510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K234143

B Applicant

Abbott Point of Care Inc.

C Proprietary and Established Names

i-STAT TBI Cartridge with the i-STAT Alinity System

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QAT	Class II	21 CFR 866.5830 - Brain Trauma Assessment Test	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase-L1 (UCH-L1)

C Type of Test:

Automated enzyme-linked immunosorbent assay, semi-quantitative

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The i-STAT TBI test is a panel of in vitro diagnostic immunoassays for the quantitative measurements of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in whole blood and a semi-quantitative interpretation of test results derived from these measurements, using the i-STAT Alinity instrument. The interpretation of test results is used, in conjunction with other clinical information, to aid in the evaluation of patients, 18 years of age or older, presenting with suspected mild traumatic brain injury (Glasgow Coma Scale score 13-15), which may include one of the following four clinical criteria: 1) any period of loss of consciousness, 2) any loss of memory for events immediately before and after the accident, 3) any alteration in mental state at the time of accident, and/or 4) focal neurological deficits, within 24 hours of injury, to assist in determining the need for a CT (computed tomography) scan of the head. A 'Not Elevated' test interpretation is associated with the absence of acute traumatic intracranial lesions visualized on a head CT scan.

The test is to be used with venous whole blood collected with EDTA anticoagulant in point of care or clinical laboratory settings by a healthcare professional.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For clincial laboratory or Point-of-Care (POC) settings

D Special Instrument Requirements:

i-STAT Alinity (K153357)

IV Device/System Characteristics:

A Device Description:

The i-STAT TBI cartridge is a multiplex immunoassay that contains assays for both GFAP and UCH-L1. The assays test for the presence of these biomarkers in a single whole blood sample and yield a semi-quantitative test interpretation based on measurements of both GFAP and UCH-L1 in approximately 15 minutes. The i-STAT TBI cartridge is designed to be run only on the i-STAT Alinity instrument. Each i-STAT TBI cartridge contains all the necessary reagents needed to perform the test.

The i-STAT Alinity instrument is a handheld, in vitro diagnostic device. The instrument is the main user interface of the i-STAT System and functions as the electro-mechanical interface to the test cartridge. The instrument executes the test cycle, acquires, and processes the electrical sensor signals converting the signals into quantitative results. These functions are controlled by a microprocessor.

Assayed quality control materials available for use with the i-STAT TBI cartridge include i-STAT TBI Controls and the i-STAT TBI Calibration Verification Levels 1-3.

- The i-STAT TBI Controls can be used to monitor performance of the GFAP and UCHL1 assays within the i-STAT TBI cartridge on the i-STAT Alinity instrument. The i-STAT TBI Controls have two levels: i-STAT TBI Control Level 1 and i-STAT TBI Control Level 2 and each level is packaged separately. The controls are sold separately from the cartridges.

- The i-STAT TBI Calibration Verification Levels 1-3 can be used to verify the pre-set calibration of the i-STAT TBI cartridge throughout the reportable range. Calibration verification materials are sold separately from the cartridges.

B Principle of Operation:

The i-STAT TBI cartridge consists of the UCH-L1 and GFAP assays and uses the sandwich enzyme-linked immunosorbent assay (ELISA) method with electrochemical detection of the resulting enzyme signal. The immunoassay uses anti-UCH-L1/alkaline phosphatase (ALP) and anti-GFAP/ALP antibody conjugates for labeling/detection (detection antibody-ALP conjugates) and anti-UCH-L1 and anti-GFAP monoclonal antibodies for capture (capture antibodies) that together allow for the detection and measurement of antigen in a whole blood sample. The detection and capture antibodies recognize distinct regions or epitopes on their respective antigens. All the steps of the ELISA are automated and conducted inside the test cartridge.

The multiplex design is based on the high degree of specificity inherent in antibody/antigen interactions. Additionally, the architecture of the cartridge and sensor design affords separation of these two antibody/antigen combinations such that the electrochemical signals arising from each analyte are measured independently at different areas of the biosensor chip contained within the cartridge.

The biosensor chip inside the cartridge features two electrochemical capture sensors that consist of microfabricated amperometric electrodes on a silicon substrate. After sample addition to the cartridge, the detection antibody-ALP conjugates dissolve into the sample and are released from the sensor. The capture antibodies immobilized on separate sensors on the chip capture the antigens (GFAP and UCH-L1) present in the sample that have bound to the detection antibody-ALP conjugate to form a sandwich (detection antibody-ALP conjugate/antigen/capture antibody) during an incubation period of approximately 12 minutes. After a wash step to remove excess labeling antibody conjugate and non-specifically bound proteins, the antigen is measured as electrochemical signal generated through enzymatic conversion of the ALP substrate present in the wash fluid. The current is proportional to the amount of labeled antigen (GFAP/UCH-L1 immobilized on the sensor. This electrical signal is converted into a quantitative measurement of the whole blood concentration of GFAP and UCH-L1 reported in units of pg/mL.

The user interface includes a touch screen display, audible signals, and ability to input information using a barcode reader and the touch screen. Based on the quantitative measurement of the whole blood concentration of GFAP and UCH-L1 (pg/mL), an interpretation (semi-quantitative result) is first displayed on the screen (“Elevated,” “Not Elevated,” or “Repeat Test”). A second page displays the quantitative results of each test. The table below provides the test interpretation matrix based on the GFAP and UCH-L1 assay results relative to cut-offs. The assay cut-offs were established to be 65 pg/mL for GFAP and 360 pg/mL for UCH-L1.

GFAP Assay Result (relative to cut-off of 65 pg/mL)	UCH-L1 Assay Result (relative to cut-off of 360 pg/mL)	Test Interpretation
Below	Below	Not Elevated
Below	Equal or Above	Elevated
Equal or Above	Below	Elevated
Equal or Above	Equal or Above	Elevated
Equal or Above	Not reported	Elevated
Equal or Above	****	Elevated
Below	Not reported	Repeat Test##
****	Equal or Above	Elevated
Not reported	Below	Repeat Test##
Not reported	Not reported	Repeat Test##

**** Star-out condition. "****" is displayed rather than a quantitative result. Instrument unable to determine a quantitative result due to signals received from a particular sensor on the cartridge being detected as uncharacteristic. Because the other assay provides a result at or above cut-off, a test interpretation can be reported.

Results are not available for either assay, or a result is available for one assay with the other assay providing a result below cut-off. In these circumstances, an error code "QCF125" will be displayed with instruction to repeat the test.

A "Not Elevated" test interpretation is associated with the absence of acute traumatic intracranial lesions visualized on a head CT scan. An "Elevated" test interpretation suggests further evaluation by head CT scan should be considered. A "Repeat Test" test interpretation indicates suppressed (not reported) results for both assays or suppressed results (not reported) from one assay and the other assay providing a result below cut-off. In these circumstances, an error code (Quality Check Failure, or QCF) will be displayed with instruction for the end-user to repeat the test (no more than once).

V Substantial Equivalence Information:

A Predicate Device Name(s):

i-STAT TBI Plasma cartridge with the i-STAT Alinity System

B Predicate 510(k) Number(s):

K201778

C Comparison with Predicate(s):

Device & Predicate Device(s):	K234143	K201778 (Predicate)
Device Trade Name	i-STAT TBI Cartridge with i-STAT Alinity System	i-STAT TBI Plasma Cartridge with i-STAT Alinity System
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The i-STAT TBI test is a panel of in vitro diagnostic immunoassays for the quantitative measurements of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in whole blood and a semi-quantitative interpretation of test results derived from these measurements, using the i-STAT Alinity instrument. The interpretation of test results is used, in conjunction with other clinical information, to aid in the evaluation of patients, 18 years of age or older, presenting with suspected mild traumatic brain injury (Glasgow Coma Scale score 13-15), which may include one of the following four clinical criteria: 1) any period of loss of consciousness, 2) any loss of memory for events immediately before and after the accident, 3) any alteration in mental state at the time of accident, and/or 4) focal neurological deficits, within 24 hours of injury, to assist in determining the need for a CT (computed tomography) scan of the head. A 'Not Elevated' test interpretation is associated with the absence of acute traumatic intracranial lesions visualized on a head CT scan. The test is to be used with venous whole blood collected with EDTA anticoagulant in point of care or clinical laboratory settings by a healthcare professional.	The i-STAT TBI Plasma test is a panel of in vitro diagnostic immunoassays for the quantitative measurements of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in plasma and a semi-quantitative interpretation of test results derived from these measurements, using the i-STAT Alinity instrument. The interpretation of test results is used, in conjunction with other clinical information, to aid in the evaluation of patients, 18 years of age or older, presenting with suspected mild traumatic brain injury (Glasgow Coma Scale score 13-15) within 12 hours of injury, to assist in determining the need for a CT (computed tomography) scan of the head. A 'Not Elevated' test interpretation is associated with the absence of acute traumatic intracranial lesions visualized on a head CT scan. The test is to be used with plasma prepared from EDTA anticoagulated specimens in clinical laboratory settings by a healthcare professional. The i-STAT TBI Plasma test is not intended to be used in point of care settings.
Measurands	GFAP and UCH-L1	Same
Assay Technology	Enzyme-linked immunosorbent assay	Same
Assay Format	Single-use multiplex cartridge including both GFAP and UCH-L1 assays	Same

