510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K223602

B Applicant

Abbott Laboratories

C Proprietary and Established Names

TBI

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

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

C Type of Test:

Automated chemiluminescent microparticle immunoassay, semi-quantitative

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) 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.

C Special Conditions for Use Statement(s):

For prescription use only

D Special Instrument Requirements:

Alinity i system

IV Device/System Characteristics:

A Device Description:

The TBI test consists of two reagent kits: the GFAP Reagent Kit for the GFAP assay components and the UCH-L1 Reagent Kit for the UCH-L1 assay components.

The GFAP Reagent Kit and UCH-L1 Reagent Kit are provided separately. The configurations of the two reagent kits are described below.

	GFAP Reagent Kit	UCH-L1 Reagent Kit
Tests per cartridge	100	100
Number of cartridges per kit	2	2
Tests per kit	200	200
Microparticles per cartridge	7.1 mL	7.1 mL
Conjugate per cartridge	6.4 mL	12.5 mL
Assay-specific diluent per cartridge	6.4 mL	10.5 mL

1. GFAP Reagent Kit:

    - Microparticles: Anti-GFAP (rabbit, monoclonal) coated microparticles in TRIS buffer with stabilizer and preservative.
    - Conjugate: Anti-GFAP (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with stabilizer and preservative.
    - Assay-specific diluent: TRIS buffer with stabilizer and preservative.

2. UCH-L1 Reagent Kit:

    - Microparticles: Anti-UCH-L1 (mouse, monoclonal) coated microparticles in TRIS buffer with stabilizer and preservative.
    - Conjugate: Anti-UCH-L1 (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with stabilizer and preservative.
    - Assay-specific diluent: TRIS buffer with stabilizer and preservative.

3. Materials required but not provided:

    - TBI assay file package
    - GFAP Calibrators: 6 levels (0, 50, 400, 2000, 20000, and 50000 pg/mL)
    - GFAP Controls: 3 levels (25, 500, and 30000 pg/mL)
    - UCH-L1 Calibrators: 6 levels (0, 200, 500, 1000, 5000, and 25000 pg/mL)
    - UCH-L1 Controls: 3 levels (250, 2000, and 15000 pg/mL)
    - Alinity Pre-Trigger Solution
    - Alinity Trigger Solution
    - Alinity i-series Concentrated Wash Buffer

B Principle of Operation:

The TBI test is a panel of *in vitro* diagnostic quantitative measurements of GFAP and UCH-L1 in human plasma and serum using an automated, two-step chemiluminescent microparticle immunoassay (CMIA) on the Alinity i system, and provides a semi-quantitative interpretation based on the results of GFAP and UCH-L1.

For GFAP, 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.

For UCH-L1, 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.

The assay cut-offs 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 Alinity i system software provides a TBI interpretation relative to the respective cut-off values as shown in the following table.

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

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

GFAP Assay Result (relative to 35.0 pg/mL cut-off)*	UCH-L1 Assay Result (relative to 400.0 pg/mL cut-off)*	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 cut-off. Below means less than the cut-off.

** 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 cut-off 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 cut-off 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 cut-off and a result flagged “<” should be considered below the cut-off.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Banyan BTI

B Predicate 510(k) Number(s):

DEN170045

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device K223602	Predicate DEN170045
Device Trade Name	TBI test	Banyan BTI
General Device Characteristic Similarities		
Intended Use/ 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.

Device & Predicate Device(s):	Device K223602	Predicate DEN170045
Intended Use Setting	Clinical laboratory	Same
Measurands	GFAP and UCH-L1	Same
Assay Technology	Immunoassay (Chemiluminescent microparticle immunoassay)	Immunoassay (Enzyme-linked immunosorbent assay)
Calibrators	GFAP: 6 levels UCH-L1: 6 levels	Same
Reportable Result	Quantitative results for GFAP and UCH-L1 and semi-quantitative interpretation for TBI	Same
Assay Format	Two separate test kits	Same
Detection Technology	Chemiluminescence	Same
General Device Characteristic Differences		
Platform	Alinity i system	Synergy 2 Multi-mode Reader (BioTek Instruments, Inc.)
Specimen Type	Serum and plasma	Serum
Controls	GFAP: 3 levels (25, 500, and 30000 pg/mL) UCH-L1: 3 levels (250, 2000, and 15000 pg/mL)	GFAP: 2 levels (Control 1-Low, Control 2-High) UCH-L1: 2 levels (Control 1-Low, Control 2-High)
Sample Volume	GFAP: 200 µL UCH-L1: 150 µL	GFAP: 150 µL UCH-L1: 100 µL
Time to Result	Approximately 18 minutes	Approximately 4 hours
Analytical Measurement Interval	GFAP: 6.1 – 42,000.0 pg/mL UCH-L1: 26.3 – 25,000.0 pg/mL	GFAP: 10 – 320 pg/mL UCH-L1: 80 – 2560 pg/mL
Cut-off	GFAP: 35.0 pg/mL UCH-L1: 400.0 pg/mL	GFAP: 22 pg/mL UCH-L1: 327 pg/mL
Assay Procedure	Automated immunoassay	Manual ELISA
Result Interpretation	Software	Performed by user

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06, 2nd ed.: Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition; Approved Guideline – Second Edition
- CLSI EP07 Ed3: Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

- CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- CLSI EP35 Ed1: Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures; Approved Guideline – First Edition
- CLSI EP37 Ed1: Supplemental Tables for Interference Testing in Clinical Chemistry – First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

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

1. Precision/Reproducibility:

a. Within-laboratory precision

A study was conducted per CLSI guideline EP05-A3 to evaluate the within-laboratory precision of the GFAP and UCH-L1 assays.

