March 2, 2023

Abbott Laboratories
Lisa Kelly
Regulatory Affairs Associate Director
100 Abbott Park Road
Abbott Park, Illinois 60064

Re: K223602
Trade/Device Name: TBI
Regulation Number: 21 CFR 866.5830
Regulation Name: Brain trauma assessment test
Regulatory Class: Class II
Product Code: QAT
Dated: November 30, 2022
Received: December 2, 2022

Dear Lisa Kelly:

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,

Ying Mao -S

Ying 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: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K223602

Device Name
TBI

Indications for Use *(Describe)*
The TBI test is a panel of in vitro diagnostic chemiluminescent microparticle immunoassays (CMIA) used for the quantitative measurements of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in human plasma and serum and provides a semi-quantitative interpretation of test results derived from these measurements using the Alinity i system.

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 negative test result is associated with the absence of acute intracranial lesions visualized on a head CT scan.

The TBI test is intended for use in clinical laboratory settings by healthcare professionals.

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.

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510(k) Summary (Summary of Safety and Effectiveness)

This summary of the 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR § 807.92.

I. Applicant Name

Abbott Diagnostics
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Date Summary Prepared: November 30, 2022

II. Device Name

TBI

Reagents

Trade Name: Glial fibrillary acidic protein (GFAP)
Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1)
Device Classification: Class II (Special controls)
Classification Name: Brain trauma assessment test
Governing Regulation: 21 CFR § 866.5830
Product Code: QAT

III. Predicate Device

Banyan Brain Trauma Indicator (BTI) (DEN170045)

IV. Description of Device

A. Reagents

The kit configurations of the GFAP and UCH-L1 reagent kits are described below.

	List Number	
	GFAP 04W1722	**UCH-L1 04W1922**
Tests per cartridge	100	100
Number of cartridges per kit	2	2
Tests per kit	200	200
Microparticles	7.1 mL	7.1 mL
Conjugate	6.4 mL	12.5 mL
Assay Specific Diluent	6.4 mL	10.5 mL

GFAP Reagent Kit

Microparticles:	Anti-GFAP (rabbit, monoclonal) coated microparticles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.05% solids. Preservative: ProClin 300.
Conjugate:	Anti-GFAP (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 0.2 mg/L. Preservative: ProClin 300.
Assay Specific Diluent:	TRIS buffer with protein (bovine) stabilizer. Preservative: ProClin 300.

UCH-L1 Reagent Kit

Microparticles:	Anti-UCH-L1 (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.05% solids. Preservative: sodium azide.
Conjugate:	Anti-UCH-L1 (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 0.2 mg/L. Preservative: ProClin 300.
Assay Specific Diluent:	TRIS buffer with protein (bovine) stabilizer. Preservative: sodium azide.

B. Biological Principles of the Procedure

The TBI test is a panel of in vitro diagnostic quantitative measurements of GFAP and UCH-L1 and provides a semi-quantitative interpretation of GFAP and UCH-L1 in human plasma and serum.

GFAP

This assay is an automated, two-step immunoassay for the quantitative measurement of GFAP in human plasma and serum using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, anti-GFAP coated paramagnetic microparticles, and assay specific diluent are combined and incubated. The GFAP present in the sample binds to the anti-GFAP coated microparticles. The mixture is washed. Anti-GFAP acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of GFAP in the sample and the RLU detected by the system optics.

UCH-L1

This assay is an automated, two-step immunoassay for the quantitative measurement of UCH-L1 in human plasma and serum using CMIA technology.

Sample, anti-UCH-L1 coated paramagnetic microparticles, and assay specific diluent are combined and incubated. The UCH-L1 present in the sample binds to the anti-UCH-L1 coated microparticles. The mixture is washed. Anti-UCH-L1 acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as an RLU. There is a direct relationship between the amount of UCH-L1 in the sample and the RLU detected by the system optics.

C. Interpretation of Results

The assay cutoffs were established to be 35.0 pg/mL (35.0 ng/L) for GFAP and 400.0 pg/mL (400.0 ng/L) for UCH-L1.

The GFAP and UCH-L1 results are reported separately and the software provides a TBI interpretation relative to the respective cutoff values as shown in the following table.

Specification for Constituent Assay Results	TBI Result	TBI Interpretation
GFAP and UCH-L1 below (<) cutoff	0	Negative
GFAP and/or UCH-L1 above (≥) cutoff	1	Positive

The following table provides a detailed summary of the TBI interpretation based on potential results.

GFAP Assay Result (Relative to Cutoff of 35.0 pg/mL [35.0 ng/L])*	UCH-L1 Assay Result (Relative to Cutoff of 400.0 pg/mL [400.0 ng/L])*	TBI Interpretation**
Below	Below	Negative
Below	Above	Positive
Above	Below	Positive
Above	Above	Positive
No result	Below	Not reportable***
No result	Above	Positive***
Below	No result	Not reportable***
Above	No result	Positive***
No result	No result	Not reportable***

* Above means greater than or equal to the cutoff. Below means less than the cutoff.

** The GFAP and UCH-L1 results can be found on the Result Details screen under Constituent Information on the User Interface.

*** An automated TBI interpretation will not be reported for specimens without a result for GFAP and/or UCH-L1. The GFAP and/or UCH-L1 assay(s) may be retested if needed to obtain a result and a manual TBI interpretation may be required. The TBI interpretation for a specimen is considered positive if the result for either constituent assay (GFAP or UCH-L1) is greater than or equal to the cutoff and no result is obtained for the other assay. The TBI interpretation for a specimen is not reportable if the result for either constituent assay is less than the cutoff and no result is obtained for the other assay.

In the case of a flagged ">" or "<" result for either assay, the TBI interpretation should be evaluated manually. A result flagged “>” should be considered above the cutoff and a result flagged “<” should be considered below the cutoff.

V. Intended Use of the Device

The TBI test is a panel of *in vitro* diagnostic chemiluminescent microparticle immunoassays (CMIA) used for the quantitative measurements of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in human plasma and serum and provides a semi-quantitative interpretation of test results derived from these measurements using the Alinity i system.

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 negative test result is associated with the absence of acute intracranial lesions visualized on a head CT scan.

The TBI test is intended for use in clinical laboratory settings by healthcare professionals.

VI. Comparison of Technological Characteristics

The TBI test is a panel of *in vitro* diagnostic quantitative measurements of GFAP and UCH-L1 and provides a semi-quantitative interpretation of GFAP and UCH-L1 in human plasma and serum.

The GFAP assay (subject device) is an automated immunoassay for the quantitative measurement of GFAP in plasma and serum using chemiluminescent microparticle immunoassay (CMIA) technology on the Alinity i system.

The UCH-L1 assay (subject device) is an automated immunoassay for the quantitative measurement of UCH-L1 in plasma and serum using chemiluminescent microparticle immunoassay (CMIA) technology on the Alinity i system.

The similarities and differences between the subject device and the predicate device are presented in the following table.