Detection Technology	Electrochemical	Same
Sample Volume	20 µL	Same
Automation	Test and wash cycles are fully automated after sample loading step	Same
Time to Result	15 minutes	Same
Reportable Result	Quantitative results for GFAP and UCH-L1 and semi-quantitative interpretation	Same
Instrument Platform	i-STAT Alinity	Same
Calibration	No calibration needed by the end user; calibration is pre-set during manufacture of the cartridge	Same
Controls	i-STAT TBI Controls (Levels 1 and 2) i-STAT TBI Calibration Verification Materials (Levels 1, 2, 3)	Same
Traceability	GFAP and UCH-L1 values assigned to i-STAT controls and calibration verification materials are traceable to Abbott's working calibrators prepared using recombinant GFAP and UCH-L1 (expressed and purified from E. coli).	Same
General Device Characteristic Differences		
Intended Use Setting	Point of care and clinical laboratory	Clinical laboratory
Analytical Measuring Interval	GFAP: 47 − 10000 pg/mL UCH-L1: 87 − 3200 pg/mL	GFAP: 30 − 10000 pg/mL UCH-L1: 200 − 3200 pg/mL
Assay Cut-off	GFAP: 65 pg/mL UCH-L1: 360 pg/mL	GFAP: 30 pg/mL UCH-L1: 360 pg/mL
Specimen Type	Venous whole blood	Plasma

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines and standards/guidance documents were used:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06-2nd Ed: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP07 Ed3: Interference Testing in Clinical Chemistry – Third Edition

- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- CLSI EP37 Ed1: Supplemental Tables for Interference Testing in Clinical Chemistry – First Edition
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices – Guidance for Industry and FDA Staff, issued May 11, 2005
- IEC 60601-1-2:2014 Medical electrical equipment – Part 1-2: General requirements for basic safety and essential performance – Collateral Standard: Electromagnetic disturbances – Requirements and tests

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

All results met the manufacturer's pre-determined acceptance criteria.

1. Precision/Reproducibility:

The precision studies were conducted based on the CLSI guideline EP05-A3. Samples in the test panel evaluated in each precision study were generated to cover the measuring interval of the GFAP and UCH-L1 assays. All studies except whole blood precision used plasma samples as the protocol required testing over multiple days which is not possible with whole blood samples. Pooled plasma samples were generated from whole blood collected in K_2EDTA tubes from normal healthy donors. Plasma samples near GFAP and UCH-L1 cut-offs were spiked with native antigen from plasma of subjects with traumatic brain injury (TBI) to mimic clinical specimens more closely. Other samples evaluated in the study were spiked with recombinant GFAP and UCH-L1.

a. *Within-laboratory precision:*

The study was conducted to evaluate within-laboratory precision of the GFAP and UCH-L1 assays within the i-STAT TBI cartridge on the i-STAT Alinity Instrument using plasma samples. The test panel consisted of five samples for GFAP and six samples for UCH-L1. Each panel member was tested at a single site for at least 20 days, by at least two operators, with two runs per day, two replicates per run, on three lots of i-STAT TBI cartridges, using multiple i-STAT Alinity instruments, to generate a total of 240 replicates per panel member, respectively. Runs were separated by a minimum of 2 hours. Two levels of i-STAT TBI control material, L1 (i-STAT TBI Control Level 1) and L2(i-STAT TBI Control Level 2), were also run in the study.

The data were analyzed in both quantitatively and qualitatively. The results are summarized in the tables below for the GFAP and UCH-L1 assays.

Quantitative analysis:

GFAP Assay												
Sample	N	Mean	Repeatability		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1*	240	78.8	3.0	3.9	0.9	1.1	0.6	0.7	2.2	2.8	3.9	5.0
2*	240	98.6	6.0	6.1	1.4	1.4	0.7	0.7	2.6	2.6	6.8	6.9
3!	240	880.6	21.3	2.4	15.8	1.8	1.7	0.2	9.8	1.1	28.8	3.3
4!	240	4415.3	144.7	3.3	67.3	1.5	17.3	0.4	135.6	3.1	212.2	4.8
5!	240	8346.7	285.0	3.4	151.1	1.8	56.7	0.7	347.6	4.2	479.5	5.7
L1	240	161.2	6.8	4.2	1.8	1.1	1.8	1.1	2.5	1.6	7.8	4.8
L2	240	4645.0	166.4	3.6	45.0	1.0	45.9	1.0	148.7	3.2	234.6	5.1

* Pooled plasma from normal donors spiked with <5% v/v GFAP from pooled TBI patient plasma
! Pooled plasma from normal donors spiked with <5% v/v recombinant GFAP antigen

UCH-L1 Assay												
Sample	N	Mean	Repeatability		Between-run		Between-day		Between-lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1!	240	159.9	11.9	7.5	3.4	2.2	0.8	0.5	4.9	3.1	13.5	8.5
2!	240	255.7	18.1	7.1	5.0	1.9	3.2	1.2	6.2	2.4	20.3	8.0
3*	240	488.8	26.5	5.4	15.0	3.1	5.9	1.2	11.6	2.4	33.5	6.9
4!	240	826.2	49.4	6.0	23.1	2.8	12.7	1.5	24.4	3.0	61.9	7.5
5!	240	1763.7	100.6	5.7	26.6	1.5	29.6	1.7	84.6	4.8	138.9	7.9
6!	240	2190.3	126.9	5.8	46.8	2.1	23.7	1.1	105.1	4.8	176.3	8.1
L1	240	466.2	28.6	6.1	7.3	1.6	6.4	1.4	16.6	3.6	34.8	7.5
L2	240	1597.6	94.0	5.9	39.2	2.5	19.8	1.2	66.0	4.1	124.9	7.8

! Pooled plasma from normal donors spiked with <5% v/v recombinant UCH-L1 antigen
* Pooled plasma from normal donors spiked with <5% v/v UCH-L1 from pooled TBI patient plasma

Qualitative analysis:

GFAP Assay (Cut-Off: 65 pg/mL)				
Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Elevated/Total Replicates)	%Correct Call
1[b]	78.8	240	239/240	99.6*
2[c]	98.6	240	240/240	100.0
3[c]	880.6	240	240/240	100.0
4[c]	4415.3	240	240/240	100.0
5[c]	8346.7	240	240/240	100.0
L1[c]	161.2	240	240/240	100.0
L2[c]	4645.0	240	240/240	100.0

[b] Near cut-off
[c] Above cut-off
*Replicates for sample with mean near cut-off can have replicates below cut-off or at/above cut-off

UCH-L1 Assay (Cut-Off: 360 ng/mL)				
Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Elevated/Total Replicates)	%Correct Call
1[a]	159.9	240	0/240	100.0
2[a]	255.7	240	0/240	100.0
3[b]	488.8	240	240/240	100.0*
4[c]	826.2	240	240/240	100.0
5[c]	1763.7	240	240/240	100.0
6[c]	2190.3	240	240/240	100.0
L1[c]	466.2	240	240/240	100.0
L2[c]	1597.6	240	240/240	100.0

[a] Below cut-off
[b] Near cut-off
[c] Above cut-off
* Replicates for sample with mean value near cut-off can have replicates below cut-off or at/above cut-off

b. *Instrument-to-instrument precision*

The study was conducted to evaluate instrument-to-instrument precision of the GFAP and UCH-L1 assays in the i-STAT TBI cartridge on the i-STAT Alinity instrument using plasma samples. The test panel that included four samples of GFAP and five samples of UCH-L1 were tested using one lot of i-STAT TBI cartridges on three i-STAT Alinity instruments with five replicates once per day for five days, to generate a total of 75 replicates per sample. The data were analyzed quantitively and qualitatively. The results are summarized in the tables below.

Quantitative analysis:

Sample	N†	Mean (pg/ml)	Within-run		Between-Instrument		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	% CV
GFAP Assay										
1**	75	68.6	4.8	6.9	1.2	1.7	0.9	1.4	5.0	7.3
2**	75*	84.3	4.5	5.3	1.2	1.4	0.8	1.0	4.7	5.6
3!	75	828.4	37.9	4.6	9.1	1.1	4.5	0.6	39.2	4.7
4!	75	8164.9	504.0	6.2	98.2	1.2	100.5	1.2	523.2	6.4
UCH-L1 Assay										
1!!	75	145.9	10.8	7.4	2.1	1.4	2.1	1.4	11.2	7.7
2!!	75	238.3	17.1	7.2	3.9	1.6	4.0	1.7	18.0	7.6
3***	75	467.2	28.4	6.1	6.6	1.4	7.5	1.6	30.1	6.4
4!!	75	789.5	53.8	6.8	11.6	1.5	14.3	1.8	56.8	7.2
5!!	75	2228.8	163.5	7.3	34.6	1.6	34.7	1.6	170.7	7.7

* One outlier identified; analysis with outlier included
! Pooled plasma from normal donors spiked with <5% v/v recombinant GFAP antigen
** Pooled plasma from normal donors spiked with <5% v/v GFAP from pooled TBI patient plasma
!! Pooled plasma from normal donors spiked with <5% v/v recombinant UCH-L1 antigen
***Pooled plasma from normal donors spiked with <5% v/v UCH-L1 from pooled TBI patient plasma

Qualitative analysis:

Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Elevated/Total Replicates)	%Correct Call
GFAP Assay				
1[b]	68.6	75	66/75	88.0*
2[c]	84.3	75	75/75	100.0
3[c]	828.4	75	75/75	100.0
4[c]	8164.9	75	75/75	100.0
UCH-L1 Assay				
1[a]	145.9	75	0/75	100.0
2[a]	238.3	75	0/75	100.0
3[b]	467.2	75	75/75	100.0*
4[c]	789.5	75	75/75	100.0
5[c]	2228.8	75	75/75	100.0

[a] Below cut-off
[b] Near cut-off
[c] Above cut-off
* Replicates for sample with mean value near cut-off can have replicates below cut-off or at/above cut-off

c. *Multi-site reproducibility*

The site-to-site reproducibility of the GFAP and UCH-L1 assays within the i-STAT TBI cartridge on the i-STAT Alinity instrument was evaluated using plasma samples at three clinical sites. The medical facilities in the study were representative of the POC setting in which the i-STAT TBI test is intended to be used and the two operators at each site, who performed the testing, were representative of end-users of the i-STAT System. At each of three sites, the test panel that included six GFAP samples and six UCH-L1 samples were tested once per day for five days by two operators, with each operator using three i-STAT TBI cartridges on three i-STAT Alinity instruments, to generate a total of 90 replicates per sample. The data were analyzed quantitatively and qualitatively. The results are summarized in the tables below for the GFAP and UCH-L1 assays.

