DxDATA™ Trauma Brain Injury (TBI) Rapid In Vitro Diagnostic Test

White Paper

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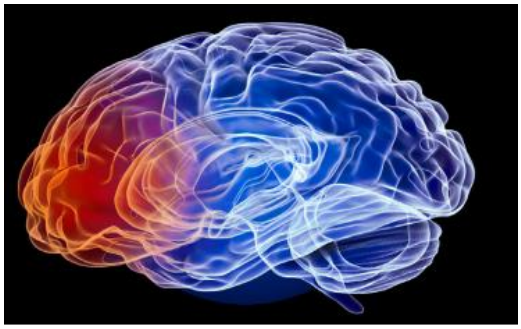

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Summary

Abstract

Traumatic brain injury (TBI) is a major global neurological disorder. The heterogeneity of TBI pathogenesis poses a diagnostic challenge to healthcare professionals. The use of computed tomography (CT) scan for diagnosing mild TBI (mTBI) has been proven to be ineffective and wasteful as most CT scan results are negative. Immunoassay tests of two blood proteins, UCH-L1 and GFAP have been approved by the FDA to aid in the evaluation of mTBI. Expanding the availability of automated, cost effective rapid in vitro diagnostic tests will make this important diagnostic tool accessible to a larger number of physicians who treat these patients. In this White paper we provide comprehensive review of DRG development of a rapid and sensitive in vitro diagnostic TBI test on the DxDATA platform.

Introduction

More than any other traumatic insult Trauma Brain Injury (TBI) is a significant cause of death and disability worldwide. It is estimated that 69 million individuals suffer TBI each year, with the Southeast Asian and Western Pacific regions experiencing the greatest overall burden of disease [1]. There were over 69,000 TBI-related deaths in the United States in 2021[2]. In the European Union, there are approximately 57,000 TBI-related deaths yearly [3]. People most commonly get TBIs from a fall, firearm-related injury, motor vehicle crash, or an assault. Depending on the severity of the injury, those who get a TBI may face health problems that last a few days or the rest of their lives.

Patients with a suspected head injury are examined using a neurological scale, called Glasgow Coma Scale (GCS). Severe injury is defined as GCS of 3-8, moderate with 9-12 and mild with 13-15. mTBI (also known as a concussion) comprises most of the TBI cases (over 90%). The selection of patients for computed tomography (CT) scan is an important decision in mild TBI since it allows early identification of brain tissue damage, or intracranial lesions, that may require treatment. However, 90-95% of scanned patients show no internal intracranial injury but being subjected to radiation risks which may elevate a person's lifetime risk of developing cancer [4]. Addressing this concern, the FDA launched in 2010 an initiative to reduce unnecessary radiation exposure from medical imaging. In addition, availability of a blood test for mTBI/concussion will likely increase saving the patient and health care system the cost of often unnecessary neuroimaging tests.

In 2018 the FDA authorized the first biomarker test that predicts absence of intracranial injury. The deNovo test was developed by Banyan Biomarkers Inc. The enzyme-linked immunosorbent immunoassay (ELISA) measures the levels of two proteins; Ubiquitin C-terminal hydrolase L1 (UCH-L1), also known as PGP9.5, and Glial fibrillary acidic protein (GFAP). UCH-L1 is a neuronal protein that is abundant in that brain and GFAP is the main intermediate filament protein in mature astrocytes. Several seminal studies demonstrated that serum levels of GFAP and UCH-L1 are elevated in patients within hours of mTBI [5,6]. This increase was associated with

detection of intracranial lesions by a CT scan. The immunoassay test is approved to aid in the evaluation of 18 years or older with suspected mTBI patients using serum collected within 12 hours of injury. Results of the GFAP and UCH-L1 are combined to determine the outcome, only when both biomarker concentrations are below cutoff is the absence of intracranial injury predicted and thus the need for CT scan. The assay is a manual with results available within 3-4 hrs.