Comparison of Subject Device (TBI for Alinity i) to Predicate Device (Banyan BTI)

Similarities and Differences Between Subject & Predicate Device		
	Subject Device: TBI	**Predicate Device: Banyan BTI (DEN170045)**
General Device Characteristic Similarities		
Intended Use and Indications for Use	The TBI test is a panel of *in vitro* diagnostic chemiluminescent microparticle immunoassays (CMIA) used for the quantitative measurements of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in human plasma and serum and provides a semi-quantitative interpretation of test results derived from these measurements using the Alinity i system. 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 negative test result is associated with the absence of acute intracranial lesions visualized on a head CT scan. The TBI test is intended for use in clinical laboratory settings by healthcare professionals.	The Banyan BTI is an in vitro diagnostic chemiluminescent enzyme-linked immunosorbent assay (ELISA). The assay provides a semi-quantitative measurement of the concentrations of ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) in human serum and is used with the Synergy 2 Multi-mode Reader. The assay results obtained from serum collected within 12 hours of suspected head injury are used, along with other available clinical information, to aid in the evaluation of patients 18 years of age and older with suspected traumatic brain injury (Glasgow Coma Scale score 13-15). A negative assay result is associated with the absence of acute intracranial lesions visualized on a head CT (computed tomography) scan.
Intended Use Setting	Clinical Laboratory	Same
Measurands	GFAP and UCH-L1	Same
Assay Technology	Chemiluminescent microparticle immunoassays (CMIA)	Enzyme-linked immunosorbent assay

Similarities and Differences Between Subject & Predicate Device		
	Subject Device: TBI	**Predicate Device: Banyan BTI (DEN170045)**
Reportable Result	Quantitative results for GFAP and UCH--L1 and semi-quantitative interpretation for TBI	Same
Assay Format	Two separate test kits – one for GFAP and one for UCH-L1	Two test kits on separate 96-well microtiter plates – one for GFAP and one for UCH-L1
Detection Technology	Chemiluminescence	Same
General Device Characteristic Differences		
Platform	Alinity i	Synergy 2 Multi-mode Reader (BioTek Instruments, Inc.)
Specimen Type	Serum and Plasma	Serum
Sample Volume	GFAP kit: 200 µL UCH-L1 kit: 150 µL	GFAP kit: 150 µL UCH-L1 kit: 100 µL
Time to Result	Approximately 18 minutes	Approximately 4 hours
Reportable Interval	Analytical Measuring Interval: GFAP: 6.1 – 42,000.0 pg/mL UCH-L1: 26.3 – 25,000.0 pg/mL Reportable Interval: GFAP: 3.2 - 42,000.0 pg/mL UCH-L1: 18.3 - 25,000.0 pg/mL	GFAP: 10 - 320 pg/mL UCH-L1: 80 - 2560 pg/mL
GFAP Cutoff	35.0 pg/mL	22 pg/mL
UCH-L1 Cutoff	400.0 pg/mL	327 pg/mL

VII. Summary of Nonclinical Performance

A. Reportable Interval

Based on representative data, the ranges over which results can be reported are provided below according to the definitions from CLSI EP34, 1st ed.*

	GFAP (pg/mL, ng/L)	UCH-L1 (pg/mL, ng/L)
Analytical Measuring Interval (AMI)[a]	6.1 – 42,000.0	26.3 – 25,000.0
Reportable Interval[b]	3.2 – 42,000.0	18.3 – 25,000.0

[a] AMI: The AMI extends from the limit of quantitation (LoQ) to the upper limit of quantitation (ULoQ). This is determined by the range of values in pg/mL (ng/L) that demonstrated acceptable performance for linearity, imprecision, and bias.

[b] The reportable interval extends from the limit of detection (LoD) to the upper limit of the AMI.

NOTE: The default Low Linearity value of the assay file corresponds to the lower limit of the reportable interval.

B. Within-Laboratory Precision

GFAP

A study was performed based on guidance from CLSI EP05-A3.† Testing was conducted using 2 lots of the GFAP reagents, 2 lots of the GFAP Calibrators, 1 lot of the GFAP Controls, and 2 instruments. Three controls and eight human plasma panels (one native panel and seven panels supplemented with GFAP analyte) were tested in a minimum of 2 replicates at 2 separate times per day on 20 days on 4 reagent lot/calibrator lot/instrument combinations, where a unique reagent lot and a unique calibrator lot are paired with both instruments. The performance is shown in the following table.

* Clinical and Laboratory Standards Institute (CLSI). *Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking.* 1st ed. CLSI Guideline EP34. Wayne, PA: CLSI; 2018.

† Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline—Third Edition*. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.

Sample	N	Mean (pg/mL, ng/L)	Within-Run		Between-Run		Between-Day		Between-Lot		Between-Instrument		Overall Within-Laboratory[a]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	479	25.4	0.76	3.0	0.70	2.7	0.12	0.5	0.43	1.7	0.53	2.1	1.24	4.9
Medium Control	480	504.8	12.89	2.6	11.67	2.3	0.00	0.0	1.40	0.3	6.38	1.3	18.58	3.7
High Control	479	31608.5	758.80	2.4	846.98	2.7	288.19	0.9	968.97	3.1	423.35	1.3	1579.35	5.0
Panel 1	479	20.4	0.69	3.4	0.64	3.1	0.36	1.8	0.02	0.1	0.19	0.9	1.03	5.0
Panel 2 (Native)	474	37.7	1.11	2.9	0.80	2.1	0.37	1.0	0.15	0.4	0.20	0.5	1.43	3.8
Panel 3	479	40.2	1.20	3.0	1.03	2.6	0.42	1.0	0.19	0.5	0.00	0.0	1.65	4.1
Panel 4	480	95.6	2.41	2.5	2.34	2.4	0.00	0.0	0.36	0.4	1.29	1.3	3.62	3.8
Panel 5	480	3097.2	72.89	2.4	63.34	2.0	55.85	1.8	55.24	1.8	36.55	1.2	129.74	4.2
Panel 6	478	7586.3	168.16	2.2	187.23	2.5	84.73	1.1	177.85	2.3	48.77	0.6	323.30	4.3
Panel 7	480	15462.7	346.62	2.2	389.78	2.5	289.53	1.9	441.47	2.9	92.99	0.6	747.96	4.8
Panel 8	478	36874.7	969.22	2.6	1233.45	3.3	0.00	0.0	1435.60	3.9	509.41	1.4	2186.60	5.9

[a] Overall within-laboratory variability contains within-run, between-run, between-day, between-lot, and between-instrument variance components.

Qualitative Precision: Results Relative to Cutoff

The qualitative analysis of precision results relative to the cutoff (35.0 pg/mL) was performed using the precision data generated for all instruments and reagent lots.

The GFAP mean (pg/mL), number of results greater than or equal to the cutoff, and % correct call for each GFAP panel and GFAP control levels for all instruments and reagent lots are presented in the table below.

Sample	N	Mean (pg/mL)	# of Results ≥ 35.0 (pg/mL) / N	% of Correct Call[a]
Low Control[b]	479	25.4	0 / 479	100.0
Medium Control[d]	480	504.8	480 / 480	100.0
High Control[d]	479	31,608.5	479 / 479	100.0
Panel 1[b]	479	20.4	0 / 479	100.0
Panel 2 (Native)[c]	474	37.7	462 / 474	100.0
Panel 3 [c]	479	40.2	479 / 479	100.0
Panel 4[d]	480	95.6	480 / 480	100.0
Panel 5[d]	480	3097.2	480 / 480	100.0
Panel 6[d]	478	7586.3	478 / 478	100.0
Panel 7[d]	480	15,462.7	480 / 480	100.0
Panel 8[d]	478	36,874.7	478 / 478	100.0

[a] Replicates for positive samples should always be ≥ cutoff, replicates for negative samples should always be < cutoff, and replicates for samples near medical decision points can have replicates < cutoff or ≥ cutoff.