Quantitative analysis:

GFAP Assay														
Sample	N	Mean (pg/mL)	Within-Day		Between-Day		Between-Operator		Within-Site		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1†	90	66.4	3.9	5.8	0.3	0.4	0.6	0.9	3.9	5.9	0.0	0.0	3.9	5.9
2*	90	86.0	5.9	6.9	0.0	0.0	0.2	0.2	6.0	6.9	0.6	0.7	6.0	7.0
3*	90	980.9	35.4	3.6	18.0	1.8	8.1	0.8	40.5	4.1	13.9	1.4	42.8	4.4
4*	90	2785.5	59.6	2.1	22.6	0.8	26.0	0.9	68.8	2.5	0.0	0.0	68.8	2.5
5*	90	5357.3	135.4	2.5	120.9	2.3	54.2	1.0	189.4	3.5	46.1	0.9	194.9	3.6
6*	90	7652.6	166.9	2.2	84.2	1.1	0.0	0.0	186.9	2.4	114.1	1.5	218.9	2.9

*pooled plasma spiked with <5% v/v recombinant GFAP antigen
†pooled plasma spiked with <5% v/v GFAP from TBI patient plasma

UCH-L1Assay														
Sample	N	Mean (pg/mL)	Within-Day		Between-Day		Between-Operator		Within-Site		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1*	90	206.5	13.3	6.4	4.6	2.2	10.1	4.9	17.3	8.4	0.0	0.0	0.0	8.4
2†	90	384.1	21.0	5.5	0.0	0.0	22.8	5.9	31.0	8.1	0.0	0.0	0.0	8.1
3*	90	681.8	35.9	5.3	16.1	2.4	35.7	5.2	53.1	7.8	0.0	0.0	0.0	7.8
4*	90	1225.9	82.6	6.7	0.0	0.0	9.3	0.8	83.2	6.8	31.6	2.6	2.6	7.3
5*	90	2051.2	98.9	4.8	25.0	1.2	99.0	4.8	142.2	6.9	0.0	0.0	0.0	6.9
6*	90	2851.8	13.3	6.4	4.6	2.2	10.1	4.9	17.3	8.4	0.0	0.0	0.0	8.4

*pooled K2EDTA plasma spiked with <5% v/v recombinant UCH-L1 antigen
†pooled K2EDTA plasma spiked with <5% v/v UCH-L1 from TBI patient plasma

Qualitative analysis:

Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Elevated/Total Replicates)	%Correct Call
GFAP Assay				
1[b]	66.4	90	74/90	82.2*
2[c]	86.0	90	89/90	98.9
3[c]	980.9	90	90/90	100.0
4[c]	2785.5	90	90/90	100.0
5[c]	5357.3	90	90/90	100.0
6[c]	7652.6	90	90/90	100.0
UCH-L1 Assay				
1[a]	206.5	90	0/90	100.0
2[b]	384.1	90	83/90	89.3*
3[c]	681.8	90	90/90	100.0
4[c]	1225.9	90	90/90	100.0
5[c]	2051.2	90	90/90	100.0
6[c]	2851.8	90	90/90	100.0

[a] Below Cut-off
[b] Near cut-off (mean ±25% of the cut-off value)
[c] Above cut-off
*Replicates for sample with mean value near cut-off can have replicates below cut-off or at/above cut-off

d. *Whole Blood precision study*

The precision of GFAP and UCH-L1 assays within the i-STAT TBI cartridge on the i-STAT Alinity instrument using whole blood was evaluated at three clinical POC sites representative of the POC setting in which the i-STAT TBI test is intended to be used. The two operators representing the end-users of the i-STAT System performed the testing at each site. At each site, test samples were prepared by spiking prospectively collected venous whole blood specimens with recombinant GFAP and/or UCH-L1 or human plasma sample from TBI patients with native GFAP and UCH-L1. The whole blood test samples included at least six samples of GFAP and six samples of UCH-L1 at each site to evaluate the precision performance of each assay. At each site and for each level, a minimum of one whole blood specimen was prepared and tested in three runs, by two

different operators with each operator using four i-STAT TBI cartridges on four i-STAT Alinity instruments (one replicate/instrument/run) for a total of 24 replicates/specimen. The results are summarized in the tables below for the GFAP and UCH-L1 assays.

Quantitative analysis:

GFAP										
Sample	N	Mean (pg/mL)	Repeatability		Between-Instrument		Between-Operator		Within-Site	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Site 1										
1†	24	63.3	10.8	16.9	4.7	7.4	3.7	5.7	12.3	19.3
2†	23‡	64.3	11.7	18.2	6.8	10.5	4.3	6.6	14.2	22.1
3*	24	103.5	10.9	10.5	0.0	0.0	2.22	2.2	11.1	10.7
4*	23‡	128.5	14.5	11.3	0.0	0.0	0.0	0.0	14.51	11.3
5*	24	986.3	88.5	9.0	0.0	0.0	0.0	0.0	88.5	9.0
6*	24	3431.6	338.5	9.9	0.0	0.0	104.4	3.0	354.2	10.3
7*	24	6371.3	637.4	10.0	0.0	0.0	163.0	2.6	657.9	10.3
8*	24	7836.9	730.9	9.3	0.0	0.0	103.0	1.3	738.1	9.4
Site 2										
1†	24	57.7	7.2	12.6	4.6	8.0	5.3	9.2	10.1	17.5
2†	24	60.9	11.1	18.2	0.0	0.0	2.2	3.5	11.3	18.5
3*	24	83.7	7.0	8.3	0.0	0.0	0.0	0.0	7.0	8.3
4*	24	148.1	12.1	8.2	0.0	0.0	0.0	0.0	12.1	8.2
5*	24	900.6	28.9	3.2	10.8	1.2	0.0	0.0	30.9	3.4
6*	24	3731.1	161.6	4.3	0.0	0.0	121.3	3.3	202.1	5.4
7*	24	5762.3	289.2	5.0	0.0	0.0	0.0	0.0	289.2	5.0
8*	24	8310.3	499.5	6.0	0.0	0.0	0.0	0.0	499.5	6.0
Site 3										
1†	23‡	58.9	4.5	7.6	2.6	4.4	0.0	0.0	5.2	8.8
2†	22§	67.2	16.5	24.6	0.0	0.0	0.0	0.0	16.5	24.6
3*	24	145.4	10.5	7.3	0.0	0.0	3.3	2.3	11.0	7.6
4*	24	962.1	56.8	5.9	24.5	2.6	0.0	0.0	61.9	6.4
5*	24	2954.5	167.4	5.7	0.0	0.0	3.1	0.1	167.4	5.7
6*	24	6226.4	246.5	4.0	18.2	0.3	20.7	0.3	248.0	4.0
7*	23¶	8366.9	502.6	6.0	0.0	0.0	168.2	2.0	530.0	6.3

*prospectively collected K2EDTA venous whole blood spiked with <5% v/v recombinant GFAP antigen
†prospectively collected K2EDTA venous whole blood spiked with <5% v/v GFAP from TBI patient plasma
‡one result not obtained due to a quality check failure (QCF) or star-out error
§two results not obtained due to a quality check failure (QCF) or star-out error
¶one result not obtained due to operator error

UCH-L1										
Sample	N	Mean (pg/mL)	Repeatability		Between-Instrument		Between-Operator		Within-Site	
			SD	% CV	SD	%CV	SD	%CV	SD	%CV
Site 1										
1*	23‡	215.7	16.6	7.7	0.0	0.0	0.0	0.0	16.6	7.69
2*	24	243.5	19.4	8.0	0.0	0.0	11.1	4.6	22.3	9.17
3†	24	333.7	28.7	8.6	12.8	3.8	17.2	5.1	35.8	10.7
4†	22	438.9	55.8	12.7	0.0	0.0	0.0	0.00	55.8	12.7
5*	24	486.7	25.6	5.3	6.4	1.3	9.3	1.9	28.0	5.7
6*	24	1451.4	106.3	7.3	0.0	0.0	70.0	4.8	127.3	8.8
7*	24	1746.3	96.1	5.5	0.0	0.0	0.0	0.0	96.1	5.5
8*	22‡¶	3020.3	146.1	4.8	20.2	0.7	58.0	1.9	158.5	5.3
Site 2										
1*	24	183.0	15.3	8.4	2.9	1.6	0.0	0.0	15.6	8.5
2*	24	220.2	19.8	9.0	0.0	0.0	0.0	0.0	19.8	9.0
3*	24	232.3	15.7	6.8	0.0	0.0	0.0	0.0	15.7	6.8
4†	24	360.8	27.0	7.5	18.3	5.1	0.0	0.0	32.6	9.0
5†	24	413.0	38.2	9.3	10.7	2.6	5.5	1.3	40.0	9.7
6*	24	535.1	61.4	11.5	0.0	0.0	10.8	2.0	62.3	11.6
7*	23‡	630.5	49.9	7.9	0.0	0.0	6.0	1.0	50.3	8.0
8*	24	675.0	50.5	7.5	20.7	3.1	0.0	0.0	54.6	8.1
9*	23‡	935.1	62.8	6.7	20.1	2.2	0.0	0.0	66.0	7.1
10*	21§	1114.1	59.4	5.3	0.0	0.0	0.0	0.0	59.4	5.3
11*	23‡	2286.3	121.2	5.3	0.0	0.0	0.0	0.0	121.2	5.3
12*	24	2319.1	139.4	6.0	42.5	1.8	66.9	6.9	160.3	6.9
13*	21‖	2945.8	141.7	4.8	0.0	0.0	0.0	0.0	141.7	4.8
Site 3										
1*	24	182.5	14.2	7.8	0.0	0.0	0.5	0.3	14.3	7.8
2*	24	204.2	16.5	8.1	0.0	0.0	11.7	5.7	20.2	9.9
3†	24	357.1	35.5	9.9	0.0	0.0	5.7	1.6	35.9	10.1
4†	24	392.8	39.5	10.1	12.2	3.1	0.0	0.0	41.4	10.5
5*	24	522.6	42.4	8.1	0.0	0.0	9.6	1.8	43.5	8.3
6*	24	1213.4	54.2	4.5	34.7	2.9	0.0	0.0	64.4	5.3
7*	24	1947.1	118.9	6.1	0.0	0.0	0.0	0.0	118.9	6.1
8*	21‡¶#	2829.4	150.9	5.3	59.8	2.1	81.5	2.9	181.6	6.4

