March 27, 2024

Abbott Point of Care
Brian Ma
Principal Specialist, Regulatory Affairs
400 College Road East
Princeton, New Jersey 08540

Re: K234143
Trade/Device Name: i-STAT TBI 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 28, 2023
Received: December 29, 2023

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 (the 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 available 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.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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 Part 803) for devices or postmarketing safety reporting (21 CFR Part 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 Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 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,

Ying Mao -S

Ying (Katelin) Mao, Ph.D.
Branch Chief
Division of Immunology and Hematology Devices
OHT7: Office of In Vitro Diagnostics
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: 07/31/2026
See PRA Statement below.

Submission Number *(if known)*

K234143

Device Name

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

Indications for Use *(Describe)*

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

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

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.

Abbott

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 Principal Specialist Regulatory Affairs Phone: 613-688-5949 Secondary: Mojgan Soleimani Associate Director, Regulatory Affairs Phone: 613-295-0932
Date Prepared	March 25, 2024

2. Device Information

Proprietary Name: *i-STAT TBI* cartridge with the *i-STAT Alinity System*

Common Name: Glial fibrillary acidic protein (GFAP)
Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1)

510(k) Number K234143

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

510(k) Number K201778

Product Code	Device Classification Name	Regulation Number	Class	Panel
QAT	Brain trauma assessment test	866.5830	II (special controls)	Immunology

4. Device Description

The *i-STAT TBI* 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 whole blood 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* 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. The instrument is the main user interface of the *i-STAT Alinity 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).

Assayed quality control materials are also available for use with the *i-STAT TBI* cartridge and include *i-STAT TBI* Control Level 1, *i-STAT TBI* Control Level 2, and the *i-STAT TBI* Calibration Verification Levels 1-3.

The *i-STAT TBI* Controls are available to monitor the performance of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) assays on the *i-STAT Alinity* instrument.

The *i-STAT TBI* Calibration Verification Materials are available to verify the calibration of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) assays throughout the reportable range on the *i-STAT Alinity* instrument.

5. Intended Use Statement

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

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

6. Summary Comparison of Technological Characteristics

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

Similarities and Differences: System (Test and Instrument)		
Feature or Characteristic	**Candidate Device: i-STAT TBI cartridge with the i-STAT Alinity System**	**Predicate Device: i-STAT TBI Plasma cartridge with the i-STAT Alinity System (K201778)**
Intended Use Setting	Clinical Laboratory and Point of Care	Clinical Laboratory
Measurands	GFAP and UCH-L1	Same
Assay Technology	Enzyme-linked immunosorbent assay	Same
Assay Format	Single use multiplex cartridge (both assays (GFAP and UCH-L1) in one cartridge)	Same
Detection Technology	Electrochemical	Same
Sample Type	Whole Blood	Plasma
Sample Volume	20 µL	Same
Automation	Test and wash cycles are fully automated after sample loading step	Same
Analytical Measuring Interval	GFAP: 47 - 10,000 pg/mL UCH-L1: 87 - 3,200 pg/mL	GFAP: 30 - 10,000 pg/mL UCH-L1: 200 - 3,200 pg/mL
Time to Result	15 minutes	Same
Reportable Result	Quantitative results for GFAP and UCH-L1 and semi-quantitative interpretation	Same
Instrument Platform	i-STAT Alinity	Same
Calibration	No calibration needed by the end user, calibration is pre-set during manufacture of the cartridge	Same
Controls	GFAP and UCH-L1 combined: i-STAT TBI Controls (Levels 1 and 2) i-STAT TBI Calibration Verification Materials (Levels 1, 2, 3)	Same
Traceability	GFAP and UCH-L1 values assigned to i-STAT controls and calibration verification materials are traceable to Abbott's working calibrators prepared using recombinant GFAP and UCH-L1 (expressed and purified from E. coli).	Same
Assay Cut-off	GFAP: 65 pg/ml UCH-L1: 360 pg/ml	GFAP: 30 pg/ml UCH-L1: 360 pg/ml

7. Performance Characteristics

A. Analytical Performance

a. Precision/Reproducibility:

Semi-quantitative 20-day precision: The precision of the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge with the *i-STAT Alinity System* was evaluated using plasma samples spiked with native or recombinant GFAP and UCH-L1 antigens at various levels across the reportable range of the GFAP and UCH-L1 assays, and two (2) controls (*i- STAT TBI* Control L1 and Control L2). The study was executed over 20 non-consecutive days, two (2) runs per day that were separated by minimum two (2) hours, by at least two (2) operators using three (3) lots of *i-STAT TBI* cartridges. Due to the inability to store or freeze whole blood samples to maintain sample stability over multiple days, plasma samples were used for this study. The study followed the standard single-site 20x2x2 experimental design based on guidance provided in CLSI EP05-A3; Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. The components of variability were estimated for GFAP and UCH-L1 and the precision results for the plasma panel are shown in **Table 1**, **Table 2**, and for the *i-STAT TBI* Controls in **Table 3**.

Table 1: Estimate of GFAP Assay Precision

Plasma 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 [B]	240	78.8	3.04	3.86	0.85	1.07	0.57	0.72	2.17	2.76	3.90	4.95
2 [B]	240	98.6	6.03	6.12	1.40	1.42	0.72	0.73	2.57	2.61	6.78	6.87
3 [A]	240	880.6	21.29	2.42	15.78	1.79	1.66	0.19	9.76	1.11	28.79	3.27
4 [A]	240	4415.3	144.73	3.28	67.27	1.52	17.25	0.39	135.59	3.07	212.16	4.81
5 [A]	240	8346.7	285.03	3.41	151.07	1.81	56.69	0.68	347.63	4.16	479.49	5.74

[A] Pooled plasma from normal donors spiked with <5% v/v recombinant GFAP antigen
[B] Pooled plasma from normal donors spiked with <5% v/v GFAP from pooled TBI patient plasma

Table 2: Estimate of UCH-L1 Assay Precision

Plasma 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 [A]	240	159.9	11.91	7.45	3.44	2.15	0.76	0.48	4.92	3.08	13.54	8.47
2 [A]	240	255.7	18.11	7.08	4.97	1.94	3.17	1.24	6.21	2.43	20.33	7.95
3 [B]	240	488.8	26.47	5.42	15.02	3.07	5.93	1.21	11.56	2.37	33.53	6.86
4 [A]	240	826.2	49.40	5.98	23.07	2.79	12.72	1.54	24.38	2.95	61.92	7.49
5 [A]	240	1763.7	100.60	5.70	26.56	1.51	29.57	1.68	84.62	4.80	138.92	7.88
6 [A]	240	2190.3	126.88	5.79	46.79	2.14	23.66	1.08	105.13	4.80	176.27	8.05

