510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K240279

B Applicant

bioMérieux, Inc.

C Proprietary and Established Names

VIDAS TBI (GFAP, UCH-L1)

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 C-terminal Hydrolase (UCH-L1)

C Type of Test:

Automated Enzyme Linked Fluorescent Assay (ELFA), Quantitative

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The VIDAS TBI (GFAP, UCH-L1) test is composed of two automated assays - VIDAS TBI (GFAP) and VIDAS TBI (UCH-L1) - to be used on the VIDAS 3 instrument for the quantitative measurement of Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-terminal Hydrolase (UCH-L1) in human serum using the ELFA (Enzyme Linked Fluorescent Assay) technique. The results of both assays are required to obtain an overall qualitative test interpretation.

The overall qualitative VIDAS TBI (GFAP, UCH-L1) test result is used, in conjunction with clinical information, to aid in the evaluation of patients (18 years of age or older), presenting within 12 hours of suspected mild traumatic brain injury (Glasgow Coma Scale score 13-15), to assist in determining the need for a Computed Tomography (CT) scan of the head. A negative interpretation of VIDAS TBI (GFAP, UCH-L1) test is associated with the absence of acute intracranial lesions visualized on a head CT scan.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For in vitro diagnostic use

D Special Instrument Requirements:

VIDAS 3 instrument (K141133)

IV Device/System Characteristics:

A Device Description:

The VIDAS TBI (GFAP, UCH-L1) test is composed of two assays: VIDAS TBI (GFAP) and VIDAS TBI (UCH-L1), each with sufficient reagents for 60 determinations.

VIDAS TBI (GFAP) kit contains the following:

- GFAP Strips (STR) (60): Ready-to-use.
- GFAP solid phase receptacles (SPR) (2x30): coated with mouse monoclonal IgG anti-GFAP antibodies. Ready-to-use.
- GFAP Calibrators (S1) (1x2.4 mL): Recombinant human GFAP diluted in buffer + stabilizer + preservative.
- GFAP Control (C1) (1x1.6 mL): Recombinant human GFAP diluted in buffer + stabilizer + preservative.

VIDAS TBS (UCH-L1) kit contains the following:

- UCH Strips (STR) (60): Ready-to-use.

- UCH Solid Phase Receptacles (SPR) (2x30): coated with mouse monoclonal IgG anti-UCH-L1 antibodies. Ready-to-use.
- UCH Calibrators (S1) (1x2.4 mL): Recombinant human UCH-L1 diluted in buffer + stabilizer + preservative.
- UCH-L1 Control (C1) (1x1.6 mL): Recombinant human UCH-L1 diluted in buffer + stabilizer + preservative.

The master lot entry (MLE) barcode printed on the outer label of the packaging contains specifications for assay calibration and concentration information of calibrator (S1) and control (C1) for each assay.

Both assays are to be used on VIDAS 3 instrument.

B Principle of Operation:

The VIDAS TBI (GFAP, UCH-L1) test is composed of two assays, VIDAS TBI (GFAP) and VIDAS (UCH-L1). Each combines a three-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). All the assay steps are performed automatically by the VIDAS 3 instrument. The sample is transferred into the well containing sample diluent. The sample/diluent mixture is cycled in and out of the Solid Phase Receptable (SPR) device several times to allow the antigen (GFAP or UCH-L1) to bind with the immunoglobulins that are fixed to the interior wall of the SPR device. The bounded complex is presented to antibodies labeled with biotin (conjugate) to form a sandwich immunocomplex. For the third immunological step, the complex is presented to anti-biotin antibodies labeled with alkaline phosphatase (tracer) using the SPR and form the final immunological complex "Antibody-Analyte-Conjugate-Tracer". Unbound tracer is eliminated during washing steps. During the final detection step, the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR device. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigen in the sample. At the end of each assay, separate results of VIDAS TBI (GFAP) and VIDAS TBI (UCH-L1) are automatically calculated by the instrument.

Final VIDAS TBI (GFAP, UCH-L1) test interpretation is performed manually by the user. The results interpretation is shown in the following table:

GFAP Assay Result (relative to cut-off)[A]	**UCH-L1 Assay Result (relative to cut-off)[B]**	**VIDAS TBI (GFAP, UCH-L1) Interpretation**
Negative	Negative	Negative
Negative, Invalid	**Positive**	**Positive**
Positive	Negative, Invalid	**Positive**
Positive	**Positive**	**Positive**
Invalid	Negative	Not Reportable[C]
Negative	Invalid	Not Reportable[C]
Invalid	Invalid	Not Reportable[C]

[A] "**Positive**" means the GFAP concentration is equal to or above the cut-off value of 22.0 pg/mL and "**Negative**" means the GFAP concentration is below the cut-off value of 22.0 pg/mL.

[B] "**Positive**" means the UCH-L1 concentration is equal to or above the cut-off value of 327.0 pg/mL and "**Negative**" means the UCH-L1 concentration is below the cut-off value of 327.0 pg/mL.

[C] Samples with "**Invalid"** results that yield a "**Not Reportable"** VIDAS TBI (GFAP, UCH-L1) result may be retested once to obtain a "**Negative"** or "**Positive"** result.

A positive clinical interpretation of VIDAS TBI (GFAP, UCH-L1), in conjunction with clinical assessment, can suggest and support the need for a head CT (Computed Tomography) scan. In contrast, a negative clinical interpretation is associated with absence of acute intracranial lesions visualized on a head CT scan.

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):	K240279	DEN170045 (Predicate)
Device Trade Name	VIDAS TBI (GFAP, UCH-L1)	Banyan BTI
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The VIDAS TBI (GFAP, UCH-L1) test is composed of two automated assays - VIDAS TBI (GFAP) and VIDAS TBI (UCH-L1) - to be used on the VIDAS 3 instrument for the quantitative measurement of Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-terminal Hydrolase (UCH-L1) in human serum using the ELFA (Enzyme Linked Fluorescent Assay) technique. The results of both assays are required to obtain an overall qualitative test interpretation. The overall qualitative VIDAS TBI (GFAP, UCH-L1) test result is used, in conjunction with clinical information, to aid in the evaluation of patients (18 years of age or older), presenting within 12 hours of suspected mild traumatic brain injury (Glasgow Coma Scale	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

	score 13-15), to assist in determining the need for a Computed Tomography (CT) scan of the head. A negative interpretation of VIDAS TBI (GFAP, UCH-L1) test is associated with the absence of acute intracranial lesions visualized on a head CT scan.	acute intracranial lesions visualized on a head CT Computed Tomography) scan. The Banyan BTI is for prescription use only.
Intended Use Setting	Clinical laboratory	Same
Measurands	GFAP and UCH-L1	Same
Assay Technology	Enzyme-linked fluorescent assay	Chemiluminescent immunoassay
Reportable Result	Quantitative results for GFAP and UCH-L1 with a qualitative interpretation of test results	Same
Assay Format	Two separate test kits	Same
Specimen Type	Serum	Same
Analytical Measuring Interval	GFAP: 10.0 – 320.0 pg/mL UCH-L1: 80.0 – 2560.0 pg/mL	Same
Assay Cut-off	GFAP: 22.0 pg/mL UCH-L1: 327.0 pg/mL	Same
General Device Characteristic Differences		
Assay Procedure	Automated immunoassay	Manual ELISA
Sample Volume	200 µL for VIDAS TBI (GFAP) 200 µL for VIDAS TBI (UCH-L1)	150 µL for Banyan GFAP 100 µL for Banyan UCH-L1
Time to result	~ 40 min	~ 4 hours