K2 EDTA human plasma samples in the test panels were prepared to achieve target concentrations of GFAP and UCH-L1 that cover the respective measuring ranges of the individual assays and tested at one site using two reagent lots of each assay and two Alinity i instruments. The panel consisted of a native plasma sample near the assay cut-off (panel member 2) and pooled plasma samples (panel members 1 and 3–8) spiked with recombinant analytes (< 0.02% of sample volume). The panel members were tested along with three controls in three replicates in two runs per day for 20 days (3 x 2 x 20 =120 replicates per reagent lot/instrument combination) on the four reagent lot/instrument combinations to generate a total of 480 replicates per test panel (i.e., N=120 for lot 1/instrument 1 combination, N=120 for lot 2/instrument 1 combination, N=120 for lot 2/instrument 1 combination, and N=120 for lot 2/instrument 2 combination). The performance across the four reagent lots/instrument combinations were analyzed.

In addition, the qualitative analysis of precision results relative to the cut-offs (35.0 pg/mL for GFAP and 400 pg/mL for UCH-L1) was performed using the same 20-day within-laboratory precision study data. The % correct call was calculated for each plasma sample and was based on the number of replicates providing the expected GFAP / UCH-L1 results (Not Elevated or Elevated) based on the mean analyte concentration for the sample (either below cut-off or at/above cut-off, respectively).

Results are summarized in the tables below for each assay.

i. GFAP:

GFAP Assay - Within-Laboratory Precision (Quantitative Analysis)														
Panel Member	**N**	**Mean (pg/mL)**	**Within-Run**		**Between-Run**		**Between-Day**		**Between-Lot**		**Between-Instrument**		**Within-Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
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
2	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
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
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
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
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
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
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
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

GFAP Assay - Within-Laboratory Precision (Qualitative Analysis)				
Panel Member [a]	**Replicates (N)**	**Mean (pg/mL)**	**Elevated Results (at/above cut-off) / Total Replicates (n/N)**	**% of Correct Call [b]**
1	479	20.4	0 / 479	100.0
2	474	37.7	462 / 474	100.0
3	479	40.2	479 / 479	100.0
4	480	95.6	480 / 480	100.0
5	480	3097.2	480 / 480	100.0
6	478	7586.3	478 / 478	100.0
7	480	15,462.7	480 / 480	100.0
8	478	36,874.7	478 / 478	100.0
Low Control	479	25.4	0 / 479	100.0
Medium Control	480	504.8	480 / 480	100.0
High Control	479	31,608.5	479 / 479	100.0

[a] Panel 1: negative; Panel 2 (native) and 3: Samples near medical decision point; Panel 4-8: positive; Low Control: negative; Medium and High Controls: positive.

[b] 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.

ii. UCH-L1

UCH-L1 Assay - Within-Laboratory Precision (Quantitative Analysis)														
Panel Member	**N**	**Mean (pg/mL)**	**Within-Run**		**Between-Run**		**Between-Day**		**Between-Lot**		**Between-Instrument**		**Within-Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
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
2	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
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
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
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
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
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
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
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

UCH-L1 Assay - Within-Laboratory Precision (Qualitative Analysis)				
Panel Member [a]	**Replicates (N)**	**Mean (pg/mL)**	**Elevated Results (at/above cut-off) / Total Replicates (n/N)**	**% of Correct Call [b]**
1	480	177.6	0 / 480	100.0
2	471	391.8	164 / 471	100.0
3	479	419.8	391 / 479	100.0
4	477	823.8	477 / 477	100.0
5	476	1553.8	476 / 476	100.0
6	479	4793.5	479 / 479	100.0
7	480	7974.6	480 / 480	100.0
8	472	19,165.5	472 / 472	100.0
Low Control	479	247.5	0 / 479	100.0
Medium Control	479	2019.6	479 / 479	100.0
High Control	477	15,179.4	477 / 477	100.0

[a] Panel 1: negative; Panel 2 (native) and 3: Samples near medical decision point; Panel 4-8: positive; Low Control: negative; Medium and High Controls: positive.

