was tested with both the 30 µL and 10 µL test volumes. To further demonstrate precision performance, SD and %CV of the within-run, between-run, within-day, between-instruments, between-operator, and between-day precision calculations were performed. The test results demonstrate that the %CV specifications were met for the Low, Mid, and High Level cell concentrations for both the TNC and RBC assays on the GloCyte® Automated Cell Counter System, at test volumes of 10 µL or 30 µL.

Reproducibility Performance – In-house Study

In-house				Within-Run		Between-Run		Between-Instrument		Between-Operator		Between-Day		Total	
Cell Type	Level	Test Volume	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
TNC	Low	30	10.8	1.1	10.0	0.0	0.0	0.1	0.2	0.0	0.0	0.3	2.4	1.1	10.3
	Mid	10	42.9	3.3	7.7	0.2	0.4	0.6	1.4	1.1	2.5	1.5	3.4	3.8	8.9
		30	41.1	2.1	5.2	0.5	1.1	0.0	0.0	0.5	1.3	1.3	3.2	2.6	6.3
	High	10	122.3	6.4	5.3	0.9	0.7	0.0	0.0	3.6	2.9	2.2	1.8	7.7	6.3
RBC	Low	30	12.3	1.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.5	1.0	8.4
	Mid	10	46.1	3.3	7.1	0.0	0.0	0.0	0.0	1.0	2.2	1.3	2.8	3.7	7.9
		30	45.0	2.1	4.7	0.4	0.9	0.2	0.5	1.1	2.4	1.1	2.4	2.7	5.9
	High	10	133.6	7.2	5.4	0.0	0.0	0.4	0.3	2.9	2.2	1.3	1.0	7.8	5.9

b. Linearity/assay reportable range:

The linearity was conducted in-house using manipulated human CSF samples with concentrations spanning the entire reportable intervals (RI) of both TNC and RBC cell types on the GloCyte® Automated Cell Counter System. A total of 14 concentration levels ranging from roughly 0–8,000 TNCs/µL were tested for the TNC and a total of 15 concentration levels ranging from roughly 0–800,000 RBCs/µL for the RBC were tested on three GloCyte® Automated Cell Counter Systems by three operators. The RI consists of both the analytical measurement range (sample levels that do not require a dilution to be measured on the GloCyte® Automated Cell Counter System) and the extended measuring interval (sample levels that require dilution to be measured on the GloCyte® Automated Cell Counter System). Dilution guidelines based on the visual appearance of the samples as well as dilution/test volume recommendations provided by the GloCyte® Automated Cell Counter System software were used to run each of the test samples tested in the linearity study. The results of the GloCyte® Automated Cell Counter System linearity study support the following linearity claims:

Analytical Measuring Range (AMR)

Cell Type	AMR GloCyte® Automated Cell Counter System [cells/µL]
TNC	3–123
RBC	2–123

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

GloCyte® Low and High Level Controls Value Assignment

Value assignment was conducted by two operators on two GloCyte® Automated Cell Counter Systems with three vials of each control level. Each vial is tested four times by each of the two operators. The vials are stored at 2–8ºC and brought to room temperature for 15 minutes prior to testing. The mean and standard deviation for the low and high controls are calculated from the total number of tests for each control level. The final value assignment for each lot of controls is based on the mean count ±3 standard deviations.

Quality Control Stability

To determine closed vial stability, three control lots were analyzed at several time points (0, 1, 3, 4, 5, 6, 7, 8, 9, 11, 13 months). The unopened GloCyte® Low and High Level Control vials from each lot were removed from 2–8°C storage conditions and tested with both TNC Reagent (10 replicates) and RBC Reagent (10 replicates) on a GloCyte® Automated Cell Counter System. GloCyte® Low and High Level Controls closed vial stability study data support a stability claim of 7 months when stored at

2–8°C.

To determine open vial stability two lots of GloCyte® Low and High Level Controls were tested over a 15 day period, with three replicates tested from each of three vials for a total of nine replicates for both TNC and RBC assays. The opened vial stability claim has been established at 8 days after opening when stored at 2–8°C after each use.

CSF Sample Stability

The sample stability study was conducted at three clinical laboratory sites by testing nine total nucleated cell (TNC) CSF samples and seven (7) red blood cell (RBC) CSF samples over the course of 5 hours, with three replicates tested each hour on the GloCyte® Automated Cell Counter System. The data collected in the CSF stability study was normalized due to the varying cell concentrations among samples tested by converting the cell counts into percent recoveries based on the cell count obtained for each sample at time zero. The result from each individual replicate tested beyond time zero was divided by the mean cell count produced at time zero and multiplied by 100%. The results of the CSF specimen stability study support a 4-hour timeframe for testing CSF specimens upon delivery to the clinical laboratory.

