May 24, 2022

Abbott Laboratories
Brian Ma
Sr. Regulatory Affairs Specialist
400 College Road East
Princeton, New Jersey 08540

Re: K201778
Trade/Device Name: i-STAT TBI Plasma cartridge with the i-STAT Alinity System
Regulation Number: 21 CFR 866.5830
Regulation Name: Brain trauma assessment test
Regulatory Class: Class II
Product Code: QAT

Dear Brian Ma:

The Food and Drug Administration (FDA) is sending this letter to notify you of an administrative change related to your previous substantial equivalence (SE) determination letter for your device cleared on January 8, 2021. Specifically, FDA is updating this SE Letter due to a typographical error in the clearance date, which was incorrectly dated January 8, 2020.

Please note that the 510(k) submission was not re-reviewed. For questions regarding this letter please contact Ying (Katelin) Mao, OHT7: Office of In Vitro Diagnostics and Radiological Health, 301-796-6635, ying.mao@fda.hhs.gov .

Sincerely,

Ying Mao -S

Ying (Katelin) Mao, Ph.D.
Chief
Division of Immunology
and Hematology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

January 8, 2020

Abbott Laboratories
Brian Ma
Sr. Regulatory Affairs Specialist
400 College Road East
Princeton, New Jersey 08540

Re: K201778
Trade/Device Name: i-STAT TBI Plasma cartridge with the i-STAT Alinity System
Regulation Number: 21 CFR 866.5830
Regulation Name: Brain Trauma Assessment Test
Regulatory Class: Class II
Product Code: QAT
Dated: December 4, 2020
Received: December 7, 2020

Dear Brian Ma:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Leonthena R. Carrington -S

Lea Carrington
Director
Division of Immunology
and Hematology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K201778

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

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) Summary

The information in this 510(k) summary is being submitted in accordance with the requirements of 21 CFR 807.92.

1. Submitter Information

Owner	Abbott Point of Care Inc. 400 College Road East Princeton, NJ 08540
Contact	Primary: Brian Ma, PhD Senior Specialist Regulatory Affairs Phone: 613-688-5949 Secondary: Susan Tibedo Director Regulatory Affairs Phone: 609-213-8514
Date Prepared	January 6, 2021

2. Device Information

Proprietary Name	i-STAT TBI Plasma Cartridge with the i-STAT Alinity System
Common Name	Glial fibrillary acidic protein (GFAP) Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1)

Product code	Device Classification name	Regulation Number	Class	Panel
QAT	Brain trauma assessment test	866.5830	II (Special controls)	Immunology

3. Predicate Device

Proprietary Name Banyan Brain Trauma Indicator (BTI)

510(k) Number DEN170045

Product code	Device Classification name	Regulation Number	Class	Panel
QAT	Brain trauma assessment test	866.5830	II (Special controls)	Immunology (82)

4. 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.

The i-STAT Alinity instrument 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 i-STAT Alinity System is comprised of the i-STAT Alinity instrument, the i-STAT test cartridges and accessories (i-STAT Alinity Base Station, Electronic Simulator and Printer).

5. Intended Use Statement

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.

6. Summary Comparison of Technological Characteristics

Similarities and Differences: System (Test and Instrument)		
Feature or Characteristic	**i-STAT TBI Plasma Cartridge (Candidate)**	**Banyan BTI (DEN170045) (Predicate)**
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 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 User	Clinical Laboratory	Clinical Laboratory
Measurands	GFAP and UCH-L1	GFAP and UCH-L1
Assay Technology	Enzyme-linked immunosorbent assay	Enzyme-linked immunosorbent assay
Assay Format	Single use multiplex cartridge (both assays (GFAP and UCH-L1) in one cartridge)	Two test kits on separate 96-well microtiter plates – one for GFAP and one for UCH-L1
Detection Technology	Electrochemical	Chemiluminescence
Specimen Type	Plasma	Serum
Sample Volume	20 µL	GFAP kit: 150 µL UCH-L1 kit: 100 µL

Similarities and Differences: System (Test and Instrument)		
Feature or Characteristic	**i-STAT TBI Plasma Cartridge (Candidate)**	**Banyan BTI (DEN170045) (Predicate)**
Preparation	Ready to Use	Manual preparation of reagents and plate.
Automation	Test and wash cycles are fully automated after sample loading step	Manual sample loading, reagent mixing, as well as transfer from incubation and washing to read steps
Analytical Measuring Interval	GFAP: 30 – 10,000 pg/mL UCH-L1: 200 – 3,200 pg/mL	GFAP: 10 – 320 pg/mL UCH-L1: 80 – 2,560 pg/mL
Time to Result	~ 15 minutes	~ 4 hours
Reportable Result	Quantitative results for GFAP and UCH-L1 and semi-quantitative interpretation	Quantitative results for GFAP and UCH-L1 and semi-quantitative interpretation
Instrument Platform	i-STAT Alinity	Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments, Inc.)
Controls	GFAP and UCH-L1 combined: 2 levels (Control 1, Control 2)	GFAP: 2 levels (Control 1, Control 2) UCH-L1: 2 levels (Control 1, Control 2)
Calibration	No calibration needed by the end user, calibration is pre-set during manufacture of the cartridge	Calibration curve generated by end user for each run using six standards

7. Performance Characteristics

1. Analytical Performance

a. Precision/Reproducibility

Semi-quantitative precision: The precision of the GFAP and UCH-L1 assays in the i-STAT TBI plasma cartridge with the i-STAT Alinity System was evaluated using plasma samples representing nine (9) levels of GFAP and seven (7) levels of UCH-L1 spanning the reportable range as well as the i-STAT TBI Controls (L1 and L2). This single-site study was based on CLSI document EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition.* Each sample was tested for at least 20 days with two (2) runs per day and two (2) results per run for a total of 80 measurements per sample per cartridge lot. Runs were separated by a minimum of 2 hours. The plasma samples were made of pooled plasma from healthy normal donors spiked with recombinant GFAP and UCH-L1 antigens or spiked with

native antigens from pooled TBI patient plasma as shown in the tables below. The components of variability were estimated for GFAP and UCH-L1 and the precision results are shown in **Table 1, Table 2,** and **Table 3**.

