510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K201778

B Applicant

Abbott Laboratories

C Proprietary and Established Names

i-STAT TBI Plasma 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:

Clearance of a new device

B Measurand:

Ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP)

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 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 semiquantitative 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.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For clinical laboratory setting

D Special Instrument Requirements:

i-STAT Alinity

IV Device/System Characteristics:

A Device Description:

The i-STAT TBI Plasma cartridge is a multiplex immunoassay that contains assays for both ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein (GFAP). The assays test for the presence of these biomarkers in a plasma sample and yield a semi-quantitative test interpretation based on measurements of both UCH-L1 and GFAP in approximately 15 minutes. The i-STAT TBI Plasma cartridge is designed to be run only on the i-STAT Alinity instrument, previously cleared under K153357.

The i-STAT TBI Plasma cartridge is a single use test cartridge. The cartridge contains a biosensor chip, all reagents, a sample chamber, a waste chamber, an air bladder and the fluid conduits, vents, and valve elements required to execute the test cycle. Reagents included in the cartridge are buffer and preservative, as well as anti-GFAP/alkaline phosphatase and anti-UCH-

L1/alkaline phosphatase antibody conjugates for labeling/detection, and anti-GFAP and anti-UCH-L1 monoclonal capture antibodies. All fluid movements (test sample or reagent) are automatically controlled by the i-STAT Alinity instrument by electro-mechanical interaction with the cartridge.

B Principle of Operation:

The i-STAT TBI Plasma 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 plasma 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. 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 (UCH-L1/GFAP) immobilized on the sensor. This electrical signal is converted into a quantitative measurement of the plasma concentration of UCH-L1 and GFAP reported in units of pg/mL.

The i-STAT TBI Plasma cartridge is designed to be run only on the i-STAT Alinity instrument. The i-STAT Alinity instrument (previously cleared under K153357) is a handheld, in vitro diagnostic device designed to run only i-STAT test cartridges. 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.

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 plasma 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 30 pg/mL for GFAP and 360 pg/mL for UCH-L1.

GFAP Assay Result (relative to 30 pg/mL cut-off)	UCH-L1 Assay Result (relative to 360 pg/mL cut-off)	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	***†	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):

Banyan BTI

B Predicate 510(k) Number(s):

DEN170045

C Comparison with Predicate(s):

Device & Predicate Device(s):	K201778	DEN170045
Device Trade Name	i-STAT TBI Plasma Cartridge with the i-STAT Alinity System	Banyan BTI
General Device		

Characteristic Similarities		
Intended Use/Indications For Use	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.	The Banyan BTI is an in vitro diagnostic chemiluminescent enzyme-linked immunosorbent assay (ELISA). The assay provides a semi-quantitative measurement of the concentrations of ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) in human serum and is used with the Synergy 2 Multi-mode Reader. The assay results obtained from serum collected within 12 hours of suspected head injury are used, along with other available clinical information, to aid in the evaluation of patients 18 years of age and older with suspected traumatic brain injury (Glasgow Coma Scale score 13-15). A negative assay result is associated with the absence of acute intracranial lesions visualized on a head CT (computed tomography) scan.
Intended Use Setting	Clinical laboratory	Same
Measurands	GFAP and UCH-L1	Same
Assay technology	Enzyme-linked immunosorbent assay	Same
Reportable result	Quantitative results for GFAP and UCH-L1 with a semi-quantitative interpretation of results	Same

General Device Characteristic Differences		
Platform	i-STAT Alinity	Synergy 2 Multi-mode Reader, model SL (BioTek Instruments, Inc.)
Assay format	Single-use multiplex cartridge containing both GFAP and UCH-L1 assays	Separate test kits for GFAP and UCH-L1 run on separate 96-well plates
Detection technology	Electrochemical	Chemiluminescence
Specimen type	Plasma	Serum
Sample volume	20 µL/cartridge	GFAP kit: 150 µL UCH-L1 kit: 100 µL
Time to result	~15 minutes	~4 hours
Reportable range	GFAP: 30–10,000 pg/mL UCH-L1: 200–3200 pg/mL	GFAP: 10–320 pg/mL UCH-L1: 80–2560 pg/mL
GFAP cut-off	30 pg/mL	22 pg/mL
UCH-L1 cut-off	360 pg/mL	327 pg/mL

VI Standards/Guidance Documents Referenced:

21 CFR 866.5830 Special Controls for Brain trauma assessment test

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

CLSI EP06-A: 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 study designs and analyses for precision studies were based on the CLSI guideline EP05-A3. Samples in the test panel evaluated in each precision study were generated to cover the measuring range of the GFAP and UCH-L1 assays. 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 more closely mimic clinical specimens. Other samples evaluated in the study were spiked with recombinant GFAP and UCH-L1.

a. *Within-laboratory precision:*

Semi-quantitative precision: The purpose of this study was to evaluate within-laboratory precision of GFAP and UCH-L1 assays within the i-STAT TBI cartridge on the i-STAT Alinity instrument using plasma samples. Plasma samples consisted of nine plasma samples for GFAP and seven plasma samples for UCH-L1. Each panel member was tested at a single site for at least 20 days, by multiple operators, with two runs per day, two replicates per run, on two or three lots of i-STAT TBI cartridges, using multiple i-STAT Alinity instruments, to generate a total of 160 or 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 results are summarized in the tables below for the GFAP and UCH-L1 assays.

GFAP Assay												
Panel Member	N	Mean (pg/mL)	Repeatability		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
PLS8[A]	240	17.1	1.76	10.3	1.16	6.8	0.62	3.6	1.41	8.2	2.60	15.2
PLS1[B]	240	30.9	2.67	8.6	0.50	1.6	0.35	1.1	0.39	1.3	2.77	9.0
PLS2[B]	240	65.5	3.37	5.1	0.69	1.1	1.15	1.8	0.44	0.7	3.66	5.6
PLS9[A]	241*	104.7	3.94	3.8	1.82	1.7	0.00	0.0	1.72	1.6	4.67	4.5
PLS6[A]	240	964.2	24.75	2.6	16.41	1.7	17.83	1.8	21.05	2.2	40.35	4.2
PLS3[A]	160	2029.5	39.18	1.9	26.30	1.3	19.10	0.9	94.89	4.7	107.69	5.3
PLS7[A]	240	3139.5	75.98	2.4	35.92	1.1	49.34	1.6	97.09	3.1	137.57	4.4
PLS4[A]	161*	5707.1	155.36	2.7	59.87	1.0	61.31	1.1	177.85	3.1	251.22	4.4
PLS5[A]	160	7544.9	153.15	2.0	136.90	1.8	34.40	0.5	196.91	2.6	286.63	3.8
L1	241*	197.3	10.48	5.3	5.51	2.8	0.54	0.3	5.16	2.6	12.93	6.6
L2	242*	5153.8	236.89	4.6	94.93	1.8	28.10	0.5	183.00	3.6	315.29	6.1

[A] Pooled plasma from normal donors spiked with recombinant GFAP and UCH-L1 antigens
[B] Pooled plasma from normal donors spiked with antigens from pooled TBI patient plasma
* Additional GFAP result(s) was/were obtained due to cartridge re-run because of an UCH-L1 star-out