[b] Negative samples

[c] Samples near medical decision point

[d] Positive samples

UCH-L1

A study was performed based on guidance from CLSI EP05-A3.* Testing was conducted using 2 lots of the UCH-L1 reagents, 2 lots of the UCH-L1 Calibrators, 1 lot of the UCH-L1 Controls, and 2 instruments. Three controls and eight human plasma panels (one native panel and seven panels supplemented with UCH-L1 analyte) were tested in a minimum of 2 replicates at 2 separate times per day on 20 days on 4 reagent

* Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline—Third Edition*. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.

lot/calibrator lot/instrument combinations, where a unique reagent lot and a unique calibrator lot are paired with both instruments. The performance is shown in the following table.

Sample	N	Mean (pg/mL, ng/L)	Within-Run		Between-Run		Between-Day		Between-Lot		Between-Instrument		Overall Within-Laboratory[a]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	479	247.5	9.16	3.7	4.99	2.0	3.13	1.3	1.62	0.7	1.07	0.4	11.06	4.5
Medium Control	479	2019.6	47.72	2.4	28.81	1.4	19.81	1.0	17.23	0.9	0.00	0.0	61.62	3.1
High Control	477	15179.4	367.43	2.4	219.00	1.4	120.20	0.8	0.00	0.0	59.99	0.4	448.35	3.0
Panel 1	480	177.6	7.20	4.1	5.15	2.9	1.68	0.9	0.00	0.0	5.59	3.1	10.60	6.0
Panel 2 (Native)	471	391.8	15.53	4.0	4.50	1.1	7.18	1.8	1.27	0.3	14.89	3.8	23.15	5.9
Panel 3	479	419.8	14.47	3.4	8.81	2.1	6.95	1.7	0.00	0.0	14.86	3.5	23.58	5.6
Panel 4	477	823.8	27.42	3.3	21.32	2.6	8.46	1.0	0.00	0.0	25.33	3.1	43.81	5.3
Panel 5	476	1553.8	53.15	3.4	28.09	1.8	25.67	1.7	9.83	0.6	21.27	1.4	69.44	4.5
Panel 6	479	4793.5	164.54	3.4	138.24	2.9	0.00	0.0	0.00	0.0	115.93	2.4	244.18	5.1
Panel 7	480	7974.6	269.27	3.4	161.73	2.0	72.92	0.9	21.25	0.3	145.84	1.8	354.54	4.4
Panel 8	472	19165.5	619.10	3.2	428.85	2.2	162.96	0.9	0.00	0.0	290.87	1.5	823.62	4.3

[a] Overall within-laboratory variability contains within-run, between-run, between-day, between-lot, and between-instrument variance components.

Qualitative Precision: Results Relative to Cutoff

The qualitative analysis of precision results relative to the cutoff (400.0 pg/mL) was performed using the precision data generated for all instruments and reagent lots.

The UCH-L1 mean (pg/mL), number of results greater than or equal to the cutoff, and % correct call for each UCH-L1 panel and UCH-L1 control levels for all instruments and reagent lots are presented in the table below.

Sample	N	Mean (pg/mL)	# of Results ≥ 400.0 (pg/mL) / N	% of Correct Call[a]
Low Control[b]	479	247.5	0 / 479	100.0
Medium Control[d]	479	2019.6	479 / 479	100.0
High Control[d]	477	15,179.4	477 / 477	100.0
Panel 1[b]	480	177.6	0 / 480	100.0
Panel 2 (Native)[c]	471	391.8	164 / 471	100.0
Panel 3[c]	479	419.8	391 / 479	100.0
Panel 4[d]	477	823.8	477 / 477	100.0
Panel 5[d]	476	1553.8	476 / 476	100.0
Panel 6[d]	479	4793.5	479 / 479	100.0
Panel 7[d]	480	7974.6	480 / 480	100.0
Panel 8[d]	472	19,165.5	472 / 472	100.0

[a] Replicates for positive samples should always be ≥ cutoff, replicates for negative samples should always be < cutoff, and replicates for samples near medical decision points can have replicates < cutoff or ≥ cutoff.

[b] Negative samples

[c] Samples near medical decision point

[d] Positive samples

C. Lower Limits of Measurement

The claimed limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) values are summarized in the table below. The LoD is in alignment with the low end of the reportable interval and the LoQ is in alignment with the low end of the AMI for GFAP and UCH-L1.

	GFAP	UCH-L1
	pg/mL, ng/L	**pg/mL, ng/L**
LoB	1.6	6.1
LoD	3.2	18.3
LoQ	6.1	26.3

A study was performed based on guidance from CLSI EP17-A2.* Testing was conducted using 3 lots of the GFAP and UCH-L1 reagents on each of 2 instruments over a minimum of 3 days.

- The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples. The observed GFAP LoB was 1.6 pg/mL (1.6 ng/L). The observed UCH-L1 LoB was 6.1 pg/mL (6.1 ng/L).
- The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples. The observed GFAP LoD was 2.2 pg/mL (2.2 ng/L). The observed UCH-L1 LoD was 16.1 pg/mL (16.1 ng/L).
- The LoQ is defined as the lowest concentration at which a maximum allowable precision of 20.0 %CV was met and was determined from n ≥ 60 replicates of low-analyte level samples. The observed GFAP LoQ was 2.4 pg/mL (2.4 ng/L). The observed UCH-L1 LoQ was 16.1 pg/mL (16.1 ng/L).

D. Linearity

GFAP

A study was performed based on guidance from CLSI EP06, 2nd ed.†

This assay is linear across the analytical measuring interval of 6.1 to 42,000.0 pg/mL (6.1 to 42,000.0 ng/L).

UCH-L1

A study was performed based on guidance from CLSI EP06, 2nd ed.‡

This assay is linear across the analytical measuring interval of 26.3 to 25,000.0 pg/mL (26.3 to 25,000.0 ng/L).

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* Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition*. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.

† Clinical and Laboratory Standards Institute (CLSI). *Evaluation of the Linearity of Quantitative Measurement Procedures.* 2nd ed. CLSI Guideline EP06. Wayne, PA: CLSI; 2020.

‡ Clinical and Laboratory Standards Institute (CLSI). *Evaluation of the Linearity of Quantitative Measurement Procedures.* 2nd ed. CLSI Guideline EP06. Wayne, PA: CLSI; 2020.

E. Analytical Specificity

Interference

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07, 3rd ed.* Each substance was tested at 2 levels of the GFAP analyte (approximately 25 pg/mL and 10,000 pg/mL) and at 2 levels of the UCH-L1 analyte (approximately 280 pg/mL and 5000 pg/mL).

No significant interference (interference within ± 10.0%) was observed at the following concentrations.

No Significant Interference (Interference within ± 10.0%)		
	Interferent Level	
Potentially Interfering Substance	**GFAP**	**UCH-L1**
Conjugated Bilirubin	40 mg/dL	40 mg/dL
Unconjugated Bilirubin	40 mg/dL	20 mg/dL
Hemoglobin	1000 mg/dL	1000 mg/dL
Intralipid	1500 mg/dL	2000 mg/dL
Total Protein	10 g/dL	9 g/dL
Glucose	1000 mg/dL	1000 mg/dL
Heterophilic Antibodies (HAMA)	80x activity	80x activity
Rheumatoid Factor (RF)	500 IU/mL	500 IU/mL

* Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry.* 3rd ed. CLSI Guideline EP07. Wayne, PA: CLSI; 2018.

Interference beyond ± 10.0% (based on 95% Confidence Interval [CI]) was observed at the concentrations shown below for the following substances.