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06-Ed2, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- 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, Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures – First Edition
- CLSI EP37, 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 VIDAS TBI (GFAP) and VIDAS TBI (UCH-L1) assays. Two separate panels, one for each assay and each consisting of five human serum samples, were tested over the course of 20 days at one site using one VIDAS 3 instrument and one assay lot. Each panel includes two serum samples close to the respective assay cut-off value, and three other samples to cover the analytical measuring interval of each assay. Samples were tested each day with two runs per day, two replicate measurements per run for a total of 80 replicates per sample.

The data were analyzed quantitatively and qualitatively. The results are summarized in the tables below.

Quantitative analysis:

GFAP Assay										
Sample	N	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP01[A]	80	16.39	0.85	5.2	0.00	0.0	0.26	1.6	0.89	5.4
PP02[A]	80	32.45	1.49	4.6	0.00	0.0	0.48	1.5	1.56	4.8
PP03[A]	80	82.63	2.06	2.5	2.45	3.0	0.00	0.0	3.21	3.9
PP04[B]	80	209.22	5.83	2.8	0.00	0.0	2.69	1.3	6.42	3.1
PP05[B]	80	298.17	9.52	3.2	4.51	1.5	2.14	0.7	10.74	3.6

[A] Native human sera from a subject with traumatic brain injury
[B] Native human sera from subject with meningeal hemorrhage

UCH-L1 Assay										
Sample	N	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP06[A]	80	136.11	7.86	5.8	1.98	1.5	3.84	2.8	8.97	6.6
PP06[*]	79	135.24	6.41	4.7	0.00	0.0	3.18	2.4	7.15	5.3
PP07[A]	80	270.37	10.48	3.9	6.49	2.4	2.76	1.0	12.63	4.7
PP08[A]	80	380.79	14.88	3.9	0.00	0.0	5.29	1.4	15.79	4.1
PP09[C]	80	821.76	22.80	2.8	0.00	0.0	11.93	1.5	25.73	3.1
PP10[C]	80	2200.11	67.63	3.1	52.66	2.4	31.31	1.4	91.25	4.1

[A] Native human sera from a subject with traumatic brain injury
[C] Native human sera spiked with recombinant protein
[*] Analysis without the outliers

Qualitative analysis:

GFAP Assay (Cut-Off: 22.0 pg/mL)				
Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Positives/Total Replicates)	%Correct Call
PP01[A]	16.39	80	0/80	100
PP02[C]	32.45	80	80/80	100
PP03[C]	82.63	80	80/80	100
PP04[C]	209.22	80	80/80	100
PP05[C]	298.17	80	80/80	100

[A] Negative (GFAP concentration is below the cut-off value)
[C] Positive (GFAP concentration is equal to or above the cut-off value)

UCH-L1 Assay (Cut-Off: 327.0 pg/mL)				
Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Positives/Total Replicates)	%Correct Call
PP06[A]	136.11	80	0/80	100
PP06[*]	135.24	79	0/79	100
PP07[B]	270.37	80	0/80	100
PP08[B]	380.79	80	80/80	100
PP09[C]	821.76	80	80/80	100
PP10[C]	2200.11	80	80/80	100

[A] Negative (UCH-L1 concentration is below the cut-off value)
[B] Near cut-off (mean ±25% of the cut-off value)
[C] Positive (UCH-L1 concentration is equal to or above the cut-off value)
[*] Analysis without the outliers

b. Lot-to-Lot Precision

A study was conducted per the CLSI guideline EP05-A3 to evaluate the effects of between-lot variability. The same two sample panels evaluated in the within-laboratory

precision study were tested in five replicates, one run per day, during five days, on three different lots of the VIDAS TBI (GFAP) assay and three different lots of the VIDAS TBI (UCH-L1) assay using one VIDAS 3 instrument. A total of 75 replicates were obtained per panel member. The data were analyzed quantitively and qualitatively. The results are summarized in the tables below.

Quantitative analysis:

GFAP Assay										
Sample	N	Mean (pg/mL)	Within-Run		Between-Run/Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP01[A]	75	15.63	0.93	5.9	0.00	0.0	0.62	4.0	1.11	7.1
PP02[A]	75	31.03	1.16	3.7	0.12	0.4	1.56	5.0	1.95	6.3
PP03[A]	75	75.29	4.69	6.2	0.00	0.0	4.62	6.1	6.58	8.7
PP04[B]	75	199.54	5.93	3.0	2.47	1.2	10.47	5.2	12.28	6.2
PP05[B]	75	282.23	8.23	2.9	2.36	0.8	16.08	5.7	18.22	6.5

[A] Native human sera from a subject with traumatic brain injury
[B] Native human sera from subject with meningeal hemorrhage

UCH-L1 Assay										
Sample	N	Mean (pg/mL)	Within-Run		Between-Run/Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP06[A]	75	141.30	6.58	4.7	4.28	3.0	1.14	0.8	7.93	5.6
PP07[A]	75	274.52	10.72	3.9	2.61	1.0	3.42	1.2	11.55	4.2
PP08[A]	75	388.91	14.54	3.7	0.00	0.0	7.74	2.0	16.47	4.2
PP09[C]	75	837.10	30.20	3.6	0.00	0.0	16.75	2.0	34.54	4.1
PP10[C]	75	2253.47	104.87	4.7	0.00	0.0	86.28	3.8	135.80	6.0

[A] Native human sera from a subject with traumatic brain injury
[C] Native human sera spiked with recombinant protein

Qualitative analysis:

GFAP Assay (Cut-Off: 22.0 pg/mL)				
Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Positives/Total Replicates)	%Correct Call
PP01[A]	15.63	75	0/75	100
PP02[C]	31.03	75	75/75	100
PP03[C]	75.29	75	75/75	100
PP04[C]	199.54	75	75/75	100
PP05[C]	282.23	75	75/75	100