Table 1: Estimate of GFAP Assay Precision

Sample	N	Mean (pg/mL)	Repeatability		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
1	238‡	17.0	1.76	10.4%	0.91	5.4%	0.61	3.6%	1.31	7.7%	2.46	14.5%
2	238‡	30.8	2.49	8.1%	0.00	0.0%	0.00	0.0%	0.52	1.7%	2.55	8.3%
3	238‡	65.6	3.21	4.9%	0.87	1.3%	1.03	1.6%	0.62	0.9%	3.54	5.4%
4	238*§	104.9	3.37	3.2%	2.08	2.0%	0.00	0.0%	1.50	1.4%	4.24	4.0%
5	238‡	962.9	22.42	2.3%	13.61	1.4%	17.33	1.8%	21.17	2.2%	37.90	3.9%
6	160	2029.5	39.18	1.9%	26.30	1.3%	19.10	0.9%	94.89	4.7%	107.69	5.3%
7	240	3139.5	75.98	2.4%	35.92	1.1%	49.34	1.6%	97.09	3.1%	137.57	4.4%
8	160*†	5713.3	143.96	2.5%	42.68	0.7%	65.72	1.2%	170.29	3.0%	236.36	4.1%
9	159†	7537.2	129.57	1.7%	133.30	1.8%	35.89	0.5%	187.57	2.5%	266.51	3.5%

*Additional GFAP result(s) was obtained due to cartridge re-run due to a UCH-L1 star-out

†one (1) outlier removed from analysis

‡two (2) outliers removed from analysis

§three (3) outliers removed from analysis

Table 2: Estimate of UCH-L1 Assay Precision

Sample	N	Mean (pg/mL)	Repeatability		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
1	238‡	72.5	4.88	6.7%	1.73	2.4%	0.00	0.0%	3.93	5.4%	6.50	9.0%
2	239†	300.1	15.12	5.0%	5.94	2.0%	0.00	0.0%	15.54	5.2%	22.48	7.5%
3	240	519.9	29.56	5.7%	1.54	0.3%	13.38	2.6%	8.21	1.6%	33.51	6.4%
4	238‡	1058.9	56.88	5.4%	22.59	2.1%	15.13	1.4%	33.6	3.2%	71.44	6.7%
5	159*	1639.6	91.57	5.6%	8.72	0.5%	15.74	1.0%	28.46	1.7%	97.56	6.0%
6	240	2067.4	111.09	5.4%	54.99	2.7%	46.01	2.2%	15.00	0.7%	133.06	6.4%
7	239†	2849.7	145.4	5.1%	100.56	3.5%	0.00	0.0%	15.16	0.5%	177.44	6.2%

*one (1) result was unavailable due to star-out

†one (1) outlier removed from analysis

‡two (2) outliers removed from analysis

Table 3: Estimate of GFAP and UCH-L1 Assay Precision with i-STAT TBI Controls												
Sample	N	Mean (pg/mL)	Repeatability		Between-Run		Between-Day		Between-Lot**		Within-Laboratory	
			SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
GFAP Assay												
L1	238*§	196.7	9.94	5.1%	2.69	1.4%	2.25	1.1%	5.70	2.9%	11.98	6.1%
L2	242*	5153.8	236.89	4.6%	94.93	1.8%	28.10	0.5%	183.00	3.6%	315.29	6.1%
UCH-L1 Assay												
L1	239†	562.6	29.79	5.3%	9.57	1.7%	11.92	2.1%	13.21	2.3%	36.00	6.4%
L2	240	1624.7	90.14	5.5%	53.68	3.3%	0.00	0.0%	32.25	2.0%	109.76	6.8%

* Additional GFAP result was obtained due to cartridge re-run due to a UCH-L1 star-out
†one (1) outlier removed from analysis
‡two (2) outliers removed from analysis
§three (3) outliers removed from analysis
**This refers to precision estimates calculated between cartridge lots. A single lot of i-STAT TBI controls was used for this study.

Qualitative precision: The qualitative agreement of cartridge results relative to the expected sample result (mean) was evaluated for the 80 measurements per sample per cartridge lot for each assay from the semi-quantitative analysis above. The mean, total number of replicates, total number of elevated results, and % correct call for each sample level are presented in **Table 4** for GFAP and **Table 5** for UCH-L1.

Table 4: GFAP assay results for qualitative precision analysis					
Plasma Sample	Lot	Mean (pg/mL)	Total Number of Replicates	Total Number of Results at or above the cut-off	% Correct Call
1[A]	A	18.5	80	0	100%
	B	15.6	80	0	100%
	C	17.1	80	0	100%
2[B]	D	30.9	80	59	74%
	E	30.5	80	50	63%
	F	31.5	80	69	86%
3[C]	D	65.7	80	80	100%
	E	64.8	80	80	100%
	F	66.0	80	80	100%
4[C]	A	106.3	80	80	100%
	B	102.7	80	80	100%
	C	105.1	81	81	100%
5[C]	A	939.3	80	80	100%
	B	979.6	80	80	100%
	C	973.8	80	80	100%
6[C]	G	2096.8	80	80	100%
	H	1962.2	80	80	100%
7[C]	A	3050.2	80	80	100%

Table 4: GFAP assay results for qualitative precision analysis					
Plasma Sample	**Lot**	**Mean (pg/mL)**	**Total Number of Replicates**	**Total Number of Results at or above the cut-off**	**% Correct Call**
	B	3244.7	80	80	100%
	C	3123.6	80	80	100%
8[C]	G	5579.9	80	80	100%
	H	5832.8	83	83	100%
9[C]	G	7404.2	80	80	100%
	H	7685.7	80	80	100%
i-STAT TBI Controls					
L1[C]	A	203.4	81	81	100%
	B	193.3	80	80	100%
	C	195.1	80	80	100%
L2[C]	A	5162.0	82	82	100%
	B	5335.2	80	80	100%
	C	4964.2	80	80	100%