[A] Pooled plasma from normal donors spiked with <5% v/v recombinant UCH-L1 antigen
[B] Pooled plasma from normal donors spiked with <5% v/v UCH-L1 from pooled TBI patient plasma

Table 3: Estimate of GFAP and UCH-L1 Assay Precision with i-STAT TBI Controls												
Control Level	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	240	161.2	6.80	4.22	1.77	1.10	1.78	1.11	2.54	1.57	7.76	4.81
L2	240	4645.0	166.40	3.58	45.04	0.97	45.91	0.99	148.68	3.20	234.60	5.05
UCH-L1 Assay												
L1	240	466.2	28.55	6.12	7.27	1.56	6.43	1.38	16.60	3.56	34.75	7.45
L2	240	1597.6	93.98	5.88	39.23	2.46	19.79	1.24	65.97	4.13	124.87	7.82

Qualitative 20-day 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 above. The mean, total number of replicates, total number of elevated results, and % correct call for each sample level is presented in **Table 4** for GFAP and **Table 5** for UCH-L1.

Table 4: GFAP Assay Results for Qualitative Precision Analysis					
Plasma Sample	Mean (pg/mL)	Total Number of Replicates	Qualitative Agreement		
			Total Number of Results at or Above the Cut-off	Total Number of Results Below the Cut-off	%Correct Call
1[b]	78.8	240	239	1	99.6%*
2[c]	98.6	240	240	0	100.0%
3[c]	880.6	240	240	0	100.0%
4[c]	4415.3	240	240	0	100.0%
5[c]	8346.7	240	240	0	100.0%
i-STAT TBI Control					
L1[c]	161.2	240	240	0	100.0%
L2[c]	4645.0	240	240	0	100.0%

[a] Below cut-off; [b] Near cut-off; [c] Above cut-off

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

Table 5: UCH-L1 Assay Results for Qualitative Precision Analysis					
Plasma Sample	Mean (pg/mL)	Total Number of Replicates	Qualitative Agreement		
			Total Number of Results at or Above the Cut-off	Total Number of Results Below the Cut-off	%Correct Call
1[a]	159.9	240	0	240	100.0%
2[a]	255.7	240	0	240	100.0%

Table 5: UCH-L1 Assay Results for Qualitative Precision Analysis					
Plasma Sample	Mean (pg/mL)	Total Number of Replicates	Qualitative Agreement		
			Total Number of Results at or Above the Cut-off	Total Number of Results Below the Cut-off	%Correct Call
3[b]	488.8	240	240	0	100.0%
4[c]	826.2	240	240	0	100.0%
5[c]	1763.7	240	240	0	100.0%
6[c]	2190.3	240	240	0	100.0%
i-STAT TBI Control					
L1[b]	466.2	240	240	0	100.0%
L2[c]	1597.6	240	240	0	100.0%

[a] Below cut-off; [b] Near cut-off; [c] Above cut-off

Semi-quantitative multi-site precision: The precision performance of the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge on the i-*STAT Alinity System* was evaluated using an 8-member panel of plasma-based test materials spiked with native or recombinant sourced antigens, which included six (6) levels of GFAP and six (6) levels of UCH-L1. The test materials were tested in point-of-care settings at three (3) clinical sites. At each site, each of the test materials was tested once per day for five (5) days by two (2) different operators, with each operator using three (3) *i-STAT TBI* cartridges on three (3) *i-STAT Alinity* instruments. Due to the inability to store or freeze whole blood samples to maintain sample stability over multiple days, plasma samples were used for this study. The study followed a 3x5x2x3 design based on CLSI EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition*. The estimates of GFAP and UCH-L1 precision are shown in **Table 6** and **Table 7**.

Table 6: i-STAT TBI Multi-site Precision – GFAP (pg/mL) Assay – All Sites														
Test Material	N	Mean (Min, Max)	Repeatability		Between-Day		Between-Operator		Within-Site		Between-Site		Reproducibility	
			SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV
2†	90	66.4 (50, 77)	3.85 (3.57, 4.17)	5.79	0.28 (0.20, 0.46)	0.42	0.56 (0.43, 0.81)	0.85	3.90 (3.63, 4.21)	5.87	0.00 (0.00, 0.00)	0.00	3.90 (3.63, 4.21)	5.87
4*	90	86.0 (60, 109)	5.94 (5.52, 6.44)	6.91	0.00 (0.00, 0.00)	0.00	0.17 (0.13, 0.25)	0.20	5.95 (5.54, 6.42)	6.92	0.61 (0.32, 3.82)	0.71	5.98 (5.57, 6.46)	6.95
5*	90	980.9 (912, 1147)	35.38 (32.86, 38.33)	3.61	18.04 (12.93, 29.78)	1.8	8.11 (6.24, 11.59)	0.83	40.53 (36.55, 45.50)	4.13	13.86 (7.21, 87.09)	1.41	42.84 (36.65, 51.55)	4.37
6*	90	2785.5 (2617, 2941)	59.55 (55.29, 64.51)	2.14	22.55 (16.17, 37.23)	0.81	26.04 (20.03, 37.21)	0.93	68.79 (62.80, 76.07)	2.47	0.00 (0.00, 0.00)	0.00	68.79 (62.76, 76.12)	2.47
7*	90	5357.3 (4873, 5927)	135.38 (125.71, 146.68)	2.53	120.86 (86.67, 199.51)	2.26	54.17 (41.68, 77.41)	1.01	189.4 (160.74, 230.58)	3.54	46.07 (23.99, 289.52)	0.86	194.92 (162.82, 242.89)	3.64
8*	90	7652.6 (7192, 8239)	166.85 (154.93, 180.77)	2.18	84.16 (60.35, 138.92)	1.10	0.00 (0.00, 0.00)	0.00	186.87 (168.34, 210.02)	2.44	114.05 (59.38, 716.75)	1.49	218.92 (167.49, 316.14)	2.86