GloCyte Reagent Stability

Reagent stability was assessed using simulated spinal fluid (SSF). SSF samples were created on each day of testing using whole blood diluted into a simulated CSF matrix. The stability of the GloCyte TNC and RBC Reagents was established using both closed reagent vials (shelf life stability) and open reagent vials (in-use stability).

i. TNC Reagent Stability

Closed vial stability

Three vials from each of three lots of TNC Reagent that were stored at two temperatures (4°C and 10°C) and tested at various time intervals (0, 1, 2, 3, 4, 5, 6, 7, 9 or 10, 13, 15 and 16 months). TNC Reagent vials were removed from their respective storage conditions at each time point and brought to room temperature before testing for a total of five replicates per TNC Reagent vial on the GloCyte® Automated Cell Counter System. The TNC closed vial study data supports stability claim of 13 months when stored at 2–8°C.

Open vial stability

Six TNC Reagent vials from two lots of reagent were brought to room temperature from 4°C storage conditions and tested on the GloCyte® Automated Cell Counter System at each test point (0, 4, 7, 13, and 15 months). Three replicates from each vial were tested with the normal saline and simulated spinal fluid samples. Three of the six TNC Reagent vials were then stored at 4°C and the other three TNC Reagent vials were stored at 22°C to simulate refrigerated and room temperature storage conditions, respectively. These opened TNC Reagent vials from both storage conditions were tested weekly for a total of 5 weeks. The

TNC opened vial stability claim has been established at 5 days after opening when stored at room temperature. All acceptance criteria for the closed and open vial stability were met for the TNC Reagent.

ii. RBC Reagent Stability

Closed vial stability

Three vials from each of three lots of RBC Reagent that were stored at two temperatures (4°C and 10°C) and tested at various time intervals (0, 1, 2, 3, 4, 5, 6, 7, 10, 13, 15 and 17 months). The RBC Reagent vials were removed from their respective storage conditions at each time point and brought to room temperature before testing on the GloCyte® Automated Cell Counter System. Normal saline and simulated spinal fluid samples were tested with the RBC Reagents for a total of five replicates per RBC Reagent vial on the GloCyte System. The RBC closed vial study data supports a preliminary stability claim of 13 months when stored at 2–8°C.

Open vial stability

Six RBC Reagent vials each from each of two lots of RBC Reagent were brought to room temperature from 4°C storage conditions and tested on the GloCyte® Automated Cell Counter System with normal saline and simulated spinal fluid at each test point (0, 4, 7, 13, and 15 months). Three replicates from each vial were tested with the normal saline and simulated spinal fluid samples. Three of the six RBC Reagent vials were then stored at 4°C and the other three RBC Reagent vials were stored at 22°C to simulate refrigerated and room temperature storage conditions, respectively. These opened RBC Reagent vials from both storage conditions were tested weekly for a total of five weeks. The RBC opened vial stability claim has been established at 5 days after opening when stored at room temperature. All acceptance criteria for the closed and open vial stability were met for the RBC Reagent.

GloCyte Test Cartridge Stability

To determine closed vial stability, three lots of GloCyte test cartridges were evaluated on the GloCyte System for both TNC and RBC assays at three different storage temperatures (8°C, 22°C, and 42°C). Testing was performed at various time points (0, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 13 months). GloCyte test cartridges were brought to room temperature from the different storage conditions prior to testing on the GloCyte at each time point. The GloCyte test cartridges were tested with normal saline and simulated spinal fluid (both nucleated cells and red blood cells) for a total of 72 cartridges per cell type. The GloCyte test cartridge study data supports a stability claim of 10 months when stored at 10°C – 40°C.

d. Detection limit:

Limit of Blank (LoB) testing was performed using five blank human CSF samples with low concentrations (1–2 cells/µL) of TNCs and/or RBCs on two GloCyte®

Automated Cell Counter Systems. Samples were tested four times with one reagent lot and one cartridge lot and four times with a second reagent and cartridge lot for a total of 40 tests per day. A total of 120 tests were performed on each instrument per assay (TNC or RBC) over a testing period of 3 days.

LoD testing was performed using CSF specimens with low (RBC or TNC) count levels (1–2 cells/µL). Six RBC and six TNC CSF specimens were tested on each of two GloCyte instruments. The reference cell counts for each low-count CSF specimen were determined by manual hemocytometer cell counting. Each low-count CSF specimen was tested 10 times with one reagent lot and one cartridge lot and 10 times with a second reagent and cartridge lot. A total of 120 tests were performed on each instrument per assay.