UCH-L1 Assay												
Panel Member	N	Mean (pg/mL)	Repeatability		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
PLS1[B]	240†	73.8	18.25	24.7	3.14	4.3	0.00	0.0	2.38	3.2	18.67	25.3
PLS1[B]	238‡	72.5	4.88	6.7	1.73	2.4	0.00	0.0	3.93	5.4	6.50	9.0
PLS2[B]	240	300.7	18.13	6.0	5.13	1.7	0.00	0.0	14.74	4.9	23.92	8.0
PLS8[A]	240	519.9	29.56	5.7	1.54	0.3	13.38	2.6	8.21	1.6	33.51	6.4
PLS6[A]	240	1059.2	58.82	5.6	28.23	2.7	17.49	1.7	33.14	3.1	75.24	7.1
PLS3[A]	159**	1639.6	91.57	5.6	8.72	0.5	15.74	1.0	28.46	1.7	97.56	6.0
PLS9[A]	240	2067.4	111.09	5.4	54.99	2.7	46.01	2.2	15.00	0.7	133.06	6.4
PLS7[A]	240	2846.7	148.04	5.2	105.16	3.7	20.9	0.7	2.05	0.1	182.80	6.4
L1	240	561.5	35.69	6.4	7.44	1.3	7.87	1.4	11.14	2.0	38.93	6.9
L2	240	1624.7	90.14	5.5	53.68	3.3	0.00	0.0	32.25	2.0	109.76	6.8

[A] Pooled plasma from normal donors spiked with recombinant GFAP and UCH-L1 antigens
[B] Pooled plasma from normal donors spiked with antigens from pooled TBI patient plasma
** One result was unavailable because of an UCH-L1 star-out
†Two outliers identified in data set; analysis with outliers included
‡Two outliers removed from analysis

Qualitative precision: A total of 160 – 240 replicates of each panel member were tested across two or three lots of i-STAT TBI cartridges to evaluate qualitative precision. The panel members were identical to those in the 20-day within-laboratory semi-quantitative precision study above. The % correct call was calculated for each plasma sample and was based on the number of replicates providing the expected GFAP/UCH-L1 result (Not Elevated or Elevated) based on the mean antigen concentration for the sample (either below cut-off or at/above cut-off, respectively). Results are summarized in the tables below for each assay.

GFAP Assay				
			Qualitative Agreement	
Panel Member	Mean (pg/mL)	Total Number Replicates	Number of Elevated Results (At/Above Cut-off) / Total Replicates	%Correct Call
PLS8[A]	17.1	240	0/240	100
PLS1[B]	30.9	240	178/240	100‡
PLS2[C]	65.5	240	240/240	100
PLS9[C]	104.7	241*	241/241	100
PLS6[C]	964.2	240	240/240	100
PLS3[C]	2029.5	160	160/160	100
PLS7[C]	3139.5	240	240/240	100
PLS4[C]	5707.1	161*	161/161	100
PLS5[C]	7544.9	160	160/160	100
L1[C]	197.3	241*	241/241	100
L2[C]	5153.8	242*	242/242	100

[A] Not Elevated
[B] Near cut-off (mean ±25% of the cut-off value)
[C] Elevated
* Additional GFAP result(s) was/were obtained due to cartridge re-run because of an UCH-L1 star-out
‡ Replicates for sample with mean value near cut-off can have replicates below cut-off or at/above cut-off

UCH-L1 Assay				
			Qualitative Agreement	
Panel Member	Mean (pg/mL)	Total Number Replicates	Number of Elevated Results (At/Above Cut-off) / Total Replicates	%Correct Call
PLS1[A]	73.8	240	0/240	100
PLS2[B]	300.7	240	1/240	100‡
PLS8[C]	519.9	240	240/240	100
PLS6[C]	1059.2	240	240/240	100
PLS3[C]	1639.6	159**	159/159	100
PLS9[C]	2067.4	240	240/240	100
PLS7[C]	2846.7	240	240/240	100
L1[C]	561.5	240	239/240	99.6
L2[C]	1624.7	240	240/240	100

[A] Not Elevated
[B] Near cut-off (mean ±25% of the cut-off value)
[C] Elevated
** One result was unavailable because of an UCH-L1 star-out
‡ 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*

Semi-quantitative precision: The purpose of this study was to evaluate instrument-to-instrument precision of GFAP and UCH-L1 assays in the i-STAT TBI cartridge on the i-STAT Alinity instrument using plasma samples. Eight levels of GFAP and six UCH-L1 levels 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. Results are summarized in the tables below.

Assay	Panel Member	N†	Mean (pg/mL)	Within-run		Between-Instrument		Between-Day		Within-Laboratory	
				SD	% CV	SD	% CV	SD	% CV	SD	% CV
GFAP	PLS10[A]	75	19.5	1.91	9.8	0.38	1.9	1.62	8.3	2.54	13.0
	PLS1[B]	75	29.8	1.95	6.5	0.54	1.8	0.40	1.3	2.06	6.9
	PLS2[B]	75	63.1	3.60	5.7	0.79	1.3	1.29	2.0	3.90	6.2
	PLS9[A]	76*	102.5	4.28	4.2	0.91	0.9	2.35	2.3	4.97	4.8
	PLS6[A]	75	890.2	30.77	3.5	7.78	0.9	17.32	1.9	36.16	4.1
	PLS3[A]	75	2130.5	68.91	3.2	16.37	0.8	13.1	0.6	72.03	3.4
	PLS4[A]	75	5387.9	199.07	3.7	42.23	0.8	50.32	0.9	209.63	3.9
	PLS5[A]	74**	7158.1	269.82	3.8	60.98	0.9	103.92	1.5	295.50	4.1
UCH-L1	PLS1[B]	75	65.7	4.64	7.1	1.76	2.7	0.93	1.4	5.04	7.7
	PLS2[B]	75	274.9	16.31	5.9	4.47	1.6	5.81	2.1	17.88	6.5
	PLS10[A]	75	540.6	31.18	5.8	5.68	1.1	6.63	1.2	32.38	6.0
	PLS6[A]	75	1038.0	45.85	4.4	11.89	1.1	25.43	2.4	53.76	5.2
	PLS9[A]	75	1932.6	81.78	4.2	29.19	1.5	39.62	2.1	95.44	4.9
	PLS3[A]	75	2765.2	105.94	3.8	23.18	0.8	19.44	0.7	110.18	4.0

[A] Pooled plasma from normal donors spiked with recombinant GFAP and UCH-L1 antigens
[B] Pooled plasma from normal donors spiked with antigens from pooled TBI patient plasma
* Additional GFAP result(s) was/were obtained due to cartridge re-run because of an UCH-L1 star-out
** One result was unavailable because of a star-out
† All sample replicates were run by a single operator for samples PLS1, PLS2, PLS3, PLS4, and PLS5. Sample replicates were run by two operators for samples PLS10, PLS9, and PLS6.