Interference beyond ± 10.0% (based on 95% CI)				
Assay	**Potentially Interfering Substance**	**Interferent Level**	**Analyte Level**	**% Interference (95% CI)**
GFAP	Intralipid	2000 mg/dL	10,000 pg/mL	-10.3% (-11.3%, -9.3%)
GFAP	Total Protein	15 g/dL	10,000 pg/mL	-15.6% (-16.7%, -14.6%)
UCH-L1	Unconjugated Bilirubin	40 mg/dL	280 pg/mL	9.2% (7.7%, 10.7%)
UCH-L1	Total Protein	15 g/dL	280 pg/mL	-19.0% (-21.5%, -16.5%)
UCH-L1	Total Protein	15 g/dL	5000 pg/mL	-16.9% (-18.5%, -15.4%)

Potentially Interfering Drugs

A study was performed based on guidance from CLSI EP07, 3rd ed.* Each substance was tested at 2 levels of the GFAP analyte (approximately 25 pg/mL and 10,000 pg/mL) and at 2 levels of the UCH-L1 analyte (approximately 280 pg/mL and 5000 pg/mL).

No significant interference (interference within ± 10.0%) was observed at the following concentrations.

No Significant Interference (Interference within ± 10.0%)		
	Interferent Level	
Potentially Interfering Substance	**GFAP**	**UCH-L1**
Acetaminophen	20 mg/dL	20 mg/dL
Acetylcysteine	15 mg/dL	9 mg/dL
Acetylsalicylic Acid	65 mg/dL	65 mg/dL
Amphetamine	33 µg/dL	33 µg/dL
Ampicillin-Na	7.5 mg/dL	7.5 mg/dL
Ascorbic Acid	5.25 mg/dL	5.25 mg/dL
Benzoylecgonine	200 µg/dL	200 µg/dL
Biotin	4250 ng/mL	4250 ng/mL
Brivaracetam	1.05 mg/dL	1.05 mg/dL
Calcium dobesilate	6 mg/dL	2 mg/dL
Cannabinoids	50 ng/mL	50 ng/mL
Carbamazepine	4.5 mg/dL	4.5 mg/dL
Cefoxitin	660 mg/dL	660 mg/dL
Celecoxib	879 µg/dL	879 µg/dL
Clopidogrel (Plavix)	9 µg/mL	9 µg/mL
Codeine	141 µg/dL	141 µg/dL
Cyclobenzaprine	10.2 µg/dL	10.2 µg/dL
Cyclosporine	0.18 mg/dL	0.18 mg/dL

* Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry.* 3rd ed. CLSI Guideline EP07. Wayne, PA: CLSI; 2018.

No Significant Interference (Interference within ± 10.0%)		
	Interferent Level	
Potentially Interfering Substance	**GFAP**	**UCH-L1**
Diazepam	3 mg/dL	3 mg/dL
Doxycycline	1.8 mg/dL	1.8 mg/dL
EDDP	318 µg/dL	318 µg/dL
Ethanol	3000 mg/dL	1000 mg/dL
Fentanyl	0.03 mg/dL	0.03 mg/dL
Heparin	330 U/dL	330 U/dL
Ibuprofen	50 mg/dL	50 mg/dL
Imipramine	0.0315 mg/dL	0.0315 mg/dL
Levodopa	0.75 mg/dL	0.75 mg/dL
Methadone	318 µg/dL	318 µg/dL
d-Methamphetamine	400 µg/dL	400 µg/dL
Methaqualone	200 µg/dL	200 µg/dL
Methyldopa	2.25 mg/dL	2.25 mg/dL
Methylenedioxy methamphetamine (MDMA)	500 ng/mL	500 ng/mL
Metoprolol	0.5 mg/dL	0.5 mg/dL
Metronidazole	12.3 mg/dL	12.3 mg/dL
Morphine	780 µg/dL	780 µg/dL
Naproxen	36 mg/dL	36 mg/dL
Nicardipine	46.5 µg/dL	46.5 µg/dL
Ondansetron	34.2 µg/dL	34.2 µg/dL
Oxazepam	425 µg/dL	432 µg/dL
Phencyclidine	20 µg/dL	20 µg/dL
Propoxyphene	321 µg/dL	321 µg/dL
Rifampicin	4.8 mg/dL	4.8 mg/dL
Secobarbital	1.59 mg/dL	1.59 mg/dL
Theophylline	6 mg/dL	6 mg/dL
Warfarin (Coumadin)	7.5 mg/dL	7.5 mg/dL

Interference beyond ± 10.0% (based on 95% CI) was observed at the concentrations shown below for the following substances.

Interference beyond ± 10.0% (based on 95% CI)				
Assay	**Potentially Interfering Substance**	**Interferent Level**	**Analyte Level**	**% Interference (95% CI)**
GFAP	Ethanol	5000 mg/dL	25 pg/mL	-9.3% (-10.2%, -8.4%)
GFAP	Ethanol	5000 mg/dL	10,000 pg/mL	-13.0% (-13.9%, -12.2%)
UCH-L1	Acetylcysteine	13 mg/dL	280 pg/mL	10.7% (9.4%, 12.1%)
UCH-L1	Acetylcysteine	15 mg/dL	5000 pg/mL	8.4% (6.1%, 10.8%)
UCH-L1	Calcium dobesilate	6 mg/dL	280 pg/mL	13.0% (11.8%, 14.2%)
UCH-L1	Calcium dobesilate	6 mg/dL	5000 pg/mL	11.9% (10.2%, 13.5%)
UCH-L1	Ethanol	5000 mg/dL	280 pg/mL	14.9% (12.9%, 17.0%)
UCH-L1	Ethanol	5000 mg/dL	5000 pg/mL	10.9% (8.6%, 13.2%)

Cross-Reactants

GFAP

A study was performed based on guidance from CLSI EP07, 3rd ed.* Samples containing the cross-reactants listed below were prepared in GFAP depleted plasma and tested with the GFAP assay on the Alinity i system. The % cross-reactivity results are shown below.

* Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry.* 3rd ed. CLSI Guideline EP07. Wayne, PA: CLSI; 2018.

Cross-Reactant	Cross-Reactant Concentration	% Cross-Reactivity (95% CI)
Desmin	130,000 pg/mL	0.0% (0.0%, 0.0%)
Internexin	80,000 pg/mL	0.0% (0.0%, 0.0%)
Keratin type II	12,000 pg/mL	0.0% (0.0%, 0.0%)
Neurofilament light	70 pg/mL	-0.4% (-0.5%, -0.2%)
Neurofilament medium	9000 pg/mL	0.0% (0.0%, 0.0%)
Neurofilament heavy	80,000 pg/mL	0.0% (0.0%, 0.0%)
Peripherin	6000 pg/mL	0.0% (0.0%, 0.0%)
Vimentin	360,000 pg/mL	0.0% (0.0%, 0.0%)

UCH-L1

A study was performed based on guidance from CLSI EP07, 3rd ed.* A sample containing the cross-reactant listed below was prepared in UCH-L1 depleted plasma and tested with the UCH-L1 assay on the Alinity i system. The % cross-reactivity results are shown below.

Cross-Reactant	Cross-Reactant Concentration	% Cross-Reactivity (95% CI)
Ubiquitin carboxyl-terminal hydrolase L3 (UCH-L3)	360,000 pg/mL	0.0% (0.0%, 0.0%)

* Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry.* 3rd ed. CLSI Guideline EP07. Wayne, PA: CLSI; 2018.