DRG aims to develop rapid in vitro diagnostic chemiluminescence linked immunoassay (CLIA) blood test. The test is magnetic bead based and is used to aid in the evaluation of patients 18 years of age and older suspected with mTBI using serum samples collected within 12 hours of injury. The assay provides a quantitative measurement of the concentration of UCH-L1 and GFAP in human serum and is used with DRG's DxDATA. DxDATA is an automated CLIA reader utilizing a single disposable cartridge for each of the biomarkers with test results available in 30 min for both biomarkers simultaneously. The results of both tests are required to obtain an overall qualitative (Positive/Negative) test interpretation. Results of this DxDATA™ TBI will help health care professionals determine the need for a CT scan in patients suspected of having mTBI and help prevent unnecessary neuroimaging. The test will provide an affordable cost-effective alternative, expanding access to an important healthcare tool.

Here we describe in detail the analytical studies of DxDATA™ TBI and method comparison with the FDA approved Banyan ELISA.

Methods

A. Antibodies

The monoclonal antibodies (mAbs) used in the DRG TBI assays are shown in **Table 1**. For the UCH-L1 assay, two capture mAbs are coated onto magnetic beads and for the GFAP assay only one. Assay detection mAbs are linked to alkaline phosphatase (AP).

Table 1. Recommended capture and detection mAbs for UCH-L1 detection. The mAbs were purified on Protein A followed by size exclusion S-300 chromatography for purity of ≥ 95%.

UCH-L1		
Use	**Clone**	**Part Number**
Capture mAb	2D3	70763
Capture mAb	10D1	70764
Detection mAb	1016	70765
GFAP		
Use	**Clone**	**Part Number**
Capture mAb	12H2	70766
Detection mAb	203	70767

B. DxDATA Assays

The DxDATA instrument (Fig. 1) is a fully automated chemiluminescence immunoassay (CLIA) IVD analyzing system using magnetic-particle technology utilizes 16 well cartridges to run single-use assay (Fig. 2) Each cartridge holds the needed reagents and sampling materials, with a well designated to add the corresponding samples, and each cartridge fits into an 8-cartridge rack that is placed into the instrument and held at 37°C for testing. GFAP and UCH-L1 cartridges are run in parallel with results for both biomarkers are available within approximately 30 minutes. The DxDATA™ TBI are immunoassay sandwich assays that utilize capture mAbs coupled to magnetic beads (mAb-MB) and detection mAbs conjugated to AP (mAb-AP) to separate and quantify the amount of antigen in solution. During the assay's runtime, the 120 µL serum sample is mixed with the coupled mAb-MB and mAb-AP reagents for 10 min before being magnetically separated and washed 3 times, then added to a dark-well containing substrate for chemiluminescent detection. The emitted light is proportional to the amount of antigen detected.

Figure 1. DxDATA

Figure 2. All-in-one single use cartridge

C. Data Analysis Software

Microsoft Excel and Analyze-It Standard Edition software were used for data analysis for the studies performed on the DxDATA™ TBI assays.

Results

A. Assay Calibration and Quality Control

Calibration curve samples for each assay are made by spiking the corresponding calibrator diluent with the respective purified human protein for each assay (UCH-L1; 2560 pg/mL, GFAP; 320 pg/mL) and lyophilized. These lyophilized samples are resuspended in water and serially diluted 2-fold with calibrator diluent and are run 3 times on the DxDATA machine to generate three calibration curves ranging 10-320 pg/mL for GFAP and 80-2560 pg/mL for UCH-L1. These curves are inputted into the software to generate a master curve. A set of calibrator and QC samples made with the same process are then generated (2 calibrator samples, 2 QC samples) to calibrate the master curve monthly.