Qualitative precision: A total of 75 replicates of each plasma sample were tested across three instruments to evaluate qualitative precision. The plasma samples were identical to those in the instrument-to-instrument semi-quantitative precision study above. The % correct call was calculated for each plasma sample and was based on the number of replicates providing the expected GFAP/UCH-L1 result (Not Elevated or Elevated) based on the mean antigen concentration for the sample (either below cut-off or at/above cut-off, respectively). Results are summarized in the table below for each assay.

Assay	Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
				Number of Elevated Results (At/Above Cut-off) / Total Replicates	%Correct Call
GFAP	PLS10[A]	19.5	75	0/75	100
	PLS1[B]	29.8	75	44/75	100‡
	PLS2[C]	63.1	75	75/75	100
	PLS9[C]	102.5	76*	76/76	100
	PLS6[C]	890.2	75	75/75	100
	PLS3[C]	2130.5	75	75/75	100
	PLS4[C]	5387.9	75	75/75	100
	PLS5[C]	7158.1	74**	74/74	100
UCH-L1	PLS1[A]	65.7	75	0/75	100
	PLS2[B]	274.9	75	0/75	100‡
	PLS10[C]	540.6	75	75/75	100
	PLS6[C]	1038.0	75	75/75	100
	PLS9[C]	1932.6	75	75/75	100
	PLS3[C]	2765.2	75	75/75	100

[A] Not Elevated
[B] Near cut-off (mean ±25% cut-off value)
[C] Elevated
* Additional GFAP result(s) was/were obtained due to cartridge re-run because of an UCH-L1 star-out

** One result was unavailable because of a star-out
‡ Replicates for sample with mean near cut-off can have replicates below cut-off or at/above cut-off

c. *Multi-site precision study*

Semi-quantitative precision: The purpose of this study was to evaluate precision of GFAP and UCH-L1 assays within the i-STAT TBI cartridge on the i-STAT Alinity instrument using plasma samples at three clinical sites. The medical facilities in the study were representative of the setting in which the i-STAT TBI Plasma 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 site, a panel of six GFAP samples and a panel of five UCH-L1 samples, were tested once per day for five days by two operators at each of the three sites, and three replicates per operator run, to generate a total of 90 replicates per panel member. Each operator ran each panel member on three i-STAT Alinity instruments. All panel members at all sites were evaluated using a single cartridge lot and each site evaluated the same panel members (which were provided to each site). The results are summarized in the tables below for the GFAP and UCH-L1 assays.

GFAP Assay														
Panel Member	N	Mean (pg/mL)	Within-Day$^{\delta}$		Between-Day		Between-Operator		Within-Site†		Between-Site		Overall‡	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
5[A]	90	19.4	2.6	13.4	0.0	0.0	0.0	0.0	2.6	13.4	2.9	14.9	3.9	20.0
9[B]	90	30.8	2.2	7.0	0.6	1.8	0.2	0.7	2.2	7.2	2.5	8.1	3.4	10.9
10[B]	90	66.2	3.0	4.5	0.9	1.3	0.0	0.0	3.1	4.7	4.0	6.0	5.1	7.6
8[A]	90	150.1	4.5	3.0	2.2	1.4	1.4	0.9	5.0	3.3	4.1	2.7	6.5	4.3
6[A]	90	4504.7	88.8	2.0	52.3	1.2	0.0	0.0	103.0	2.3	74.3	1.7	127.1	2.8
7[A]	90	9196.8	193.9	2.1	189.1	2.1	63.2	0.7	270.8	2.9	167.7	1.8	318.5	3.5

[A] Pooled plasma from normal donors spiked with recombinant GFAP and UCH-L1 antigens
[B] Pooled plasma from normal donors spiked with antigens from pooled TBI patient plasma
δ Within-day variability includes within-operator and between-operator variance components
† Within-site variability is the summation of within-day and between-day variance components
‡Overall variability is the summation of the between-site, within-day, and between-day variance components

UCH-L1 Assay														
Panel Member	N	Mean (pg/mL)	Within-Day$^{\delta}$		Between-Day		Between-Operator		Within-Site†		Between-Site		Overall‡	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
5[A]	90	60.5	6.0	10.0	1.6	2.6	2.7	4.4	6.2	10.3	0.0	0.0	6.2	10.3
11[C]	90	297.9	16.5	5.6	5.0	1.7	3.7	1.2	17.3	5.8	9.2	3.1	19.6	6.6
8[A]	90	538.0	28.9	5.4	9.5	1.8	17.2	3.2	30.4	5.7	3.3	0.6	30.6	5.7
6[A]	90	5143.2	311.6	6.1	58.8	1.1	148.4	2.9	317.1	6.2	137.4	2.7	345.6	6.7
7[A]	90	9538.7	516.4	5.4	107.7	1.1	236.1	2.5	527.5	5.5	351.7	3.7	634.0	6.6

[A] Pooled plasma from normal donors spiked with recombinant GFAP and UCH-L1 antigens
[C] Pooled plasma from normal donors and TBI patient
δ Within-day variability includes within-operator and between-operator variance components
† Within-site variability is the summation of within-day and between-day variance components
‡Overall variability is the summation of the between-site, within-day, and between-day variance components

Qualitative precision: A total of 90 replicates of each plasma sample were tested across three sites to evaluate qualitative precision. The plasma samples were identical to those in

the multi-site semi-quantitative precision study above. The % correct call was calculated for each plasma sample and was based on the number of replicates providing the expected GFAP/UCH-L1 result (Not Elevated or Elevated) based on the mean antigen concentration for the sample (either below cut-off or at/above cut-off, respectively). Results are summarized in the table below for each assay.

Assay	Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
				Number of Elevated Results (At/Above Cut-off) / Total Replicates	%Correct Call
GFAP	5[A]	19.4	90	0/90	100
	9[B]	30.8	90	60/90	100‡
	10[C]	66.2	90	90/90	100
	8[C]	150.1	90	90/90	100
	6[C]	4504.7	90	90/90	100
	7[C]	9196.8	90	90/90	100
UCH-L1	5[A]	60.5	90	0/90	100
	11[B]	297.9	90	0/90	100‡
	8[C]	538.0	90	90/90	100
	6[C]	5143.2	90	90/90	100
	7[C]	9538.7	90	90/90	100

[A] Not Elevated
[B] Near cut-off (mean ±25% of the cut-off value)
[C] Elevated
‡ Replicates for sample with mean value near cut-off can have replicates below cut-off or at/above cut-off

2. Linearity:

a. *Linearity*

The purpose of this study was 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-A. 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, plasma 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 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, seven sample levels of the native antigen samples and seven sample levels of the recombinant antigen samples were generated. Plasma samples used for testing were prepared by separating the plasma portion of the whole blood samples from the red blood cells via centrifugation. The expected values for GFAP and UCH-L1 of each plasma sample were calculated based on add-mix ratios of the high and low plasma levels with known concentration, measured by the i-STAT TBI cartridges on i-STAT Alinity instruments. Six

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. The % recovery was calculated using the means of the GFAP and UCH-L1 assay results and the expected values of the plasma samples.