[A] Below cut-off
[B] Near cut-off (mean ± 25%)
[C] Above cut-off

Table 5: UCH-L1 assay results for qualitative precision analysis					
Plasma Sample	**Lot**	**Mean (pg/mL)**	**Total Number of Replicates**	**Total Number of Results at or above the cut-off**	**% Correct Call**
1[A]	D	70.7	80	0	100%
	E	73.5	80	0	100%
	F	77.1	80	0	100%
2[B]	D	295.6	80	0	100%
	E	289.0	80	1	99%
	F	317.5	80	0	100%
3[C]	A	525.1	80	80	100%
	B	509.1	80	80	100%
	C	525.6	80	80	100%
4[C]	A	1039.8	80	80	100%
	B	1039.0	80	80	100%
	C	1098.8	80	80	100%
5[C]	G	1617.9	79	79	100%
	H	1661.0	80	80	100%
6[C]	A	2078.7	80	80	100%
	B	2040.2	80	80	100%
	C	2083.2	80	80	100%
7[C]	A	2865.0	80	80	100%
	B	2819.5	80	80	100%
	C	2855.7	80	80	100%
i-STAT TBI Controls					
L1[C]	A	556.8	80	80	100%
	B	575.2	80	80	100%
	C	552.6	80	80	100%
L2[C]	A	1601.2	80	80	100%
	B	1664.7	80	80	100%

Table 5: UCH-L1 assay results for qualitative precision analysis					
Plasma Sample	**Lot**	**Mean (pg/mL)**	**Total Number of Replicates**	**Total Number of Results at or above the cut-off**	**% Correct Call**
	C	1608.1	80	80	100%

[A] Below cut-off
[B] Near cut-off (mean ± 25%)
[C] Above cut-off

Semi-quantitative multi-site precision: The precision performance of the GFAP and UCH-L1 assays in the i-STAT TBI Plasma cartridge on the i-STAT Alinity System was evaluated using seven (7) test materials representing six (6) levels of GFAP and five (5) levels of UCH-L1 at three (3) external clinical sites. At each site, each test material was tested once per day for five (5) days by two (2) different operators, with each operator using three (3) i-STAT Alinity Instruments. The plasma samples were made of pooled plasma from healthy normal donors spiked with recombinant GFAP and UCH-L1 antigens or spiked with native antigens from pooled TBI patient plasma as shown in the tables below. Thirty (30) replicates were obtained for the applicable assay analyzed for each test material at each of the three (3) sites for a total of 90 replicates for each test material. The estimates of GFAP and UCH-L1 precision are shown in **Table 6** and **Table 7**.

Table 6: i-STAT TBI Plasma Multi-site Precision - GFAP (pg/mL) - All Sites														
Test Material	N	Mean (Min, Max)	Within-Day		Between-Day		Between-Operator		Within-Site (Total)		Between-Site		Overall	
			SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV
5[A]	90	19.4 (7, 26)	2.60 (2.14, 3.37)	13.4%	0.00 (0.00, 0.00)	0.0%	0.00 (0.00, 0.00)	0.0%	2.60 (2.32, 2.97)	13.4%	2.88 (1.66, 12.71)	14.9%	3.88	20.0%
9[B]	90	30.8 (23, 36)	2.15 (1.76, 2.77)	7.0%	0.55 (0.41, 0.83)	1.8%	0.20 (0.13, 0.59)	0.7%	2.22 (1.97, 2.53)	7.2%	2.51 (1.45, 11.07)	8.1%	3.35	10.9%
10[B]	90	66.2 (54, 77)	2.97 (2.44, 3.84)	4.5%	0.86 (0.65, 1.30)	1.3%	0.00 (0.00, 0.00)	0.0%	3.09 (2.76, 3.54)	4.7%	4.00 (2.31, 17.64)	6.0%	5.05	7.6%
8[A]	90	150.1 (137, 168)	4.48 (3.73, 5.64)	3.0%	2.18 (1.64, 3.30)	1.4%	1.41 (0.88, 4.13)	0.9%	4.98 (4.38, 5.78)	3.3%	4.09 (2.37, 18.08)	2.7%	6.45	4.3%
6[A]	90	4504.7 (4257, 4813)	88.75 (72.82, 114.75)	2.0%	52.32 (39.53, 79.29)	1.2%	0.00 (0.00, 0.00)	0.0%	103.03 (90.40, 120.20)	2.3%	74.34 (42.95, 328.25)	1.7%	127.05	2.8%
7[A]	90	9196.8 (8575, 10099)	193.85 (161.79, 243.68)	2.1%	189.14 (142.89, 286.60)	2.1%	63.22 (39.17, 184.60)	0.7%	270.84 (229.00, 33.56)	2.9%	167.65 (96.86, 740.24)	1.8%	318.53	3.5%

[A] pooled normal donor plasma spiked with recombinant GFAP and UCH-L1 antigen
[B] pooled normal donor plasma spiked with antigens from pooled TBI patient sample

Table 7: i-STAT TBI Plasma Multi-site Precision - UCH-L1 (pg/mL) - All Sites														
Test Material	N	Mean (Min, Max)	Within-Day		Between-Day		Between-Operator		Within-Site (Total)		Between-Site		Overall	
			SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV
5[a]	90	60.5 (44, 83)	6.03 (5.10, 7.44)	10.0%	1.57 (1.19, 2.38)	2.6%	2.69 (1.67, 7.85)	4.4%	6.23 (5.52, 7.18)	10.3%	0.00 (0.00, 0.00)	0.0%	6.23	10.3%
11[c]	90	297.9 (257, 348)	16.53 (13.68, 21.08)	5.6%	5.01 (3.79, 7.59)	1.7%	3.71 (2.30, 10.84)	1.2%	17.28 (15.36, 19.79)	5.8%	9.22 (5.33, 40.71)	3.1%	19.58	6.6%
8[a]	90	538.0 (467, 613)	28.88 (24.66, 35.04)	5.4%	9.52 (7.19, 14.43)	1.8%	17.19 (10.65, 50.19)	3.2%	30.41 (26.63, 35.57)	5.7%	3.25 (1.88, 14.36)	0.6%	30.58	5.7%
6[a]	90	5143.2 (4417, 6539)	311.62 (263.93, 382.79)	6.1%	58.76 (44.39, 89.03)	1.1%	148.37 (91.93, 433.25)	2.9%	317.11 (280.91, 365.15)	6.2%	137.41 (79.39, 606.70)	2.7%	345.60	6.7%
7[a]	90	9538.7 (7946, 11016)	516.39 (436.60, 636.06)	5.4%	107.70 (81.36, 163.20)	1.1%	236.10 (146.28, 689.40)	2.5%	527.50 (467.50, 607.02)	5.5%	351.68 (203.19, 1552.81)	3.7%	633.99	6.6%