[b] 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. Multi-site reproducibility

A study was conducted per CLSI guideline EP05-A3 to evaluate the multi-site reproducibility precision of the GFAP and UCH-L1 assays. Two separate test panels, each consisting of seven human K2 EDTA plasma samples with levels of GFAP or UCH-L1 that cover the measuring range of the respective assays, were tested along with three controls. The panel included one native sample near the assay cut-off (panel member 2) and pooled plasma samples (panel members 1 and 3–7) spiked with recombinant analytes. Each sample was tested in four replicates per run, two runs per day, for five days at three sites in the U.S. with one instrument at each site, using three reagent lots to generate a total of 360 replicates (i.e., 4 x 2 x 5 x 3 x 3).

The quantitative and qualitative analysis were conducted as described above. Results are summarized in the tables below for each assay.

i. GFAP

GFAP Assay - Multi-Site Reproducibility (Quantitative Analysis)

Panel Member	N	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Between-Lot		Between-Site		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
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
2	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
3	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
4	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
5	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
6	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
7	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
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

GFAP Assay - Multi-Site Reproducibility (Qualitative Analysis)				
Panel Member [a]	**Replicates (N)**	**Mean (pg/mL)**	**Elevated Results (at/above Cut-off) / Total Replicates (n/N)**	**% of Correct Call [b]**
1	360	20.4	0 / 360	100.0
2	360	37.4	337 / 360	100.0
3	360	94.9	360 / 360	100.0
4	360	3072.8	360 / 360	100.0
5	360	7449.5	360 / 360	100.0
6	360	15269.2	360 / 360	100.0
7	360	36101.1	360 / 360	100.0
Low Control	360	24.9	0 / 360	100.0
Medium Control	360	494.6	360 / 360	100.0
High Control	360	30520.7	360 / 360	100.0

[a] Panel 1: negative; Panel 2: Samples near medical decision point; Panel 3-7: positive; Low Control: negative; Medium and High Controls: positive.

[b] 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.

ii. UCH-L1

UCH-L1 Assay - Multi-Site Reproducibility (Quantitative Analysis)														
Panel Member	**N**	**Mean (pg/mL)**	**Within-Run**		**Between-Run**		**Between-Day**		**Between-Lot**		**Between-Site**		**Reproducibility**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
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
2	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
3	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
4	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
5	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
6	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
7	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
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

UCH-L1 Assay - Multi-Site Reproducibility (Qualitative Analysis)				
Panel Member [a]	**Replicates (N)**	**Mean (pg/mL)**	**Elevated Results (at/above cut-off) / Total Replicates (n/N)**	**% of Correct Call [b]**
1	360	187.3	0 / 360	100.0
2	360	402.4	230 / 360	100.0
3	359 *	838.9	359 / 359	100.0
4	360	1568.0	360 / 360	100.0
5	360	4792.8	360 / 360	100.0
6	360	8011.9	360 / 360	100.0
7	360	19606.6	360 / 360	100.0
Low Control	360	249.9	0 / 360	100.0
Medium Control	360	1983.7	360 / 360	100.0
High Control	360	14945.1	360 / 360	100.0

[a] Panel 1: negative; Panel 2: Samples near medical decision point; Panel 3-7: positive; Low Control: negative; Medium and High Controls: positive.

[b] 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.

***** One (1) no result with aspiration error message code

2. Linearity:

The linearity of the GFAP and UCH-Ll assays on the Alinity i system was evaluated in accordance with the CLSI guideline EP6 2nd Edition. Two studies for each assay were conducted in parallel and the data from the studies were analyzed for evaluating linearity for each assay.

a. GFAP

The linear range of the GFAP assay was established using a native human plasma sample set and three recombinant GFAP sample sets. One unique native sample set was prepared using a native high K2 EDTA plasma combined with analyte-depleted plasma to create a series of 10 dilutions with GFAP concentrations spanning from 3.8 to 23868.7 pg/mL. Each sample dilution was tested in five replicates using one reagent lot. Three recombinant sample sets (A, B, and C) were prepared using a high recombinant GFAP stock diluted with three unique sets of analyte-depleted plasma to create a series of dilutions with GFAP concentrations spanning from 5.2 to 53720.5 pg/mL for set A, from 4.8 to 49245.4 pg/mL for set B, and from 5.1 to 42556.9 pg/mL for set C. Each recombinant sample set consisted of at least nine dilutions, each tested in ten replicates. Deviations from linearity were calculated for the pooled results of the native human plasma sample set and each recombinant sample set individually (i.e., native set and recombinant set A, native set and recombinant set B, and native set and recombinant set C). The representative deviation from linearity analysis using the native set and recombinant set A is shown in the table below.