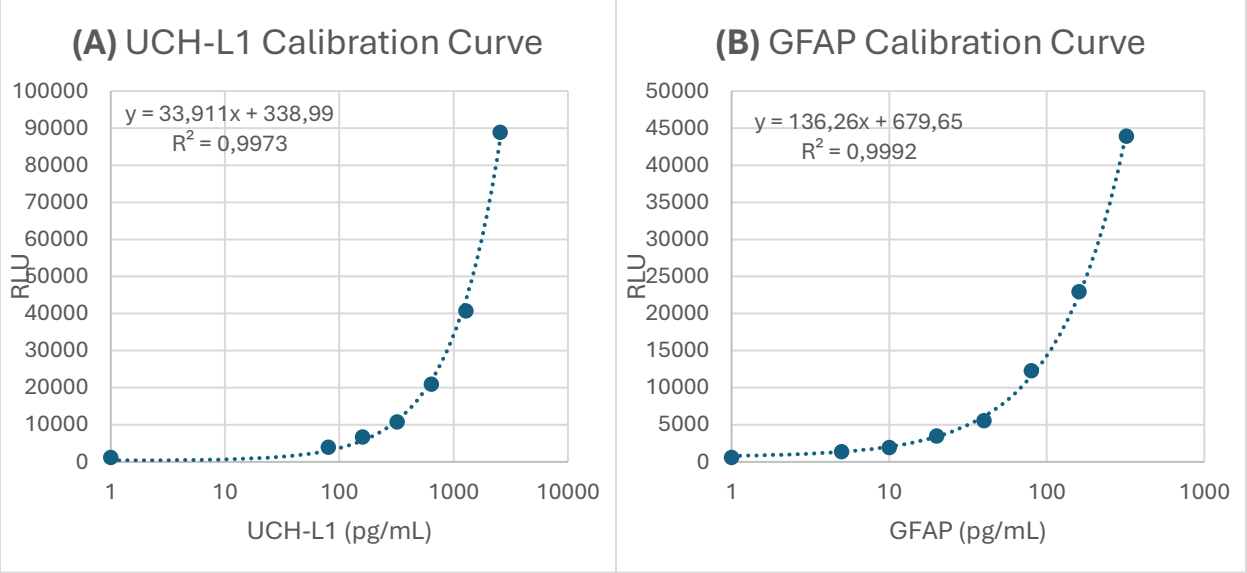

Figure 3: (A) UCH-L1 Calibration Curve range is 80-2560 pg/mL. **(B)** GFAP Calibration Curve range is 10-320 pg/mL.

B. Limit of Blank, Limit of Detection and Limit of Quantification

The Limit of Blank (LoB) and Limit of Detection (LoD) of the two TBI assays were determined on the basis of guideline CLSI EP17-A2 (Table 2). For the LoB, at least 24 replicates of "blank" unspiked pooled normal human serum samples were run on multiple reagent lots to quantify the limit of blank samples using the calculations laid out by CLSI. The LoD was then found using a series of four low level samples (no more than 5x the LoB) run in at least eight replicates and quantified via CLSI calculation guidelines. LLoQ and LLoQ were set according to the correct call of the quantitative clinical data (see Tables 3 and 4).

Table 2: DxDATA™ TBI LoB, LoD, LLoQ, and ULOQ. Each limiting concentration was determined on the basis of guideline CLSI EP17-A2.

	UCH-L1	GFAP
LoB	12.4 pg/mL	0.4 pg/mL
LoD	18.1 pg/mL	2.3 pg/mL
LLoQ	80.0 pg/mL	10.0 pg/mL
ULoQ	2560 pg/mL	320 pg/mL

C. Linearity and Reportable Range

The linearity of the TBI kits were evaluated via the guidelines set by CLSI's EP6-A. The assay studies measure the linear relationship between the observed values and the true concentrations of the analyte when spiked in the sample matrix (serum). Linearity is important for clinical evaluation of the measured analyte in matrix within the detection range. The study was performed by first combining pooled human serum of both high/low concentrations of analyte in an incremental fashion, then testing each combination of serum across the measuring range of the assay to determine the recovery to the expected result. Standard linear regression was used to calculate % Recovery for each assay (Figure 4). Linearity was seen throughout the measuring range of the UCH-L1 and GFAP.