GFAP Assay					
Antigen Source	**Panel Member**	**N**	**Expected Mean GFAP (pg/mL)**	**Measured Mean GFAP (pg/mL)**	**%Recovery**
Native	P1	6	3245.5	3245.5	100.0
Native	P2	6	2599.6	2607.8	100.3
Native	P3	6	1953.6	1990.3	101.9
Native	P4	6	1307.7	1353.2	103.5
Native	P5	6	661.7	634.8	95.9
Native	P6	6	338.7	346.7	102.4
Native	P7	6	177.3	169.5	95.6
Native	P8	6	96.5	98.9	102.5
Native	P9	6	56.1	49.4	88.1
Native	P10	6	15.8	15.8	100.0
Recombinant	P1	6	10686.5	10686.5	100.0
Recombinant	P2	6	9618.9	9612.2	99.9
Recombinant	P3	6	8551.3	8581.2	100.3
Recombinant	P4	6	7483.8	7630.5	102.0
Recombinant	P5	6	6416.1	6117.7	95.3
Recombinant	P6	6	5348.6	5653.3	105.7
Recombinant	P7	6	4281.0	4539.1	106.0
Recombinant	P8	6	3213.4	3595.3	111.9
Recombinant	P9	6	2145.8	2355.7	109.8
Recombinant	P10	6	1078.3	1206.6	111.9
Recombinant	P11	6	608.6	571.8	94.0
Recombinant	P12	6	309.6	294.5	95.1
Recombinant	P13	6	160.2	153.6	95.9
Recombinant	P14	6	85.4	81.5	95.4
Recombinant	P15	6	47.7	39.4	82.6
Recombinant	P16	6	29.2	23.5	80.5
Recombinant	P17	6	19.9	16.4	82.4

UCH-L1 Assay					
Antigen Source	**Sample**	**N**	**Expected Mean UCH-L1 (pg/mL)**	**Measured Mean UCH-L1 (pg/mL)**	**%Recovery**
Native	P1	6	2286.6	2286.6	100.0
Native	P2	6	1844.1	1945.6	105.5
Native	P3	6	1401.6	1408.6	100.5
Native	P4	6	959.2	1012.6	105.6
Native	P5	6	516.7	453.2	87.7
Native	P6	6	295.4	296.4	100.3
Native	P7	6	184.8	183.1	99.1
Recombinant	P7	6	4298.9	4950.8	115.2
Recombinant	P8	6	3245.7	3598.3	110.9
Recombinant	P9	6	2192.4	2492.8	113.7
Recombinant	P10	6	1139.1	1216.9	106.8
Recombinant	P11	6	651.4	592.1	90.9
Recombinant	P12	6	368.6	345.1	93.6
Recombinant	P13	6	227.2	213.1	93.8
Recombinant	P14	6	156.5	154.4	98.7

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. Linearity was demonstrated throughout the measurable ranges for both GFAP and UCH-L1 assays (23–10,000 pg/mL for GFAP and 70–3200 pg/mL for UCH-LI) with a deviation from linearity within 15%.

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, a plasma sample that was prepared from K_2EDTA venous whole blood collected from one donor and 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 plasma 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 two or three 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 (39–54 pg/mL) and high (120–180 pg/mL) GFAP, and low (468–648 pg/mL) and high (1440–2160 pg/mL) UCH-L1. Plasma prepared from K_2EDTA venous whole blood sourced 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, and listed below. Each test and control sample was 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: ± the greater of 6 pg/mL or 10% of the control sample mean for GFAP, ± 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 30 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 albumin	15 g/dL
Triglycerides	4747 mg/dL*
Intralipid 20%	3000 mg/dL

*Test concentration as per DEN170045

Exogenous Substances Not Found to Interfere	
Potentially interfering substance	**Test concentration (µmol/L unless specified)†**
Acetylsalicylic acid (aspirin)	3620*
Acetaminophen	1324
Amphetamine	2.44
Benzoylecgonine	8.64**
Caffeine	556
Clopidogrel	21.4*
Chloramphenicol	241
Cocaine	11.41**
Diazepam	105
Diclofenac	81
Dopamine	4.06
EDDP[A] perchlorate	0.331***
Erythromycin	188
Ibuprofen	2425*
d-Methamphetamine	1.865δ
Methadone	10.3
Methaqualone	32.36‡
Metoprolol tartrate	18.7*
Morphine	27.3
Nicardipine hydrochloride	0.97
Nicotine	5.97
Oxazepam	15.1
Phencyclidine	0.0357δ
Phenytoin	238
Propoxyphene	9.46*
Secobarbital	66.8
Sodium ascorbate	298
Warfarin	243

[A] 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
†Test concentration as per CLSI EP37 unless otherwise noted
*Test concentration as per DEN170045
**Test concentration as per Therapeutic Drug Monitoring. October 2010. Vol. 32(5): 628 – 637
***Test concentration as per Anesthesiology. December 2011. Vol. 115(6): 1153 – 1161
δ Test concentration as per Karch's Pathology of Drug Abuse, Fourth Edition. 2008. CRC Press.
‡ Test concentration as per Journal of Pharmaceutical Sciences. February 1983. Vol. 72(2)

With the exception of HAMA and RF, none of the endogenous and exogenous substances evaluated at the concentrations indicated in the table above demonstrated interference with the GFAP or UCH-L1 assays at low or high concentrations of GFAP and UCH-L1. Only one exogenous substance – ethanol – was found to interfere with the UCH-L1 assay. Neither HAMA nor RF was found to interfere with the GFAP assay at the concentrations tested. However, both HAMA and RF were found to interfere with the UCH-L1 assay. A dose-response study was performed to determine the highest test concentrations of HAMA, RF, and ethanol at which no interference could be demonstrated with the UCH-L1 assay using samples with high and low levels of UCH-L1. The table below summarizes the results of the HAMA, RF, and ethanol interference study on the GFAP and UCH-L1 assays.

Substances Found to Interfere with i-STAT TBI test				
Interfering Substance	**Assay**	**Test concentrations**	**Highest concentration tested at which NO interference is observed**	**Effect of interference**
Human anti-mouse antibody (HAMA)	GFAP	> 160X*	N/A, No interference	N/A
	UCH-L1	40X, 80X, 120X, >160X	40X	Decrease in UCH-L1 quantitative results
Rheumatoid factor (RF)	GFAP	1000 IU/mL	N/A, No interference	N/A
	UCH-L1	250 IU/mL 500 IU/mL 750 IU/mL 1000 IU/mL	500 IU/mL	Decrease in UCH-L1 quantitative results
Ethanol	GFAP	130mmol/L	N/A, No interference	N/A
	UCH-L1	32.5 mmol/L 65 mmol/L 97.5 mmol/L 130 mmol/L	65 mmol/L	Decrease in UCH-L1 quantitative results

*The "X" factor listed indicates the number of times of activity in crosslinking antibodies in a mouse system assay