F. Tube Type

A study was performed to evaluate the suitability of specific blood collection tube types for use with the GFAP and UCH-L1 assays. Samples were collected/obtained from a minimum of 70 donors and evaluated across tube types. The following blood collection tube types were determined to be acceptable for use with the GFAP and UCH-L1 assays:

Specimen Types	Collection Tubes
Serum	Serum Serum separator
Plasma	Dipotassium EDTA Tripotassium EDTA Lithium heparin Lithium heparin separator

G. Expected Values (Reference Intervals) (GFAP, UCH-L1, TBI)

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

A reference interval study was performed based on guidance from CLSI C28-A3c* with a US-based general population from apparently healthy individuals (≥ 18 years old). The specimens were tested with both the GFAP and UCH-L1 assays on the Alinity i system. Based on the results, a 95% reference interval of an apparently healthy population of each assay was determined to be as follows:

Assay	n	Mean (pg/mL, ng/L)	SD	Median (pg/mL, ng/L)	Reference Interval (2.5th to 97.5th Percentile) (pg/mL, ng/L)
GFAP	160	23.5	13.79	20.5	6.6, 70.9
UCH-L1	160	108.1	45.28	98.0	44.7, 226.8

* Clinical and Laboratory Standards Institute (CLSI). *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition*. CLSI Document C28-A3c. Wayne, PA: CLSI; 2008.

The TBI interpretation based on the GFAP and UCH-L1 results shown above for apparently healthy individuals (≥ 18 years old) are summarized below.

GFAP Result (Relative to Cutoff of 35.0 pg/mL)[a]	UCH-L1 Result (Relative to Cutoff of 400.0 pg/mL)[a]	TBI Interpretation	N (Percentage)
Above	Above	Positive	0 (0/160 = 0.0%)
Below	Above	Positive	0 (0/160 = 0.0%)
Above	Below	Positive	21 (21/160 = 13.1%)[b]
Below	Below	Negative	139 (139/160 = 86.9%)

[a] Above means greater than or equal to the cutoff. Below means less than the cutoff.

[b] Although 13.1% of an apparently healthy population was found to have a positive TBI interpretation, it is important to note GFAP and UCH-L1 cutoffs were optimized in a population of patients with head injury.

H. Specimen Storage

A study was performed to evaluate serum and plasma specimens when subjected to various conditions (2 to 8°C storage, room temperature [15 to 25°C], -20°C or colder) and tested with GFAP and UCH-L1 assays.

The results support the use of serum and plasma specimens that have been stored at the following conditions:

- Room temperature (15 to 25°C) for up to 8 hours on or off the clot, red blood cells, or separator gel.
- 2 to 8°C for up to 8 hours on the clot, red blood cells, or separator gel and up to 7 days off the clot, red blood cells, or separator gel. Note: Recentrifuge after 8 hours of storage.
- -20°C or colder for up to 1 month off the clot, red blood cells, or separator gel with up to 1 freeze/thaw.

I. Assay Cutoff

The assay cutoffs were determined by analyzing a training set with GFAP and UCH-L1 results from a total of 354 with 132 CT positive subjects with suspected mild traumatic brain injury (TBI; Glasgow Coma Scale score of 13-15). Subjects who had blood drawn within 12 hours of injury, a head CT scan determination, and were 18 years or older at

the time of injury were included in the analysis. Using a 10-fold cross validation and bootstrapping method, the cutoff values of 35.0 pg/mL (GFAP assay) and 400.0 pg/mL (UCH-L1 assay) were selected for the Alinity i using the selection criteria with an adjusted Negative Predictive Value (NPV) (prevalence 6%) ≥ 99%, sensitivity ≥ 96% and specificity ≥ 37%.

VIII. Summary of Clinical Performance

A. System Reproducibility

GFAP

A study was performed based on guidance from CLSI EP05-A3.* Testing was conducted using 3 lots of the GFAP reagents, 3 lots of the GFAP Calibrators, 3 lots of the GFAP Controls, and 1 instrument at each of the 3 testing sites. Three controls and seven human plasma panels (one native panel and six panels supplemented with GFAP analyte) were tested in 4 replicates at 2 separate times per day on 5 different days.

* Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline—Third Edition.* CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.

Sample	N	Mean (pg/mL, ng/L)	Within-Run		Between-Run		Between-Day		Between-Lot		Between-Site		Overall Reproducibility[a]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	360	24.9	0.61	2.4	0.18	0.7	0.57	2.3	0.26	1.1	0.37	1.5	0.97	3.9
Medium Control	360	494.6	8.33	1.7	4.46	0.9	8.06	1.6	3.46	0.7	0.94	0.2	12.93	2.6
High Control	360	30520.7	630.70	2.1	404.86	1.3	230.74	0.8	252.37	0.8	428.65	1.4	928.64	3.0
Panel 1	360	20.4	0.61	3.0	0.26	1.3	0.53	2.6	0.19	0.9	0.34	1.7	0.94	4.6
Panel 2 (Native)	360	37.4	0.74	2.0	0.49	1.3	0.91	2.4	0.29	0.8	0.83	2.2	1.54	4.1
Panel 4	360	94.9	1.66	1.8	0.89	0.9	1.87	2.0	0.00	0.0	1.26	1.3	2.94	3.1
Panel 5	360	3072.8	50.95	1.7	33.14	1.1	43.49	1.4	17.37	0.6	11.66	0.4	77.60	2.5
Panel 6	360	7449.5	135.20	1.8	84.45	1.1	102.16	1.4	0.00	0.0	0.00	0.0	189.34	2.5
Panel 7	360	15269.2	252.80	1.7	152.99	1.0	207.63	1.4	68.84	0.5	227.57	1.5	432.38	2.8
Panel 8	360	36101.1	852.46	2.4	368.77	1.0	568.78	1.6	781.48	2.2	1037.82	2.9	1695.28	4.7

[a] Overall Reproducibility includes within-run, between-run, between-day, between-lot, and between-site variance components

Qualitative Precision: Results Relative to Cutoff

The GFAP mean (pg/mL), number of results greater than or equal to the cutoff, and % correct call for each GFAP reproducibility panel members and 3 GFAP control levels for all 3 sites are shown in the following table.

Site	Sample	N	Mean (pg/mL)	# of Result ≥ 35.0 (pg/mL) / N	% of Correct Call[a]
All Sites	Low Control[b]	360	24.9	0 / 360	100.0
	Medium Control[d]	360	494.6	360 / 360	100.0
	High Control[d]	360	30,520.7	360 / 360	100.0
	Panel 1[b]	360	20.4	0 / 360	100.0
	Panel 2 (Native)[c]	360	37.4	337 / 360	100.0
	Panel 4[d]	360	94.9	360 / 360	100.0
	Panel 5[d]	360	3072.8	360 / 360	100.0
	Panel 6[d]	360	7449.5	360 / 360	100.0
	Panel 7[d]	360	15,269.2	360 / 360	100.0
	Panel 8[d]	360	36,101.1	360 / 360	100.0

[a] Replicates for positive samples should always be ≥ cutoff, replicates for negative samples should always be < cutoff, and samples near the medical decision point (GFAP Panel 2) can have replicates < cutoff or ≥ cutoff.

[b] Negative samples

[c] Samples near medical decision point

[d] Positive samples

UCH-L1

A study was performed based on guidance from CLSI EP05-A3.* Testing was conducted using 3 lots of the UCH-L1 reagents, 3 lots of the UCH-L1 Calibrators, 3 lots of the UCH-L1 Controls, and 1 instrument at each of the 3 testing sites. Three controls and seven human plasma panels (one native panel and six panels supplemented with UCH-L1 analyte) were tested in 4 replicates at 2 separate times per day on 5 different days.

* Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline—Third Edition.* CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.