*pooled K_2EDTA plasma spiked with <5% v/v recombinant GFAP antigen

†pooled K_2EDTA plasma spiked with <5% v/v GFAP from TBI patient plasma

Table 7: i-STAT TBI Multi-site Precision - UCH-L1 (pg/mL) Assay – All Sites														
Test Material	N	Mean (Min, Max)	Repeatability		Between-Day		Between-Operator		Within-Site		Between-Site		Reproducibility	
			SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV	SD (95% CI)	%CV
1*	90	206.5 (163, 251)	13.25 (12.31, 14.36)	6.42	4.60 (3.30, 7.59)	2.23	10.14 (7.80, 14.49)	4.91	17.31 (15.35, 19.85)	8.38	0.00 (0.00, 0.00)	0.00	17.31 (15.35, 19.85)	8.38
3†	93	384.1 (284, 460)	21.02 (19.96, 22.20)	5.47	0.00 (0.00, 0.00)	0.00	22.75 (17.51, 32.52)	5.92	30.98 (26.50, 37.28)	8.06	0.00 (0.00, 0.00)	0.00	30.98 (26.64, 37.01)	8.06
4*	90	681.8 (575, 800)	35.91 (33.34, 38.90)	5.27	16.13 (11.57, 26.63)	2.37	35.67 (27.44, 50.98)	5.23	53.12 (46.02, 62.85)	7.79	0.00 (0.00, 0.00)	0.00	53.12 (45.98, 62.92)	7.79
5*	90	1225.9 (1028, 1460)	82.64 (76.74, 89.54)	6.74	0.00 (0.00, 0.00)	0.00	9.25 (7.12, 13.22)	0.75	83.16 (77.46, 89.77)	6.78	31.55 (16.43, 198.30)	2.57	88.94 (77.63, 104.14)	7.26
6*	90	2051.2 (1646, 2403)	98.89 (91.83, 107.14)	4.82	24.98 (17.91, 41.24)	1.22	99.04 (76.20, 141.54)	4.83	142.17 (122.50, 169.43)	6.93	0.00 (0.00, 0.00)	0.00	142.17 (122.83, 168.80)	6.93
8*	90	2851.8 (2488, 3331)	137.56 (127.73, 149.03)	4.82	39.27 (28.16, 64.83)	1.38	110.46 (84.99, 157.86)	3.87	180.74 (159.43, 208.68)	6.34	0.00 (0.00, 0.00)	0.00	180.74 (159.60, 208.38)	6.34

*pooled K_2EDTA plasma spiked with <5% v/v recombinant UCH-L1 antigen

†pooled K_2EDTA plasma spiked with <5% v/v UCH-L1 from TBI patient plasma

Qualitative multi-site precision: The qualitative agreement of cartridge results relative to the expected sample result was evaluated for all measurements per test material for each assay above. The mean value of the test material was used as the expected result to classify the sample as below cut-off, near cut-off (overall mean ± 25% of cut-off), or above cut-off for each assay. The mean, total number of replicates, total number of elevated results, and % correct call for each test material is 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)	N	Qualitative Agreement		
			Total # of results at or above the assay cut-off	Total # of results below the assay cut-off	% Correct call
2[B]	66.4	90	74	16	82.22*
4[C]	86.0	90	89	1	98.89
5[C]	980.9	90	90	0	100.00
6[C]	2785.5	90	90	0	100.00
7[C]	5357.3	90	90	0	100.00
8[C]	7652.6	90	90	0	100.00

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

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

Table 9: Qualitative Precision Analysis - UCH-L1 Assay – All Sites					
Test Material	Mean (pg/mL)	N	Qualitative Agreement		
			Total # of results at or above the cut-off	Total # of results below the assay cut-off	% Correct call
1[A]	206.5	90	0	90	100.00
3[B]	384.1	93	83	10	89.25*
4[C]	681.8	90	90	0	100.00
5[C]	1225.9	90	90	0	100.00
6[C]	2051.2	90	90	0	100.00
8[C]	2851.8	90	90	0	100.00

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

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

Semi-quantitative whole blood precision: The precision performance of the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge on the *i-STAT Alinity System* was evaluated in point of care settings at three (3) clinical sites following a modified design based on CLSI EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition*. At each site, test samples across the reportable ranges of each assay were prepared by spiking prospectively collected venous whole blood specimens with recombinant GFAP and/or UCH-L1 or human plasma sample from traumatic brain injury (TBI) patients with native GFAP and UCH-L1. Eight (8) GFAP and eight (8) UCH-L1 samples were prepared at Site 1; eight (8) GFAP and 13 UCH-L1 samples were prepared at Site 2; seven (7) GFAP and eight (8) UCH-L1 samples were prepared at Site 3 At each site, each whole blood sample was tested in three (3) runs, by two (2) different operators, each operator using four (4) *i-STAT TBI* cartridges on four (4) *i-STAT Alinity* instruments (1 replicate/instrument/run) for a total of 24 replicates/specimen. For samples with target ranges near the GFAP and UCH-L1 assay cut-offs, a minimum of 2 samples were prepared and tested using the *i-STAT TBI* cartridges at each clinical site. The estimates of GFAP and UCH-L1 precision are shown in **Table 10** and **Table 11**.