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 cartridges, plasma samples (prepared from K_2EDTA venous whole blood from one donor) spiked to achieve the targeted concentrations of GFAP (~40 pg/mL) and UCH-L1 (~500 pg/mL), and multiple i-STAT Alinity instruments. The concentrations of GFAP and UCH-L1

selected were representative of a low positive concentration 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 was evaluated with a similar number of replicates, with at least 10 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				
GFAP control	N/A	10	40.9	7.42
Desmin	127,000[1]	10	43.5	8.33
Internexin	77,000[B]	10	43.4	7.33
Keratin type II	10,000[2]	10	43.8	5.97
Neurofilament light	68[3]	10	43.1	6.60
Neurofilament medium	8,600[4]	10	42.8	6.76
Neurofilament heavy	77,000[5]	10	44.1	4.56
Vimentin	354,000[6]	10	41.2	7.73
UCH-L1 Assay				
UCH-L1 control	N/A	19*	497.0	50.65
UCH-L3	354,000[B]	20	500.8	38.39

[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
*One replicate resulted in a Quality Check Failure (QCF), so no result was reported

c. *Cross-talk*

The purpose of this study was 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, plasma samples prepared from K_2EDTA venous whole blood collected from healthy donors and spiked to low and high GFAP and UCH-L1 levels and multiple i-STAT Alinity instruments. Two levels (low and high) for both GFAP (85–117 pg/mL and 585–715 pg/mL) and UCH-L1 (468–648 pg/mL and 3240–3960 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 and Level Tested	Control or Test	Antigen Tested	Antigen Test Concentration (pg/mL)	N	Mean (pg/mL)	SD
GFAP Assay						
GFAP High	Control	N/A	N/A	10	576.0	8.99
GFAP High	Test	UCH-L1	100,000	10	593.0	15.49
GFAP Low	Control	N/A	N/A	10	94.8	3.98
GFAP Low	Test	UCH-L1	100,00	10	103.4	6.40
UCH-L1 Assay						
UCH-L1 High	Control	N/A	N/A	10	3294.6	98.53
UCH-L1 High	Test	GFAP	100,000	10	3381.3	90.95
UCH-L1 Low	Control	N/A	N/A	10	507.7	30.16
UCH-L1 Low	Test	GFAP	100,000	10	523.8	17.17

4. Assay Reportable Range:

   The reportable range for GFAP is 30–10,000 pg/mL and the reportable range for UCH-L1 is 200–3,200 pg/mL. Assay results may be preceded by the symbol for greater than (>) or less than (<) if the result is outside of the reportable range.

   GFAP concentrations below 30 pg/mL and UCH-L1 concentrations below 200 pg/mL can be reliably measured by each assay. See Section A.2.a Linearity and Section A.6 Detection Limit.

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

   i. 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 Plasma 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.

   ii. Quality Control: The i-STAT TBI Controls may be used to monitor performance of the GFAP and UCHL1 assays within the i-STAT TBI Plasma cartridge on the i-STAT Alinity instrument. The controls are packaged and sold separately from the cartridges. 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.

   iii. Calibration Verification Material: Calibration verification material may be used to verify the pre-set calibration of the i-STAT TBI Plasma cartridge throughout the reportable range. Calibration verification materials are available to meet clinical laboratory and regulatory requirements and are sold separately from the cartridges.

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

v. Sample Stability and Storage: In order to evaluate fresh plasma stability, plasma samples targeting three levels of GFAP and UCH-L1 that covered the reportable ranges of the antigens were evaluated. Plasma obtained from approximately 10 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 and 10 unique samples for each GFAP and UCH-L1 level. The results from the study support the following stability claims: immediate use of plasma preparation or room temperature storage for up to 2 hours. Specifically, if plasma testing is not planned for immediately after plasma preparation, remove the top 1/3 of the separated plasma. Place the plasma in an aliquot tube, cover and store at room temperature for up to 2 hours.

vi. Temperature Operating Range: In order to evaluate the performance of the GFAP and UCH-L1 assays within the i-STAT TBI Plasma cartridge when operated at an elevated temperature, a study was performed in which GFAP and UCH-L1 samples near their respective cut-offs (30 pg/mL and 360 pg/mL, respectively) were tested on i-STAT Alinity instruments at room temperature (24.4°C/75.9°F) compared to samples tested on i-STAT Alinity instruments at elevated temperature (tests run in a temperature chamber at 30.8°C/87.4°F). A total of approximately 120 cartridges were run at each temperature condition. This study demonstrated that the average relative percent difference in performance is -8.6% for the GFAP assay with testing at elevated temperature and an average relative percent difference in performance of -5.1% for the UCH-L1 assay with testing at the elevated temperature. The results from this study are provided in the table below.

Temperature Operating Study Results									
Condition	**N***	**GFAP Mean (pg/mL)**	**GFAP %CV**	**GFAP Mean Bias (pg/mL)**	**GFAP Mean % Bias**	**UCH-L1 Mean (pg/mL)**	**UCH-L1 %CV**	**UCH-L1 Bias (pg/mL)**	**UCH-L1 Mean % Bias**
Elevated Temp.	116	33.4	7.5	-3.1	-8.6%	404.0	5.9	-21.8	-5.1%
Room Temp.	118	36.5	5.3	N/A	N/A	425.8	4.9	N/A	N/A

*There were six QCFs (suppressed/no results reported for cartridge) in study, four at elevated temperature and two at room temperature.

6. Detection Limit:

The purpose of this study was to determine the Limit of Quantitation (LoQ) of the GFAP and UCH-L1 assays within the i-STAT TBI Plasma cartridge using the i-STAT Alinity instrument. The study design was based on the CLSI guideline EP17-A2 and the LoQ of each assay was determined using the precision profile approach as described in this guideline. The testing was conducted on five days using four lots of i-STAT TBI cartridges and pooled plasma samples prepared from K_2EDTA venous whole blood collected from 11 donors and

spiked with antigen from a patient plasma pool to achieve six target low antigen levels of GFAP and UCH-L1. Ten replicates of each sample were run on each of the four lots of cartridges every day for five days, generating a total of 50 replicates per sample for each cartridge lot, and ~200 replicates per sample across all four lots of cartridges. The within-laboratory precision (as %CV) was calculated for each assay and sample tested. The criteria for determination of the LoQ for each assay was based on the requirement for within-laboratory precision ≤ 15%CV as evaluated in the LoQ study and a deviation from linearity ≤ 15% as determined by the linearity study (see Section A.2.a). The LoQ for each assay should also be least 20% below the assay cut-off. Based on these criteria, the LoQ for GFAP was determined to be 23 pg/mL and the LoQ for UCH-L1 was determined to be 70 pg/mL.