Analyte Source	Sample No.	N	Mean Observed (pg/mL)	Expected (pg/mL)	Predicted Value (pg/mL)	Deviation from Linearity (pg/mL)	%Deviation from Linearity
Native	1	5	23868.7	23868.7	22731.8	1136.9	5.0
Native	2	5	19233.7	19095.0	18185.5	1048.2	5.8
Native	3	5	14498.4	14321.2	13639.1	859.3	6.3
Native	4	5	9582.6	9547.5	9092.7	489.9	5.4
Native	5	5	4739.0	4773.7	4546.4	192.7	4.2
Native	6	5	2136.1	2100.4	2000.4	135.7	6.8
Native	7	5	230.7	238.7	227.3	3.4	1.5
Native	8	5	57.9	59.7	56.8	1.1	1.8
Native	9	5	12.8	14.3	13.6	-0.8	-6.2
Native	10	5	3.8	4.8	4.5	-0.8	*
Recombinant	1	10	53720.5	53720.5	51161.7	2558.8	5.0
Recombinant	2	10	42269.4	43647.9	41568.9	700.5	1.7
Recombinant	3	10	31945.8	33575.3	31976.1	-30.2	-0.1
Recombinant	4	10	19557.5	20145.2	19185.6	371.8	1.9
Recombinant	5	10	9603.3	10072.6	9592.8	10.4	0.1
Recombinant	6	10	3093.7	3357.5	3197.6	-103.9	-3.3
Recombinant	7	10	312.1	335.8	319.8	-7.6	-2.4
Recombinant	8	10	73.9	83.9	79.9	-6.1	-7.6
Recombinant	9	10	18.6	21.0	20.0	-1.4	-6.8
Recombinant	10	10	5.2	5.2	5.0	0.2	*

* Not applicable for % bias as acceptance criteria is: ± 1.0 pg/mL for samples less than 10.0 pg/mL and ± 10.0% for samples greater than or equal to 10.0 pg/mL

The study demonstrated linearity across a series of dilutions spanning an interval of 3.8 to 42,556.9 pg/mL. The claimed linearity of the GFAP assay is 6.1 to 42,000.0 pg/mL based on the pooled analysis of native and non-native samples.

b. UCH-L1

The linear range of the UCH-L1 assay was established using a native human plasma sample set. One unique sample set was prepared using a native high K2 EDTA plasma combined with analyte-depleted plasma to create a series of 10 dilutions of UCH-L1 concentrations that span across the analytical measuring interval of the assay. Each sample dilution was tested in five replicates using one reagent lot. For each sample, the mean value of the measured values, predicted value and the deviation from linearity were calculated.

Analyte Source	Sample No.	N	Mean Observed (pg/mL)	Expected (pg/mL)	Predicted Value (pg/mL)	Deviation from Linearity (pg/mL)	%Deviation from Linearity
Native	1	5	23235.1	28235.1	27517.9	717.2	2.6
Native	2	5	22054.2	22588.1	22014.3	39.9	0.2
Native	3	5	16514.3	16941.1	16510.7	3.6	0.0
Native	4	5	10774.9	11294.0	11007.2	-232.2	-2.1
Native	5	5	5492.3	5647.0	5503.6	-11.2	-0.2
Native	6	5	2367.5	2484.7	2421.6	-54.1	-2.2
Native	7	5	271.7	282.4	275.2	-3.5	-1.3
Native	8	5	66.6	70.6	68.8	-2.2	-3.2
Native	9	5	17.6	16.9	16.5	1.0	6.4
Native	10	5	10.1	8.5	8.3	1.9	*

* Not applicable for % bias as acceptance criteria: ± 16.0 pg/mL for samples less than 160.0 pg/mL and ± 10.0% for samples greater than or equal to 160.0 pg/mL

The study demonstrated linearity across a series of dilutions produced with a native specimen and spanning an interval of 10.1 to 28,235.1 pg/mL. The claimed linearity of the UCH-L1 assay is 26.3 to 25,000 pg/mL.

3. Analytical Specificity/Interference:

a. Interference

Assay interference was assessed in accordance with the CLSI guideline EP07-3rd Edition by testing pooled human plasma spiked with either recombinant GFAP at final concentrations of 25 and 10,000 pg/mL or recombinant UCH-L1 at final concentrations of 280 pg/mL and 5000 pg/mL. Test samples were created by spiking with the potentially interfering substances. Control samples were spiked only with the appropriate solvent used to create the interfering substances panel. Test and control samples were analyzed in replicates of five, in one assay run, with one reagent lot of each assay on the Alinity i system. The %Interference of each analyte was calculated by determining the percent difference between the test and control samples: $\%Interference = [(Mean_{test} - Mean_{control})/ Mean_{control}] \times 100\%$. No significant interference was observed (≤10% difference from the control sample) for the GFAP and UCH-L1 assays up to the concentrations of the endogenous and exogenous substances tested as shown in the tables below:

Potentially Endogenous Interfering Substance	Interferent Level	
	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
Human Albumin	10 g/dL	9 g/dL
Glucose	1000 mg/dL	1000 mg/dL
Human anti-mouse antibody (HAMA)	80x activity	80x activity
Rheumatoid Factor (RF)	500 IU/mL	500 IU/mL

Potentially Exogenous Interfering Substance	Interferent Level	
	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
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

Potentially Exogenous Interfering Substance	Interferent Level	
	GFAP	UCH-L1
Methaqualone	200 µg/dL	200 µg/dL
Methyldopa	2.25 mg/dL	2.25 mg/dL
Methylenedioxy methamphetamine	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

*2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

b. Cross-reactivity

To assess test performance in the presence of putative cross-reactants, a panel comprised of proteins that have significant homology to either GFAP or UCH-L1 was evaluated. Samples containing the cross-reactants at the concentrations listed in the table below were prepared in GFAP-depleted plasma or UCH-L1 depleted plasma and tested with the GFAP and UCH-L1 assays on the Alinity i system in 30 replicates. The % cross-reactivity was calculated, and the results showed none of the potentially cross-reactants tested were found to interfere with the GFAP and UCH-L1 assays.