[A]Negative (GFAP concentration is below the cut-off value)
[C]Positive (GFAP concentration is equal to or above the cut-off value)

UCH-L1 Assay (Cut-Off: 327.0 pg/mL)				
Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Positives/Total Replicates)	%Correct Call
PP06[A]	141.30	75	0/75	100
PP07[B]	274.52	75	0/75	100
PP08[B]	388.91	75	75/75	100
PP09[C]	837.10	75	75/75	100
PP10[C]	2253.47	75	75/75	100

[A] Negative (UCH-L1 concentration is below the cut-off value)
[B] Near cut-off (mean ±25% of the cut-off value)
[C] Positive (UCH-L1 concentration is equal to or above the cut-off value)

c. Multi-Site Reproducibility

A study was conducted per CLSI guideline EP05-A3 to evaluate the effects of site-to-site variability. The same two sample panels evaluated in the within-laboratory precision study were tested in triplicate, two runs per day with at least two hours between runs, during five days in three different laboratories including two in the U.S. and one OUS. One lot of the VIDAS TBI (GFAP) assay, one lot of the VIDAS TBI (UCH-L1) assay and one VIDAS 3 instrument at each site was used. Combining all sites, a total of 90 replicates were obtained per panel member. The data were analyzed quantitively and qualitatively. The results are summarized for each assay in the table below.

Quantitative analysis:

GFAP Assay										
Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP01[A]	90	15.41	0.92	6.0	0.67	4.3	1.44	9.4	1.97	12.8
PP01[*]	89	15.49	0.82	5.3	0.59	3.8	1.37	8.8	1.83	11.8
PP02[A]	90	31.38	1.22	3.9	0.57	1.8	1.29	4.1	2.07	6.6
PP03[A]	90	76.35	2.92	3.8	0.73	1.0	4.60	6.0	6.17	8.1
PP04[B]	90	200.60	5.24	2.6	3.08	1.5	8.98	4.5	11.20	5.6
PP05[B]	90	289.00	8.24	2.9	0.76	0.3	9.49	3.3	13.55	4.7

[A] Native human sera from a subject with traumatic brain injury
[B] Native human sera from subject with meningeal hemorrhage
[*] Analysis without the outliers

UCH-L1 Assay										
Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP06[A]	90	141.60	5.92	4.2	1.72	1.2	2.86	2.0	7.67	5.4
PP06[*]	89	141.25	4.68	3.3	2.09	1.5	3.13	2.2	6.99	5.0
PP07[A]	90	278.43	17.10	6.1	0.00	0.0	7.73	2.8	19.07	6.8
PP07[*]	89	279.86	9.73	3.5	0.00	0.0	7.19	2.6	13.43	4.8

UCH-L1 Assay										
Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP08[A]	90	391.28	10.88	2.8	0.00	0.0	6.95	1.8	14.12	3.6
PP09[C]	90	844.83	24.51	2.9	8.75	1.0	5.67	0.7	26.64	3.2
PP10[C]	90	2266.22	67.51	3.0	41.26	1.8	18.76	0.8	83.70	3.7

[A] Native human sera from a subject with traumatic brain injury
[C] Native human sera spiked with recombinant protein
[*] Analysis without the outliers

Qualitative precision:

GFAP Assay (Cut-Off: 22.0 pg/mL)				
Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Positives/Total Replicates)	%Correct Call
PP01[A]	15.41	90	0/90	100
PP01[*]	15.49	89	0/89	100
PP02[C]	31.38	90	90/90	100
PP03[C]	76.35	90	90/90	100
PP04[C]	200.60	90	90/90	100
PP05[C]	289.00	90	90/90	100

[A] Negative (GFAP concentration is below the cut-off value)
[C] Positive (GFAP concentration is equal to or above the cut-off value)
[*]Analysis without the outliers

UCH-L1 Assay (Cut-Off: 327.0 pg/mL)				
Panel Member	Mean (pg/mL)	Total Number Replicates	Qualitative Agreement	
			n/N (Positives/Total Replicates)	%Correct Call
PP06[A]	141.60	90	0/90	100
PP06[*]	141.25	89	0/89	100
PP07[B]	278.43	90	0/90	100
PP07[*]	279.86	89	0/89	100
PP08[B]	391.28	90	90/90	100
PP09[C]	844.83	90	90/90	100
PP10[C]	2266.22	90	90/90	100

[A] Negative (UCH-L1 concentration is below the cut-off value)
[B] Near cut-off (mean ±25% of the cut-off value)
[C] Positive (UCH-L1 concentration is equal to or above the cut-off value)
[*]Analysis without the outliers

2. Linearity:

The linearity of the VIDAS TBI (GFAP) and VIDAS TBI (UCH-L1) assays on the VIDAS 3 instrument was evaluated in accordance with CLSI guideline EP06-Ed2. Two separate dilution series, one for each assay, and each composed of 11 sample pools, were prepared by mixing a High sample and a Low sample to cover the analytical measuring interval (AMI) of

each assay. The High sample for each assay was generated by spiking positive serum sample into a negative serum sample (for GFAP) or pools (for UCH-L1). The Low sample for each assay was analyte depleted pooled serum. With the exception of the low concentration sample pool, which was tested in eight replicates, all other sample pools in the dilution series were tested in four replicates using two lots of each assay. The observed values were evaluated against the expected values and weighted least squares linear regression was performed. The %CV of the concentrations measured, the predicted values, deviations from linearity, and % deviations from the regression line were calculated for each sample. The results are summarized in the table below:

Assay	Lot #	Test Range (pg/mL)	Slope (95% CI)	Intercept (95% CI)	R^2	% Deviation
GFAP	1	6.7 – 354.5	1.024 [0.999; 1.050]	-0.716 [-1.722; 0.289]	0.9988	-2.15 to 9.07%
UCH-L1	1	58.9 – 3034.0	1.023 [1.006; 1.040]	-1.857 [-5.165; 1.452]	0.9995	-6.23 to 1.72%
	2	40.2 – 2769.1	0.980 [0.958; 1.002]	6.818 [3.472; 10.164]	0.9990	-13.05 to 1.75%

The study supports the linear range of 6.7–354.5 pg/mL for the GFAP assay and 58.9–2769.1 pg/mL for the UCH-L1 assay, with a deviation from linearity within 10% (except the Low sample with the UCH-L1 value of 40.2 pg/mL has a deviation of -13.05% from linearity). The results support the linearity throughout the reportable ranges of 10.0–320.0 pg/mL for GFAP and 80.0 – 2560.0 pg/mL for UCH-LI.