[a] pooled normal donor plasma spiked with recombinant GFAP and UCH-L1 antigen
[c] pooled TBI patient and normal donor plasma

Qualitative multi-site precision: The qualitative agreement of cartridge results relative to the expected sample result (mean) was evaluated for the 90 measurements per test material for each assay from the semi-quantitative analysis above. The mean, total number of replicates, total number of elevated results, and % correct call for each test material are presented in **Table 8** for GFAP and **Table 9** for UCH-L1.

Table 8: Qualitative Precision Analysis - GFAP Assay - All Sites

Test Material	Mean (pg/mL)	Total Number of Replicates	Total Number of GFAP Results at or above the cut-off	% Correct Call
5 [A]	19.4	90	0	100%
9 [B]	30.8	90	60	67%*
10 [C]	66.2	90	90	100%
8 [C]	150.1	90	90	100%
6 [C]	4504.7	90	90	100%
7 [C]	9196.8	90	90	100%

[A] Below cut-off; [B] Near cut-off (overall mean +/- 25%); [C] Above cut-off
*Test Material range includes cut-off. % Correct call based on test material overall mean for all sites.

Table 9: Qualitative Precision Analysis - UCH-L1 Assay - All Sites

Test Material	Mean (pg/mL)	Total Number of Replicates	Total Number of UCH-L1 Results at or above the cut-off	% Correct Call
5 [A]	60.5	90	0	100%
11 [B]	297.9	90	0	100%*
8 [C]	538.0	90	90	100%
6 [C]	5143.2	90	90	100%
7 [C]	9538.7	90	90	100%

[A] Below cut-off; [B] Near cut-off (overall mean +/- 25%); [C] Above cut-off
*Test Material range includes cut-off. % Correct call based on test material overall mean for all sites.

b. Linearity/assay reportable range

Linearity: The linearity of the GFAP and UCH-L1 assays in the i-STAT TBI Plasma cartridge with the i-STAT Alinity System was established by testing plasma samples of varying antigen levels that range from below the lower limit of the reportable range to above the upper reportable range for both GFAP and UCH-L1. The study was designed based on CLSI EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures.* The study was conducted using plasma samples spiked with either native antigens from a TBI patient sample exhibiting high levels of both GFAP and UCH-L1 or with recombinant GFAP and UCH-L1 antigens. Plasma samples of varying GFAP and UCH-L1 levels were prepared through proportional mixing of low and high antigen concentration samples.

The regression equation for the linear range of the GFAP assay is y=1.02x-6.7. The regression equation for the linear range of the UCH-L1 assay is y=1.04x-17.7. Regression summary of the results obtained for each assay in the i-STAT TBI Plasma cartridge (y-

axis) versus the expected values (x-axis) is provided in **Table 10**.

Table 10: Linearity Across Reportable Range				
Assay	**Reportable Range (pg/mL)**	**Slope**	**Intercept**	**r^2**
GFAP	30 – 10000	1.02	-6.7	0.9985
UCH-L1	200 – 3200	1.04	-17.7	0.9869

Recovery: The recovery evaluation was performed using data collected as part of the linearity studies. The % recovery was calculated using the means of the GFAP and UCH-L1 assay results and the expected values of the plasma samples as determined by the i-STAT Alinity instruments. The % recovery is presented in **Table 11** for the GFAP assay and **Table 12** for the UCH-L1 assay.

Table 11: GFAP Assay % Recovery				
Antigen Source	**N**	**Mean GFAP (pg/mL)**	**GFAP Expected (pg/mL)**	**% Recovery**
Native Plasma	6	3245.5	3245.5	100.0%
	6	2607.8	2599.6	100.3%
	6	1990.3	1953.6	101.9%
	5	1353.2	1307.7	103.5%
	6	634.8	661.7	95.9%
	6	346.7	338.7	102.4%
	6	169.5	177.3	95.6%
	6	98.9	96.5	102.5%
	6	49.4	56.1	88.1%
	6	15.8	15.8	100.0%
Recombinant Plasma	5	10686.5	10686.5	100.0%
	6	9612.2	9618.9	99.9%
	6	8581.2	8551.3	100.3%
	6	7630.5	7483.8	102.0%
	6	6117.7	6416.1	95.3%
	6	5653.3	5348.6	105.7%
	6	4539.1	4281.0	106.0%
	6	3595.3	3213.4	111.9%
	6	2355.7	2145.8	109.8%
	6	1206.6	1078.3	111.9%
	6	571.8	608.6	94.0%
	6	294.5	309.6	95.1%
	5	153.6	160.2	95.9%
	5	81.5	85.4	95.4%
	6	39.4	47.7	82.6%
	6	23.5	29.2	80.5%
	6	16.4	19.9	82.4%