7. Assay Cut-Off:

The GFAP and UCH-L1 assay cut-offs were determined by analyzing a training dataset from samples collected from a completely independent population distinct from subjects evaluated in the pivotal study to validate the assay cut-offs. Samples to establish the cut-offs were collected as part of the TRACK-TBI (Transforming Research and Clinical Knowledge in Traumatic Brain Injury) study. A total of 420 subjects (65.2% male, 34.8% female) with Glasgow Coma Scale (GCS) scores between 13–15 who had blood specimens collected within 12 hours from the time of suspected head injury were included in the training dataset. Of the 420 subjects, 18% had a positive computed tomography (CT) result. Using a 10-fold cross validation and bootstrapping method, the optimal cut-off values were selected as 360 pg/mL for UCH-L1 and 30 pg/mL for GFAP.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

a. *Clinical performance with frozen plasma specimens from the ALERT-TBI study*

The ability of the i-STAT TBI Plasma test to assist physicians in determining the need for a computed tomography (CT) scan of the head in conjunction with other clinical information was evaluated by testing frozen plasma specimens that were previously collected by Banyan Biomarkers (San Diego, CA) from subjects participating in their study "A Prospective Clinical Evaluation of Biomarkers of Traumatic Brain Injury" (ALERT-TBI, protocol ATO-06). A total of 1901 frozen and de-identified plasma samples these subjects were tested at three (3) clinical sites by operators representative of end-users of the i-STAT System. Each

specimen was associated with a standard of care computed tomography (CT) scan of the head from the same study subject. Under the ALERT-TBI study protocol, the CT scans were independently reviewed by a panel of radiologists and a subject's CT scan was classified as positive if intracranial lesions were present. The standard of care head CT scans were previously classified as positive or negative for intracranial lesions as part of the ALERT-TBI study. Additional details regarding the evaluation of CT scans and definition of intracranial lesion can be found in DEN170045. The demographic characteristics of the enrolled subjects evaluated with the i-STAT TBI Plasma test are presented in the table below.

ALERT-TBI study			
Characteristic	**Head CT scan results**		**Total**
	Positive	**Negative**	
N	120	1781	1901
Age [A] (Years)			
Mean (SD)	58.8	48.5	49.1
Median	58.5	48.0	49.0
Range (min, max)	(20, 95)	(18, 98)	(18, 98)
Gender, N (%)			
Male	70 (58.3%)	1005 (56.4%)	1075 (56.5%)
Female	50 (41.7%)	776 (43.6%)	826 (43.5%)
Ethnicity, N (%)			
Hispanic or Latino	1 (0.8%)	89 (5.0%)	90 (4.7%)
Not Hispanic or Latino	118 (98.3%)	1691 (94.9%)	1809 (95.2%)
Not Reported	1 (0.8%)	1 (0.1%)	2 (0.1%)
Race [B], N (%)			
White	98 (81.7%)	1245 (69.9%)	1343 (70.6%)
Black or African American	16 (13.3%)	483 (27.1%)	499 (26.2%)
Asian	5 (4.2%)	24 (1.3%)	29 (1.5%)
Native Hawaiian/Pacific Islander	1 (0.8%)	2 (0.1%)	3 (0.2%)
American Indian or Alaska Native	1 (0.8%)	9 (0.5%)	10 (0.5%)
Unknown	1 (0.8%)	27 (1.5%)	28 (1.5%)

[A] Age was calculated relative to the date of informed consent
[B] Subjects could have indicated more than one race. Totals may thus be greater than number of enrolled subjects, though percentages are calculated based on enrolled number.

The following 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 3.5 hours. Most subjects had a GCS score of 15 (94/120 or 78.3% in CT scan-positive subjects and 1695/1781 or 95.2% 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.

ALERT-TBI study			
Characteristic	**Head CT scan results**		**Total**
	Positive	**Negative**	
N	120	1781	1901
Time from head injury to examination (hours) [A]			
Mean (SD)	1.9 (1.73)	1.6 (1.71)	1.6 (1.71)
Median	1.2	1.0	1.1
Range (min, max)	(0.3, 7.8)	(0.1, 10.7)	(0.1, 10.7)
Time from head injury to CT scan (hours) [A]			
Mean (SD)	2.8 (1.95)	2.7 (1.93)	2.7 (1.93)
Median	2.1	2.2	2.1
Range (min, max)	(0.5, 8.9)	(0.2, 13.3)	(0.2, 13.3)
Time from head injury to blood draw (hours) [A]			
Mean (SD)	3.8 (1.91)	3.5 (1.88)	3.5 (1.89)
Median	3.3	3.1	3.2
Range (min, max)	(0.3, 9.3)	(0.3, 11.9)	(0.3, 11.9)
GCS score			
13	7 (5.8%)	15 (0.8%)	22 (1.1%)
14	19 (15.8%)	71 (4.1%)	90 (4.7%)
15	94 (78.3%)	1695 (95.2%)	1789 (94.1%)
Neurological assessment - Number (%) of subjects experiencing:			
Loss of Consciousness (LOC)	82 (68.3%)	721 (40.5%)	803 (42.2%)
Alteration of Consciousness (AOC)	92 (76.7%)	978 (54.9%)	1070 (56.3%)
Confusion	44 (36.7%)	313 (17.6%)	357 (18.8%)
Vomiting	14 (11.7%)	128 (7.2%)	142 (7.5%)
Post traumatic Amnesia (PTA)	81 (67.5%)	546 (30.7%)	627 (33.0%)
Post Traumatic Seizures	2 (1.7%)	11 (0.6%)	13 (0.7%)

Subjects with Drug or Alcohol Intoxication at Time of Presentation to Facility	33 (27.5%)	369 (20.7%)	402 (21.1%)
Dangerous Mechanism of Injury [B]	27 (22.5%)	369 (20.7%)	396 (20.8%)
Physical Evidence [C]			
Visible Trauma Above the Clavicle	101 (84.2%)	1102 (61.9%)	1203 (63.3%)
Suspected Open or Depressed Skull Fracture	14 (11.7%)	46 (2.6%)	60 (3.2%)
Signs of Basal Skull Fracture	10 (8.3%)	26 (1.5%)	36 (1.9%)
Presence of Neurosurgical Lesions	5 (4.2%)	0 (0.0%)	5 (0.3%)

[A] Time since head injury calculated relative to time that subject was first examined by medical personnel at facility
[B] Dangerous mechanism of injury was pedestrian struck by a motor vehicle, an occupant ejected from a motor vehicle, or a fall from an elevation of 3 or more feet or 5 stairs
[C] Prior to head CT

The most common head CT findings in the 120 subjects with CT-positive scans were scalp injury (96.7%), subarachnoid hemorrhage (59.2%), the presence of incidental findings (57.5%), and acute subdural hematoma (47.5%). Other frequently reported findings included cranial fractures (26.7%), parenchymal hematoma (20.0%), facial fractures (16.7%), skull based fractures (15.0%), and indeterminate extra-axial lesions (15.0%). All other findings occurred in less than 10% of CT-positive subjects.