Hook effect

The potential hook effect was evaluated. A serum sample spiked to a high antigen level was used to prepare a dilution series with 12 samples. Each dilution was tested in triplicate for each assay using one lot of VIDAS TBI (GFAP) and one lot of VIDAS TBI (UCH-L1) assay on one VIDAS 3 instrument. No hook effect was observed for each assay using serum samples with antigen concentrations exceeding 200,000 pg/mL GFAP and 400,000 pg/mL UCH-L1, when tested on the VIDAS 3 instrument.

3. Analytical Specificity/Interference:

Studies to evaluate the analytical specificity/interference were conducted using at least one lot of VIDAS TBI (GFAP) assay, one lot of VIDAS TBI (UCH-L1) assay and one VIDAS 3 instrument. Two levels of GFAP and UCH-L1 in pooled serum samples were evaluated in the studies: low (17.0−27.0 pg/mL) and high (180.0−220.0 pg/mL) for GFAP, and low (300.0–350.0 pg/mL) and high (1300.0–1700.0 pg/mL) for UCH-L1. The low GFAP and UCH-L1 level corresponds to the concentration close to the respective clinical decision threshold. The high UCH-L1 serum sample was spiked with recombinant UCH-L1 to achieve the targeted concentration.

a. Endogenous and exogenous interference

Test samples were created by spiking the pooled serum samples with the potentially interfering substances listed in the table below at test concentrations recommended in CLSI EP37 Ed1. Control samples were spiked only with the appropriate solvent used to prepare the stock solution of the potentially interfering substance. Each test and control sample were evaluated with five to seven replicates in one assay run for each potentially interfering substance. The %recovery was calculated by comparing measurements of the test and control samples. A substance was identified as an interferent if the difference in the means between the test and control samples was not within 10% of the control sample mean for GFAP or UCH-L1. For any substances identified as an interferent at the initial concentration tested, a dose response analysis was performed.

Endogenous Substances Not Found to Interfere		
	VIDAS TBI (GFAP)	**VIDAS TBI (UCH-L1)**
Potentially interfering substance	**Concentration**	
Bilirubin (conjugated)	0.4 g/L	0.4 g/L
Bilirubin (unconjugated)	0.4 g/L	0.4 g/L
Biotin	3840 ng/mL	3510 ng/mL
Hemoglobin	10 g/L	0.6 g/L
Human Albumin	60 g/L	60 g/L
Human anti-mouse antibody (HAMA)	2000 ng/mL	2000 ng/mL
Rheumatoid factor	802 IU/mL	175 IU/mL
Triglycerides	30 g/L	30 g/L
Total proteins	120 g/L	120 g/L

Exogenous Substances Not Found to Interfere	
Potentially interfering substance	**Concentration**
Acetaminophen	15.6 mg/dL
Acetylsalicylic acid (Aspirin)	3.0 mg/dL
Benzoylecgonine tetrahydrate	37.5 ng/mL
Cardene (Nicardipine)	0.047 mg/dL
Clopidogrel hydrogensulfate (Plavix)	4.5 mg/dL
Coumadin (Warfarin)	7.5 mg/dL
EDDP[A]	125 ng/mL
Ethanol	600.1 mg/dL
Ibuprofen	21.9 mg/dL
Lopressor (Metroprolol + tartrate salt)	18.7 µmol/L
Methadone hydrochloride	0.318 mg/dL
d-Methamphetamine	125 ng/mL
Methaqualone	37.5 ng/mL
Morphine monohydrate	0.78 mg/dL
Metoclopramide	0.225 mg/dL
Ondansetron hydrochloride dihydrate	0.96 mg/dL
Oxazepam	0.435 mg/dL
Phencyclidine hydrochloride	3.1 ng/mL
Propoxyphene	0.321 mg/dL
Secobarbital	1.59 mg/dL

[A] 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

b. Cross-reactivity

For cross-reactivity, each potential cross-reacting substance was tested at a concentration that corresponds to its highest reported physiological level reported in circulation according to literature, as applicable, indicated in the table below. The effect of each potential cross-reactant was evaluated by comparing the test results from a control sample, spiked with the appropriate solvent used to create the stock solution of the potential cross-reactant (Control Pool), with the test results from a sample spiked with the potentially cross-reacting substance (Test Pool). Each test and control sample for each potentially cross-reacting substance was evaluated with five replicates. The %recovery was calculated by comparing measurements of the test and control samples. A substance was identified as an interferent if the difference in the means between the test and control samples was not within 10% of the control sample mean for GFAP or UCH-L1. None of the tested cross-reacting substances were found to interfere with the VIDAS TBI (GFAP) and VIDAS TBI (GFAP) assays.

Concentrations Found Not to Interfere	
Potential Cross-Reactant	**No Interference Observed Up To**
GFAP Assay	
Desmin	127 ng/mL
Internexin	77 ng/mL
Keratin type II	10 ng/mL
Neurofilmament light	68 pg/mL
Neurofilmament medium	8.6 ng/mL
Neurofilmament heavy	77 ng/mL
Peripherin	5 ng/mL
Vimentin	354 ng/mL
UCH-L1 Assay	
UCH-L3	354 ng/mL

4. Assay Reportable Range:

   The reportable range for both VIDAS TBI (GFAP) and VIDAS TBI (UCH-L1) is the same as the analytical measuring interval for both assays.

   Reportable range: VIDAS TBI (GFAP): 10.0 –320.0 pg/mL
   VIDAS TBI (UCH-L1): 80.0 –2560.0 pg/mL

   Assay results are preceded by the symbol for greater than (>) or less than (<) if the result is outside of the reportable range.

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

   a. Traceability

   There are no internationally recognized reference standards for GFAP and UCH-L1. Calibration of the VIDAS TBI (GFAP, UCH-L1) assay is traceable to in-house reference calibrators for each of the two proteins.

   b. Kit Stability

   Shelf-life stability: Reagent shelf-life of the VIDAS TBI (GFAP, UCH-L1) was determined on three VIDAS 3 instruments using three reagent lots of each assay. One kit lot was exposed to a series of thermal shocks to simulate variations in temperature during shipping. A panel of four pooled serum samples covering the respective AMIs of each assay were tested. The mean value for each sample at T0, and sequent timepoints (3, 6, 9, 12 and 13 months) were determined in triplicate in three runs. The absolute and relative recovery of the test samples with respect to the initial measurement at T0 was evaluated. The results support that the VIDAS TBI (GFAP, UCH-L1) is stable up to 8 months when stored at 2–8°C.

   In-use stability: Stability of the VIDAS TBI (GFAP, UCH-L1) after first opening was evaluated for kit components including SPR (storage pouch opened, then tightly closed),

Calibrator S1 and Control C1 (up to four cycles of successive opening and closing of the vials) and the reagent kit (12 cycles of 70 minutes at 30°C ± 2°C). A panel of four pooled serum samples covering the respective AMIs of each assay were tested in triplicate at test timepoints. The results support the following in-use stability claims:

- SPRs were stable up to 8 months after first opening and stored at 2–8°C.
- Reagent kit was stable up to12 cycles of 70 minutes at 30°C and back to 2–8°C.
- Calibrator S1 and Control C1 were stable up to four cycles from 2–8°C to 25°C.