* prospectively collected K2EDTA venous whole blood spiked with <5% v/v recombinant UCH-L1 antigen

† prospectively collected K2EDTA venous whole blood spiked with <5% v/v UCH-L1 from TBI patient plasma

‡ one result not obtained due to a quality check failure (QCF) or star-out error

§ three results not obtained due to a quality check failure (QCF) or star-out error

¶ one result not measurable due to being above the measurement range

‖ three results not measurable due to being above the measurement range

one result not obtained due to operator error

Qualitative analysis:

Sample	Mean (pg/mL)	Total Number of Replicates	Qualitative Agreement	
			n/N (Elevated/Total Replicates)	%Correct Call
Site 1				
1[b]	61.0^	24	8/24	66.7*
2[b]	66.0^	23	16/23	69.6*
3[c]	103.5	24	24/24	100.0
4[c]	128.5	24	24/24	100.0
5[c]	986.3	24	24/24	100.0
6[c]	3431.6	24	24/24	100.0
Site 2				
1[b]	58.5^	24	5/19	79.2*
2[c]	62.5^	24	9/24	62.5*
3[c]	83.7	24	24/24	100.0
4[c]	148.1	24	24/24	100.0
5[c]	900.6	24	24/24	100.0
6[c]	3731.1	24	24/24	100.0
7[c]	5762.3	24	24/24	100.0
8[c]	8310.3	24	24/24	100.0
Site 3				
1[b]	60.0^	23	1/23	95.7*
2[b]	67.0^	22	14/22	63.6*
3[c]	145.4	24	24/24	100.0
4[c]	962.1	24	24/24	100.0
5[c]	2954.5	24	24/24	100.0
6[c]	6226.4	24	24/24	100.0
7[c]	8366.9	23	23/23	100.0

[b] Near cut-off (overall mean ± 25%)

[c] Above cut-off

*Determination of correct call based on test material median. Replicates for sample with median near cut-off can have replicates below cut-off or at/above cut-off.

^ Median Value shown

Sample	Mean (pg/mL)	Total Number of Replicates	Qualitative Agreement	
			n/N (Elevated/Total Replicates)	%Correct Call
Site 1				
1[a]	215.7	23	0/23	100.0
2[a]	243.5	24	0/24	100.0
3[a]	333.7	24	5/19	79.2*
4[b]	438.9	22	22/22	100.0
5[c]	486.7	24	24/24	100.0
6[c]	1451.4	24	24/24	100.0
7[c]	1746.3	24	24/24	100.0
8[c]	3020.3	22	22/22	100.0

Sample	Mean (pg/mL)	Total Number of Replicates	Qualitative Agreement	
			n/N (Elevated/Total Replicates)	%Correct Call
Site 2				
1[a]	183.0	24	0/24	79.2
2[a]	220.2	24	0/24	62.5
3[a]	232.3	24	0/24	100.0
4[b]	360.8	24	13/24	100.0
5[c]	413.0	24	22/24	100.0
6[c]	535.1	24	24/24	100.0
7[c]	630.5	23	23/23	100.0
8[c]	675.0	24	24/24	100.0
9[c]	935.1	23	23/23	100.0
10[c]	1114.1	21	21/21	100.0
11[c]	2286.3	23	23/23	100.0
12[c]	2319.1	24	24/24	100.0
13[c]	2945.8	21	21/21	100.0
Site 3				
1[a]	182.5	24	0/24	100.0
2[a]	204.2	24	0/24	100.0
3[b]	357.1	24	11/13	54.2[*]
4[b]	392.8	24	21/24	87.5[*]
5[c]	522.6	24	24/24	100.0
6[c]	1213.4	24	24/24	100.0
7[c]	1947.1	24	24/24	100.0
8[c]	2829.4	21	21/21	100.0

[a] Below cut-off
[b] Near cut-off (overall mean ± 25%)
[c] Above cut-off
[*]Determination of correct call based on test material median. Replicates for sample with median near cut-off can have replicates below cut-off or at/above cut-off.

2. Linearity:

a. *Linearity*

The study was conducted to evaluate the linearity of the GFAP and UCH-L1 assays within the i-STAT TBI cartridge on the i-STAT Alinity instrument over the reportable range of each assay per the CLSI guideline EP06-2nd Ed. Two studies for each assay were conducted in parallel and the data from the studies were combined to evaluate the linearity for each assay. One study used the native antigen, and the other study used the recombinant antigen at concentrations that overlap with those of the native antigen. In each study, whole blood samples of varying GFAP and UCH-L1 levels were prepared through proportional mixing of low and high antigen concentration samples. The highest concentration sample with the native antigen was created by spiking an aliquot of K_2EDTA whole blood with plasma (<5% v/v) from a severe TBI patient exhibiting high levels of both GFAP and UCH-L1 antigens. The highest concentration sample with the recombinant antigen was created by spiking whole blood with recombinant GFAP and UCH-L1(<5% v/v) to target ranges. Samples with

intermediate levels of native and recombinant GFAP and UCH-L1 antigens were obtained through a process of proportional mixing of low and high antigen concentration samples. For evaluation of GFAP assay linearity, 10 sample levels of the native antigen samples and 17 sample levels of the recombinant antigen samples were generated. For evaluation of UCH-L1 assay linearity, 10 sample levels of the native antigen samples and 16 sample levels of the recombinant antigen samples were generated. Ten replicates of each sample were tested in the study, which was run over the course of a single day, using one cartridge lot and multiple i-STAT Alinity instruments.

To evaluate linearity, weighted least squares regression analyses were performed using replicates from all sample levels (native and recombinant) for both GFAP and UCH-L1 antigens, and an assessment of the deviation from linearity was determined for each sample level for GFAP and UCH-L1. The results are summarized in the table below:

Assay	Test Range (pg/mL)	Slope (95% CI)	Intercept (95% CI)	R^2 (95% CI)	% Deviation
GFAP	17.6 -11303.9	1.0 (0.99; 1.02)	-3.5 (-6.74; -0.23)	0.99 (0.998; 1.000)	8.0 to -13.4%
UCH-L1	86.4 - 3281.5	1.0 (0.96; 0.99)	2.7 (-3.54; 8.86)	0.99 (0.995; 0.999)	-0.8 to 2.6%

The study supports the linear range of 24.3 pg/mL to 11303.9 pg/mL for the GFAP and 86.4 pg/mL to 3281.5 pg/mL for the UCH-L1 assay with a deviation from linearity within 15%. The results support the linearity throughout the reportable ranges of 65–10,000 pg/mL for GFAP and 360–3200 pg/mL for UCH-LI.

b. *Hook effect*

To show that a clinical specimen with very high concentrations of antigen would not cause false negative results ("Not Elevated" result) hook effect studies were performed. The testing was conducted using one lot of i-STAT TBI cartridges, whole blood samples spiked to a high antigen level for each assay (GFAP and UCH-L1), and multiple i-STAT Alinity instruments. No hook effect was observed for the GFAP and UCH-L1 assays within the i-STAT TBI cartridge using whole blood samples with antigen concentrations exceeding 100,000 pg/mL GFAP and 100,000 pg/mL UCH-L1, respectively, when tested on the i-STAT Alinity instrument.

3. Analytical Specificity/Interference:

Studies to evaluate the analytical specificity/interference were conducted using four lots of i-STAT TBI cartridges and multiple i-STAT Alinity instruments. Two levels of GFAP and UCH-L1 samples were evaluated in the studies: low (98–130 pg/mL) and high (585–715 pg/mL) GFAP, and low (540–720 pg/mL) and high (1440–2160 pg/mL) UCH-L1. Venous whole blood samples collected in K_2EDTA anticoagulant from normal healthy donors was used to create low-positive (low) and moderate-positive (high) GFAP and UCH-L1 base pools for the preparation of the control samples and the potentially interfering test samples. Recombinant GFAP and UCH-L1 was added to achieve the targeted GFAP and UCH-L1 concentrations.

a. *Endogenous and exogenous interference*

Each potentially interfering substance was tested at the toxic/pathological concentration based on CLSI guideline EP37 Ed1, as applicable, and indicated in the tables below. The effect of each substance at each level of GFAP and UCH-L1 was evaluated by comparing the test results from a control sample, spiked with the appropriate solvent used to prepare the stock solution of the potentially interfering substance, with the test results from a sample spiked with a solution containing the potentially interfering substance as per CLSI guideline EP07 Ed3. Each test and control sample were evaluated with at least 15 replicates for each potentially interfering substance. A substance was identified as an interferent if the difference in the means between the control and test samples was not within: ± 13 pg/mL or 10% of the control sample mean for GFAP, and ± the greater of 40 pg/mL or 10% of the control sample mean for UCH-L1. For any substances identified as an interferent at the initial concentration tested, a dose response analysis was performed.