Sample	N	Mean (pg/mL, ng/L)	Within-Run		Between-Run		Between-Day		Between-Lot		Between-Site		Overall Reproducibility[a]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	360	249.9	5.83	2.3	1.63	0.7	4.85	1.9	1.62	0.6	4.29	1.7	9.01	3.6
Medium Control	360	1983.7	36.07	1.8	14.64	0.7	14.46	0.7	2.40	0.1	31.50	1.6	52.18	2.6
High Control	360	14945.1	243.83	1.6	124.28	0.8	187.94	1.3	75.42	0.5	183.94	1.2	386.96	2.6
Panel 1	360	187.3	5.29	2.8	2.03	1.1	4.45	2.4	1.68	0.9	9.93	5.3	12.39	6.6
Panel 2 (Native)	360	402.4	10.91	2.7	4.55	1.1	3.58	0.9	5.52	1.4	13.24	3.3	18.93	4.7
Panel 4	359	838.9	20.67	2.5	6.18	0.7	7.22	0.9	13.02	1.6	32.20	3.8	41.52	4.9
Panel 5	360	1568.0	40.83	2.6	21.71	1.4	3.97	0.3	15.74	1.0	49.33	3.1	69.54	4.4
Panel 6	360	4792.8	125.95	2.6	48.58	1.0	61.63	1.3	23.27	0.5	112.59	2.3	187.72	3.9
Panel 7	360	8011.9	188.84	2.4	62.49	0.8	77.68	1.0	30.53	0.4	192.57	2.4	289.16	3.6
Panel 8	360	19606.6	513.60	2.6	197.00	1.0	252.28	1.3	201.66	1.0	571.99	2.9	856.78	4.4

[a] Overall Reproducibility includes within-run, between-run, between-day, between-lot, and between-site variance components

Qualitative Precision: Results Relative to Cutoff

The UCH-L1 mean (pg/mL), number of results greater than or equal to the cutoff, and % correct call for each UCH-L1 reproducibility panel members and 3 UCH-L1 control levels for all 3 sites are shown in the following table.

Site	Sample	N	Mean (pg/mL)	# of Result ≥ 400.0 (pg/mL) / N	% of Correct Call[a]
All Sites	Low Control[b]	360	249.9	0 / 360	100.0
	Medium Control[d]	360	1983.7	360 / 360	100.0
	High Control[d]	360	14,945.1	360 / 360	100.0
	Panel 1[b]	360	187.3	0 / 360	100.0
	Panel 2 (Native)[c]	360	402.4	230 / 360	100.0
	Panel 4[d]	359	838.9	359 / 359	100.0
	Panel 5[d]	360	1568.0	360 / 360	100.0
	Panel 6[d]	360	4792.8	360 / 360	100.0
	Panel 7[d]	360	8011.9	360 / 360	100.0
	Panel 8[d]	360	19,606.6	360 / 360	100.0

[a] Replicates for positive samples should always be ≥ cutoff, replicates for negative samples should always be < cutoff, and samples near the medical decision point (UCH-L1 Panel 2) can have replicates < cutoff or ≥ cutoff.

[b] Negative samples

[c] Samples near medical decision point

[d] Positive samples

B. Clinical Performance

A pivotal study using prospectively collected and archived (frozen) plasma specimens was conducted to establish the clinical performance of the TBI test on the Alinity i system. 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 a

* Bazarian JJ, Biberthaler P, Welch RD, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. *Lancet Neurol* 2018;17:782-789.

health care facility (HCF) or emergency department (ED) with suspected TBI with initial Glasgow Coma Scale (GCS) scores of 9-15 and who had a 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 dipotassium EDTA 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 enrolled in the original study with GCS scores of 13 to 15, 72 subjects were not included in the study due to lack of consent for future testing, withdrawn consent, and no specimen available. Specimens from 23 subjects were not included in the analysis due to unreadable, inconclusive, or no CT scan results; unknown time of blood draw or blood draw more than 12 hours after injury; and/or no recorded time of injury. Specimens from 1899 subjects were included in the analysis.

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

Demographic Characteristics	Head CT Scan Result		Total
	Positive	Negative	
N	120 (6.3%)	1779 (93.7%)	1899
Age (Years)[a]			
Mean (SD)	58.8 (18.29)	48.5 (21.01)	49.1 (20.99)
Median	58.5	48.0	49.0
Range (minimum, maximum)	(20, 95)	(18, 98)	(18, 98)
Gender, N (%)			
Male	70 (58.3%)	1003 (56.4%)	1073 (56.5%)
Female	50 (41.7%)	776 (43.6%)	826 (43.5%)
Ethnicity, N (%)			
Hispanic or Latino	1 (0.8%)	89 (5.0%)	90 (4.7%)
Not Hispanic or Latino	118 (98.3%)	1689 (94.9%)	1807 (95.2%)
Not Reported	1 (0.8%)	1 (0.1%)	2 (0.1%)
Race, N (%)			
White	97 (80.8%)	1237 (69.5%))	1334 (70.2%)
Black or African American	16 (13.3%)	477 (26.8%)	493 (26.0%)
Asian	4 (3.3%)	24 (1.3%)	28 (1.5%)
Native Hawaiian or other Pacific Islander	0 (0.0%)	2 (0.1%)	2 (0.1%)
American Indian or Alaska Native	0 (0.0%)	4 (0.2%)	4 (0.2%)
White/American Indian or Alaska Native [b]	1 (0.8%)	4 (0.2%)	5 (0.3%)
White/Black or African American [b]	0 (0.0%)	3 (0.2%)	3 (0.2%)
White/Black or African American/American Indian or Alaska Native [b]	0 (0.0%)	1 (0.1%)	1 (0.1%)
Asian/Native Hawaiian or other Pacific Islander [b]	1 (0.8%)	0 (0.0%)	1 (0.1%)
Unknown	1 (0.8%)	27 (1.5%)	28 (1.5%)

[a] Age was calculated relative to the date of informed consent.

[b] Subjects indicated more than 1 race.

The head injury characteristics of the subjects represented by the 1899 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 below.

Head Injury Characteristics	Head CT Scan Result		Total
	Positive	Negative	
Time from head injury to examination (hours)[a]			
N	120	1779	1899
Mean (SD)	1.9 (1.73)	1.6 (1.71)	1.6 (1.71)
Median	1.2	1.0	1.1
Range (minimum, maximum)	(0.3, 7.8)	(0.1, 10.7)	(0.1, 10.7)
Time from head injury to CT scan (hours)[a]			
N	120	1779	1899
Mean (SD)	2.8 (1.95)	2.7 (1.93)	2.7 (1.93)
Median	2.1	2.2	2.1
Range (minimum, maximum)	(0.5, 8.9)	(0.2, 13.3)	(0.2, 13.3)
Time from head injury to blood draw (hours)[a]			
N	120	1779	1899
Mean (SD)	3.8 (1.91)	3.5 (1.88)	3.5 (1.89)
Median	3.3	3.1	3.2
Range (minimum, maximum)	(0.3, 9.3)	(0.3, 11.9)	(0.3, 11.9)
GCS score			
13	7 (5.8%)	15 (0.8%)	22 (1.2%)
14	19 (15.8%)	71 (4.0%)	90 (4.7%)
15	94 (78.3%)	1693 (95.2%)	1787 (94.1%)
Neurological assessment **Number (%) of subjects experiencing**			
Loss of Consciousness (LOC)	82 (68.3%)	720 (40.5%)	802 (42.2%)
Confusion	44 (36.7%)	312 (17.5%)	356 (18.7%)
Alteration of Consciousness (AOC)	92 (76.7%)	976 (54.9%)	1068 (56.2%)

Head Injury Characteristics	Head CT Scan Result		Total
	Positive	Negative	
Vomiting	14 (11.7%)	128 (7.2%)	142 (7.5%)
Vomiting Two or More Episodes	10 (8.3%)	60 (3.4%)	70 (3.7%)
Post Traumatic Amnesia (PTA)	81 (67.5%)	544 (30.6%)	625 (32.9%)
Post Traumatic Seizures	2 (1.7%)	11 (0.6%)	13 (0.7%)
Subjects with Drug or Alcohol Intoxication at Time of Presentation to Facility	33 (27.5%)	369 (20.7%)	402 (21.2%)
Dangerous Mechanism of Injury[b]	27 (22.5%)	369 (20.7%)	396 (20.9%)
Physical Evidence[c]			
Visible Trauma Above the Clavicle	101 (84.2%)	1102 (61.9%)	1203 (63.3%)
Suspected Open or Depressed Skull Fracture	14 (11.7%)	46 (2.6%)	60 (3.2%)
Signs of Basal Skull Fracture	10 (8.3%)	26 (1.5%)	36 (1.9%)
Presence of Neurosurgical Lesions	5 (4.2%)	0 (0.0%)	5 (0.3%)

[a] Time since head injury calculated relative to time that the subject was first examined by medical personnel at facility.