Table 12: UCH-L1 Assay % Recovery				
Antigen Source	**N**	**Mean UCH-L1 (pg/mL)**	**UCH-L1 Expected (pg/mL)**	**% Recovery**
Native Plasma	6	2286.6	2286.6	100.0%
	6	1945.6	1844.1	105.5%
	6	1408.6	1401.6	100.5%
	5	1012.6	959.2	105.6%
	6	453.2	516.7	87.7%
	6	296.4	295.4	100.3%
	6	183.1	184.8	99.1%
Recombinant Plasma	6	4950.8	4298.9	115.2%
	6	3598.3	3245.7	110.9%
	6	2492.8	2192.4	113.7%
	6	1216.9	1139.1	106.8%
	5	592.1	651.4	90.9%
	6	345.1	368.6	93.6%
	5	213.1	227.2	93.8%
	5	154.4	156.5	98.7%

Hook Effect: The GFAP and UCH-L1 assays in the i-STAT TBI Plasma cartridge on the i-STAT Alinity System were evaluated for high dose hook effect. The testing was conducted using plasma samples spiked to a high antigen level for each assay (>100,000 pg/mL). Each sample was tested to verify that the measured current response (nA)[1] is greater than that of a nominal GFAP target of 10,000 pg/mL and a nominal UCH-L1 target of 4,000 pg/mL. Hook effect was not observed for the GFAP and UCH-L1 assays as the current responses of high dose samples were significantly greater than 10,000 pg/mL for the GFAP assay and 4,000 pg/mL for the UCH-L1 assay.

c. Traceability, Calibration, and Reference Interval

Traceability and Calibration: The i-STAT System test for glial fibrillary acidic protein (GFAP) or ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) measures GFAP and UCH-L1 amount-of-substance concentration in plasma (units of measure: pg/mL) for *in vitro* diagnostic use.

There are no internationally recognized standard reference materials available for either glial fibrillary acidic protein (GFAP) or ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1). GFAP and UCH-L1 values assigned to i-STAT controls and calibration verification materials are traceable to Abbott Point of Care's working calibrators prepared using recombinant GFAP and UCH-L1 (expressed and purified from *E. coli*). The working calibrators are traceable to an in-house Reference Standard prepared from recombinant GFAP and UCH-L1 (expressed and purified from *E. coli*).

[1] The i-STAT TBI Plasma cartridge measures assay current response in nanoamps (nA).

i-STAT System controls and calibration verification materials are validated for use only with the i-STAT System and assigned values may not be commutable with other methods.

Reference Interval: A reference interval study was conducted with a US-based general population. Plasma specimens from 225 self-declared healthy subjects between the ages of 18 and 79 years reporting no history of neurological disease within 1 year were tested with the i-STAT TBI Plasma cartridge with the i-STAT Alinity system to determine GFAP and UCH-L1 levels. Based on the test results, a 95% reference interval of an apparently healthy population of each biomarker was determined to be as follows:

Table 13: Reference Interval

Biomarker	N	Mean (pg/mL)	SD (pg/mL)	Median (pg/mL)	Reference Interval (2.5^{th} to 97.5^{th} Percentile) (pg/mL)
GFAP	225	19	16.2	15	2 – 51
UCH-L1	225	81	42.4	71	21 – 204

d. Detection Limit

Limit of Quantitation (LoQ): The LoQ was determined for the GFAP and UCH-L1 assays in the i-STAT TBI Plasma cartridge in a study based on CLSI EP17-A2: *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline–Second Edition.* The testing was conducted on five (5) days using four (4) lots of i-STAT TBI cartridges with plasma from normal donors containing six (6) low levels of GFAP and UCH-L1. The LoQ for the GFAP and UCH-L1 assays in the i-STAT TBI Plasma cartridge tested on i-STAT Alinity instruments was determined to be below the lower limit of the reportable range for each assay as shown in **Table 14**.

Table 14: LoQ Results for the GFAP and UCH-L1 Assays

Assay	Lower limit of the reportable range (pg/mL)	LoQ (pg/mL)
GFAP	30	23
UCH-L1	200	70

e. Analytical Specificity

Endogenous and Exogenous Interferences: The interference performance of the GFAP and UCH-L1 assays in the i-STAT TBI Plasma cartridge on the i-STAT Alinity System was evaluated using plasma samples in a study based on CLSI EP07 ED3: *Interference Testing in Clinical Chemistry, Third Edition*. The effect of each substance was evaluated by comparing the performance of a control sample, spiked with blank solvent solution, with the test results from a sample spiked with the potentially interfering substance at the

toxic/pathological concentration based on CLSI EP37 ED1: *Supplemental Tables for Interference Testing in Clinical Chemistry, First Edition*, as applicable. A substance was identified as an interferent if the difference between the control and test samples was outside of a pre-determined acceptable range for each assay. **Table 15** contains the list of potentially interfering substances tested for the GFAP and UCH-L1 assays and the interference results.

Table 15: Interfering Substance Testing

Substance	Test Concentration		Assay	Interference (Yes/No)	Interference Results
	µmol/L	mg/dL			
Albumin	150 g/L	15 g/dL	GFAP	No	
			UCH-L1	No	
Bilirubin	684	40	GFAP	No	
			UCH-L1	No	
Bilirubin (conjugated)	475	40	GFAP	No	
			UCH-L1	No	
Hemoglobin	10 g/L	1000	GFAP	No	
			UCH-L1	No	
Human anti-mouse antibody (HAMA) [a]	> 160x [b]	N/A	GFAP	No	
			UCH-L1	Yes	Highest concentration tested where no interference observed: 40x Testing above this level showed decreased results[c]
Intralipid (Intralipid 20%)	N/A	4747	GFAP	No	
			UCH-L1	No	
Rheumatoid Factor (RF) [a]	1000 IU/mL	N/A	GFAP	No	
			UCH-L1	Yes	Highest concentration tested where no interference observed: 500 IU/mL Testing above this level showed decreased results[c]
Triglycerides [a]	33.88 mmol/L	3000	GFAP	No	
			UCH-L1	No	
Acetaminophen [a]	1.324 mmol/L	15.6	GFAP	No	
			UCH-L1	No	
Sodium Ascorbate	298	5.25	GFAP	No	
			UCH-L1	No	
Caffeine	556	10.8	GFAP	No	
			UCH-L1	No	
Clopidogrel [a]	21.4	9 µg/mL	GFAP	No	
			UCH-L1	No	
Dopamine	4.06	0.114	GFAP	No	
			UCH-L1	No	
Ethanol	130 mmol/L	600	GFAP	No	
			UCH-L1	Yes	Highest concentration