Table 10: Whole Blood Precision at Point-of-Care Sites – GFAP Assay											
Site	Whole Blood Sample	N	Mean	Repeatability		Between-Instrument		Between-Operator		Within-Site	
				SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
01	1†	24	63.3	9.84	15.53	0.00	0.00	2.98	4.70	10.28	16.23
	2†	23‡	64.3	11.72	18.23	6.76	10.51	4.25	6.61	14.18	22.06
	3*	24	103.5	10.85	10.48	0.00	0.00	2.22	2.14	11.07	10.70
	4*	23‡	128.5	14.51	11.29	0.00	0.00	0.00	0.00	14.51	11.29
	5*	24	986.3	88.48	8.97	0.00	0.00	0.00	0.00	88.48	8.97
	6*	24	3431.6	338.46	9.86	0.00	0.00	104.36	3.04	354.19	10.32
	7*	24	6371.3	637.41	10.00	0.00	0.00	162.96	2.56	657.91	10.33
	8*	24	7836.9	730.91	9.33	0.00	0.00	102.96	1.31	738.13	9.42
02	1†	24	57.7	7.24	12.56	4.60	7.97	5.28	9.16	10.07	17.47
	2†	24	60.9	11.08	18.18	0.00	0.00	2.15	3.53	11.28	18.52
	3*	24	83.7	6.98	8.34	0.00	0.00	0.00	0.00	6.98	8.34
	4*	24	148.1	12.08	8.16	0.00	0.00	0.00	0.00	12.08	8.16
	5*	24	900.6	28.89	3.21	10.84	1.20	0.00	0.00	30.85	3.43
	6*	24	3731.1	161.63	4.33	0.00	0.00	121.29	3.25	202.08	5.42
	7*	24	5762.3	289.18	5.02	0.00	0.00	0.00	0.00	289.18	5.02
	8*	24	8310.3	499.50	6.01	0.00	0.00	0.00	0.00	499.50	6.01
03	1†	23‡	58.9	4.47	7.59	2.60	4.41	0.00	0.00	5.17	8.78
	2†	22§	67.2	16.54	24.62	0.00	0.00	0.00	0.00	16.54	24.62
	3*	24	145.4	10.54	7.25	0.00	0.00	3.28	2.26	11.03	7.59
	4*	24	962.1	56.81	5.90	24.53	2.55	0.00	0.00	61.88	6.43
	5*	24	2954.5	167.36	5.66	0.00	0.00	3.12	0.11	167.39	5.67
	6*	24	6226.4	246.48	3.96	18.23	0.29	20.69	0.33	248.02	3.98
	7*	23¶	8366.9	502.57	6.01	0.00	0.00	168.21	2.01	529.97	6.33

*prospectively collected K_2EDTA venous whole blood spiked with <5% v/v recombinant GFAP antigen
†prospectively collected K_2EDTA venous whole blood spiked with <5% v/v GFAP from TBI patient plasma
‡ one (1) result not obtained due to a quality check failure (QCF) or star-out error
§ two (2) results not obtained due to a quality check failure (QCF) or star-out error
¶ one (1) result not obtained due to operator error

Table 11: Whole Blood Precision at Point-of-Care Sites – UCH-L1 Assay											
Site	Whole Blood Sample	N	Mean	Repeatability		Between-Instrument		Between-Operator		Within-Site	
				SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
01	1*	23‡	215.7	16.58	7.69	0.00	0.00	0.00	0.00	16.58	7.69
	2*	24	243.5	19.35	7.95	0.00	0.00	11.14	4.57	22.33	9.17
	3†	24	333.7	28.69	8.60	12.79	3.83	17.15	5.14	35.79	10.7
	4†	22	438.9	55.79	12.71	0.00	0.00	0.00	0.00	55.79	12.7
	5*	24	486.7	25.57	5.25	6.37	1.31	9.33	1.92	27.96	5.74
	6*	24	1451.4	106.30	7.32	0.00	0.00	70.00	4.82	127.28	8.77
	7*	24	1746.3	96.10	5.50	0.00	0.00	0.00	0.00	96.10	5.50
	8*	22‡‖	3020.3	146.12	4.84	20.19	0.67	57.99	1.92	158.50	5.25
02	1*	24	183.0	15.28	8.35	2.89	1.58	0.00	0.00	15.55	8.50
	2*	24	220.2	19.75	8.97	0.00	0.00	0.00	0.00	19.75	8.97
	3*	24	232.3	15.71	6.76	0.00	0.00	0.00	0.00	15.71	6.76
	4†	24	360.8	26.99	7.48	18.27	5.06	0.00	0.00	32.59	9.03
	5†	24	413.0	38.18	9.25	10.68	2.59	5.52	1.34	40.03	9.69

Table 11: Whole Blood Precision at Point-of-Care Sites – UCH-L1 Assay											
Site	Whole Blood Sample	N	Mean	Repeatability		Between-Instrument		Between-Operator		Within-Site	
				SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
	6*	24	535.1	61.35	11.47	0.00	0.00	10.79	2.02	62.29	11.64
	7*	23‡	630.5	49.93	7.92	0.00	0.00	5.97	0.95	50.29	7.98
	8*	24	675.0	50.54	7.49	20.74	3.07	0.00	0.00	54.63	8.09
	9*	23‡	935.1	62.83	6.72	20.06	2.15	0.00	0.00	65.95	7.05
	10*	21§	1114.1	59.38	5.33	0.00	0.00	0.00	0.00	59.38	5.33
	11*	23‡	2286.3	121.21	5.30	0.00	0.00	0.00	0.00	121.21	5.30
	12	24	2319.1	139.38	6.01	42.48	1.83	66.90	6.91	160.34	6.91
	13	21#	2945.8	141.67	4.81	0.00	0.00	0.00	0.00	141.67	4.81
03	1*	24	182.5	14.24	7.80	0.00	0.00	0.48	0.26	14.25	7.81
	2*	24	204.2	16.51	8.08	0.00	0.00	11.65	5.71	20.21	9.90
	3†	24	357.1	35.46	9.93	0.00	0.00	5.71	1.60	35.92	10.06
	4†	24	392.8	39.52	10.06	12.15	3.09	0.00	0.00	41.35	10.53
	5*	24	522.6	42.40	8.11	0.00	0.00	9.55	1.83	43.46	8.32
	6*	24	1213.4	54.20	4.47	34.69	2.86	0.00	0.00	64.35	5.30
	7*	24	1947.1	118.94	6.11	0.00	0.00	0.00	0.00	118.94	6.11
	8*	21‡‖¶	2829.4	150.88	5.33	59.80	2.11	81.45	2.88	181.59	6.42

*prospectively collected K_2EDTA venous whole blood spiked with <5% v/v recombinant UCH-L1 antigen

†prospectively collected K_2EDTA venous whole blood spiked with <5% v/v UCH-L1 from TBI patient plasma

‡ one (1) result not obtained due to a quality check failure (QCF) or star-out error

§ three (3) results not obtained due to a quality check failure (QCF) or star-out error

¶ one (1) result not obtained due to operator error

‖ one (1) result not measurable due to being above the measurement range

three (3) results not measurable due to being above the measurement range

Qualitative Whole Blood Precision: The qualitative agreement of cartridge results relative to the expected sample result was evaluated for all measurements per test material for each assay above. The mean (or median) value of the test material was used as the expected result to classify the sample as below cut-off, near cut-off (overall mean ± 25% of cut-off), or above cut-off for each assay. The mean (or median), total number of replicates, total number of elevated results, and % correct call for each test material is presented in **Table 12** for GFAP and **Table 13** for UCH-L1.