To estimate clinical performance characteristics, the i-STAT TBI Plasma test results were compared to the consensus head CT scan result for each patient. The performance estimates are summarized in the 2x2 table below. Of the 1901 evaluable subjects, 120 had positive CT scan results. Of the 120 subjects with positive CT scan results, 115 had an Elevated i-STAT TBI test result (sensitivity = 95.8%). The remaining five CT scan positive subjects had Not Elevated results from the i-STAT TBI Plasma test. The rate of false negative (FN) results was 4.2% (5/120). None of the five subjects identified with a lesion requiring surgical intervention had a FN result suggesting that i-STAT TBI Plasma test correctly classified all these five CT-positive subjects with an Elevated test result. Of the 1781 subjects with negative CT scan results, 720 had a Not Elevated i-STAT TBI Plasma test result (specificity = 40.4%). The rate of False Positive (FP) results was 59.6% (1061/1781). Overall, there were 725 subjects with Not Elevated i-STAT TBI Plasma test results. Of these, 720 had negative CT scan results. The Negative Predictive Value (NPV) of the assay was 99.3% (720/725). The potential benefit of the assay would be a reduction in unnecessary CT scans by approximately 40% (40.4% or 720 of 1781 subjects had true negative assay results). The Positive Predictive Value (PPV) of the assay was 9.8%. The Likelihood Ratio Negative (LRN) of the assay was 0.10 (95% confidence interval [CI]: 0.04; 0.23). The Likelihood Ratio positive (LRP) of the assay was 1.61 (95%CI:1.51; 1.69). The results showed that the clinical performance of the i-STAT TBI Plasma test is characterized by high clinical sensitivity and high NPV comparable to that demonstrated by the Banyan BTI (DEN170045; clinical sensitivity = 97.5%, clinical specificity = 36.5%, NPV = 99.6%, PPV = 9.2%), which supports clinical utility as an aid in the evaluation of the need for a CT scan in subjects presenting with a GCS score of 13 to 15 and a Not Elevated i-STAT TBI Plasma test result.

ALERT-TBI study				
		Head CT scan result		Total
		Positive	Negative	
i-STAT TBI Plasma Test result	Elevated	115	1061	1176
	Not Elevated	5	720	725
	Total	120	1781	1901

Sensitivity = 95.8% (115/120); 95% CI: 90.6%−98.2%

Specificity = 40.4% (720/1781); 95% CI: 38.2%−42.7%

Negative predictive value (NPV)† = 99.3% (720/725); 95% CI: 98.5%−99.7%

Positive predictive value (PPV)‡ = 9.8% (115/1176); 95% CI: 9.2%−10.2%

Likelihood Ratio Negative (LRN) = 0.10; 95% CI: 0.04–0.23

Likelihood Ratio Positive (LRP) = 1.61; 95% CI: 1.51–1.69

CT scan positive prevalence rate in study = 6.3% (120/1901)

†Adjusted NPV for 6% CT scan positive prevalence rate (DEN170045) = 99.3%; 95% CI: 98.5%–99.7%

‡Adjusted PPV for 6% CT scan positive prevalence rate (DEN170045) = 9.3%; 95% CI: 8.8%–9.7%

Analyses of assay performance by gender and time from injury relative to blood draw are shown in the table below. There was little variation in NPV and PPV between males and females and with increasing time from injury. These data indicate that gender differences and differences between head injury characteristics did not translate into statistically significant differences in assay performance.

ALERT-TBI study				
	Sensitivity N (%) (95% CI)	**Specificity** N (%) (95% CI)	**NPV** N (%) (95% CI)	**PPV** N (%) (95%CI)
All subjects N=1901	115/120 (95.8%) (90.6−98.2)	720/1781 (40.4%) (38.2−42.7)	720/725 (99.3%) (98.4−99.7)	115/1176 (9.8%) (8.2−11.6)
Gender				
Male N=1075 (56.5%)	67/70 (95.7%) (88.1−98.5)	403/1005 (40.1%) (37.1−43.2)	403/406 (99.3%) (97.9−99.7)	67/669 (10.0%) (8.0−12.5)
Female N=826 (43.5%)	48/50 (96.0%) (86.5−98.9)	317/776 (40.9%) (34.4−44.3)	317/319 (99.4%) (97.7−99.8)	48/507 (9.5%) (7.2−12.3)
Time from injury to blood draw				
0−4 hours N=1445 (76.0%)	83/86 (96.5%) (90.2−98.8)	541/1359 (39.8%) (37.2−42.4)	541/544 (99.4%) (98.4−99.8)	83/901 (9.2%) (7.5−11.3)
>4−8 hours N=378 (19.9%)	27/28 (96.4%) (82.3−99.4)	152/350 (43.4%) (38.3−48.7)	152/153 (99.3%) (96.4−99.9)	27/225 (12.0%) (8.4−16.9)
0−8 hours N=1823 (95.9%)	110/114 (96.5%) (91.3−98.6)	693/1709 (40.6%) (38.2−42.9)	693/697 (99.4%) (98.5−99.8)	110/1126 (9.8%) (8.2−11.6)
>8−12 hours N=78 (4.1%)	5/6 (83.3%) (43.6−97.0)	27/72 (37.5%) (27.2−49.0)	27/28 (96.4%) (82.3−99.4)	5/50 (10.0%) (4.3−21.4)

Since specimens from the ALERT-TBI study were archived frozen samples, a specimen stability study was conducted to demonstrate the integrity of clinical samples, as per special control b(1)(ii)(i) of 21 CFR 866.5830. The study demonstrated stability of plasma samples covering a range of GFAP and UCH-L1 antigen levels stored frozen at -70° C.

b. *Clinical performance with fresh plasma samples from the TRACK-TBI Phase 2 study*

A supplemental clinical validation of the GFAP and UCH-L1 cut-offs was performed for fresh plasma samples, the intended use matrix for the i-STAT TBI Plasma test. In order to validate the GFAP and UCH-L1 cut-offs in fresh plasma samples, an additional clinical validation study was performed using fresh plasma samples prepared from K_3EDTA venous whole blood prospectively collected from subjects participating in the Traumatic Brain Injury Specimen Collection and Testing Study (TRACK) TBI Phase 2 study (Protocol Number 9XY-02-TBI06). Only the plasma specimens which were collected in accordance with the proposed intended use of the i-STAT TBI Plasma test were included in the analysis (n=88). The demographic characteristics of the evaluable subjects are presented in the table below.

TRACK-TBI Phase 2 study			
Characteristic	**Head CT scan results**		**Total**
	Positive	**Negative**	

N	29	59	88
Age (Years)			
Mean (SD)	49.2 (16.9)	39.3 (15.4)	42.5 (16.5)
Median	47	36	41
Range (Min, Max)	(24, 85)	(18, 76)	(18, 85)
Gender			
Male	23	40	63
Female	6	19	25
Time from head injury to CT scan (hours)			
Mean (SD)	2.5 (1.8)	2.2 (1.4)	2.3 (1.5)
Median	2.0	1.9	1.9
Range (min, max)	(0.7, 8.7)	(0.7, 7.5)	(0.7, 8.7)
Time from head injury to blood draw (hours)			
Mean (SD)	6.6 (2.9)	4.4 (2.0)	5.1 (2.5)
Median	6.0	3.9	4.3
Range (min, max)	(2.3, 11.8)	(2.0, 9.9)	(2.0, 11.8)
GCS score*			
13	1 (1.1%)	0 (0.0%)	1 (1.1%)
14	6 (6.8%)	9 (10.2%)	15 (17.0%)
15	22 (25.0%)	50 (56.8%)	72 (81.8%)
Neurological assessment Number (%) of subjects experiencing:			
Loss of Consciousness (LOC)	23 (79.3%)	37 (62.7%)	60 (68.2%)
Confusion	19 (65.5%)	40 (67.8%)	59 (67.0%)
Vomiting**	--	--	--
Post traumatic Amnesia (PTA)	22 (75.9%)	38 (64.4%)	60 (68.2%)
Post Traumatic Seizures	0 (0.0%)	0 (0.0%)	0 (0.0%)
Subjects with Drug Intoxication at Time of Presentation to Site	3 (10.3%)	2 (3.4%)	5 (5.7%)
Subjects with Alcohol Intoxication at Time of Presentation to Site	6 (20.7%)	4 (6.8%)	10 (11.4%)
Physical Evidence			
Signs of Skull Fracture	9 (31.0%)	1 (1.7%)	10 (11.4%)
Mechanism of Injury			
Acceleration/Deceleration	24 (82.8%)	41 (69.5%)	65 (73.9%)