A study was also performed to determine the stability of each assay from storage at 2–8°C or after subjecting to different temperature conditions before testing. The results support that assay reagents could remain at 18–25°C for up to 4 hours before testing.

On-board stability: A study was performed to determine the stability of the reagents, i.e., SPR and strips, inside the VIDAS 3 instrument. A panel of four samples that includes Calibrator S1, Control C1 and two samples, one with analyte concentration close to the cut-off of the respective assay, was tested at the following time intervals using reagent on-board: 0 min, 6 min ± 1, 22 min ± 1, and 32 min ± 2. The results support the stability of the reagents in the VIDAS 3 instrument for up to 8 hours at 37°C and storage of samples in the strip in the VIDAS 3 instrument up to 20 minutes at 37°C before assaying.

c. Sample Stability

A study was performed to evaluate the stability of fresh serum specimens in primary tubes using one lot of the VIDAS TBI (GFAP) assay and one lot of the VIDAS TBI (UCH-L1) assay on one VIDAS 3 instrument. Forty specimens with concentrations of GFAP and UCH-L1 covering the respective assays were collected in serum tubes with separator gel and coagulation activator SST II Advance (SST). All specimens were centrifuged and then tested in duplicate with both assays at different timepoints under storage conditions to mimic customer use. Passing and Bablok regression was used to compare the result of the reference condition to the result of the other conditions. In addition, stability of the sample under freeze/thaw cycles was also evaluated by testing 40 specimens collected in SST tubes using one lot of the VIDAS TBI (GFAP) assay and two lots of the VIDAS TBI (UCH-L1) assay on two VIDAS 3 instruments.

The results support the sample is stable:

- up to three hours at 18–25°C after centrifugation
- up to 43 hours at 2–8°C in closed primary tubes
- for one free/thaw cycle (when stored at -19 to -31°C or -60°C for 5 months)

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted in accordance with CLSI guideline EP17-A2. The studies evaluated two lots of VIDAS TBI (GFAP) assay and two lots of VIDAS TBI (UCH-L1) assay on one VIDAS 3 instrument.

For LoB, four blank samples corresponding to very low concentrations of native GFAP were tested in duplicate over 8 days on one VIDAS 3 instrument using two reagent lots of the VIDAS TBI (GFAP) assay. The five blank samples corresponding to very low concentrations of native UCH-L1 were tested in duplicate over 8 days on one VIDAS 3 instrument using two reagent lots of the VIDAS TBI (UCH-L1) assay. The LoB was evaluated for each lot of reagent and the final LoB was determined as the greater of the LoB values obtained for each reagent lot for each assay.

For LoD, seven low-level of GFAP serum samples were tested in five replicates per day for 8 days using two lots of the VIDAS TBI (GFAP) assay on one VIDAS 3 instrument to obtain 40 measurements for each sample, totaling 280 measurements per assay lot. The sample protocol was used to test six low-level of UCH-L1 serum samples using two lots of the VIDAS TBI (UCH-L1) assay, resulting a total of 240 measurements per assay lot. The final LoD was determined as the greater of the LoD values obtained for each reagent lot for each assay.

For LoQ, the data generated from the LoD study above was estimated based on the lowest concentration quantifiable with an intermediate precision of ≤15% CV and ≥ LoD for each assay.

The results are summarized in the table below:

Assay	LoB (pg/mL)	LoD (pg/mL)	LoQ (pg/mL)	Lower Limit of AMI/ Reportable Range (pg/mL)
GFAP	4.4	5.4	5.4	10.0
UCH-L1	41.8	48.1	48.1	80.0

7. Assay Cut-Off:

VIDAS TBI (GFAP): 22.0 pg/mL
VIDAS TBI (UCH-L1): 327.0 pg/mL

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable

2. Matrix Comparison:

Sample matrix equivalence was evaluated for the VIDAS TBI (GFAP, UCH-L1) in accordance with CLSI guideline EP35 1st Ed for serum samples collected with three different blood collection tube types: serum tube with coagulation activator (CAT), serum tube with separator gel and coagulation activator SST II Advance (SST) and Rapid Serum tube with thrombin-based clot activator and separator gel (RST). Blood from donors was drawn on each of the tested blood collection tubes.

CAT vs SST

A set of samples was prepared to cover the AMI of the respective assay. All specimens were centrifuged and then tested in duplicate on one VIDAS 3 instrument using one assay lot for each assay. Passing-Bablok regression analysis was performed to compare the result of the SST to the result of the CAT used as reference. Results are summarized in the table below for each assay.

Assay	N	SST (pg/mL)	CAT (pg/mL)	Slope (95% CI)	Intercept (95% CI)	R
GFAP	31	10.4 – 314.2	11.6 – 307.7	1.02 [0.99; 1.04]	-0.4 [-1.1; 1.2]	0.997
UCH-L1	35	95.2 – 2106.0	97.3 – 2395.2	1.00 [0.94; 1.05]	-5.9 [-18.9; 16.7]	0.994

SST vs RST

A separate study was performed using a set of samples that cover the entire AMI of the respective assay to validate the use of RST tube. All samples were tested in duplicate. Passing-Bablok regression analysis was performed to compare the result of the RST to the result of the SST used as reference. Results are summarized in the table below for each assay.

Assay	N	SST (pg/mL)	RST (pg/mL)	Slope (95% CI)	Intercept (95% CI)	R
GFAP	41	10.0 – 326.4	12.1 – 279.9	1.02 [0.99; 1.04]	0.8 [0.0; 1.8]	0.992
UCH-L1	46	60.0 – 1764.7	55.7 – 1348.5	0.98 [0.92; 1,04]	9.8 [-7.4; 26.0]	0.988

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity:

The performance of the VIDAS TBI (GFAP, UCH-L1) as an aid to assist physicians in determining the need for a computed tomography (CT) scan of the head was evaluated in two studies: the first study used samples from the ALERT-TBI study, the second study used samples from the BRAINI study.