The LoQ was determined by graphically plotting the estimated percent total error (%TE) of the GloCyte® Automated Cell Counter System along the y-axis and the "true" manual count for each sample along the x-axis and then performing a linear regression. The LoQ is the manual count at which the linear regression intersects the 20% maximum allowable %TE acceptance criteria.

Analyte	LoB (Cells/µL)	LoD (Cells/µL)	LoQ (Cells/µL)
RBC	<1	1	2
TNC	<1	1	3

These results indicate that the estimated LoB, LoD and LoQ for the GloCyte® Automated Cell Counter System TNC and RBC assays meet the expected performance criteria for background limits and low limits of the measuring ranges.

e. Analytical specificity:

The interference study was conducted to evaluate the potential of various endogenous substances present in CSF to affect RBC and TNC counts. Human whole blood was drawn on days of testing and mixed with pooled human CSF to create the Matrix/Base Pools containing RBCs or TNCs at two different cell concentrations: low (approximately 10 cells/µL) and high (approximately 120 cells/µL). A total of four Matrix/Base Pools were created, two per assay cell type (RBC or TNC cells/µL). Samples for interference testing were created using these Base Pools by spiking in a fixed volume of either the potential interferent or the solvent used to prepare the potential interferent stock solution. All the potential interferents, with the exception of nucleated red blood cells (NRBCs), were tested at high, "worst case" concentrations that a typical laboratory would observe among patient specimens submitted for analysis, and lower concentrations that are more prevalent in a normal population or in patients with only slightly elevated levels of that substance. Interference from NRBCs were evaluated using six clinical peripheral blood samples with varying NRBC concentrations used to create manipulated CSF samples to be tested using the TNC Reagent on the GloCyte® Automated Cell Counter System.

Each NRBC test sample was run for a total of 10 replicates. The GloCyte® Automated Cell Counter System RBC and TNC Assays count immature cells including NRBCs.

Interference Testing Summary

Potential Interferent	GloCyte Assay	Highest Concentration at which No Interference was observed
Conjugated Bilirubin	TNC	307.8 µmol/L (18.0 mg/dL)
	RBC	307.8 µmol/L (18.0 mg/dL)
Unconjugated Bilirubin	TNC	323.2 µmol/L (18.9 mg/dL)
	RBC	323.2 µmol/L (18.9 mg/dL)
Hemolytic Hemoglobin	TNC	1.1 g/dL
	RBC	NONE
Protein	TNC	59.0 g/L
	RBC	59.0 g/L
Lactate	TNC	13.2 mmol/L
	RBC	13.2 mmol/L
Haemophilus influenzae	TNC	10^8 CFU/mL
	RBC	10^8 CFU/mL
Streptococcus pneumoniae	TNC	10^8 CFU/mL
	RBC	10^8 CFU/mL
Neisseria lactamica	TNC	10^7 CFU/mL
	RBC	10^8 CFU/mL
Escherichia coli	TNC	10^8 CFU/mL
	RBC	10^8 CFU/mL
Candida albicans	TNC	10^8 CFU/mL
	RBC	10^8 CFU/mL
Platelets	TNC	14.7×10^3 Platelets/µL
	RBC	14.7×10^3 Platelets/µL
Nonspecific FcR Binding (Monocytes)	RBC	1.7 x Monocytes/µL
RBC Fragments	RBC	Could Not be Quantified
Nucleated RBCs	TNC	None

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A total of 321 CSF specimens (203 adult specimens and 118 pediatric specimens) for TNC and a total of 422 CSF specimens (243 adult specimens and 179 pediatric specimens) for RBC were analyzed at five clinical sites in the U.S. The CSF samples were tested for TNC and RBC counts using the Neubauer hemocytometer (reference method). Each sample was analyzed in duplicate on the Neubauer hemocytometer and on the GloCyte® Automated Cell Counter System. Results were compared by regressing the first of the duplicate measurements for the GloCyte® Automated Cell Counter System against the mean of the duplicate counts for the hemocytometer.

An in-house method comparison was also performed by using manipulated CSF samples (31 samples for TNC and 41 samples for RBC) that were created by diluting human TNC and RBC into pooled blank human CSF. Pediatric samples (pediatric venous blood and pediatric CSF) and adult samples (adult venous blood and adult CSF) were contrived to allow for separate analyses of pediatric and adult data. Test sample levels were randomly tested. Two manual counts were performed using the Neubauer hemocytometer and two counts were performed using the GloCyte® Automated Cell Counter System.

The external and in-house method comparison slope and intercept results of all regression analyses met the acceptance criteria. The TNC and RBC results demonstrate that the GloCyte Automated Cell Counter System yields results equivalent to manual counts using the Neubauer hemocytometer for the intended use, in both pediatric and adult populations.