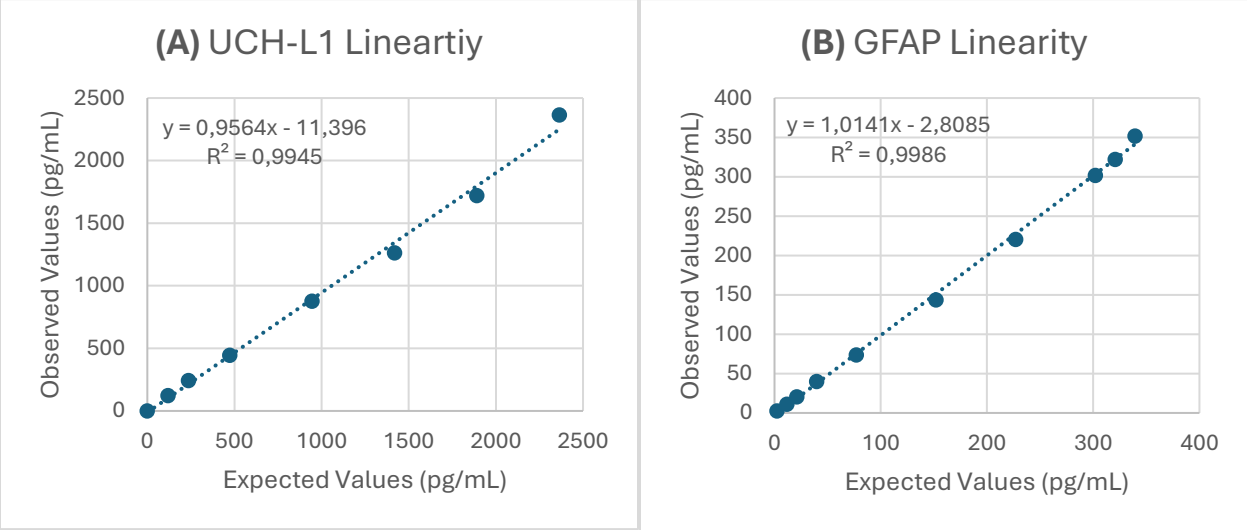

Figure 4: (A) UCH-L1 linearity plot spanning 123-2364 pg/mL with an R-squared value of 0.9945. **(B)** GFAP linearity plot spanning 10-320 pg/mL with an R-squared value of 0.9986.

D. Precision and Reproducibility

Quantitative precision:

Following the CLSI guidelines laid out in EP05-A3, studies were carried out to assess the within-laboratory precision of the UCH-L1 and GFAP DxDATA™ TBI assays, thus ensuring that the kits are working as intended on a day-to-day basis. Two serum sample panels were generated for the respective assay and made to cover the range of detection for each assay. The GFAP panel was generated by combining pools of low/healthy patient serum and spiking with the serum of patients with TBI diagnoses. The UCH-L1 panel was generated by spiking normal human serum with recombinant mammalian human UCH-L1. These panels were aliquoted and tested over the course of 20 days on one machine, using one reagent lot, and at one lab. The assay was run twice per day (once in morning and once in the afternoon), and each sample was run in duplicate for a total of 80 data points per panel sample (Table 3).

Table 3: 20 Day Precision study quantitative results for both UCH-L1 and GFAP assays.

UCH-L1 Repeatability			Within Run		Between Run		Between Day		Within Lab	
Panel Member	Mean Conc. (pg/mL)	N	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1[A]	88.5	80	0.0	0%	21.6	24%	8.6	10%	23.3	26%
2[A]	227.6	80	0.0	0%	16.2	7%	7.7	3%	18.0	8%
3[B]	443.4	80	0.0	0%	23.8	5%	0.0	0%	23.8	5%
4[B]	1343.8	80	17.8	1%	76.7	6%	0.0	0%	76.7	6%
5[B]	2456.1	80	58.3	2%	147.8	6%	49.8	2%	155.9	6%

GFAP Repeatability			Within Run		Between Run		Between Day		Within Lab	
Panel Member	Mean Conc. (pg/mL)	N	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1[A]	11.2	77.0[C]	1.1	10.3	0.8	7.3	0.4	3.2	1.2	10.7
2[A]	14.9	77.0[C]	1.2	8.4	0.0	0.00	0.7	4.9	1.4	9.7
3[B]	26.1	80.0	1.6	6.0	0.0	0.00	0.5	1.8	1.6	6.3
4[B]	69.8	77.0[C]	3.2	4.5	2.4	3.4	1.2	1.8	3.4	4.9
5[B]	157.0	77.0[C]	9.8	6.2	7.0	4.4	0.0	0.0	9.8	6.2

[A]Below cut-off; [B]Above cut-off; [C]A total of 12 measurements were invalid and excluded from analysis.

Qualitative precision:

The data of the 80 replicates from the quantitative study were then used to assign "positive/negative" results to determine the qualitative precision of the assays using Banyan cut-off values. Each panel member was given a "% Correct" value based on the clinical accuracy of each sample reading against the expected reading (Table 4). For example, all measurements of UCH-L1 panel number 1 with mean value of 88.5pg/mL were below the cut-off allowing 100% clinical correct call.