Table 12: Qualitative Precision Analysis – GFAP Assay						
Site	Sample	Mean/ Median (pg/mL)	N	Qualitative Agreement		
				# of GFAP Results at or Above the Cut-off	# of GFAP Results Below the Cut-off	% Correct Call
01	1 [B]	61.0†	24	8	16	66.67%*
	2 [B]	66.0†	23	16	7	69.57%*
	3 [C]	103.5	24	24	0	100.00%
	4 [C]	128.5	23	23	0	100.00%
	5 [C]	986.3	24	24	0	100.00%
	6 [C]	3431.6	24	24	0	100.00%

Table 12: Qualitative Precision Analysis – GFAP Assay						
Site	Sample	Mean/ Median (pg/mL)	N	Qualitative Agreement		
				# of GFAP Results at or Above the Cut-off	# of GFAP Results Below the Cut-off	% Correct Call
	7 [C]	6371.3	24	24	0	100.00%
	8 [C]	7836.9	24	24	0	100.00%
02	1 [B]	58.5†	24	5	19	79.17%*
	2 [B]	62.5†	24	9	15	62.50%*
	3 [C]	83.7	24	24	0	100.00%
	4 [C]	148.1	24	24	0	100.00%
	5 [C]	900.6	24	24	0	100.00%
	6 [C]	3731.1	24	24	0	100.00%
	7 [C]	5762.3	24	24	0	100.00%
	8 [C]	8310.3	24	24	0	100.00%
03	1 [B]	60.0†	23	1	22	95.65%*
	2 [B]	67.0†	22	14	8	63.64%*
	3 [C]	145.4	24	24	0	100.00%
	4 [C]	962.1	24	24	0	100.00%
	5 [C]	2954.5	24	24	0	100.00%
	6 [C]	6226.4	24	24	0	100.00%
	7 [C]	8366.9	23	23	0	100.00%

†Median value shown.

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

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

Table 13: Qualitative Precision Analysis – UCH-L1 Assay						
Site	Sample	Mean (pg/mL)	N	Qualitative Agreement		
				# of UCH-L1 Results at or Above the Cut-off	# of UCH-L1 Results Below the Cut-off	% Correct Call
01	1 [A]	215.65	23	0	23	100.00%
	2 [A]	243.54	24	0	24	100.00%
	3 [B]	438.91	22	22	0	100.00%
	4 [B]	333.71	24	5	19	79.17%*
	5 [C]	486.71	24	24	0	100.00%
	6 [C]	1451.38	24	24	0	100.00%
	7 [C]	1746.33	24	24	0	100.00%
	8 [C]	3020.27	22	22	0	100.00%
02	1 [A]	182.96	24	0	24	100.00%
	2 [A]	220.17	24	0	24	100.00%
	3 [A]	232.33	24	0	24	100.00%
	4 [B]	360.75	24	13	11	54.17%*
	5 [B]	413.00	24	22	2	91.67%*
	6 [C]	675.04	24	24	0	100.00%
	7 [C]	630.52	23	23	0	100.00%

Table 13: Qualitative Precision Analysis – UCH-L1 Assay						
Site	Sample	Mean (pg/mL)	N	Qualitative Agreement		
				# of UCH-L1 Results at or Above the Cut-off	# of UCH-L1 Results Below the Cut-off	% Correct Call
	8 [C]	535.13	24	24	0	100.00%
	9 [C]	1114.14	21	21	0	100.00%
	10 [C]	935.13	23	23	0	100.00%
	11 [C]	2286.26	23	23	0	100.00%
	12 [C]	2319.13	24	24	0	100.00%
	13 [C]	2945.81	21	21	0	100.00%
03	1 [A]	204.21	24	0	24	100.00%
	2 [A]	182.46	24	0	24	100.00%
	3 [B]	357.08	24	11	13	54.17%*
	4 [B]	392.75	24	21	3	87.50%*
	5 [C]	522.63	24	24	0	100.00%
	6 [C]	1213.38	24	24	0	100.00%
	7 [C]	1947.08	24	24	0	100.00%
	8 [C]	2829.38	21	21	0	100.00%

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

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

b. Linearity/assay reportable range:

i. Linearity

The linearity of the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge on the *i-STAT Alinity System* was established by testing whole blood samples of varying GFAP and UCH-L1 levels over the reportable range of each assay. The study was designed based on CLSI EP06-Ed2: *Evaluation of the Linearity of Quantitative Measurement Procedures, 2nd Edition*. The study was conducted using whole blood samples of varying GFAP and UCH-L1 levels prepared by admixture with either native antigens from a TBI patient sample or prepared by admixture with recombinant antigens. The regression summary of the results obtained for each assay in the *i-STAT TBI* cartridge (y-axis) versus the expected values (x-axis) is provided in **Table 14** below.

Table 14: Linearity Across Reportable Range					
Assay	Test Sample Range (pg/mL)	Reportable Range (pg/mL)	Slope	Intercept (pg/mL)	R^2
GFAP	17.6 – 11303.9	47 – 10000	1.01	-3.49	0.9990
UCH-L1	86.4 – 3281.5	87 – 3200	0.98	2.66	0.9977

ii. Hook Effect

The GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge on the *i-STAT Alinity* System were evaluated for high dose hook effect. The testing was conducted using whole blood samples spiked to a high antigen level for each assay. Each sample was tested to verify that the measured signal is greater than that of a nominal GFAP target of 10,000 pg/mL and a nominal UCH-L1 target of 4000 pg/mL. Hook effect was not observed for the GFAP and UCH-L1 assays using whole blood samples with antigen concentrations exceeding 100,000 pg/mL.

c. Traceability, Calibration and Reference Interval

i. Traceability and Calibration

There are no internationally recognized standard reference materials available for either glial fibrillary acidic protein (GFAP) or ubiquitin carboxy-terminal hydrolase L1 (UCH-L1). Therefore, the traceability of both the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge has been established against reference materials created using recombinant GFAP and UCH-L1 antigens (expressed and purified from *E. coli*) as the metrologically highest-level material in the metrological calibration hierarchy.

ii. Reference Interval

A reference interval study was conducted with a general population. Whole blood specimens from 150 apparently healthy subjects, aged ≥ 18 years of age reporting no history of neurological disease were tested with the *i-STAT TBI* 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 shown in **Table 15** below.