Summary of Method Comparison Clinical Data by Site

Cell Type	Population	N	Range [cells/µL]	Slope (95% CI)	Intercept (95% CI)	Differences within 95% CI
Site 1						
TNC	Pediatric	59	0 – 1,329	1.066 (1.000 – 1.250)	0.000 (-0.250 – 0.000)	93%
	Adult	2	NA	NA	NA	NA
RBC	Pediatric	88	0 – 758,125	0.874 (0.858 – 0.929)	0.000 (-0.107 – 0.170)	95%
	Adult	4	NA	NA	NA	NA
Site 2						
TNC	Pediatric	19	0 – 2,513	0.822 (0.740 – 0.890)	1.000 (-0.781 – 1.781)	95%
	Adult	90	0 - 601	0.962 (0.903 – 1.000)	0.038 (0.000 – 0.193)	96%
RBC	Pediatric	21	0 – 25,695	0.929 (0.876 – 1.039)	0.000 (-1.039 – 0.000)	95%
	Adult	111	0 – 49,000	0.943 (0.914 – 0.999)	0.000 (0.000 – 0.086)	98%

Cell Type	Population	N	Range [cells/µL]	Slope (95% CI)	Intercept (95% CI)	Differences within 95% CI
Site 3						
TNC	Pediatric	9	0 - 261	1.000 (0.333 – 1.423)	0.000 (-1.423 – 0.667)	100%
	Adult	32	0 – 1,472	1.030 (0.969 – 1.167)	0.864 (-0.167 – 1.031)	97%
RBC	Pediatric	19	0 – 4,886	0.932 (0.823 – 1.074)	0.000 (-1.074 – 4.354)	95%
	Adult	39	0 – 59,722	1.029 (0.982 – 1.088)	0.000 (-0.088 – 0.018)	95%
Site 4						
TNC	Pediatric	14	0 - 209	1.047 (0.933 – 1.458)	-0.047 (-1.687 – 0.067)	93%
	Adult	77	0 – 6,306	1.042 (1.000 – 1.134)	-0.042 (-0.137 – 0.000)	97%
RBC	Pediatric	16	0 – 251,112	0.895 (0.8202 – 1.074)	1.053 (0.131 – 3.478)	94%
	Adult	87	0 – 58,417	1.068 (1.021 – 1.114)	0.000 (0.000 – 0.000)	97%
Site 5						
TNC	Pediatric	17	0 - 975	0.933 (0.831 – 1.147)	0.067 (-7.448 – 4.471)	94%
	Adult	2	NA	NA	NA	NA
RBC	Pediatric	35	0 – 11,000	0.910 (0.835 – 0.935)	-0.281 (-1.442 – 1.000)	97%
	Adult	2	NA	NA	NA	NA

Summary of Method Comparison Studies

Parameter		Range (cells/µL)	Slope (95% CI)	Intercept (95% CI)
RBC	Pediatric	0 – 817,500	0.910 (0.885 0.935)	0.000 (-0.045, 0.058)
	Adult	0 – 901,250	1.000 (0.986, 1.007)	0.000 (0.000, 0.014)
TNC	Pediatric	0 – 7,672	0.963 (0.909, 1.000)	0.037 (0.000, 0.182)
	Adult	0 – 9,900	1.000 (1.000, 1.003)	0.000 (-0.003, 0.000)

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:
The reference ranges were based on the existing medically accepted published reference ranges.

CSF Normal Reference Ranges

Demographic	Total WBC	RBC
Neonates (<1 year)	0–30 cells/µL	None
Ages 1 to 4 years	0–20 cells/µL	None
Ages 5 to puberty (18 years)	0–10 cells/µL	None
Adults (>18 years)	0–5 cells/µL	None

N. Instrument Name:

GloCyte® Instrument

O. System Descriptions:

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:

   The personal computer and application software provide the primary user interface for interactions with the GloCyte Instrument. Test information and user commands are entered into the instrument program using a standard keyboard and mouse/pointing device.

4. Specimen Sampling and Handling:

   The Sample Preparation Tray includes locations for patient specimen tubes, dilution tubes, staining tubes, cartridges, controls, and either TNC or RBC reagents. CSF is stained with TNC or RBC reagents. The stained CSF sample is deposited manually onto the test cartridge, using either the 10 or 30 μL fixed volume pipette. The test cartridge is used in conjunction with the Vacuum Station, which draws the stained test sample liquid through the test cartridge membrane, and is analyzed in the GloCyte Instrument.

5. Calibration:

   Not applicable

6. Quality Control:

   GloCyte® Low and High Level Controls

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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