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

c. Carryover / cross-contamination

A study was conducted to evaluate if cross-contamination and/or carryover occurs between samples during the assay procedure by comparing the result of a protected low sample (analyte-depleted pooled plasma sample before a high sample) to that of an unprotected low sample (analyte-depleted pooled plasma sample after a high sample). The high sample was a pooled plasma sample spiked with recombinant GFAP analyte stock targeted to greater than or equal to 500,000 pg/mL and UCH-L1 analyte stock targeted to greater than or equal to 100,000 pg/mL. There were four testing steps (wash buffer – protected low sample – high sample – unprotected) in each testing iteration and the cycle of the four testing steps was repeated for a total of 27 iterations across five runs on the Alinity i system. No sample carryover was observed and the GFAP and UCH-L1 assays are not susceptible to sample carryover.

4. Assay Reportable Range:

The analytical measuring internal (AMI) is 6.1–42,000 pg/mL for GFAP and 26.3–25,000 pg/mL for UCH-L1.

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

Traceability

The GFAP and UCH-L1 Calibrators are manufactured gravimetrically and are referenced to an internal reference standard at each concentration level. The internal reference standards are stored frozen at ≤ -70°C. The GFAP and UCH-L1 Controls are traceable to an internal reference standard at each concentration level.

Stability

a. Sample storage stability

A study was performed to evaluate serum and plasma specimens when subjected to various storage conditions (-28 to -20°C, 2 to 8°C, and 15 to 25°C) and tested with the GFAP and UCH-L1 assays. Native specimens spanning the respective AMI of each assay were collected from patients with suspected TBI in either K2 EDTA or serum separator tubes and tested in two or three replicates to determine sample storage stability. For the on-the-cell storage conditions, testing was performed directly from the blood collection tubes. For the off-the-cell storage conditions, multiple collection tubes were obtained from each subject and their respective serum or plasma was pooled prior to baseline testing. Sample storage stability was evaluated individually for the K2 EDTA or the serum separator tube type. The results support the use of the GFAP and UCH-L1 assays when testing serum and plasma specimens that have been stored at the following conditions:

- room temperature (15 to 25°C): up to 8 hours on and off-the-cells
- 2 to 8°C: up to 8 hours on-the-cells/clot/gel and up to 7 days off-the-cells/clot/gel

- -28 to -20°C: up to 1 month off-the-cells/clot/gel with up to 1 freeze/thaw cycle

The TBI Package Insert 'Limitations of the Procedure' states 'Avoid more than 1 freeze/thaw cycle'.

b. On-board sample stability

A study was performed to evaluate the stability of the samples when stored on the Alinity i system and tested with the GFAP and UCH-L1 assays using two levels of samples (GFAP at 25 and 10,000 pg/mL, UCH-L1 at 280 and 5000 pg/mL). Minimum sample volume was 200 µL for the GFAP assay and 150 µL for the UCH-L1 assay. Samples may be stored on the Alinity i system for up to 2 hours.

c. Assay kit stability

An assay kit stability study was performed using a real-time stability study design in accordance with the CLSI guideline EP25-A. Three lots of each GFAP and UCH-L1 assay kit were stored at 2 to 8°C for the evaluation of real-time stability. An additional lot of each GFAP and UCH-L1 assay kit was opened and stored in the Alinity i system for the evaluation of on-board stability. Plasma samples at three analyte levels for each GFAP (~11, ~43, and ~ 26,000 pg/mL) and UCH-L1 (~90, ~400, and ~15,000 pg/mL) were tested in a minimum of 10 replicates at each testing time point from the baseline time point (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 months for real-time stability; at 0, 2, 4, 6, 8, 10, 12, and 13 months for on-board stability). The data from the stability study support expiration dating up to 12 months at 2 to 8°C and 30 days on-board.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted in accordance with the CLSI guideline EP17-A2. The studies evaluated three reagents lots of the GFAP and UCH-L1 assays on each of two Alinity i systems. A description of each study and the results obtained are summarized below:

The LoB was determined as the 95th percentile of measurements from 120 replicates of four analyte-depleted pooled human plasma samples that were each tested in five replicates for three days in two instruments per lot. The highest observed LoB of the three lots was 1.6 pg/mL for GFAP and 6.1 pg/mL for UCH-L1. The claimed LoB is 1.6 pg/mL for GFAP and 6.1 pg/mL for UCH-L1.