Table 15: Reference Interval

Assay	N	Mean (pg/mL)	SD (pg/mL)	Median (pg/mL)	Reference Interval (2.5th to 97.5th percentile) (pg/mL)
GFAP	150	18.7	14.51	18.0	<47 - 53
UCH-L1	150	89.3	50.99	81.5	<87 - 251

Based on the test results with the *i-STAT TBI* cartridge with the *i-STAT Alinity System*, 0.7% (1/150) of the individuals from an apparently healthy population had a test interpretation of "elevated" for biomarkers.

d. Detection Limit

i. Limit of Quantitation (LoQ)

The LoQ was determined for the GFAP and UCH-L1 assays in the *i-STAT TBI* 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 six (6) days using three (3) lots of *i-STAT TBI* cartridges with fresh whole blood from 12 apparently healthy donors altered to achieve 11 low-level samples of GFAP and 12 low level samples of UCH-L1. The estimated LoQ for the *i-STAT TBI* cartridge tested on the *i-STAT Alinity* instrument from this study was 47 pg/mL for the GFAP assay and 32 pg/mL for the UCH-L1 assay. Based on the concentration range in the linearity study, the established LoQ of the UCH-L1 assay is 87 pg/mL

e. Analytical Specificity

i. Interference

The interference performance of the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge on the *i-STAT Alinity System* was evaluated using whole blood samples 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 16** below contains the list of potentially interfering substances tested for the GFAP and UCH-L1 assays and the interference results.

Table 16: Interfering Substances Testing

Substance	Test Concentration		Assay	Interference	Comment
	µmol/L	mg/dL		(Yes/No)	
Acetaminophen [a]	1324	20	GFAP	No	
			UCH-L1	No	
Acetylsalicylic acid [a]	3620	65.22	GFAP	No	
			UCH-L1	No	
Albumin	150 g/L	15 g/dL	GFAP	No	
			UCH-L1	Yes	Decreased results at >12.1 g/dL. The highest concentration in the reference interval reported by CLSI EP37 is 5.2 g/dL.
Amphetamine	2.44	0.033	GFAP	Yes	Decreased results at >1.83 µmol/L. The highest drug concentration under therapeutic treatment reported by CLSI EP37 is 0.815 µmol/L.
			UCH-L1	No	
Ascorbic acid	298	5.90	GFAP	No	
			UCH-L1	No	
Benzoylecgonine [a]	8.64	2.5 µg/mL	GFAP	No	
			UCH-L1	No	
Bilirubin	684	40	GFAP	No	
			UCH-L1	No	
Bilirubin (conjugated)	475	40	GFAP	No	
			UCH-L1	No	
Caffeine	556	10.8	GFAP	No	
			UCH-L1	No	
Chloramphenicol	241	7.79	GFAP	No	
			UCH-L1	No	
Clopidogrel [a]	21.4	0.90	GFAP	No	
			UCH-L1	No	
Cocaine [a]	3.46 µg/mL	0.346	GFAP	No	
			UCH-L1	Yes	Decreased results at >2.595 µg/mL. The mean maximum

Table 16: Interfering Substances Testing					
Substance	**Test Concentration**		**Assay**	**Interference**	**Comment**
	µmol/L	**mg/dL**		**(Yes/No)**	
					plasma concentration (Cmax) per literature is 0.115 µg/mL[c].
Diazepam	105	2.99	GFAP	No	
			UCH-L1	No	
Diclofenac	81	2.58	GFAP	No	
			UCH-L1	No	
Dopamine	4.06	0.077	GFAP	No	
			UCH-L1	No	
EDDP† [a]	0.33	125 ng/mL	GFAP	No	
			UCH-L1	No	
Erythromycin	188	13.80	GFAP	No	
			UCH-L1	No	
Ethanol	130 mmol/L	599	GFAP	No	
			UCH-L1	No	
Hemoglobin	10 g/L	1000	GFAP	No	
			UCH-L1	No	
Human anti-mouse antibodies (HAMA) [a]	>80x [b]	N/A	GFAP	No	
			UCH-L1	No	
Ibuprofen [a]	2425	50.0	GFAP	No	
			UCH-L1	No	
Intralipid (Intralipid 20%)	N/A	7075	GFAP	No	
			UCH-L1	No	
Methadone	10.3	0.319	GFAP	Yes	Decreased results at >7.725 µmol/L. The highest drug concentration under therapeutic treatment reported by CLSI EP37 is 3.43 µmol/L.
			UCH-L1	No	
d-Methamphetamine [a]	1.865	278.3 ng/mL	GFAP	Yes	Decreased results at >208.8 ng/mL. The mean maximum plasma concentration (Cmax) per literature is 92.8 ng/mL.[d]
			UCH-L1	No	
Methaqualone [a]	32.36	8.1 µg/mL	GFAP	No	
			UCH-L1	No	
Metoprolol [a]	18.7	1.28	GFAP	No	
			UCH-L1	Yes	Decreased results at >14.025 µmol/L. The highest drug concentration under therapeutic treatment

Table 16: Interfering Substances Testing					
Substance	**Test Concentration**		**Assay**	**Interference**	**Comment**
	µmol/L	**mg/dL**		**(Yes/No)**	
					reported by CLSI EP37 is 1.875 µmol/L.
Morphine	27.3	0.78	GFAP	No	
			UCH-L1	No	
Nicardipine hydrochloride	0.97	0.05	GFAP	No	
			UCH-L1	No	
Nicotine	5.97	0.0097	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	
Phenytoin	238	6.0	GFAP	No	
			UCH-L1	No	
Propoxyphene [a]	9.46	0.32	GFAP	No	
			UCH-L1	Yes	Decreased results at >7.095 µmol/L. The highest drug concentration under therapeutic treatment reported by CLSI EP37 is 3.15 µmol/L.
Rheumatoid Factor (RF) [a]	1000 IU/mL	N/A	GFAP	No	
			UCH-L1	Yes	Decreased results at >875 IU/mL
Secobarbital	66.8	1.59	GFAP	No	
			UCH-L1	No	
Triglycerides [a]	33.88 mmol/L	3000	GFAP	No	
			UCH-L1	No	
Warfarin	243	7.5	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] Scheidweiler, K. B., Spargo, E. A., Kelly, T. L., Cone, E. J., Barnes, A. J., and Huestis, M. A. (2010) Pharmacokinetics of cocaine and metabolites in human oral fluid and correlation with plasma concentrations after controlled administration. *Ther Drug Monit.* **32**, 628-637

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

ii. Cross-reactivity

The i-STAT TBI cartridge on the *i-STAT Alinity System* were evaluated in the presence of potentially cross-reactive endogenous substances in whole blood specimens based on CLSI guidance EP07-ED3: *Interference Testing in Clinical Chemistry*, 3rd edition. The effect of each

substance was evaluated by comparing the performance of a test sample spiked with a potentially cross-reactive substance and a control sample spiked with an equal volume of blank plasma diluent as per CLSI EP07 ED3. **Table 17** below lists the proteins with significant homology to GFAP and UCH-L1 that were tested at highest known physiological levels. None (0) of the nine (9) substances tested were found to cross-react with the GFAP or UCH-L1 assays in the *i-STAT TBI* cartridge tested on the *i-STAT Alinity System*.