Clinical study using samples from the ALERT-TBI study

The study was conducted by testing frozen serum specimens that were previously collected by Banyan Biomarkers (San Diego, CA) from subjects participating in their prospective multicenter study "*A Prospective Clinical Evaluation of Biomarkers of Traumatic Brain Injury*" (ALERT-TBI, protocol ATO-06). This study enrolled subjects over the age of 18 who presented to a health care facility (HCF) or emergency department (ED) with suspected head injuries and had blood withdrawn within 12 hours of head injury. A total of 1911 frozen and de-identified serum samples of the ALERT cohort were tested in singlicate at three clinical sites (that include two in the U.S.) on the VIDAS 3 instrument, using three lots and two calibrations per lot by operators representative of end-users of the VIDAS TBI (GFAP, UCH-L1) test. Each specimen was associated with a standard of care computed tomography (CT) scan of the head from the same study subject. Under the ALERT-TBI study protocol, the CT scans were independently reviewed by a panel of radiologists and a subject's CT scan was

classified as positive if intracranial lesions were present. The standard of care head CT scans were previously classified as positive or negative for intracranial lesions as part of the ALERT-TBI study. Additional details regarding the inclusion and exclusion criteria, evaluation of CT scans and definition of intracranial lesion can be found in DEN170045[1] and published study[2]. The demographic characteristics of the enrolled subjects evaluated with the VIDAS TBI (GFAP, UCH-L1) test are presented in the table below.

The demographic characteristics and CT scan results for all 1911 evaluable subjects (120 CT-positive patients and 1791 CT-negative patients) are presented in the table below.

Demographic Characteristics (ALERT TBI Cohort)	Head CT-Scan Results		Total
	Positive	Negative	
N	120	1791	1911
Age[A] (Years)			
Mean	58.8	48.5	49.1
Median	58.5	48.0	49.0
Standard Deviation	18.29	21.02	21.00
Range	20 – 95	18 – 98	18 – 98
Gender, N (%)			
Male	70 (58.3%)	1010 (56.4%)	1080 (56.5%)
Female	50 (41.7%)	781 (43.6%)	831 (43.5%)
Race[B], N (%)			
White	98 (81.7%)	1250 (69.8%)	1348 (70.5%)
Black or African American	16 (13.3%)	487 (27.2%)	503 (26.3%)
Asian	5 (4.2%)	24 (1.3%)	29 (1.5%)
Native Hawaiian/Pacific Islander	1 (0.8%)	2 (0.1%)	3 (0.2%)
American Indian or Alaska Native	1 (0.8%)	10 (0.6%)	11 (0.6%)
Unknown	1 (0.8%)	27 (1.5%)	28 (1.5%)
Ethnicity, N (%)			
Hispanic or Latino	1 (0.8%)	89 (5.0%)	90 (4.7%)
Not Hispanic or Latino	118 (98.3%)	1701 (95.0%)	1819 (95.2%)
Not Reported	1 (0.8%)	1 (0.1%)	2 (0.1%)

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

Other baseline characteristics collected for all evaluable subjects were similar in the CT scan-positive and CT scan-negative groups, except for alcohol use, which was slightly lower but not statistically significant in the CT scan-positive group (refer to DEN170045[1]).

The following head injury characteristics were collected for all evaluable subjects and summarized in the table below. The mean time from head injury to blood draw was 3.5 hours. Most subjects had a GCS score of 15 (1798/1911 or 94.1% in the total cohort, 94/120 or 78.3% in CT scan-positive subjects and 1738/1827 or 95.1% in CT scan-negative

[1] FDA. De Novo request for evaluation of automatic class III designation of the Banyan Brain Trauma Indicatory (BTI) 2018 [Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170045.pdf
[2] Bazarian JJ, Biberthaler P, Welch RD, Lewis LM, Barzo P, Bogner-Flatz V, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERTTBI): a multicentre observational study. The Lancet Neurology. 2018;17(9):782-789.

subjects). The percentage of subjects with GCS scores of 13 and 14 were higher in the CT scan-positive subjects (7/120 or 5.8% for GCS of 13 and 19/120 or 15.8% for GCS of 14) compared to the CT scan-negative subjects (15/1791 or 0.8% for GCS of 13 and 72/1791 or 4.0% for GCS of 14).

Head Injury Characteristics (ALERT TBI Cohort)	Head CT-Scan Results		Total
	Positive	Negative	
N	120	1791	1911
Glasgow Coma Score – N (%)			
13	7 (5.8%)	15 (0.8%)	22 (1.2%)
14	19 (15.8%)	72 (4.0%)	91 (4.8%)
15	94 (78.3%)	1704 (95.1%)	1798 (94.1%)
Neurological Assessment - Number (%) of Subjects Experiencing			
Loss of Consciousness	82 (68.3%)	727 (40.6%)	809 (42.3%)
Confusion	44 (36.7%)	314 (17.5%)	358 (18.7%)
Vomiting	14 (11.7%)	131 (7.3%)	145 (7.6%)
Vomiting two or more episodes	10 (8.3%)	62 (3.5%)	72 (3.8%)
Post Traumatic Amnesia (PTA)	81 (67.5%)	552 (30.8%)	633 (33.1%)
Retrograde PTA ≥ 30 min	22 (18.3%)	68 (3.8%)	90 (4.7%)
Persistent Anterograde PTA	42 (35.0%)	172 (9.6%)	214 (11.2%)
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%)	370 (20.7%)	403 (21.1%)
Dangerous Mechanism of Injury[B]	27 (22.5%)	370 (20.7%)	397 (20.8%)
Physical Evidence[C]			
Visible Trauma Above the Clavicle	101 (84.2%)	1109 (61.9%)	1210 (63.3%)
Suspected Open or Depressed Skull Fracture	14 (11.7%)	46 (2.6%)	60 (3.1%)
Signs of Basal Skull Fracture	10 (8.3%)	26 (1.5%)	36 (1.9%)
Presence of Neurosurgical Lesion	5 (4.2%)	0 (0.0%)	5 (0.3%)
Time from Head Injury to Examination, (hours)[A]			
N	1789	120	1909[D]
Mean (SD)	1.8 (1.73)	1.7 (1.91)	1.7 (1.90)
Median	1.2	1.1	1.1
Range (minimum, maximum)	(0.3, 7.8)	(0.1, 33.4)	(0.1, 33.4)
Time from Head Injury to CT-Scan, (hours)[A]			
N	1789	120	1909[D]
Mean (SD)	2.8 (2.08)	2.8 (2.10)	2.8 (2.10)
Median	2.1	2.2	2.2
Range (minimum, maximum)	(0.5, 10.9)	(0.2, 33.5)	(0.2, 33.5)
Time from Head Injury to Blood Draw, (hours)[A]			
N	1788	120	1908[E]
Mean (SD)	3.8 (1.92)	3.5 (2.06)	3.5 (2.06)
Median	3.3	3.2	3.2

Range (minimum, maximum)	(0.3, 9.3)	(0.3, 35.3)	(0.3, 35.3)[F]

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

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

[C] Prior to head CT.

[D] Two subjects did not have time from head injury to examination and CT scan recorded.

[E] Three subjects did not have time from head injury to blood draw recorded.