[b] Dangerous mechanism of injury was pedestrian struck by a motor vehicle, an occupant ejected from a motor vehicle, or a fall from an elevation of 3 or more feet or 5 stairs.

[c] Prior to head CT.

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

To estimate the clinical performance characteristics, the TBI test interpretation was compared to the adjudicated head CT scan result for each subject. The performance estimates are summarized below. Of the 1899 specimens, 120 had positive CT scan results. Of these 120 specimens, 116 had a positive TBI interpretation (Sensitivity: 96.7%; 95% CI: 91.7%, 98.7%). Four specimens associated with CT scan positive results had a negative TBI interpretation. The rate of False Negative (FN) results was 3.3% (4/120). Five subjects in the study were identified with lesion requiring surgical intervention; none of these five subjects had a FN result, suggesting that the TBI test

correctly classified all these five CT-positive subjects with a positive TBI interpretation. Of the 1779 specimens with negative CT scan results, 713 had a negative TBI interpretation (Specificity: 40.1%). The rate of False Positive (FP) results was 59.9% (1066/1779).

Overall, there were 717 specimens with a negative TBI interpretation. Of these, 713 specimens were associated with negative CT scan results. The NPV of the assay was 99.4% (713/717, 95% CI: 98.6%, 99.8%).

The Positive Predictive Value (PPV) of the assay was 9.8%. The Likelihood Ratio Negative (LR-) of the assay was 0.08 (95% CI: 0.03, 0.22). The Likelihood Ratio positive (LR+) of the assay was 1.61 (95% CI: 1.53, 1.70).

		Head CT Scan Result		
		Positive	**Negative**	**Total**
TBI Interpretation	**Positive**	116	1066	1182
	Negative	4	713	717
	Total	120	1779	1899

Sensitivity (%) = 96.7 (116 / 120); 95% CI: 91.7, 98.7
Specificity (%) = 40.1 (713 / 1779); 95% CI: 37.8, 42.4
NPV (%)[a] = 99.4 (713 / 717); 95% CI: 98.6, 99.8
PPV (%)[b] = 9.8 (116 / 1182); 95% CI: 8.2, 11.6
Likelihood Ratio Negative (LR-) = 0.08; 95% CI: 0.03, 0.22
Likelihood Ratio Positive (LR+) = 1.61; 95% CI: 1.53, 1.70

[a] Adjusted NPV (%) for 6% CT scan positive prevalence* = 99.5; 95% CI: 98.6, 99.8

[b] Adjusted PPV (%) for 6% CT scan positive prevalence* = 9.3; 95% CI: 8.9, 9.8

* Evaluation of Automatic Class III Designation for Banyan Brain Trauma Indicator. US Food and Drug Administration. Published February 2018. Accessed October 19, 2021 http://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170045.pdf

Performance by gender and time from injury to blood draw is also presented below.

ALERT-TBI study										
	Head CT Scan Results				Sensitivity (%) (N) (95% CI)	Specificity (%) (N) (95% CI)	Adj. PPV[a] (%) (95% CI)	Adj. NPV[a] (%) (95% CI)	LR+ (95% CI)	LR- (95% CI)
	Positive		Negative							
	TBI		TBI							
Category	Pos	Neg	Pos	Neg						
All subjects n = 1899	116	4	1066	713	96.7 (116 / 120) (91.7, 98.7)	40.1 (713 / 1779) (37.8, 42.4)	9.3 (8.9, 9.8)	99.5 (98.6, 99.8)	1.61 (1.53, 1.70)	0.08 (0.03, 0.22)
Gender										
Male n = 1073 (56.5%)	68	2	601	402	97.1 (68 / 70) (90.2, 99.2)	40.1 (402 / 1003) (37.1, 43.1)	9.4 (8.8, 9.9)	99.5 (98.2, 99.9)	1.62 (1.52, 1.73)	0.07 (0.02, 0.28)
Female n = 826 (43.5%)	48	2	465	311	96.0 (48 / 50) (86.5, 98.9)	40.1 (311 / 776) (36.7, 43.6)	9.3 (8.6, 10.0)	99.4 (97.6, 99.8)	1.60 (1.48, 1.74)	0.10 (0.03, 0.39)
Time from injury to blood draw										
0 - 4 hours n = 1443 (76.0%)	84	2	823	534	97.7 (84 / 86) (91.9, 99.4)	39.4 (534 / 1357) (36.8, 42.0)	9.3 (8.9, 9.8)	99.6 (98.5, 99.9)	1.61 (1.53, 1.70)	0.06 (0.01, 0.23)
> 4 - 8 hours n = 378 (19.9%)	27	1	198	152	96.4 (27 / 28) (82.3, 99.4)	43.4 (152 / 350) (38.3, 48.7)	9.8 (8.8, 10.9)	99.5 (96.5, 99.9)	1.70 (1.52, 1.91)	0.08 (0.01, 0.57)
0 - 8 hours n = 1821 (95.9%)	111	3	1021	686	97.4 (111 / 114) (92.5, 99.1)	40.2 (686 / 1707) (37.9, 42.5)	9.4 (9.0, 9.8)	99.6 (98.7, 99.9)	1.63 (1.55, 1.71)	0.07 (0.02, 0.20)
> 8 - 12 hours n = 78 (4.1%)	5	1	45	27	83.3 (5 / 6) (43.6, 97.0)	37.5 (27 / 72) (27.2, 49.0)	7.8 (5.4, 11.3)	97.2 (85.2, 99.5)	1.33 (0.89, 1.99)	0.44 (0.07, 2.73)

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

The results showed that the clinical performance of the TBI test is characterized by high clinical sensitivity and high NPV comparable to that demonstrated by the Banyan BTI (DEN170045; clinical sensitivity = 97.5%, clinical specificity = 36.5%, NPV = 99.6%, PPV = 9.2%), which supports clinical utility as an aid in the evaluation of the need for a CT scan in subjects presenting with a GCS score of 13 to 15.