Human anti-mouse antibody (HAMA) and rheumatoid factor (RF) were also evaluated as potentially interfering endogenous substances. The low- and high-level GFAP and UCH-L1 samples were initially evaluated with screening concentrations of >160X HAMA and 1000 IU/mL RF, which are the concentrations of these substances evaluated in DEN170045. Multiple test and control samples were generated for both the low- and high-level GFAP and UCH-L1 samples, and at least 15 replicates of each sample were evaluated.

Endogenous Substances Not Found to Interfere	
Potentially interfering substance	**Test concentration**
Bilirubin (unconjugated)	40 mg/dL
Bilirubin (conjugated)	40 mg/dL
Hemoglobin	1000 mg/dL
Human anti-mouse antibodies (HAMA)	>80X[a]
Triglycerides	3000 mg/dL*
Intralipid 20%	7075 mg/dL

[a] The 'x' factor listed indicates the number of times more activity than a known negative sample for its ability to crosslink antibodies in a mouse system assay. As half of the plasma from the whole blood sample was replaced with HAMA-containing plasma, the final test concentration in the reconstituted whole blood sample is half of the original value

*Test concentration as per DEN170045

Exogenous Substances Not Found to Interfere	
Potentially interfering substance	**Test concentration (µmol/L unless specified)†**
Acetaminophen	1324
Acetylsalicylic acid (aspirin)	3620*
Ascorbic acid	298
Benzoylecgonine	8.6***
Caffeine	556
Clopidogrel	21.4*
Chloramphenicol	241
Diazepam	105
Diclofenac	81
Dopamine	4.1
EDDP[A] perchlorate	0.3*
Erythromycin	188
Ethanol	130 mmol/L
Ibuprofen	2425*
Methaqualone	32.4
Morphine	27.3
Nicardipine hydrochloride	0.97
Nicotine	5.97
Oxazepam	15.1
Phencyclidine	0.0357**
Phenytoin	238
Secobarbital	66.8
Warfarin	243

* Test concentration as per DEN170045

** Karch, SB. (2008) Dissociative Anesthetics. In: Karch's Pathology of Drug Abuse. 4th ed. Boca Raton, FL: CRC Press

*** Scheidweiler, K. B., Spargo, E. A., Kelly, T. L., Cone, E. J., Barnes, A. J., and Huestis, M. A. (2010) Pharmacokinetics of cocaine and metabolites in human oral fluid and correlation with plasma concentrations after controlled administration. *Ther Drug Monit.* **32**, 628-637

[A] 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

Among the endogenous substances, only albumin and RF were found to interfere with the UCH-L1 assay and none of the endogenous substances interfered with the GFAP assay. Among the exogenous substances, amphetamine, methadone, and d-methamphetamine were found to interfere with the GFAP assay while cocaine, metoprolol, and propoxyphene interfered with the UCH-LI assay. A dose-response study was performed to determine the highest test concentrations of these exogenous and endogenous interferents at which no interference could be demonstrated with the GFAP and UCH-L1 assay using samples with high and low levels of GFAP and UCH-L1 and the results are summarized in the tables below.

Assay	Interfering Substance	Antigen Level	Interferent Testing Concentration	Highest Concentration Tested without Interference
UCH-L1	Albumin	High	15 g/dL	5.2 g/dL
	RF	Low	1000 IU/mL	875 IU/mL

Assay	Interfering Substance	Antigen Level	Interferent Testing Concentration	Highest Concentration Tested without Interference
GFAP	Amphetamine	High	2.4 µmol/L	1.8 µmol/L
	Methadone	High	10.3 µmol/L	7.7 µmol/L
	d-Methamphetamine	Low	278.4 ng/mL	208.8 ng/mL
UCH-L1	Cocaine	Low	3.5 ng/mL	2.6 ng/mL
	Metoprolol	Low	18.7 µmol/L	14.0 µmol/L
	Propoxyphene	High	9.5 µmol/L	7.1 µmol/L

b. *Cross-reactivity*

A panel comprised of proteins that have significant homology to either GFAP or UCH-L1 was evaluated for cross-reactivity. The study was conducted using one lot of i-STAT TBI cartridge, venous whole blood samples collected in K_2EDTA anticoagulant from healthy donor spiked with either recombinant GFAP and/or UCH-L1 to achieve the targeted concentrations of GFAP (98–130 pg/mL) and UCH-L1 (540–720 pg/mL), and multiple i-STAT Alinity instruments. The concentrations of GFAP and UCH-L1 selected were representative of a low positive concentration (low-positive defined as 1.5 – 2x the decision level), for each assay, to provide insight into the potential for the cross-reactant substance to affect medical decisions. Each potential cross-reacting substance was tested at a concentration that corresponds to its highest reported physiological level reported in circulation according to literature, as applicable, indicated in the table below, and as per DEN170045. The effect of each potential cross-reactant was evaluated by comparing the test results from a control sample, spiked with the appropriate solvent used to create the stock solution of the potential cross-reactant, with the test results from a sample spiked with the potentially cross-reacting substance as per CLSI guideline EP07 Ed3. Each test and control sample were evaluated with a similar number of replicates, with at least 20 replicates evaluated for each potentially cross-reacting substance.

Results as summarized in the table below demonstrate that none of the potentially cross-reacting substances tested were found to interfere with the GFAP and UCH-L1 assays.

Potential Cross-Reactant	Test Concentration (pg/mL)[A]	N	Mean (pg/mL)	SD (pg/mL)
GFAP Assay				
Desmin	127,000[1]	89	99.5	6.15
Internexin	77,000[B]	20	93.4	5.25
Keratin type II	10,000[2]	118	102.5	11.47
Neurofilament light	68[3]	35	92.9	6.73
Neurofilament medium	8,600[4]	30	91.4	5.35
Neurofilament heavy	77,000[5]	30	95.1	4.15

Peripherin	5000	20	94.9	4.39
Vimentin	354,000[6]	74	94.9	11.93
UCH-L1 Assay				
UCH-L3	354,000[B]	20	547.3	29.69

[A] In alignment with DEN170045, concentrations of all potential cross-reactants (except for internexin and UCH-L3) are based on the highest concentration of each protein in circulation as reported in: [1]Ma *et al*. (2009) *Mol Cell Proteomics* 8.8:1878, [2]Sundstrom *et al.* (1990) *Int J Cancer* 46:604, [3]Giottino *et al*. (2013) *PLOS One* 8: e75091, [4]Martinez-Morillo *et al*. (2015) *Clin Chem Lab Med* 53:1575, [5]Lu *et al.* (2015) *J Neurol Neurosurg Psychiatry* 86:565, [6]Sun *et al.* (2010) *J Proteome Research* 9:1923.

[B] Concentration as tested in DEN170045

c. *Cross-talk*

The study was conducted to evaluate the GFAP and UCH-L1 assays within the i-STAT TBI cartridge on the i-STAT Alinity instrument for potential cross-talk. Cross-talk is the potential for high levels of the antigen (GFAP or UCH-L1) of one assay to impact the result of the other assay. The study was conducted using one lot of i-STAT TBI cartridges, venous whole blood collected in K_2EDTA anticoagulant from healthy donors and spiked to low and high GFAP and UCH-L1 levels, and multiple i-STAT Alinity instruments. Two levels (low positive and moderate positive) for both GFAP (98–130 pg/mL and 585–715 pg/mL) and UCH-L1 (540–720 pg/mL and 1440–2160 pg/mL) were tested in the absence (control sample) and presence (test sample) of a single high-level concentration of the other antigen (at 100,000 pg/mL) for potential crosstalk. The potential for cross-talk was evaluated by comparing the result from the control sample with the result from the corresponding test sample containing the high level of the other antigen. Cross-talk is a subset of cross-reactivity and, therefore, % cross-talk is based on the measure of % cross-reactivity described in CLSI guideline EP07 Ed3. Results summarized in the table below show no cross-talk effect was observed between the GFAP and UCH-L1 antigens.

Sample Level Tested	Control or Test	Antigen Tested	Antigen Test Concentration (pg/mL)	N	Mean (pg/mL)	SD
GFAP Assay						
GFAP Low Positive	Control	N/A	N/A	10	98.5	3.6
	Test	UCH-L1	100,000	8	120.4	13.7
GFAP Moderate Positive	Control	N/A	N/A	10	632.2	14.4
	Test	UCH-L1	100,00	8	624.3	23.4
UCH-L1 Assay						
UCH-L1 Low Positive	Control	N/A	N/A	10	629.3	34.2
	Test	GFAP	100,000	10	613.6	37.0
UCH-L1 Moderate Positive	Control	N/A	N/A	10	1837.6	116.0
	Test	GFAP	100,000	10	1897.3	67.7

4. Assay Reportable Range:

The reportable range for GFAP is 65 – 10,000 pg/mL
The reportable range for UCH-L1 is 360 – 3,200 pg/mL.