Blow to Head	4 (13.8%)	8 (13.6%)	12 (13.6%)
Head Against Object	24 (82.8%)	42 (71.2%)	66 (75.0%)
Fall	19 (65.5%)	21 (35.6%)	40 (45.5%)
* Percent based on total subjects **Information not collected			

Samples were evaluated at four clinical sites in the U.S., with operators of the test blinded to the CT results associated with the study subjects. The subject's CT scan images were independently evaluated by a minimum of two U.S. board-certified radiologists blinded to the i-STAT TBI Plasma result and each other's evaluation to determine the presence or absence of an acute intracranial lesion. If consensus was not established, the head CT scan images for those subjects were evaluated independently by a third U.S. board-certified radiologist in order to make a final determination of the CT scan result. A head CT scan result of a subject was classified as positive if intracranial lesions were present. The intracranial lesions were defined as any trauma-induced or related finding visualized upon head CT scan, and may have included acute epidural hematomas, acute subdural hematomas, indeterminate extra-axial lesions, cortical contusions, parenchymal hematomas, non-hemorrhagic contusions, ventricle compression, ventricular trapping, brain herniation, intraventricular hemorrhage, hydrocephalus, subarachnoid hemorrhage, petechial hemorrhage, global or focal brain edema and post traumatic ischemia.

To estimate clinical performance characteristics, the i-STAT TBI Plasma test result was compared to the consensus head CT scan result for each patient. The performance estimates are summarized in the 2x2 table below. Of the 88 evaluable subjects, 29 had positive CT scan results. Of the 29 subjects with positive CT scan results, 29 had an Elevated i-STAT TBI test result (clinical sensitivity = 100%). The rate of false negative (FN) results was 0% (0/29). Of the 59 subjects with negative CT scan results, 14 had a Not Elevated i-STAT TBI Plasma test result (14/59, clinical specificity = 23.7%). The rate of False Positive (FP) results was 76.3% (45/59). Overall, there were 14 subjects with Not Elevated i-STAT TBI Plasma test results, all of which had negative CT scan results. The Negative Predictive Value (NPV) of the assay was 100% (14/14). The potential benefit of the assay would be a reduction in unnecessary CT scans by approximately 24% (23.7% or 14 of 59 subjects had true negative assay results). The Positive Predictive Value (PPV) of the assay was 39.2%. The results show that the i-STAT TBI Plasma test, when evaluated with fresh plasma samples, is characterized by high sensitivity and NPV supportive of its clinical utility as an aid in the evaluation of the need for a CT scan in subjects presenting with a GCS score of 13 to 15 and a negative i-STAT TBI Plasma test.

TRACK-TBI Phase 2 study				
		Head CT scan result		Total
		Positive	Negative	
i-STAT TBI Plasma Test result	Elevated	29	45	74
	Not Elevated	0	14	14
	Total	29	59	88

Sensitivity = 100% (29/29); 95% CI: 88.3%−100.0%

Specificity = 23.7% (14/59); 95% CI: 14.7%−36.0%

Negative predictive value (NPV)† = 100% (14/14); 95% CI: 80.2%−100.0%

Positive predictive value (PPV)† = 39.2% (29/74); 95% CI: 35.9%−43.4%

Likelihood Ratio Negative (LRN) = 0.00; 95% CI: 0.00–0.50

Likelihood Ratio Positive (LRP) = 1.31; 95% CI: 1.14–1.56

CT scan positive prevalence rate in study = 33.0% (29/88)

†NPV and PPV are estimated at 33.0% prevalence of CT scan positive rate for suspected mild TBI subjects in TRACK-TBI Phase 2 study cohort. If NPV and PPV are adjusted to 6% CT scan positive prevalence rate (comparable to ALERT-TBI study cohort and DEN170045), NPV = 100.0% (95% CI: 96.9%–100.0%), and PPV = 7.7% (95% CI: 6.8%–9.1%).

2. Clinical Specificity:

   Refer to Clinical Sensitivity section, above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable

D Clinical Cut-Off:

Refer to Assay Cut-Off section.

E Expected Values/Reference Range:

The expected values from 225 self-declared apparently healthy donors ranging in age from 18 to 79 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 biomarkers. The 2.5^{th} and 97.5^{th} percentiles (95% reference interval) and their respective 95% confidence intervals for both biomarkers were calculated non-parametrically. The mean (SD) age was 43.3 (17.2) years. The mean (SD) concentration for GFAP was 19.0 (16.2) pg/mL, and the median was 15.0 pg/mL. The 95% reference intervals for GFAP was 2–51 pg/mL. The mean (SD) concentration

for UCH-L1 was 80.7 (42.2) pg/mL, and the median was 71.3 pg/mL. The 95% reference intervals for UCH-L1 was 21–204 pg/mL.

Reference Interval					
Biomarker	**N**	**Mean (pg/mL)**	**SD (pg/mL)**	**Median (pg/mL)**	**Reference Interval (2.5th to 97.5th percentile) (pg/mL)**
GFAP	225	19	16.2	15	2 – 51
UCH-L1	225	81	42.2	71	21 – 204

There were 36 healthy donors who tested positive for GFAP only and no donors were positive for both GFAP and UCH-L1. The results summarized in the table below show that 84% have a Not Elevated i-STAT TBI Plasma test result and 16% have an Elevated positive i-STAT TBI Plasma test result.

UCH-L1 result (relative to cut-off)*	**GFAP result** (relative to cut-off)**	**i-STAT TBI Plasma Test Interpretation Result**	**All subjects (N=225)**
Below	Below	Not Elevated	189 (84.0%)
Above	Above	Elevated	0 (0.0%)
Below	Above	Elevated	36 (16.0%)
Above	Below	Elevated	0 (0.0%)

* "Above" indicated for UCH-L1 denotes the UCH-L1 concentration is $\geq$ 360 pg/mL. Below denotes the UCH-L1 concentration is < 360 pg/mL
** "Above" indicated fir GFAP denotes the GFAP concentration $\geq$ 30 pg/mL. Below denotes the GFAP concentration < 30 pg/mL

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.