Table 15: Interfering Substance Testing					
Substance	**Test Concentration**		**Assay**	**Interference (Yes/No)**	**Interference Results**
	µmol/L	**mg/dL**			
					tested where no interference observed: 65 mmol/L [d] Testing above this level showed decreased results
Erythromycin	188	0.720	GFAP	No	
			UCH-L1	No	
Nicotine	5.97	0.00240	GFAP	No	
			UCH-L1	No	
Metoprolol Tartrate [a]	18.7	128.06	GFAP	No	
			UCH-L1	No	
Acetylsalicylic acid [a]	3.62 mmol/L	6521.79	GFAP	No	
			UCH-L1	No	
Chloramphenicol	241	7.80	GFAP	No	
			UCH-L1	No	
Diclofenac	81	2.40	GFAP	No	
			UCH-L1	No	
Ibuprofen [a]	2.425 mmol/L	50.0	GFAP	No	
			UCH-L1	No	
Phenytoin	238	6	GFAP	No	
			UCH-L1	No	
Amphetamine	2.44	0.033	GFAP	No	
			UCH-L1	No	
Benzoylecgonine [a]	8.64	2.5 µg/mL	GFAP	No	
			UCH-L1	No	
Nicardipine hydrochloride	0.97	0.0465	GFAP	No	
			UCH-L1	No	
EDDP† perchlorate [a]	0.3308	125 ng/mL	GFAP	No	
			UCH-L1	No	
Methadone	10.3	0.318	GFAP	No	
			UCH-L1	No	
Methaqualone [a]	32.36	8.1 µg/mL	GFAP	No	
			UCH-L1	No	
d-Methamphetamine [a]	1.865	278.4 ng/mL	GFAP	No	
			UCH-L1	No	
Morphine	27.3	0.78	GFAP	No	
			UCH-L1	No	
Oxazepam	15.1	0.432	GFAP	No	
			UCH-L1	No	
Phencyclidine [a]	0.0357	8.7 ng/mL	GFAP	No	
			UCH-L1	No	
Secobarbital	66.8	159.17	GFAP	No	
			UCH-L1	No	
Cocaine [a]	11.406	3.46 µg/mL	GFAP	No	
			UCH-L1	No	
Propoxyphene [a]	9.46	32.11	GFAP	No	
			UCH-L1	No	
Warfarin	243	7.5	GFAP	No	

Table 15: Interfering Substance Testing					
Substance	Test Concentration		Assay	Interference (Yes/No)	Interference Results
	µmol/L	mg/dL			
			UCH-L1	No	
Diazepam	105	0.330	GFAP	No	
			UCH-L1	No	

† 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

[a] The test concentration used for this substance is not from CLSI guideline EP37 1st edition

[b] 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.

[c] One out of the five samples enriched for the presence of HAMA and two out of the five samples enriched for presence of RF exhibited an interference effect. See note regarding HAMA or other heterophile antibodies in Limitations of the Procedure section above.

[d] Note that the ethanol level is well above the CLSI highest therapeutic level of 43.4 mmol/L (200 mg/dL)

Cross-reactivity: The i-STAT TBI Plasma cartridge is specific to the measurement of GFAP and UCH-L1. The following proteins in **Table 16** with significant homology to GFAP or UCH-L1 were tested at highest known physiological levels and none were found to have significant cross-reactive effects on measured the GFAP or UCH-L1 levels.

Table 16: Interfering Substance Testing			
Substance	Test Concentration	Assay	Cross-reactivity (Yes/No)
	pg/mL		
Keratin type II	10 000	GFAP	No
Internexin	77 000	GFAP	No
Neurofilament medium	8600	GFAP	No
Neurofilament heavy	77 000	GFAP	No
Neurofilament light	68	GFAP	No
Peripherin	5000	GFAP	No
Desmin	127 000	GFAP	No
Vimentin	354 000	GFAP	No
Ubiquitin Carboxyl-Terminal Hydrolase L3 (UCH-L1)	354 000	UCH-L1	No

Cross-talk: The GFAP and UCH-L1 assays in the i-STAT TBI Plasma cartridge were evaluated for potential cross-talk to determine if high levels of the antigen (GFAP or UCH-L1) of one assay have potential to the impact the result of the other assay. Plasma samples spiked to low and high GFAP and UCH-L1 levels were evaluated in the presence of a single high level of the other antigen being evaluated for potential cross-talk. No cross-talk effect was observed as the results demonstrated that the GFAP result is not affected when UCH-L1 is present in a sample, and that the UCH-L1 result is not affected when GFAP is present in a sample.

Table 17: Interfering Substance Testing			
Substance	Test Concentration pg/mL	Assay	Cross-talk (Yes/No)
UCH-L1	100 000	GFAP	No
GFAP	100 000	UCH-L1	No

f. Assay cutoff

Cutoff: The assay cutoffs were determined by analyzing a training set with GFAP and UCH-L1 results from a total of 420 (274 males and 146 females) with suspected mild traumatic brain injury (TBI; Glasgow Coma Scale score of 13-15). Subjects who had blood drawn within 12 hours of injury and a head CT scan determination, were included in the analysis. Using a 10-fold cross validation and bootstrapping method, the cutoff values of 30 pg/mL (GFAP assay) and 360 pg/mL (UCH-L1 assay) were selected for the i-STAT TBI Plasma Cartridge using the selection criteria with an adjusted NPV (prevalence 6%) ≥98.5% and sensitivity ≥98%.

2. Clinical Study

Clinical Sensitivity and Specificity: A pivotal study using prospectively collected and archived (frozen) plasma specimens was conducted to establish the clinical performance of the i-STAT TBI Plasma test. The testing of the archived plasma specimens was conducted at three clinical sites in the United States.