Table 4: Qualitative 20-day precision study results. Cut-offs used for analysis were 22 pg/mL for GFAP and 327 pg/mL for UCH-L1.

UCH-L1 Repeatability Qualitative Agreement				
Panel	Mean (pg/mL)	Replicates	# Above Cut-off	% Correct Call
1[A]	88.5	80	0/80	100%
2[A]	227.6	80	0/80	100%
3[B]	443.4	80	80/80	100%
4[B]	1343.8	80	80/80	100%
5[B]	2456.1	80	80/80	100%

GFAP Repeatability Qualitative Agreement				
Panel	Mean (pg/mL)	Replicates	# Above Cut-off	% Correct Call
1[A]	11.5	77.0[C]	0/77	100%
2[A]	14.9	77.0[C]	0/77	100%
3[B]	26.1	80.0	78/80	97.5%
4[B]	69.8	77.0[C]	77/77	100%
5[B]	156.9	77.0[C]	77/77	100%

[A]Below cut-off; [B]Above cut-of; [C]A total of 12 measurements were invalid and excluded from analysis.

Quantitative internal reproducibility:

Following the CLSI guidelines laid out in EP05-A3, studies were carried out to assess the precision between instruments and reagent lots. Two serum sample panels were generated for the respective assay and made to cover the range of detection for each assay. The GFAP panel was generated by combining pools of low/healthy patient serum and spiking with the serum of patients with TBI diagnoses. The UCH-L1 panel was generated by spiking normal human serum with recombinant mammalian human UCH-L1. These panels were aliquoted and tested over the course of five and three days for GFAP and UCH-L1, respectively, on two machines, using three reagent lots, at one lab. The assay was run once per day on three machines, and each sample was run in quadruplicate (Table 5).

Table 5: Reproducibility study quantitative results.

UCH-L1 Reproducibility												
Panel	**Mean (pg/ml)**	**N**	**Repeatability**		**Between Day**		**Between Machine**		**Between Lot**		**Within Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1[A]	190.2	108.0	13.8	7.2	0.0	0.0	18.4	9.6	15.7	8.2	27.8	14.6
2[B]	378.1	108.0	18.6	4.9	0.0	0.0	24.0	6.3	22.6	5.9	37.8	10.0
3[B]	1219.2	100.0[C]	160.5	12.9	0.0	0.0	0.0	0.0	59.8	4.8	171.3	13.8
4[B]	2154.6	96.0[C]	114.5	5.2	37.5	1.7	147.3	6.7	0.0	0.0	190.3	8.7

GFAP Reproducibility												
Panel	**Mean (pg/ml)**	**N**	**Repeatability**		**Between Day**		**Between Machine**		**Between Lot**		**Within Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1[A]	12.47	120	1.1	9.1	0.0	0.0	0.0	0.0	1.0	7.7	1.5	11.9
2[A]	15.92	120	1.0	6.3	0.0	0.0	0.2	1.0	1.0	6.2	1.4	8.9
3[B]	27.09	120	1.7	6.1	0.0	0.0	0.8	3.1	1.2	4.3	2.2	8.0
4[B]	93.11	120	4.3	4.6	1.4	1.5	4.5	4.8	0.0	0.0	6.3	6.8
5[B]	233.15	120	9.0	3.9	2.4	1.0	21.5	9.2	0.0	0.0	23.5	10.1

[A]Below cut-off; [B]Above cut-of; [C]A total of 20 measurements were invalid and excluded from analysis.

Qualitative internal reproducibility:

The data of the 108 to 120 replicates from the quantitative study were then used to assign "positive/negative" results to determine the qualitative precision of the assays. Each panel member was given a "% Correct" value based of the clinical accuracy of each sample reading against the expected reading (Table 6).

Table 6: Reproducibility qualitative results.

UCH-L1 Reproducibility				
Panel	**Mean (pg/mL)**	**Replicates**	**# Above Cut-off**	**% Correct**
1[A]	190.2	108[C]	0/108	100%
2[B]	378.1	108[C]	101/108	94%
3[B]	1219.2	100[C]	108/108	100%
4[B]	2154.6	96[C]	108/108	100%

GFAP Reproducibility				
Panel	**Mean (pg/mL)**	**Replicates**	**# Above Cut-off**	**% Correct**
1[A]	12.5	120	0/120	100%
2[A]	15.9	120	0/120	100%
3[B]	27.1	120	119/120	99%
4[B]	93.1	120	120/120	100%
5[B]	233.2	120	120/120	100%

[A]Below cut-off; [B]Above cut-of; [C]A total of 20 measurements were invalid and excluded from analysis.