Assay results are preceded by the symbol for greater than (>) or less than (<) if the result is outside of the reportable range.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   a. Traceability: There are no internationally recognized standard reference materials available for GFAP or UCH-L1. The traceability of the GFAP and UCH-L1 assays within the i-STAT TBI cartridge has been established against reference material created using recombinant GFAP and UCH-L1 antigens that are expressed in and purified from Escherichia coli. The reference materials are divided into aliquots and stored frozen at -80°C.

   b. i-STAT Cartridge Stability: A real-time shelf-life stability study performed on three lots of i-STAT TBI cartridges support shelf-life claims of 6 months when stored at refrigerated temperatures (2–8°C) and 14-days when stored at room temperature (18–30°C).

   c. Sample Stability and Storage: In order to evaluate fresh venous whole blood stability, samples targeting three levels of GFAP and UCH-L1 that covered the reportable ranges of the antigens were evaluated. Venous whole blood collected in K_2EDTA from 27 apparently healthy donors were each spiked with antigen (from either pooled TBI patient samples or spinal cord lysate) to the three targeted concentration levels of GFAP and UCH-L1, thereby generating a total of 30 samples total or 10 unique samples for each GFAP and UCH-L1 level. The results for the GFAP and UCH-L1 assays in the i-STAT TBI cartridge when tested on the i-STAT Alinity System demonstrated that whole blood samples collected with EDTA anticoagulant are stable up to 60 minutes at room temperature (18–30°C).

6. Detection Limit:

   The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) of the GFAP and UCH-L1 assays in the i-STAT TBI cartridge on the i-STAT Alinity System were determined based on CLSI EP17-A2.

   For LoB, the study was conducted over four days, using three cartridge lots and fresh whole blood samples from 16 apparently healthy donors altered to achieve blank GFAP and UCH-L1 levels. The blank sample results were evaluated for each cartridge lot, and the final LoB was determined as the greater of the LoB values obtained for each of the three cartridge lots.

   For LoD, the study was conducted over four days, using three cartridge lots and fresh whole blood samples from 18 apparently healthy donors altered to achieve low GFAP and UCH-L1 levels. The low-level sample results were evaluated for each cartridge lot, and the final LoD was determined as the greater of the LoD values obtained for each of the three cartridge lots.

   For LoQ, the study was conducted over six days, using three cartridge lots and fresh whole blood from 12 apparently healthy donors altered to achieve 11 low-level samples of GFAP and 12 low level samples of UCH-L1. The low-level sample results were evaluated for each cartridge lot, and the final LoQ were determined as the greater of the LoQ results obtained for each of the three cartridge lots at a precision limit of 20 %CV.

The result for the analytical sensitivity is presented in the table below:

Assay	LoB (pg/mL)	LoD (pg/mL)	LoQ (pg/mL)	Claimed LoQ (pg/mL)	Lower Limit of Reportable Range (pg/mL)
GFAP	13	27	47	47	65
UCH-L1	0	32	32*	87	360

* The estimated LoQ for the UCH-L1 assay of 30 pg/mL was lower than the estimated LoD. Due to this, the recommended reportable LoQ for UCH-L1 assay will be the same as the LoD estimate of 32 pg/mL as per CLSI EP17-A2. The linearity study results support LoQ for UCH-L1 to be 87 pg/mL.

7. Assay Cut-Off:

The cut-off for GFAP: 65 pg/mL
The cut-off for UCH-L1: 360 pg/mL

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable

2. Matrix Comparison:

i. Plasma versus Whole Blood

The matrix equivalency between plasma and whole blood samples collected with K_2EDTA (dipotassium ethylenediaminetetraacetic acid) anticoagulant was evaluated with the GFAP and UCH-L1 assays in the i-STAT TBI cartridge on the i-STAT Alinity System according to the study design recommended in CLSI EP35. The study was conducted using three lots of i-STAT TBI cartridges and paired whole blood (primary specimen type) and plasma (candidate specimen type) samples with K_2EDTA anticoagulant representing levels across the reportable range of the GFAP and UCH-L1 assays. Venous whole blood was collected without anticoagulant and used to prepare samples for each assay (GFAP and UCH-L1) across the reportable range. Each donor specimen was used to prepare samples at five concentration levels of GFAP and five concentration levels of UCH-L1 across the reportable range to generate a total of 67 paired specimens for GFAP and 70 paired specimens for UCH-L1. The prepared whole blood samples were dispensed into K_2EDTA tubes, split for whole blood sample (primary specimen type) testing, and centrifuged to separate plasma samples (candidate specimen type) for testing. Each paired sample was tested in duplicate on the same day and the first valid replicate GFAP or UCH-L1 result of the plasma sample was compared to the first valid replicate GFAP or UCH-L1 result of the whole blood sample by Passing-Bablok regression. The results are summarized in the table below.

Assay	Whole Blood Range (pg/mL)	Plasma Range (pg/mL)	N	Correlation Coefficient (95% CI)	Slope (95% CI)	Intercept (95% CI)
GFAP	47– 9171	47 – 8426	67	0.99 (0.99; 1.00)	0.94 (0.92; 0.98)	-1.38 (-8.79; 4.13)
UCH-L1	153 –3045	156-3192	67	0.97 (0.95; 0.98)	1.07 (1.04; 1.12)	-9.83 (-48.62; 13.46)

ii. EDTA Tube Type Equivalence (K_2EDTA vs K_3EDTA)

The matrix equivalency between whole blood samples collected with K_2EDTA (dipotassium ethylenediaminetetraacetic acid) and K_3EDTA (tripotassium ethylenediaminetetraacetic acid) anticoagulants was evaluated with the GFAP and UCH-L1 assays in the i-STAT TBI cartridge on the i-STAT Alinity System. The study was conducted using three lots of i-STAT TBI cartridges and paired whole blood samples in K_2EDTA (primary specimen type) and K_3EDTA (candidate specimen type) representing levels across the reportable range of the GFAP and UCH-L1 assays. Venous whole blood was collected without anticoagulant and used to prepare samples for each assay (GFAP and UCH-L1) across the reportable range to generate a total of 67 paired specimens for GFAP and 70 paired specimens for UCH-L1. The prepared whole blood samples were transferred to tubes containing K_2EDTA and K_3EDTA anticoagulant. Each paired sample was tested in duplicate on the same day and the first valid replicate GFAP or UCH-L1 result of K_2EDTA whole blood sample was compared to the first valid replicate GFAP or UCH-L1 result of the K_3EDTA whole blood sample by Passing-Bablok regression. The slope, intercept, correlation coefficient (r), and their respective 95% confidence intervals for both assays are summarized in the table below.

Assay	K_2EDTA Range (pg/mL)	K_3EDTA Range (pg/mL)	N	Correlation Coefficient (95% CI)	Slope (95% CI)	Intercept (95% CI)
GFAP	47–9171	37–8728	67	1.00 (0.99;1.00)	0.99 (0.97;1.01)	-1.32 (-6.19; 5.53)
UCH-L1	153–3045	159–3037	67	0.99 (0.98;0.99)	0.98 (0.94;1.01)	16.64 (-9.52;41.73)

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity:

A prospective, multi-center, observational study was conducted to evaluate the clinical performance of the i-STAT TBI cartridge in classifying intended use population subjects with suspected mild TBI for the likely absence of acute intracranial lesions visualized by a head CT scan. Venous whole blood specimens were collected in EDTA within 24 hours of the head injury from prospectively enrolled subjects, 18 years of age or older, who had experienced a head injury and presented to the health care facility or the emergency department (ED) with suspected mild TBI, with a GCS score of 13–15; and who had a head CT scan ordered as part of their standard of clinical care. Each specimen was tested for GFAP and UCH-L1 using two i-STAT TBI cartridges and two i-STAT Alinity instruments. Testing was performed at 20 external point of care clinical sites across the United States. CT

scans were performed in accordance with the clinical site's standard of care. Images were transmitted to a central data capture system. Images were interpreted by at least two neuroradiologists who were masked to other clinical and laboratory data; procedures for scoring images were established before conducting image review. The clinical outcome was based on the consensus interpretation of a subject's CT scan between two neurologists. Outcomes were classified as positive or negative as defined by the presence or absence of acute traumatic intracranial lesions, respectively. Acute intracranial lesion was defined as any trauma induced or related finding visualized upon head CT scan. A total of 970 specimens were included in the study. The demographic characteristics of the enrolled subjects represented in the performance analysis are summarized in the table below.

Demographic Characteristics	Head CT Scan Results		Total
	Positive	Negative	
N	283	687	970
Age (Years)			
Mean	51.1	45.0	46.8
Median	52.0	42.0	46.0
Standard Deviation	19.7	18.9	19.3
Range	18 – 96	18 – 97	18 – 97
Gender, N (%)			
Male	187 (66.1%)	434 (63.2%)	621 (64.0%)
Female	94 (33.2%)	252 (36.7%)	346 (35.7%)
Unspecified/ Not Reported	2 (0.7%)	1 (0.1%)	3 (0.3%)
Race, N (%)			
White	224 (79.2%)	441 (64.2%)	665 (68.6%)
Black or African American	20 (7.1%)	152 (22.1%)	172 (17.7%)
Asian	11 (3.9%)	38 (5.5%)	49 (5.1%)
Native Hawaiian/Pacific Islander	4 (1.4%)	6 (0.9%)	10 (1.0%)
American Indian or Alaska Native	4 (1.4%)	8 (1.2%)	12 (1.2%)
Asian, White	2 (0.7%)	3 (0.4%)	5 (0.5%)
Asian, Black or African American	0 (0.0%)	1 (0.1%)	1 (0.1%)
Black or African American, American Indian or Alaska Native	0 (0.0%)	2 (0.3%)	2 (0.2%)
White, Black or African American	0 (0.0%)	5 (0.7%)	5 (0.5%)
Not Reported	10 (3.5%)	19 (2.8%)	29 (3.0%)
Unknown	8 (2.8%)	12 (1.7%)	20 (2.1%)
Ethnicity, N (%)			
Hispanic or Latino	67 (23.7%)	120 (17.5%)	187 (19.3%)
Not Hispanic or Latino	209 (73.9%)	552 (80.3%)	761 (78.5%)
Unknown	6 (2.1%)	6 (0.9%)	12 (1.2%)
Not Reported	1 (0.4%)	9 (1.3%)	10 (1.0%)

The head injury characteristics were collected for all evaluable subjects and summarized in the table below. The mean time from head injury to blood draw was 10.4 hours. Most

subjects had a GCS score of 15 (176/283 or 62.2% in CT scan-positive subjects and 586/1687 or 85.3% in CT scan-negative subjects). The percentage of subjects with GCS scores of 13 and 14 were higher in the CT scan-positive subjects compared to the CT scan-negative subjects. Information regarding time from head injury to exam, head injury to CT scan, and head injury to blood draw, as well as GCS, neurological assessment and physical evidence of trauma, categorized by head CT scan results, are shown in table below.