The specimens were originally collected in a prospective, multi-center clinical study that enrolled consenting men and women 18 years of age or older who presented to emergency departments (ED) with suspected traumatic brain injury with initial Glasgow Coma Scale (GCS) scores of 13-15 and who had a computed tomography (CT) scan performed per the clinical site's standard of care. Subjects were enrolled at 22 clinical sites in three countries: United States, Germany and Hungary.

CT scans were performed in accordance with the clinical site's standard of care. Images were transmitted to a central neuroimaging processing center. 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 between two neuroradiologists with adjudication by a third neuroradiologist if necessary. Outcomes were 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.

Whole blood was collected into K2EDTA blood collection tubes from each subject using venipuncture and centrifuged to obtain plasma. Specimens were collected within 12 hours of head injury. The plasma specimens were divided into aliquots and frozen in cryovials before being provided to testing sites.

Of the 1994 subjects with GCS scores of 13 to 15 enrolled in the original study, specimens from 93 subjects were not included in the performance analysis due to subject discontinuation, lack of consent for specimen archiving for future testing, inconclusive or unreadable CT scan results, and/or unknown time from injury to blood collection. Specimens from 1901 subjects were included in the analysis.

The demographic characteristics of the subjects represented in the performance analysis are summarized in **Table 18** below.

Table 18: Demographic Characteristics

Characteristic	Head CT Scan Result		Total
	Positive	Negative	
N	120	1781	1901
Age[1] (Years)			
Mean	58.8	48.5	49.1
Median	58.5	48.0	49.0
Standard Deviation	18.29	21.01	20.99
Range	(20, 95)	(18, 98)	(18, 98)
Gender, N (%)			
Male	70 (58.3%)	1005 (56.4%)	1075 (56.6%)
Female	50 (41.7%)	776 (43.6%)	826 (43.5%)
Race[2], 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%)
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%)

[1] Age was calculated relative to the date of informed consent.
[2] Subjects could have indicated more than one race.

The head injury characteristics of the subjects represented by the 1901 specimens included in the performance analysis were tabulated. 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 19**.

Table 19: Head Injury Characteristics

Characteristic	Head CT Scan Result		Total
	Positive	Negative	
N	120	1781	1901
Time from head injury to exam (hours)[1]			
Mean	1.9	1.6	1.6
Median	1.2	1.0	1.1
Standard Deviation	1.73	1.71	1.71
Range	(0.3, 7.8)	(0.1, 10.7)	(0.1, 10.7)
Time from head injury to CT scan (hours)[1]			
Mean	2.8	2.7	2.7
Median	2.1	2.2	2.1
Standard Deviation	1.95	1.93	1.93
Range	(0.5, 8.9)	(0.2, 13.3)	(0.2, 13.3)
Time from head injury to blood draw (hours)[1]			
Mean	3.8	3.5	3.5
Median	3.3	3.1	3.2
Standard Deviation	1.91	1.88	1.89
Range	(0.3, 9.3)	(0.3, 11.9)	(0.3, 11.9)
Glasgow Coma Score – N (%)			
13	7 (5.8%)	15 (0.8%)	22 (1.2%)
14	19 (15.8%)	71 (4.0%)	90 (4.7%)
15	94 (78.3%)	1695 (95.2%)	1789 (94.1%)
Neurological assessment - N (%) 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 the Time of Presentation to Facility	33 (27.5%)	369 (20.7%)	402 (21.1%)
Dangerous Mechanism of Injury[2]	27 (22.5%)	369 (20.7%)	396 (20.8%)
Physical Evidence[3]			
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%)

[1] Based on time subject was initially examined at the medical facility
[2] 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
[3] Prior to head CT

The i-STAT TBI Plasma test clinical performance estimates are shown in **Table 20**. Of the 1901 specimens, 120 were associated with positive CT scan results. Of these 120 specimens, 115 had an 'elevated' i STAT TBI Plasma test interpretation (115/120, clinical sensitivity = 95.8%). Five specimens associated with CT scan positive results had an i-STAT TBI Plasma test interpretation that was 'not elevated'. The rate of false

negative (FN) results was 4.2% (5/120). Five subjects in the study were identified with lesion requiring surgical intervention; none of these five subjects had a FN result, suggesting that the i-STAT TBI Plasma test correctly classified all these five CT-positive subjects with a test interpretation of 'elevated.' Of the 1781 specimens associated with negative CT scan results, 720 had an i-STAT TBI Plasma test interpretation that was 'not elevated' (720/1781, clinical specificity = 40.4%). The rate of False Positive (FP) results was 59.6% (1061/1781).

Overall, there were 725 specimens with i-STAT TBI Plasma test interpretations of 'not elevated'. Of these, 720 specimens were associated with negative CT scan results. The Negative Predictive Value (NPV) of the assay was 99.3% (720/725).

Table 20: Clinical Performance

i-STAT TBI Plasma Test Interpretation	Adjudicated CT Scan Result		Total
	Positive	Negative	
Elevated	115	1061	1176
Not Elevated	5	720	725
Total	120	1781	1901

Clinical Performance Parameters	N=1901	95% Confidence Interval
Clinical Sensitivity	95.8%	90.6%, 98.2%
Clinical Specificity	40.4%	38.2%, 42.7%
Negative Predictive Value (NPV)	99.3%	98.5%, 99.7%
Positive Predictive Value (PPV)	9.8%	9.2%, 10.2%
Likelihood Ratio Negative (LRN)	0.10	0.04, 0.23
Likelihood Ratio Positive (LRP)	1.61	1.51, 1.69

To supplement the results of the pivotal study (N=1901) described above, a study was conducted using freshly collected plasma specimens from consenting men and women 18 years of age or older who presented to Level 1 trauma center emergency departments (ED) with suspected traumatic brain injury, with initial Glasgow Coma Scale(GCS) scores of 13-15, and who had a computed tomography (CT) scan of the head performed per the clinical site's standard of care. A total of 88 subjects were enrolled across 4 clinical sites of the Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) study in the United States.

Similar to the pivotal study, CT scans were performed in accordance with the clinical site's standard of care. 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 between two neuroradiologists, with adjudication by a third neuroradiologist if necessary. Outcomes were 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.