E. Traceability

Traceability and value assignment:

Up to now, no international reference standards exist for either of the UCH-L1 or GFAP assays. Instead, commercially available antigens for UCH-L1 and GFAP were used to create Reference Standards for each kit, aliquoted, and stored at -70C. Additionally, these Reference Standards were used to quantify three human serum pools at Low, Medium, and High concentrations to be used as Quality Control Reference Standards using at least two developmental lots of the assay. These QC Reference Standards were also aliquoted and stored at -70°C.

Calibrators and Controls:

For both assays, calibrators and controls were created at four different levels within the standard curve of the assay (Standards 2 and 6 for the Calibrators, one QC as negative and second QC as a positive), lyophilized and stored at 2-8°C.

F. Stability

Closed assay kit stability:

A real-time stability study was performed on one lot via CLSI EP25AE guidelines for both the UCH-L1 and GFAP assay kits. DxDATA was calibrated for two years, and Cal and QC samples values were recorded with measurements of serum spikes samples. The study grants a claim of 2 years shelf-life when stored at 2-8°C and in the dark.

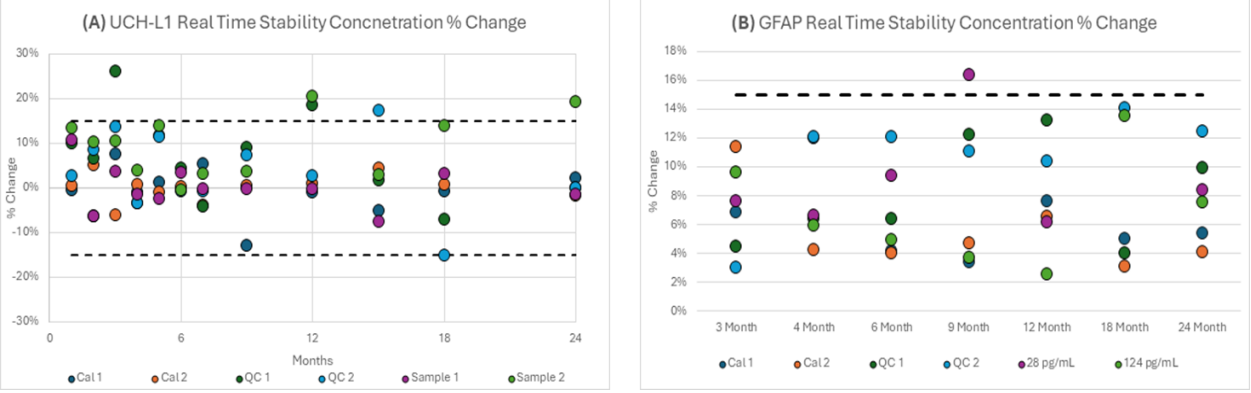

Figure 5: (A) UCH-L1 **(B)** GFAP plots plot represent the % change of calibrator/control/sample concentrations over the course of real time stability testing.

G. Endogenous and Exogenous Interference

Assay interference evaluation was conducted according to the CLSI guideline EP07-A2, using normal pooled human serum spiked with either recombinant UCH-L1 protein or purified human native GFAP. To prepare the test samples, the pooled serum was spiked with potential interfering

substances (Table 7) at concentrations recommended in CLSI EP07-A2. Control samples were spiked only with or without the 5% appropriate solvent used to prepare the interference panel. Both test and control samples were analyzed in duplicates within a single assay run using a single kit lot. Recovery was determined by comparing the measurements obtained from test samples with those from control samples.

For UCH-L1 no interference was observed in the presence of albumin (≤5g/dL), and rheumatoid factor (at ≤ 25 IU). For GFAP no interference was observed in the presence of albumin (≤5g/dL), Triglycerides (at ≤1500mg/dL), HAMA (at ≤ 20X the activity of a known negative as characterized by SunDiagnostics (New Gloucester, ME, USA)), and rheumatoid factor (at ≤ 400 IU).