Table 17 : Cross-Reactivity

Assay	Substance	Substance Test Concentration (pg/mL)	Outcome (Cross-Reactivity/ No Cross-Reactivity)
GFAP	Keratin type II	10,000	No Cross-Reactivity
	Internexin	77,000	No Cross-Reactivity
	Neurofilament Medium	8,600	No Cross-Reactivity
	Neurofilament Heavy	77,000	No Cross-Reactivity
	Neurofilament Light	68	No Cross-Reactivity
	Peripherin Protein	5,000	No Cross-Reactivity
	Desmin	127,000	No Cross-Reactivity
	Vimentin	354,000	No Cross-Reactivity
UCH-L1	Ubiquitin Carboxyl-Terminal Hydrolase L3 (UCH-L3)	354,000	No Cross-Reactivity

iii. Cross-talk

The GFAP and UCH-L1 assays in the *i-STAT TBI* 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. Whole Blood 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. As presented in **Table 18** 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 18 : Cross-talk

Assay	Level	Substance	Substance Test Concentration (pg/mL)	Cross-talk
GFAP	Low-positive	UCH-L1	100,000	No
	Moderate-positive	UCH-L1	100,000	No
UCH-L1	Low-positive	GFAP	100,000	No
	Moderate-positive	GFAP	100,000	No

f. Hematocrit Sensitivity

The effect of hematocrit on the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge was assessed across a hematocrit range of 15-60% packed cell volume (PCV). The study was conducted using two (2) lots of *i-STAT TBI* cartridges and *i-STAT Alinity* instruments. Whole blood samples from six (6) donors were altered to target three (3) GFAP and UCH-L1 levels (low, moderate and high) across the reportable range for each respective assay. Each sample was evaluated at three (3) hematocrit (HCT) levels, with the nominal hematocrit level as control condition and low and high hematocrit levels as test conditions. The hematocrit sensitivity at each GFAP and UCH-L1 level was assessed by comparing the results at the low and high hematocrit levels (test conditions) to the nominal hematocrit level (control condition). Imprecision (CV) and bias exceeding 10% were observed for low level GFAP samples with hematocrit levels above 56% PCV.

g. Assay Cut-Off

The assay cut-offs 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 cut-off values of 65 pg/mL (GFAP assay) and 360 pg/mL (UCH-L1 assay) were selected for the *i-STAT TBI* Cartridge using the selection criteria with an adjusted NPV (to 10%) ≥98.5% and sensitivity ≥97%.

B. Clinical Sensitivity and Specificity

A prospective, multi-center, observational study was conducted to evaluate the clinical performance of the *i-STAT TBI* cartridge in classifying intended use population subjects with suspected mild TBI for the likely absence of acute intracranial lesions visualized by a head CT scan. Venous whole blood specimens were used for *i-STAT TBI* cartridge testing.

Venous whole blood specimens were collected in K_2EDTA within 24 hours of the head injury from prospectively enrolled subjects, 18 years of age or older, who had experienced a head injury and presented to the health care facility or the emergency department (ED) with suspected mild TBI, with a GCS score of 13-15; and who had a head CT scan ordered as part of their standard of clinical care. Each specimen was tested for GFAP and UCH-L1 using two (2) *i-STAT TBI* cartridges and two (2) *i-STAT Alinity* instruments. Testing was performed at 20 external point of care clinical sites across the United States.

CT scans were performed in accordance with the clinical site's standard of care. Images were transmitted to a central data capture system. Images were interpreted by at least two neuroradiologists who were masked to other clinical and laboratory data; procedures for scoring images were established before conducting image review. The clinical outcome was based on the consensus interpretation between two neurologists. 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.

Specimens from 970 subjects were included in the analysis.

The demographic characteristics of the subjects represented in the clinical performance analysis are summarized in **Table 19** below.

Table 19 : Demographic Characteristics			
Characteristic	**Head CT Scan Result**		**Total**
	Positive	**Negative**	
N	283	687	970
Age (Years)			
Mean	51.1	45.0	46.8
Median	52.0	42.0	46.0
Standard Deviation	19.68	18.92	19.33
Minimum	18	18	18
Maximum	96	97	97
Gender, N (%)			
Male	187 (66.1%)	434 (63.2%)	621 (64.0%)
Female	94 (33.2%)	252 (36.7%)	346 (35.7%)
Unspecified/ Not Reported	2 (0.7%)	1 (0.1%)	3 (0.3%)
Race, N (%)			
White	224 (79.2%)	441 (64.2%)	665 (68.6%)
Black or African American	20 (7.1%)	152 (22.1%)	172 (17.7%)
Asian	11 (3.9%)	38 (5.5%)	49 (5.1%)
Native Hawaiian/Pacific Islander	4 (1.4%)	6 (0.9%)	10 (1.0%)
American Indian or Alaska Native	4 (1.4%)	8 (1.2%)	12 (1.2%)
Asian, White	2 (0.7%)	3 (0.4%)	5 (0.5%)
Asian, Black or African American	0 (0.0%)	1 (0.1%)	1 (0.1%)
Black or African American, American Indian or Alaska Native	0 (0.0%)	2 (0.3%)	2 (0.2%)
White, Black or African American	0 (0.0%)	5 (0.7%)	5 (0.5%)
Not Reported	10 (3.5%)	19 (2.8%)	29 (3.0%)
Unknown	8 (2.8%)	12 (1.7%)	20 (2.1%)
Ethnicity, N (%)			
Hispanic or Latino	67 (23.7%)	120 (17.5%)	187 (19.3%)
Not Hispanic or Latino	209 (73.9%)	552 (80.3%)	761 (78.5%)
Unknown	6 (2.1%)	6 (0.9%)	12 (1.2%)
Not Reported	1 (0.4%)	9 (1.3%)	10 (1.0%)

The head injury characteristics of the 970 subjects 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 20** below.