[F] Two subjects had blood draws taken more than 12h (18h and 35.3h) from head injury. Please note that the "efficacy" ALERT population was used for this study to be aligned with Banyan testing strategy (worst case scenario). This population included all subjects for whom head CT scan results were obtained.

To estimate clinical performance characteristics, the VIDAS TBI (GFAP, UCH-L1) test result was compared to the consensus head CT scan result for each patient. The performance estimates are summarized in the table below.

ALERT TBI Sample Cohort		**Head CT-Scan Result**		**Total**
		Positive	Negative	
VIDAS TBI (GFAP, UCH-L1)	Positive	116	1053	1169
	Negative	4	738	742
Total		120	1791	1911

Clinical Sensitivity: 96.7% (116/120) (95% CI: 91.7–99.1%)
Clinical Specificity: 41.2% (738/1791) (95% CI: 38.9–43.5%)
Negative Predictive Value (NPV)[A]: 99.5% (738/742) (95% CI: 98.6–99.9%)
Positive Predictive Value (PPV)[B]: 9.9% (116/1169) (95% CI: 8.3–11.8%)
Likelihood Ratio Negative (LRN): 0.10 (95% CI: 0.04–0.23)
Likelihood Ratio Positive (LRP): 1.6 (95% CI: 1.51–1.69)
CT scan positive prevalence rate in study: 6.3% (120/1911)

[A] Adjusted NPV for 6% CT scan positive prevalence rate (DEN170045): 99.3% (95% CI: 98.5–99.7%)
[B] Adjusted PPV for 6% CT scan positive prevalence rate (DEN170045): 9.3% (95% CI: 8.8–9.7%)

Of the 120 subjects with positive CT scan results, four had negative results from the VIDAS TBI (GFAP UCH-L1) test. The rate of false negative (FN) results was 3.33% (4/120). None of these four subjects were identified with a lesion requiring surgical intervention. Of the 1791 subjects with negative CT scan results, 738 had a negative VIDAS TBI (GFAP UCH-L1) result (clinical specificity = 41.2%). The rate of False Positive (FP) results was 58.8% (1053/1791). The Negative Predictive Value (NPV) of the assay was 99.5% (738/742). The results showed that the clinical performance of the VIDAS TBI (GFAP UCH-L1) test is characterized by high clinical sensitivity and high NPV, comparable to that demonstrated by the Banyan BTI (DEN170045) with clinical sensitivity of 97.5% and NPV of 99.6%, supporting clinical validity as an aid in the evaluation of the need for a CT scan in subjects presenting with a GCS score of 13 to 15.

Analyses of assay performance by gender, age, and time from injury relative to blood draw are shown in the table below.

	Sensitivity N (%) (95% CI)	Specificity N (%) (95% CI)	NPV N (%) (95% CI)	PPV N (%) (95%CI)
All Subjects N=1911	116/120 (96.7%) (91.7−99.1%)	738/1791 (41.2%) (38.9−43.5%)	738/742 (99.5%) (98.6−99.9%)	116/1169 (9.9%) (8.3−11.8%)
Gender				
Male N=1080 (56.5%)	67/70 (95.7%) (88.0−99.1%)	413/1010 (40.9%) (37.9−44.0%)	413/416 (99.3%) (97.9−99.9%)	67/664 (10.1%) (8.0−12.6%)
Female N=831 (43.5%)	49/50 (98.0%) (89.4−99.9%)	325/781 (41.6%) (38.2−45.1%)	325/326 (99.7%) (98.3−100.0%)	49/505 (9.7%) (7.4−12.6%)
Age				
<65 years N=1410 (73.8 %)	74/77 (96.1%) (89.0−99.2%)	674/1333 (50.6%) (48.0−53.3%)	675/678 (99.6%) (98.7−99.9%)	74/732 (10.1%) (8.1−12.5%)
≥65 years N=501 (26.2 %)	42/43 (97.7%) (87.7−99.9%)	63/458 (13.8%) (10.9−17.2%)	63/64 (98.4%) (91.6−100.0%)	42/437 (9.6%) (7.2−12.7%)
Time from Injury to Blood Draw				
0−4 hours N=1443 (75.5 %)	83/86 (96.5%) (90.1−99.3%)	545/1357 (40.2%) (37.6−42.8%)	545/548 (99.5%) (98.4−99.9%)	83/895 (9.3%) (7.5−11.4%)
4−8 hours N=386 (20.2 %)	28/28 (100.0%) (87.7−100.0%)	164/358 (45.8%) (40.7−51.0%)	164/164 (100.0%) (97.8−100.0%)	28/222 (12.6%) (8.9−17.6%)
0−8 hours N=1829 (95.7 %)	111/114 (97.4%) (92.5−99.5%)	709/1715 (41.3%) (39.0−43.7%)	709/712 (99.6%) (98.8−99.9%)	111/1117 (9.9%) (8.3−11.8%)
8−12 hours N=80 (4.2 %)	5/6 (83.3%) (43.6−97.0%)	29/74 (39.2%) (28.9−50.6%)	29/30 (96.7%) (82.8−99.9%)	5/50 (10.0%) (4.3−21.4%)

There was little variation in NPV and PPV between males and females and with increasing time from injury. However, the differences did not translate into statistically significant differences in assay performance. The analysis by age shows that the clinical specificity is lower in patients ≥ 65 years of age (13.8%) than in patients < 65 years of age (50.6%), and conversely, the clinical sensitivity is higher in the older population (97.7% versus 96.1%), respectively.

Clinical study using samples from the BRAINI study

A supplemental clinical validation of the GFAP and UCH-L1 cut-offs was performed with serum samples from subjects participating in the multi-site study within 4 years after collection and stored frozen at -60°C. The BRAINI study was designed following the main inclusion and non-inclusion criteria defined for the ALERT-TBI cohort. Only the serum

specimens collected and stored less than 3.5 years (42 months) in accordance with the proposed intended use of the VIDAS TBI (GFAP, UCH-L1) test were included in the analysis (n=562). The demographic and head injury characteristics of the evaluable subjects are presented in the tables below.