For determination of LoD, five plasma samples with low GFAP concentrations ranged 1.6 to 8.8 pg/mL and six plasma samples with low UCH-L1 concentrations ranged 10.1 to 50.7 pg/mL were measured in 20 replicates in a run per day over three days in two instruments for a total of 120 replicates per lot (60 replicates per lot / instrument) for each sample. The highest observed GFAP LoD was 2.2 pg/mL of three lots. The highest observed UCH-L1 LoD was 16.1 pg/mL of three lots. The claimed LoD is 3.2 pg/mL for GFAP and 18.3 pg/mL for UCH-L1.

The LoQ was defined as the lowest concentration at which a maximum allowable precision of 20.0 %CV and determined from 120 replicates per lot (60 replicates per lot / instrument) for each low-analyte level sample. The highest observed GFAP LoQ was 2.4 pg/mL. The highest observed UCH-L1 LoQ was 16.1 pg/mL of three lots. The claimed LoQ is 6.1 pg/mL for GFAP and 26.3 pg/mL for UCH-L1.

7. Assay Cut-Off:

The GFAP and UCH-L1 assay cut-offs were determined by analyzing a training dataset from a completely independent study population that is distinct from subjects evaluated in the pivotal study to validate the assay cut-offs. Frozen EDTA plasma samples from two study cohorts; ATO-04b (ClinicalTrials.gov Identifier: NCT01295346) and ATO-06x (ClinicalTrials.gov Identifier: NCT02439736) were utilized to establish the assay cut-offs. A total of 354 subjects with Glasgow Coma Scale (GCS) scores between 13–15 who had blood specimens collected within 12 hours from the time of suspected head injury, a head CT scan determination, and were 18 years or older at the time of injury were included in the training dataset. Of the 354 subjects, 37.3% (132/354) had a positive CT result. Using a 10-fold cross validation and bootstrapping method, the optimal cut-off values were selected as 35 pg/mL for the GFAP assay and 400 pg/mL for the UCH-L1 assay.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable

2. Matrix Comparison:

Sample matrix equivalence was evaluated in accordance with the CLSI guideline EP35 1st ed for samples collected with six different blood collection tube types: K2 EDTA, serum, serum separator tube, K3 EDTA, lithium heparin, and lithium heparin separator tube. Samples ranged across the AMI (45–47 contrived and 54–55 native samples per tube type for the GFAP assay; 46 contrived and 53–54 native samples per tube type for the UCH-L1 assay) and tested using one instrument and one reagent lot in duplicates for each sample. The test results of the samples from each tube type (except K2 EDTA) were compared to the test results of the samples from the primary tube type (K2 EDTA) ranged 12.8 to 41,121.0 pg/mL. A Passing-Bablok regression analysis was performed using the primary tube (K2 EDTA) first replicate concentration compared to other tube type first replicate concentration as summarized in the table below.

GFAP

Candidate Tube	N	Candidate Tube (pg/mL)		Intercept		Slope		Correlation Coefficient (r)
		Min	Max	Estimate	95% CI	Estimate	95% CI	
K3 EDTA	102	11.5	38808.4	0.5	(0.1, 1.1)	0.96	(0.95, 0.97)	1.000
Serum	100	13.2	44850.6	0.4	(-0.3, 1.1)	1.03	(1.02, 1.04)	0.999
Serum Separator Tube	99	12.4	41490.4	0.7	(-0.1, 1.4)	1.02	(1.02, 1.03)	1.000
Lithium Heparin	102	12.0	42579.4	0.5	(-0.1, 1.1)	1.01	(1.00, 1.01)	1.000
Li-Hep (Plasma Separator)	99	13.2	40402.5	0.7	(0.4, 1.5)	0.99	(0.99, 1.00)	1.000

UCH-L1

Candidate Tube	N	Candidate Tube (pg/mL)		Intercept		Slope		Correlation Coefficient (r)
		Min	Max	Estimate	95% CI	Estimate	95% CI	
K3 EDTA	100	46.5	18226.9	4.8	(1.7, 8.5)	0.98	(0.97, 0.99)	0.999
Serum	100	46.3	19428.8	5.7	(0.1, 10.8)	1.06	(1.04, 1.07)	0.999
Serum Separator Tube	99	57.2	18508.7	2.8	(-3.8, 7.1)	1.06	(1.05, 1.07)	0.999
Lithium Heparin	100	44.9	18632.1	2.2	(-3.2, 5.5)	1.03	(1.01, 1.04)	0.999
Li-Hep (Plasma Separator)	99	51.0	19133.1	2.0	(-2.3, 4.8)	1.02	(1.01, 1.03)	0.999

C Clinical Studies:

1. Clinical performance with frozen plasma specimens from the ALERT-TBI study:

   A pivotal study using archived (frozen) plasma specimens was conducted to evaluate the clinical performance of the TBI test on the Alinity i system. The clinical specimens tested in this study were previously collected by Banyan Biomarkers from subjects participating in their study "A Prospective Clinical Evaluation of Biomarkers of Traumatic Brain Injury" (ALERT-TBI, protocol ATO-06). The ALERT-TBI study enrolled subjects at 18 years of age or older who presented to a health care facility (HCF) or emergency department (ED) with head injuries and had a blood drawn within 12 hours of head injury and a standard of care computed tomography (CT) scan of the head. Subjects were enrolled at 22 clinical sites in three countries: United States (U.S.), Germany, and Hungary.