Table 20 : Head Injury Characteristics

Assessment	Head CT Scan Result		Total
	Positive	Negative	
N	283	687	970
Time from head injury to Initial Assessment (hours)*			
Mean	2.0	1.3	1.5
Median	1.0	0.8	0.9
Standard Deviation	2.01	1.45	1.67
Range	(1.0, 10.2)	(0.8, 10.0)	(0.8, 10.2)
Time from head injury to CT scan (hours)*			
Mean	2.6	2.5	2.6
Median	1.7	2.0	1.9
Standard Deviation	2.37	1.80	1.98
Range	(0.2, 11.4)	(0.3, 10.7)	(0.2, 11.4)
Time from head injury to blood draw (hours)*			
Mean	14.5	8.8	10.4
Median	13.5	5.8	8.1
Standard Deviation	6.65	6.43	6.99
Range	(2.0, 24.0)	(1.5, 24.0)	(1.5, 24.0)
Glasgow Coma Score – N (%)			
13	28 (9.9%)	11 (1.6%)	39 (4.0%)
14	79 (27.9%)	90 (13.1%)	169 (17.4%)
15	176 (62.2%)	586 (85.3%)	762 (78.6%)
Neurological assessment - N (%) of subjects experiencing:			
Loss of Consciousness (LOC)	225 (79.5%)	450 (65.5%)	675 (69.6%)
Confusion/Alteration of Consciousness (AOC)	195 (68.9%)	504 (73.4%)	699 (72.1%)
Vomiting	24 (8.5%)	21 (3.1%)	45 (4.6%)
Post Traumatic Amnesia (PTA)	196 (69.3%)	409 (59.5%)	605 (62.4%)
Post Traumatic Seizures	3 (1.1%)	0 (0.0%)	3 (0.3%)
Subjects with Drug Intoxication at the Time of Presentation to Facility	48 (17.0%)	66 (9.6%)	114 (11.8%)
Subjects with Alcohol Intoxication at the Time of Presentation to Facility	49 (17.3%)	61 (8.9%)	110 (11.3%)
Mechanism of Injury† - N (%) of subjects affected:			

Table 20 : Head Injury Characteristics			
Assessment	**Head CT Scan Result**		**Total**
	Positive	**Negative**	
Acceleration/Deceleration	68 (24.0%)	221 (32.2%)	289 (29.8%)
Direct Impact (blow to head)	44 (15.5%)	85 (12.4%)	129 (13.3%)
Direct Impact (head against object)	157 (55.5%)	437 (63.6%)	594 (61.2%)
Crush	0 (0.0%)	3 (0.4%)	3 (0.3%)
Blast	0 (0.0%)	1 (0.1%)	1 (0.1%)
Ground level fall	82 (29.0%)	170 (24.7%)	252 (26.0%)
Fall from Height > 1 meter (3 feet)	39 (13.8%)	79 (11.5%)	118 (12.2%)
Other	15 (2.2%)	7 (2.5%)	22 (2.3%)
Physical Evidence‡ – N (%) of subjects with:			
Visible Trauma Above Clavicle	214 (75.6%)	422 (61.4%)	636 (65.6%)
Signs of Basal Skull Fracture	37 (13.1%)	7 (1.0%)	44 (4.5%)

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

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

‡Prior to head CT scan.

Of the 970 evaluable subjects, 283 subjects had positive CT scan results. Of these 283 subjects with positive CT scan results, 273 had an 'Elevated' *i-STAT TBI* test interpretation (sensitivity = 96.5% (273/283)). Ten (10) subjects with CT scan positive results had an *i-STAT TBI* test interpretation that was 'Not Elevated'. The rate of false negative (FN) results was 3.5% (10/283). None of these ten (10) subjects with false negative results required surgical intervention related to their head injury as no neurosurgical lesions were identified by CT scan in these subjects.

Of the 687 subjects with negative CT scan results, 277 had an *i-STAT TBI* test interpretation that was 'Not Elevated' (277/687, specificity = 40.3 %). The rate of false positive (FP) results was 59.6% (410/687).

In the clinical study, the prevalence of adjudicated CT scan positive subjects was 29.2% (283/970). Overall, there were 287 subjects with *i-STAT TBI* test interpretations of 'Not Elevated'. Of these, 277 subjects had negative CT scan results. The negative predictive value (NPV) of the assay was 96.5% (277/287). **Table 21** below provides the clinical performance estimates of *i-STAT TBI* cartridge with *i-STAT Alinity* instrument.

Table 21 : Clinical Performance			
i-STAT TBI Test Interpretation	**All Evaluable Results (0-24h)**		**Total**
	Adjudicated Head CT Scan Positive	**Adjudicated Head CT Scan Negative**	
Elevated	273	410	683
Not Elevated	10	277	287
Total	283	687	970
Clinical Performance Parameters		**N=970**	**95% CI**
Clinical Sensitivity		96.5% (273/283)	(93.6%, 98.1%)†
Clinical Specificity		40.3% (277/687)	(36.7%, 44.0%)†
Negative Predictive Value (NPV) *		96.5% (277/287)	(93.7%, 98.1%)‡
Positive Predictive Value (PPV)		40% (273/683)	(38.4%, 41.5%)‡
Likelihood Ratio Negative (LRN)		0.09	(0.05, 0.16)§
Likelihood Ratio Positive (LRP)		1.62	(1.52, 1.73)§

* Adjusted NPV at 6% prevalence is 99.4% (95% CI: 99.0%, 99.7%).

†95% confidence intervals are calculated using the Wilson score method for a binomial portion (see CLSI EP12-Ed3)

‡95% confidence intervals for predictive values are calculated based on the confidence intervals of the corresponding likelihood ratios

§95% confidence intervals are calculated using asymptotic method for a ratio of two binomial proportion

8. Conclusion

The results of these studies demonstrate that performance of the GFAP and UCH-L1 assays in the *i-STAT TBI* cartridge with the *i-STAT Alinity System* are substantially equivalent to the predicate device.