Demographic Characteristics[A] (BRAINI Sub-Cohort)	Head CT-Scan Results		Total
	Positive	Negative	
N	74	488	562
Age (Years)			
Mean	54.5	48.6	49.4
Median	55.5	47.0	49.0
Standard Deviation	20.14	21.51	21.41
Range	19 – 95	18 – 100	18 – 100
Gender, N (%)			
Male	52 (70.3%)	309 (63.3%)	361 (64.2%)
Female	22 (29.7%)	179 (36.7%)	201 (35.8%)
[A]Information relating to race and ethnic origin was not collected as part of the BRAINI study			

Head Injury Characteristics (BRAINI Sub-Cohort)	Head CT-Scan Results		Total
	Positive	Negative	
N	74	488	562
Time from Head Injury to Examination (hours)			
Mean (SD)	1.5 (0.95)	1.6 (1.41)	1.6 (1.41)
Median	1.2	1.2	1.2
Range (min, max)	(0.3, 4.4)	(0.0, 10.0)	(0.0, 10.0)
Time from Head Injury to CT-Scan (hours)			
Mean (SD)	4.2 (2.10)	4.7 (2.61)	4.7 (2.55)
Median	3.7	4.2	4.2
Range (min, max)	(1.0, 12.3)	(0.1, 22.8)	(0.1, 22.8)
Time from Head Injury to Blood Draw (hours)			
Mean (SD)	5.2 (2.79)	5.0 (2.73)	5.0 (2.73)
Median	4.5	4.3	4.3
Range (min, max)	(1.4, 11.4)	(0.5, 11.9)	(0.5, 11.9)
Glasgow Coma Score			
13	0 (0.0%)	1 (0.2%)	1 (0.2%)
14	13 17.6%)	23 (4.7%)	36 (6.4%)
15	61(82.4%)	464 (95.1%)	525 (93.4%)
Neurological Assessment: Number (%) of Subjects Experiencing			
Loss of Consciousness [A]	46 (62.2%)	189 (38.7%)	235 (41.8%)
Vomiting	5 (6.8%)	19 (3.9%)	24 (4.3%)
Vomiting Two or More Episodes	9 (12.2%)	27 (5.5%)	36 (6.4%)
Post traumatic Amnesia (PTA)	43 (58.1%)	150 (30.7%)	193 (34.3%)
Seizures	4 (5.4%)	21 (4.3%)	25 (4.4%)
Subjects with Drug or Alcohol Intoxication at Time of Presentation to Facility[B]	9 (12.2%)	62 (12.7%)	71 (12.6%)

Dangerous Mechanism of Injury[C]	10 (13.5%)	18 (3.7%)	28 (5.0%)
Physical Evidence			
Suspected Open or Depressed Skull Fracture	11 (14.9%)	25 (5.1%)	36 (6.4%)
Signs of Basal Skull Fracture	3 (4.1%)	3 (0.6%)	6 (1.1%)

[A] Loss of consciousness was assessed at patient admission.
[B] Subjects with other type of intoxication were also included, such as cannabis intoxication, cocaine intoxication etc.
[C] Dangerous mechanism of injury was pedestrian hit by a car.

The clinical performance of the VIDAS TBI (GFAP, UCH-L1) was evaluated by testing 562 serum specimens of this BRAINI sub-cohort at three clinical sites including two in the U.S., with operators of the test blinded to the CT results associated with the study subjects. 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, brain herniation, intraventricular hemorrhage, subarachnoid hemorrhage, petechial hemorrhage and global or focal brain edema.

BRAINI Sub-Cohort		**Head CT-Scan Result**		**Total**
		Positive	Negative	
VIDAS TBI (GFAP, UCH-L1)	Positive	72	321	393
	Negative	2	167	169
Total		74	488	562
Clinical Sensitivity: 97.3% (72/74) (95% CI: 90.6–99.7%) Clinical Specificity: 34.2% (167/488) (95% CI: 30.1−38.5%) Negative Predictive Value (NPV)[A]: 98.8% (167/169) (95% CI: 95.8–99.9%) Positive Predictive Value (PPV)[B]: 18.3% (72/393) (95% CI: 14.8−22.4%) Likelihood Ratio Negative (LRN): 0.10 (95% CI: 0.04–0.23) Likelihood Ratio Positive (LRP): 1.5 (95% CI: 1.51–1.69) CT scan positive prevalence rate in study: 13.2% (74/562)				
[A]Adjusted NPV for 6% CT scan positive prevalence rate (DEN170045): 99.3% (95% CI: 98.5–99.7%) [B]Adjusted PPV for 6% CT scan positive prevalence rate (DEN170045): 9.3% (95% CI: 8.8–9.7%)				

The results show similar clinical performance of the VIDAS TBI (GFAP, UCH-L1) test to the predicate device, when evaluated with serum samples collected within 3.5 years in a sub-group of the BRANI study cohort.

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

   Not applicable

D Clinical Cut-Off:

Refer to assay cut-off

E Expected Values/Reference Range:

The expected values from 513 apparently healthy donors (53.8% male and 46.2% female) ranging in age from 18 to 85 were determined in accordance with CLSI guideline C28-A3c. The mean (SD) age was 47.3 (15.9) years. The races/ethnicity in the population is 25.2% Caucasian, 24.8% Hispanic, 25% Asian, and 25% African American. Data analysis was performed separately for the GFAP and UCH-L1 biomarkers. The 2.5th and 97.5th percentiles for both biomarkers were calculated non-parametrically. The results are shown below.

Biomarker	**N**	**Median** (pg/mL)	**95% Reference Interval (2.5th – 97.5th percentile)**
GFAP	513	10.4 pg/mL	<10.00 – 31.63 pg/mL
UCH-L1	513	94.9 pg/mL	< 80.00 – 249.70 pg/mL

Out of 513 samples, 470 (91. 6%) samples were tested negative by the VIDAS TBI (GFAP UCH-L1) test, and 43 (8.4 %) samples were tested positive by the VIDAS TBI (GFAP UCH-L1). Among 43 tested positive by the VIDAS TBI (GFAP, UCH-L1), one was positive for both GFAP and UCH-L1, 39 tested positive for GFAP only, and three tested positive for UCH-L1 only. Distribution of GFAP and UCH-L1 by age and gender in this study is summarized in the table below:

Distribution of GFAP and UCH-L1 by Gender within Age			
Age (years)	**Gender**	**GFAP (2.5th – 97.5th percentile)**	**UCH-L1 (2.5th – 97.5th percentile)**
≤ 30 (n=102)	Female (n=50)	<10 to 17.4 pg/mL	<80.0 – 156.8 pg/mL
	Male (n=52)	<10 to 18.4 pg/mL	<80.0 – 178.0 pg/mL
31 – 45 (n=129)	Female (n=55)	<10 to 16.8 pg/mL	<80.0 – 207.2 pg/mL
	Male (n=74)	<10 to 15.8 pg/mL	<80.0 – 216.6 pg/mL
46 – 60 (n=162)	Female (n=77)	<10 to 32.2 pg/mL	<80.0 – 206.2 pg/mL
	Male (n=85)	<10 to 30.4 pg/mL	<80.0 – 374.9 pg/mL
≥61 (n=120)	Female (n=55)	<10 to 68.6 pg/mL	<80.0 – 282.0 pg/mL
	Male (n=65)	<10 to 36.8 pg/mL	<80.0 – 458.8 pg/mL

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 and gender.

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.