Head Injury Characteristics	Head CT Scan Results		Total
	Positive	Negative	
N	283	687	970
Time from head injury to Initial Assessment (hours)*			
Mean	2.0	1.3	1.5
Median	1.0	0.8	0.9
Standard Deviation	2.01	1.45	1.67
Range	1.0 – 10.2	0.8 – 10.0	0.8 – 10.2
Time from head injury to CT scan (hours)			
Mean	2.6	2.5	2.6
Median	1.7	2.0	1.9
Standard Deviation	2.37	1.80	1.98
Range	0.2 – 11.4	0.3 – 10.7	0.2 – 11.4
Time from head injury to blood draw (hours)			
Mean	14.5	8.8	10.4
Median	13.5	5.8	8.1
Standard Deviation	6.65	6.43	6.99
Range	2.0 – 24.0	1.5 – 24.0	1.5 – 24.0
Glasgow Coma Score – N (%)			
13	28 (9.9%)	11 (1.6%)	39 (4.0%)
14	79 (27.9%)	90 (13.1%)	169 (17.4%)
15	176 (62.2%)	586 (85.3%)	762 (78.6%)
Neurological assessment - N (%) of subjects experiencing			
Loss of Consciousness (LOC)	225 (79.5%)	450 (65.5%)	675 (69.6%)
Confusion/Alteration of Consciousness (AOC)	195 (68.9%)	504 (73.4%)	699 (72.1%)
Vomiting	24 (8.5%)	21 (3.1%)	45 (4.6%)
Post Traumatic Amnesia (PTA)	196 (69.3%)	409 (59.5%)	605 (62.4%)
Post Traumatic Seizures	3 (1.1%)	0 (0.0%)	3 (0.3%)
Subjects with Drug Intoxication at the Time of Presentation to Facility	48 (17.0%)	66 (9.6%)	114 (11.8%)
Subjects with Alcohol Intoxication at the Time of Presentation to Facility	49 (17.3%)	61 (8.9%)	110 (11.3%)
Mechanism of Injury - N (%) of subjects affected**			
Acceleration/Deceleration	68 (24.0%)	221 (32.2%)	289 (29.8%)
Direct Impact (blow to head)	44 (15.5%)	85 (12.4%)	129 (13.3%)
Direct Impact (head against object)	157 (55.5%)	437 (63.6%)	594 (61.2%)
Crush	0 (0.0%)	3 (0.4%)	3 (0.3%)
Blast	0 (0.0%)	1 (0.1%)	1 (0.1%)
Ground level fall	82 (29.0%)	170 (24.7%)	252 (26.0%)
Fall from Height > 1 meter (3 feet)	39 (13.8%)	79 (11.5%)	118 (12.2%)
Other	15 (2.2%)	7 (2.5%)	22 (2.3%)

Head Injury Characteristics	Head CT Scan Results		Total
	Positive	Negative	
Physical Evidence* -N (%) of subjects with:**			
Visible Trauma above Clavicle	214 (75.6%)	422 (61.4%)	636 (65.6%)
Signs of Basal Skull Fracture	37 (13.1%)	7 (1.0%)	44 (4.5%)
Head CT Scan Findings** - N (%)**			
Acute Skull Fracture	106 (37.5%)	16 (2.3)	122 (12.6%)
Subdural Hematoma	177 (62.5%)	0 (0.0%)	177 (18.2%)
Subdural Hematoma Mixed Density*****	15 (5.3%)	2 (0.3%)	17 (1.8%)
Subarachnoid Hemorrhage	218 (77.0%)	0 (0.0%)	218 (22.5%)
Contusion	117 (41.3%)	0 (0.0%)	117 (12.1%)
Intracerebral Hemorrhage	3 (1.1%)	0 (0.0%)	3 (0.3%)
Epidural Hematoma	41 (14.5)	0 (0.0%)	41 (4.2%)
Traumatic Axonal Injury	26 (9.2%)	0 (0.0%)	26 (2.7%)
Diffuse Axonal Injury	10 (3.5%)	0 (0.0%)	10 (1.0%)
Intraventricular Hemorrhage	25 (8.8%)	0 (0.0%)	25 (2.6%)
Edema	11 (3.9%)	0 (0.0%)	11 (1.1%)
Brain Swelling	15 (5.3%)	0 (0.0%)	15 (1.5%)
Neurosurgical Lesion******	14 (4.9%)	0 (0.0%)	14 (1.4%)

* Based on time subject arrived at the study hospital for neurological assessments.

** A subject could have experienced head injury due to multiple mechanisms of injury. No subjects experienced head injury due to gunshot or fragment (including shell/shrapnel).

*** Prior to head CT scan.

**** Head CT scan findings confirmed by at least one neuroradiologist

***** Subdural Hematoma Mixed Density can be acute (i.e., acute-on-chronic Subdural hematoma) or chronic in nature. A chronic subdural hematoma can be present in a CT scan 'negative' for acute intracranial lesions

****** A Marshall CT Classification of "a High or mixed density lesion >25 mL not evacuated" indicated the presence of a neurosurgical lesion.

To estimate clinical performance characteristics, the adjudicated head CT scan classification for each evaluable subject and the first valid i-STAT TBI test interpretation obtained from blood drawn within 0–24 hours of injury were used to establish clinical performance. The results are summarized in the 2x2 table below.

i-STAT TBI Test Interpretation	All Evaluable Results (0-24h)		Total
	Adjudicated Head CT Scan Positive	Adjudicated Head CT Scan Negative	
Elevated	273	410	683
Not Elevated	10	277	287
Total	283	687	970
Clinical Performance Parameters			**95% CI**
Clinical Sensitivity		96.5%	93.6%; 98.1%
Clinical Specificity		40.3%	36.7%; 44.0%
Negative Predictive Value (NPV) *		96.5%	93.7%; 98.1%
Positive Predictive Value (PPV)^		40.0%	38.4%; 41.5%
Likelihood Ratio Negative (LRN)		0.09	0.05; 0.16
Likelihood Ratio Positive (LRP)		1.62	1.52; 1.73
Prevalence		29.2%	

* Adjusted NPV for 6% CT scan positive prevalence rate (DEN170045): 99.4% (95% CI: 99.0%, 99.7%).

^Adjusted PPV for 6% CT scan positive prevalence rate (DEN170045): 9.4% (95% CI: 8.8%, 9.9%).

Of the 283 subjects with CT scan positive results, 10 subjects had an i-STAT TBI test interpretation that was 'Not Elevated'. The rate of false negative (FN) results was 3.5% (10/283). None of these 10 subjects with false negative results required surgical intervention related to their head injury as no neurosurgical lesions were identified by CT scan in these subjects. Fourteen (14) subjects were identified with a lesion requiring neurosurgical intervention and all these subjects were correctly classified as elevated (true positive) with i-STAT TBI test interpretation. Of the 687 subjects associated with negative CT scan results, the rate of false positive (FP) results by the i-STAT TBI was 59.6% (410/687). Of these, 277 subjects were associated with negative CT scan results. The negative predictive value (NPV) of the assay was 96.5% (277/287) with prevalence of 29.2%. The potential benefit of the assay would be a reduction in unnecessary CT scans by 40.3% (277 of 687 subjects had true negative assay results). The positive predictive value (PPV) of the assay was 40% (273/683). Adjusted NPV and PPV based on 6% CT scan positive prevalence rate are 99.4% (95% CI: 99.0%; 99.7%) and 9.4% (95% CI: 8.8%; 9.9%), showing the equivalent clinical performance of the i-STAT TBI cartridge to the predicate device.

Analyses of assay performance by gender and time from injury relative to blood draw are shown in the table below. The adjudicated head CT scan classification for each evaluable subject and the first valid i-STAT TBI test interpretation obtained from blood drawn within 0–24 hours of injury were used to establish clinical performance. If results for time point T2 were not available for a subject, then the results from the blood draw within 12 hours of injury (T1) were used in lieu of the T2 result. Of the 970 subjects, the T2 result was used for 350 subjects and the T1 result was used for the remaining 620 subjects.