   Under the ALERT-TBI study protocol, CT scans were performed in accordance with the clinical site's standard of care and a subject's CT scan was classified as positive if intracranial lesions were present. The standard of care head CT scans was previously classified as positive or negative for intracranial lesions as part of the ALERT-TBI study.

Additional details regarding the evaluation of CT scans and definition of intracranial lesion can be found in DEN170045.

Of the 1994 subjects enrolled in the ALERT-TBI study with GCS scores of 13 to 15, 72 subjects (3.6%, 72/1994) were not included in the clinical performance study due to lack of consent for future testing, withdrawn consent, and no specimen available. Specimens from 23 subjects (1.2%, 23/1994) 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. A total of 1899 frozen and de-identified K2 EDTA plasma samples collected from these subjects were included in the clinical performance evaluation. The plasma specimens were divided into aliquots and frozen in cryovials before being provided to three testing sites in the U.S. Each specimen was associated with a standard of care computed tomography (CT) scan of the head from the same study subject. The demographic characteristics of the subjects enrolled subjects evaluated with the TBI test are presented in the table below.

ALERT-TBI study			
Demographic Characteristics	**Head CT Scan Results**		**Total (N)**
	Positive	**Negative**	
n (n/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.

ALERT-TBI study			
Head Injury Characteristics	**Head CT Scan Result**		**Total (N)**
	Positive	**Negative**	
n	120	1779	1899
Time from head injury to examination (hours)[a]			
Mean (SD)	1.9 (1.73)	1.6 (1.71)	1.6 (1.71)
Median	1.2	1.0	1.1
Range (minimum, maximum)	(0.3, 7.8)	(0.1, 10.7)	(0.1, 10.7)
Time from head injury to CT scan (hours)[a]			
Mean (SD)	2.8 (1.95)	2.7 (1.93)	2.7 (1.93)
Median	2.1	2.2	2.1
Range (minimum, maximum)	(0.5, 8.9)	(0.2, 13.3)	(0.2, 13.3)
Time from head injury to blood draw (hours)[a]			
Mean (SD)	3.8 (1.91)	3.5 (1.88)	3.5 (1.89)
Median	3.3	3.1	3.2
Range (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%)
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 result was compared to the adjudicated head CT scan result for each subject. The performance estimates are summarized in the 2x2 table below. Of the 1899 subjects, 120 had positive CT scan results. Of these 120 subjects with positive CT scan results, 116 had a positive TBI test result (clinical sensitivity= 96.7%). The remaining four CT scan positive subjects had a negative TBI test result. 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 test result. Of the 1779 subjects with negative CT scan results, 713 had a negative TBI interpretation (clinical specificity=40.1%). The rate of false positive (FP) results was 59.9% (1066/1779). Overall, there were 717 subjects with a negative TBI test result. Of these, 713 specimens were associated with negative CT scan results. The negative predictive value (NPV) of the assay was 99.4% (713/717). The potential benefit of the assay would be a reduction in unnecessary CT scans by approximately 40.1% (713 of 1779 subjects had true negative assay results). The positive predictive value (PPV) of the assay was 9.8% (116/1182). The Likelihood Ratio Negative (LRN) of the assay was 0.08. The Likelihood Ratio positive (LRP) of the assay was 1.61.

		Head CT scan result		**Total**
		Positive	**Negative**	
TBI test result	**Positive**	116	1066	1182
	Negative	4	713	717
	Total	120	1779	1899

Clinical sensitivity = 96.7 % (116 /120); 95% CI: 91.7 − 98.7%
Clinical specificity = 40.1 % (713 /1779); 95% CI: 37.8 − 42.4%
Negative predictive value (NPV) [a] = 99.4 % (713 /717); 95% CI: 98.6 − 99.8%
Positive predictive value (PPV) [b] = 9.8 % (116 /1182); 95% CI: 8.2 −11.6%
Likelihood ratio negative (LRN) = 0.08; 95% CI: 0.03 − 0.22
Likelihood ratio positive (LRP) = 1.61; 95% CI: 1.53 −1.70
CT scan positive prevalence rate in study = 6.3% (120/1899)

[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%

Analyses of assay performance by gender and time from injury relative to blood draw are shown in the table below. There was little variation in NPV and PPV between males and females and with increasing time from injury. These data indicate that gender differences and

differences between head injury characteristics did not translate into statistically significant differences in assay performance.