Whole blood was collected into K3EDTA blood collection tubes from each subject

using venipuncture and centrifuged to obtain plasma. Specimens were collected within 12 hours of head injury. The demographic characteristics of the subjects represented in the performance analysis are summarized in **Table 21** below:

Table 21: Demographic Characteristics - Supplemental Fresh Specimen Study			
Characteristic	**Head CT Scan Result**		**Total**
	Positive	**Negative**	
N	29	59	88
Age (Years)			
Mean	49.2	39.3	42.5
Median	47	36	41
Standard Deviation	16.92	15.43	16.52
Range	(24, 85)	(18, 76)	(18, 85)
Gender			
Male	23	40	63
Female	6	19	25

The head injury characteristics of the subjects in the supplemental plasma fresh specimen study including 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 22**.

Table 22: Head Injury Characteristics – Supplemental Fresh Specimen Study			
Characteristic	**Head CT Scan Result**		**Total**
	Positive	**Negative**	
N	29	59	88
Time from head injury to CT scan (hours)			
Mean	2.5	2.2	2.3
Median	2.0	1.9	1.9
Standard Deviation	1.76	1.39	1.51
Range	(0.7, 8.7)	(0.7, 7.5)	(0.7, 8.7)
Time from head injury to blood draw (hours)			
Mean	6.6	4.4	5.1
Median	6.0	3.9	4.3
Standard Deviation	2.93	1.96	2.54
Range	(2.3, 11.8)	(2.0, 9.9)	(2.0, 11.8)
Glasgow Coma Score – N (%)[1]			

Table 22: Head Injury Characteristics – Supplemental Fresh Specimen Study			
Characteristic	Head CT Scan Result		Total
	Positive	Negative	
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 - N (%) of subjects experiencing:			
Loss of Consciousness (LOC)	23 (79.3%)	37 (62.7%)	60 (68.2%)
Presence of Confusion	19 (65.5%)	40 (67.8%)	59 (67.0%)
Vomiting[2]	-	-	-
Post-traumatic Amnesia (PTA)	22 (75.9%)	38 (64.4%)	60 (68.2%)
Post-traumatic Seizures	0 (0%)	0 (0%)	0 (0%)
Subjects with Drug Intoxication at the Time of Presentation to Site	3 (10.3%)	2 (3.4%)	5 (5.7%)
Subjects with Alcohol Intoxication at the Time of Presentation to Site	6 (20.7%)	4 (6.8%)	10 (11.4%)
Physical Evidence - N (%)			
Signs of Skull Fracture	9 (31.0%)	1 (1.7%)	10 (11.4%)
Mechanism of Injury - N (%)			
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%)

[1] Percent based on total subjects

[2] Information not collected

The i-STAT TBI Plasma test clinical performance estimates from the supplemental fresh plasma specimen study are shown in **Table 23**. Of the 88 subjects tested, 29 were associated with positive head CT scan results. Of these 29 subjects, 29 had an 'elevated' i-STAT TBI Plasma test interpretation (29/29, clinical sensitivity = 100.0%). The rate of False Negatives (FN) was 0% (0/29). Of the 59 subjects associated with negative CT scan results, 14 had an i-STAT TBI Plasma test interpretation that was 'not elevated' (14/59, clinical specificity = 23.7%). The rate of False Positive (FP) results was 76.2% (45/59).

Overall, there were 14 specimens with i-STAT TBI Plasma test interpretations of 'not elevated'. All 14 specimens were associated with negative head CT scan results. The Negative Predictive Value (NPV) of the assay was 100% (14/14).

Table 23: Clinical Performance – Supplemental Fresh Specimen Study		
i-STAT TBI Plasma	Adjudicated CT Scan Result	Total

Test Interpretation	Positive	Negative	
Elevated	29	45	74
Not Elevated	0	14	14
Total	29	59	88

Clinical Performance Parameters	N=88	95% Confidence Interval
Clinical Sensitivity	100.0%	88.3%, 100.0%
Clinical Specificity	23.7%	14.7%, 36.0%
Negative Predictive Value (NPV)*	100.0%	80.2%, 100.0%
Positive Predictive Value (PPV)*	39.2%	35.9%, 43.4%
Likelihood Ratio Negative (LRN)	0.00	0.00, 0.50
Likelihood Ratio Positive (LRP)	1.31	1.14, 1.56

*NPV and PPV estimated at 33.0% prevalence of CT scan positive rate for suspected mild TBI subjects. Adjusted NPV and PPV at 6% prevalence (to be comparable to the pivotal study) are 100.0% (95% CI: 96.9%, 100.0%) and 7.7% (95% CI: 6.8%, 9.1%), respectively.

8. Conclusion

Per special controls requirements of 21 CFR 866.5830, clinical studies were performed to support substantial equivalence. A pivotal study (N=1901) using archived plasma specimens from the same cohort of subjects that was used to demonstrate clinical performance of the predicate device showed similar clinical specificity and specificity with a similar number of false negative results. None of the false negative results were of subjects identified with a lesion requiring surgical intervention. The i-STAT TBI Plasma test showed high clinical sensitivity when validated in a supplemental fresh plasma specimen study (N=88). The clinical data demonstrates that the i-STAT TBI Plasma cartridge with the i-STAT Alinity System performs comparably to the predicate Banyan BTI device. In addition, a benefit-risk assessment was performed to help determine substantial equivalence for the device. Although similar clinical specificity to the predicate device was observed in the pivotal study, this was not as high in the supplemental fresh plasma study. However, the device shows potential for some reduction in the number of unnecessary CT scans. The i-STAT TBI Plasma cartridge offers significant benefit over the predicate device by establishing clinical workflow efficiencies through ease of use and a reduce test time. The assessment concludes that the benefits outweighs the risks. Overall, the results of analytical and clinical studies demonstrate that performance of the GFAP and UCH-L1 assays in the i-STAT TBI Plasma cartridge with the i-STAT Alinity System are substantially equivalent to the comparative method.