	Sensitivity N (%) (95% CI)	**Specificity N (%) (95% CI)**	**PPV N (%) (95% CI)**	**NPV N (%) (95% CI)**
All Evaluable Subjects N=970	273/283 (96.5%) (93.6;98.1)	277/687 (40.3%) (36.7;44.0)	273/683 (40.0%) (38.4;41.5)	277/287 (96.5%) (93.7;98.1)
Gender				
Male N=621 (64.0%)	184/188 (97.9%) (94.7;99.2)	159/433 (36.7%) (32.3; 41.4)	184/458 (40.2%) (38.4; 42.0)	159/163 (97.5%) (93.7;99.1)
Female N=349 (36.0%)	89/95 (93.7%) (86.9;97.1)	118/254 (46.5%) (40.4;52.6)	89/225 (39.6%) (36.6;42.6)	118/124 (95.2%) (90.0;97.7)
Unspecified/ Not Reported* N=3	2/2 (100.0%) (34.2;100.0)	1/1 (100.0%) (20.7;100.0)	2/2 (100.0%) N/A	1/1 (100.0%) N/A
Age				
<65 N=766 (79.0%)	192/198 (97.0%) (93.5;98.6)	245/568 (43.1%) (39.1;47.2)	192/515 (37.3%) (35.5;39.1)	245/251 (97.6%) (94.9;98.9)
≥65	81/85	32/119	81/168	32/36

	Sensitivity N (%) (95% CI)	Specificity N (%) (95% CI)	PPV N (%) (95% CI)	NPV N (%) (95% CI)
N=204 (21.0%)	(95.3%) (88.5;98.2)	(26.9%) (19.7;35.5)	(48.2%) (45.3;51.2)	(88.9%) (74.6;95.6)
Time from injury to blood draw (T1) ^				
0-6 hours N=528	89/98 (90.8%) (83.5; 95.1)	189/430 (44.0%) (39.3;48.7)	89/330 (27.0%) (25.0; 29.1)	189/198 (95.5%) (91.8;97.5)
>6-12 hours N=412	171/173 (98.8%) (95.9; 99.7)	88/239 (36.8%) (31.0; 43.1)	171/322 (53.1%) (50.7; 55.5)	88/90 (97.8%) (91.7; 99.4)
0-12 hours N=938	259/270 (95.9%) (92.9; 97.7)	277/668 (41.5%) (37.8;45.2)	259/650 (39.8%) (38.2;41.5)	277/288 (96.2%) (93.3; 97.8)
Time from injury to blood draw (T2) ^				
>12-18 hours N=157	77/77 (100.0%) (95.2; 100.0)	23/80 (28.8%) (20.0;39.5)	77/134 (57.5%) (54.0;60.8)	23/23 (100.0%) (86.2;100.0)
>18-24 hours N=191	94/98 (95.9%) (90.0;98.4)	24/93 (25.8%) (18.0; 35.5)	94/163 (57.7%) (54.6; 60.7)	24/28 (85.7%) (68.4; 94.3)
>12-24 hours N=350	172/176 (97.7%) (94.3;99.1)	47/174 (27.0%) (21.0;34.1)	172/299 (57.5%) (55.2;59.8)	47/51 (92.2%) (81.2;97.0)

* For 'Gender Unspecified/ Not Reported': Because of the small sample size of this group, some of the values were 'not calculable' and were reported as N/A.

^ For sections "Time from injury to blood draw (T1)" and "Time from injury to blood draw (T2)", each timeframe incorporated the first valid result from all specimens collected in that timeframe, and each subject result was represented only once per timeframe. Since several subjects had both T1 and T2 specimen results, the sum of the subjects represented in these timeframes will be greater than the total number of evaluable subjects (N=970).

The data showed little variation in NPV and PPV between males and females and with increasing time from injury, indicating that gender differences and differences between head injury characteristics did not translate into statistically significant differences in assay performance.

D Clinical Cut-Off:

Refer to Assay Cut-Off.

E Reference Range:

The expected values from 150 self-declared apparently healthy donors ranging in age from 18 to 79 (with mean age of 45.6 years) in the U.S. population who did not have acute injury to the head (or history of neurological disease or disorder, neurosurgery, or motor vehicle accident or injury requiring medical attention for head/neck/spine within the last one year) were determined

in accordance with CSLI guideline EP28-A3c. Data analysis was performed separately for the GFAP and UCH-L1 assays in the i-STAT TBI Cartridge on the i-STAT Alinity System. The 2.5th and 97.5th percentiles (95% reference interval) and their respective 95% confidence intervals for both biomarkers were calculated non-parametrically. The results are summarized in the table below.

Assay	N	Reference Interval (2.5th to 97.5th percentile)
GFAP	150	< 47 – 53 pg/mL
UCH-L1	150	< 87 – 251 pg/mL

No subjects in the reference interval study were elevated for both GFAP and UCH-L1. One subject had an elevated GFAP result, and no subjects had an elevated UCH-L1 result for the data included in the 95% reference interval determination. There is little to no effect of age on the biomarker positivity.

F Other Supporting Studies

i. Temperature Operating Range Study

The performance of the GFAP and UCH-L1 assays in the i-STAT TBI cartridge using i-STAT Alinity System across its temperature operating range (16.0°C to 30.0°C) was evaluated. Whole blood samples from six apparently healthy donors were altered to target three GFAP (cut-off, moderate-positive, and high-range) and three UCH-L1 (cut-off, low -positive, and high-range) levels across the reportable range of each respective assay. Testing was performed in a controlled temperature chamber below (16.0°C) and above (30.0°C) room temperature (referred to as test condition) alongside testing in a location at controlled room temperature (21.0–25.0°C) (referred to as control condition). The GFAP and UCH-L1 assays in the i-STAT TBI cartridge with the i-STAT Alinity System demonstrated acceptable performance across the operating temperature range of 16.0°C to 30.0°C for all GFAP and UCH-L1 levels tested.

ii. Cleaning Robustness

The robustness of the GFAP and UCH-L1 assays (amperometric sensors) to cleaning and disinfection was previously demonstrated using the predicate i-STAT TBI Plasma cartridge (K201778). Based on commonality in design features to the predicate device, the previously provided studies also support the robustness of subject i-STAT TBI cartridge.

iii. Altitude Effect

The performance of the GFAP and UCH-L1 assays in the i-STAT TBI cartridge on the i-STAT *Alinity System* at an altitude of approximately 7,500 feet (ft) above sea level using whole blood specimens was evaluated across the reportable range of each assay. A barometric chamber was used to simulate an altitude of approximately 7,500 feet above sea level (target altitude as the test condition) and outside the chamber was used for sea level (approximately 403 feet elevation for Dallas, Texas) (as the control condition). Based on guidance from EP09c, a Passing-Bablok regression analysis and a predicted bias evaluation at the assay cut-offs between the two conditions were performed. The results of the study for the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge demonstrate equivalent performance between sea level (control condition) and at an altitude of approximately 7,500 feet (ft) above sea level (test condition) when evaluated with the *i-STAT Alinity System*.

iv. Tilting Study

The purpose of this study was to evaluate the robustness of the GFAP and UCH-L1 assays in the i-STAT TBI cartridge on the i-STAT Alinity System when the instrument is placed at a non-level angle during a cartridge test cycle. The study was conducted using one lot of i-STAT TBI cartridges and 30 i-STAT Alinity instruments. A whole blood sample from one donor was collected in K_2EDTA anticoagulant and altered to target three GFAP and UCH-L1 levels across the reportable range of each assay. Each sample was evaluated at five tilt angles, with a level surface 0° as a control condition and -31°, -14°, 14°, and 31° as test conditions. The instruments were tilted by inclining (screen angled towards sky) or declining (screen angled towards ground) along the longitudinal axis. The impact of instrument tilt angle during testing was assessed by comparing the GFAP and UCH-L1 results collected at the tilt angles (test conditions) to the results collected on a level surface (control condition). The results demonstrated the robustness of the fail-safe mechanism (quality check failure/QCF) that prevents erroneous results for the GFAP and UCH-L1 assays from being reported when the i-STAT TBI cartridge is tested on the i-STAT Alinity System at tilt angles of -31°, -14°, 14°, and 31°.

v. Vibration Study

The purpose of this study was to evaluate the robustness of the GFAP and UCH-L1 assays in the i-STAT TBI cartridge on the i-STAT Alinity System when the instrument is exposed to vibration during a cartridge test cycle. The study was conducted using one lot of i-STAT TBI cartridges and six i-STAT Alinity instruments and a panel of frozen spiked plasma samples consisting of three levels of GFAP and UCH-L1 across the reportable range for each assay. Each sample was evaluated at three vibration levels, with no vibration as a control condition and 4.8 mm/s and 15 mm/s as test conditions. The impact of vibration during testing was assessed by comparing the GFAP and UCH-L1 results collected in the presence of vibration (test conditions) to the results collected with no vibration (control condition). The results of the study demonstrated that the GFAP and UCH-L1 assays in the i-STAT TBI cartridge when tested on the i-STAT Alinity System are insensitive to vibration up to 15 mm/s across the range of GFAP and UCH-L1 levels tested.

vi. Hematocrit Sensitivity

The effect of hematocrit on the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge was assessed across a hematocrit (HCT) range of 15–60% packed cell volume (PCV). The study was conducted using two lots of i-STAT TBI cartridges and i-STAT Alinity instruments. Whole blood samples from six donors were altered to target three GFAP and UCH-L1 levels (low, moderate, and high) across the reportable range for each respective assay. Each sample was evaluated at three HCT levels, with the nominal HCT level as control condition and low and high HCT levels as test conditions. The HCT sensitivity at each GFAP and UCH-L1 level was assessed by comparing the results at the low and high HCT levels (test conditions) to the nominal HCT level (control condition). Imprecision (%CV) and bias exceeding 10% were observed for low level GFAP samples with hematocrit levels above 56% PCV.

VIII Proposed Labeling:

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

IX Conclusion:

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