ALERT-TBI study										
Category	**Head CT scan results**				**Sensitivity (%) (N) (95% CI)**	**Specificity (%) (N) (95% CI)**	**Adj. PPV[a] (%) (95% CI)**	**Adj. NPV[a] (%) (95% CI)**	**LRP (95% CI)**	**LRN (95% CI)**
	Positive		**Negative**							
	TBI		**TBI**							
	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 analyte levels stored frozen at -70° C.

2. Clinical performance with fresh plasma samples:

To supplement the results of the pivotal study (N=1899) using archived (frozen) plasma specimens from the ALERT-TBI described above, a prospective clinical validation study (Protocol No CS-2018-0009: Clinical Evaluation of the i-STAT TBI Test study) was conducted using freshly collected plasma specimens from consenting men and women 18

years of age or older who presented to a health care facility (HCF) or emergency department (ED) with suspected mild TBI, with initial GCS scores of 13–15, and who had a CT scan of the head performed. A total of 97 subjects were enrolled across five 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. CT adjudication was performed by an independent central panel consisting of board-certified radiologists blinded to the TBI test results and each other's evaluation to determine the presence or absence of an acute intracranial lesion. The intracranial lesions were defined as any trauma-induced or related finding visualized upon head CT scan, and may have included acute epidural hematomas, acute subdural hematomas, indeterminate extra-axial lesions, cortical contusions, parenchymal hematomas, non-hemorrhagic contusions, ventricle compression, ventricular trapping, brain herniation, intraventricular hemorrhage, hydrocephalus, subarachnoid hemorrhage, petechial hemorrhage, global or focal brain edema and post traumatic ischemia. A head CT scan result of a subject was classified as positive if intracranial lesions were present. Whole blood was collected into K2 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 are presented in the table below:

Supplemental fresh plasma specimen study			
Demographic Characteristics	**Head CT Scan Result**		**Total (N)**
	Positive	**Negative**	
n (n/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 (%)			
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.

Supplemental fresh plasma specimen study			
Head Injury Characteristics	**Head CT Scan Result**		**Total (N)**
	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%)
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)

Supplemental fresh plasma specimen study			
Head Injury Characteristics	**Head CT Scan Result**		**Total (N)**
	Positive	**Negative**	
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)

To estimate clinical performance characteristics, the TBI test results from the supplemental plasma fresh specimen study were compared to the consensus head CT scan results. The results are summarized in the table below.

		Head CT scan result		**Total**
		Positive	**Negative**	
TBI test Result	**Positive**	14	60	74
	Negative	0	23	23
	Total	14	83	97

Clinical sensitivity (%) = 100.0 (14/14); 95% CI: 78.5 −100.0 %
Clinical specificity (%) = 27.7 (23/83); 95% CI: 19.2 − 38.2 %
Negative predictive value (NPV) (%)[a] = 100.0 (23/23); 95% CI: 85.7−100.0 %
Positive predictive value (PPV) (%)[a] = 18.9 (14/74); 95% CI: 11.6 − 29.3%
Likelihood Ratio Negative (LRN) = 0.12; 95% CI: 0.01 − 1.91
Likelihood Ratio Positive (LRP) = 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 supplemental fresh plasma specimen study cohort. 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 summarized in the table above 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 and a negative TBI test.

D Clinical Cut-Off:

Refer to Assay Cut-off

E Expected Values/Reference Range:

A study was performed in accordance with the CLSI guideline C28-A3c to establish the expected values from 160 apparently healthy individuals (≥ 18 years old) in the U.S. population ranging in age from 18 to 91 years (mean 49.2 years) who did not have acute injury to the head (or history of neurological disease or disorder, neurosurgery, motor vehicle accident or injury requiring

medical attention for head/neck/spine within the last one year, or active cancer / diagnosis within the last 5 years. Frozen K2 EDTA plasma specimens stored for up to 5 months at -70°C or colder were tested with both the GFAP and UCH-L1 assays on the Alinity i system. The 2.5th and 97.5th percentiles (95% reference interval) and their respective 95% confidence intervals for GFAP and UCH-L1 were determined non-parametrically and presented in the table below:

Analyte	n	Mean (pg/mL)	SD	Median (pg/mL)	Reference Interval (2.5th to 97.5th percentile) (pg/mL)
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

There were 21 healthy donors who tested positive for GFAP only and no donors were positive for both GFAP and UCH-L1. The results summarized in the table below show that 86.9% have a negative TBI test result and 16% have a positive TBI test result.

GFAP Result (Relative to Cutoff of 35.0 pg/mL)[a]	UCH-L1 Result (Relative to Cutoff of 400.0 pg/mL)[a]	TBI test Interpretation Result	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 cut-off. Below means less than the cut-off.

[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 cut-offs were optimized in a population of patients with head injury.

It is the responsibility of each laboratory to establish its own reference ranges for the population of patients it serves, as expected values may be affected by different factors including age.

VIII Proposed Labeling:

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

IX Conclusion:

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