Fresh Specimen Study

To supplement the results of the pivotal study (N=1899) 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 a HCF or ED with suspected mild TBI, with initial GCS scores of 13-15, and who had a CT scan of the head performed per the clinical site's standard of care. A total of 97 subjects were enrolled across 5 clinical sites 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 transmitted to a central neuroimaging processing center. Images were interpreted by at least two radiologists 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 radiologists, with adjudication by a third radiologist if necessary. Outcomes were positive, negative, or inconclusive 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 dipotassium EDTA 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 below:

	Head CT Scan Result		
Demographic Characteristics	**Positive**	**Negative**	**Total**
N	14 (14.4%)	83 (85.6%)	97
Age (Years)			
Mean (SD)	41.5 (20.33)	48.4 (20.24)	47.4 (20.29)
Median	33.5	49.0	43.0
Range (minimum, maximum)	(19.0, 73.0)	(18.0, 85.0)	(18.0, 85.0)
Gender, N (%)			
Male	10 (71.4%)	51 (61.4%)	61 (62.9%)
Female	4 (28.6%)	32 (38.6%)	36 (37.1%)
Ethnicity, N (%)			

Demographic Characteristics	Head CT Scan Result		Total
	Positive	Negative	
Hispanic or Latino	4 (28.6%)	22 (26.5%)	26 (26.8%)
Not Hispanic or Latino	10 (71.4%)	60 (72.3%)	70 (72.2%)
Race, N (%)			
White	13 (92.9%)	56 (67.5%)	69 (71.1%)
Black or African American	0 (0.0%)	12 (14.5%)	12 (12.4%)
Asian	1 (7.1%)	7 (8.4%)	8 (8.2%)
Native Hawaiian or Other Pacific Islander	0 (0.0%)	1 (1.2%)	1 (1.0%)

The head injury characteristics of the subjects in the supplemental fresh plasma specimen study including information regarding time from head injury to CT scan and head injury to blood draw, as well as GCS, neurological assessment, physical evidence of trauma, and mechanism of injury, categorized by head CT scan results, are shown below.

Head Injury Characteristics	Head CT Scan Result		Total
	Positive	Negative	
N	14	83	97
Time from head injury to CT scan (hours)			
Mean (SD)	2.2 (1.47)	3.1 (1.76)	3.0 (1.74)
Median	1.8	2.7	2.6
Range (minimum, maximum)	(0.8, 6.5)	(0.8, 9.9)	(0.8, 9.9)
Time from head injury to blood draw (hours)			
Mean (SD)	8.4 (2.90)	5.9 (2.64)	6.3 (2.81)
Median	8.6	5.2	5.4
Range (minimum, maximum)	(4.1, 11.8)	(1.4, 12.0)	(1.4, 12.0)
GCS score – Number (%)			
13	1 (7.1%)	0 (0.0%)	1 (1.0%)
14	3 (21.4%)	4 (4.8%)	7 (7.2%)
15	10 (71.4%)	79 (95.2%)	89 (91.8%)
Neurological Assessment – Number (%) of subjects experiencing:			
Loss of Consciousness (LOC)	10 (71.4%)	31 (37.3%)	41 (42.3%)
Alteration of Consciousness (AOC)	11 (78.6%)	51 (61.4%)	62 (63.9%)
Vomiting	3 (21.4%)	3 (3.6%)	6 (6.2%)

Head Injury Characteristics	Head CT Scan Result		Total
	Positive	Negative	
Post Traumatic Amnesia (PTA)	10 (71.4%)	35 (42.2%)	45 (46.4%)
Subjects with Drug in System at the Time of Presentation to Facility	5 (35.7%)	8 (9.6%)	13 (13.4%)
Subjects with Alcohol in System at the Time of Presentation to Facility	7 (50.0%)	6 (7.2%)	13 (13.4%)
Physical Evidence – N (%)			
Subdural Hematoma	10 (71.4%)	0 (0.0%)	10 (10.3%)
Subarachnoid Hemorrhage	10 (71.4%)	1 (1.2%)	11 (11.3%)
Acute Skull Fracture	8 (57.1%)	1 (1.2%)	9 (9.3%)
Contusion	6 (42.9%)	0 (0.0%)	6 (6.2%)
Intracerebral Hemorrhage	1 (7.1%)	0 (0.0%)	1 (1.0%)
Epidural Hematoma	1 (7.1%)	0 (0.0%)	1 (1.0%)
Traumatic Axonal Injury	1 (7.1%)	0 (0.0%)	1 (1.0%)
Midline Shift Supratentorial	2 (14.3%)	0 (0.0%)	2 (2.1%)
Cisternal Compression	2 (14.3%)	0 (0.0%)	2 (2.1%)
Edema	1 (7.1%)	0 (0.0%)	1 (1.0%)
Brain Atrophy or Encephalomalacia	0 (0.0%)	2 (2.4%)	2 (2.1%)
Brain Swelling	2 (14.3%)	0 (0.0%)	2 (2.1%)
Visible Trauma Above the Clavicle	13 (92.9%)	55 (66.3%)	68 (70.1%)
Signs of Basal Skull Fracture	2 (14.3%)	0 (0.0%)	2 (2.1%)
Mechanism of Injury – N (%)			
Acceleration/Deceleration	2 (14.3%)	26 (31.3%)	28 (28.9%)
Direct impact: Blow to Head	4 (28.6%)	13 (15.7%)	17 (17.5%)
Direct impact: Head Against Object	11 (78.6%)	54 (65.1%)	65 (67.0%)
Fall from height > 1 meter (3 ft)	2 (14.3%)	4 (4.8%)	6 (6.2%)
Ground level fall	4 (28.6%)	26 (31.3%)	30 (30.9%)

The TBI test on the Alinity i system clinical performance estimates from the supplemental plasma fresh specimen study are shown below. Of the 97 subjects tested, 14 had positive head CT scan results. Of these 14 subjects, 14 had a positive TBI test interpretation (14 / 14, clinical sensitivity = 100.0%). The rate of False Negatives (FN) was 0.0% (0 / 14). Of the 83 subjects associated with negative CT scan results, 23 had a negative TBI test interpretation (23 / 83, clinical specificity =27.7%). The rate of False Positive (FP) results was 72.3% (60 / 83).

Overall, there were 23 specimens with a negative TBI test interpretation. All 23 specimens were associated with negative head CT scan results. The NPV of the assay was 100.0% (23 / 23).

TBI Interpretation	Head CT Scan Result		Total
	Positive	Negative	
Positive	14	60	74
Negative	0	23	23
Total	14	83	97

Sensitivity (%) = 100.0 (14 / 14); 95% CI: 78.5, 100.0

Specificity (%) = 27.7 (23 / 83); 95% CI: 19.2, 38.2

NPV (%)[a] = 100.0 (23 / 23); 95% CI: 85.7, 100.0

PPV (%)[a] = 18.9 (14 / 74); 95% CI: 11.6, 29.3

Likelihood Ratio Negative (LR-) = 0.12; 95% CI: 0.01, 1.91

Likelihood Ratio Positive (LR+) = 1.38; 95% CI: 1.21, 1.58

[a] NPV and PPV are estimated at 14.43% prevalence of CT scan positive rate for suspected mild TBI subjects in the fresh study. If NPV and PPV are adjusted to 6% prevalence rate (comparable to the pivotal study cohort), NPV = 99.2% (95% CI: 89.1,99.9), and PPV = 8.1 (95% CI: 7.2, 9.1)[*]

The results show that the TBI test, when evaluated with fresh plasma samples, is characterized by high sensitivity and NPV supportive of its clinical utility as an aid in the evaluation of the need for a CT scan in subjects presenting with a GCS score of 13 to 15.

IX. Conclusion

The information presented in this 510(k) premarket notification demonstrate that the subject device, TBI for use with Alinity i, is substantially equivalent to the predicate device, Banyan BTI (DEN170045), and meets special controls requirements of 21 CFR 866.5830.

[*] Evaluation of Automatic Class III Designation for Banyan Brain Trauma Indicator. US Food and Drug Administration. Published February 2018. Accessed October 19, 2021 http://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170045.pdf