Normal physiological concentration or activity for these substances is as follows: 3.4-5.4g/dL for albumin, <150 mg/dL for Triglycerides, 1X for HAMA, and bellow 20 IU for Rheumatoid factor. No interference was seen with the other interferents tested, as described in the table below.

Table 7: Materials Used to test interference in TBI assays.

Possible Interferents	Test Concentration	Source	Part #
Hemoglobin	500 mg/dL	Sun Diagnostics	INT-01
Bilirubin (conjugated)	20 mg/dL		
Triglycerides	3000 mg/dL		
Human Albumin	10 g/dL		
HAMA	>160XA		INT-02
Rheumatoid Factor	800 IU/mL		INT-03RF
Lopressor (Metoprolol)	18.7 µmol/L	Sigma Aldrich	M5391
Cardene (Nicardipine)	400 ng/mL		N7510
Coumadin (Warfarin)	32.5 µmol/L		A2250
Aspirin	3.62 mmol/L		A2093
Plavix (Clopidogrel)	9 ug/mL		SML0004
Acetaminophen	1324 µmol/L		A7085
Ibuprophen	2,425 µmol/L		I4883
Amphetamine	125 ng/mL	Thermo Scientific Drugs of Abuse Control Reagent	100200
EDDP	125 ng/mL		
Methadone	37.5 ng/mL		
Methaqualone	37.5 ng/mL		
Barbiturate	25 ng/mL		
Cocaine	37.5 ng/mL		
Benzodiazepine	25 ng/mL		
CEDIA™ Negative Drugs of Abuse Calibrators	0 ng/ml		1661388

[A] > 160 times more activity than a known negative for ability to crosslink in a mouse system assay

H. Cross-Reactivity

A panel of proteins with notable homology to either GFAP or UCH-L1 was tested for potential cross-reactivity. For GFAP, the panel of potential cross-reactive proteins was added to the GFAP calibrator diluent. UCH-L3 and UCHL-5 are the only proteins known to have substantial amino acid similarity to UCH-L1 (approximately 55% and 23%, respectively). The spiked calibrator diluent samples were tested in replicates using one GFAP kit lot and one UCH-L1 kit lot. Detection was below the LoD suggesting no cross-reactivity with GFAP and UCH-L1 kits (Table 8).

Table 8: Cross Reactivity study reactants and correlating test values.

Potential Cross Reactant	Test Conc.[A]
Vimentin[1]	354 ng/mL
Desmin[2]	127 ng/mL
Peripherin[3]	5 ng/mL
Neurofilament Light[4]	68 pg/mL
Neurofilament Medium[5]	8.6 ng/mL

Potential Cross Reactant	Test Conc.[A]
Neurofilament Heavy[6]	77 ng/mL
Keratin type II[7]	10 ng/mL
Internexin[B]	77 ng/mL
UCH-L3[C]	354 ng/mL
UCHL-5[C]	354 ng/mL

[A] Test concentration of the identified cross-reactants (except for Internexin and UCH-L3) are based on the highest concentration of each protein in the circulation as reported in:

[1] Sun *et al.* (2010) *J Proteome Research* 9:1923
[2] Ma *et al.* (2009) *Mol Cell Proteomics* 8.8:1878
[3] Determined at MyBioSource, email communication
[4] Giotto *et al.* (2013) *PLOS One* 8: e75091
[5] Martinez-Morillo *et al.* (2015) *CLin Chem Lab Med* 53:1575
[6] Lu *et al.* (2015) *J Neurol Neurosurg Psychiatry* 86:565
[7] Sundstrom *et al.* (1990) *Int J Cancer* 46:604

[B] For internexin, the level of 77 ng/mL was chosen because this is the highest concentration of a neurofilament (i.e. neurofilament heavy) tested in the study.

[C] For UCH-L3 and UCH-L5, the level of 354 ng/mL was chosen because this is the highest concentration of cross-reactant (Vimentin) tested with the GFAP kit.

I. Hook Effect

The hook effect describes a phenomenon that can occur in immunoassays, particularly sandwich assays, that can lead to falsely low results when the analyte concentration is extremely high. To test for this effect duplicate samples were spiked with 32 ng/ml of GFAP or 500 ng/mL for UCH-L1. These values are 100-fold and 200-fold higher than the highest calibration point, respectively. Blanks samples were controls with expected conc below 10 pg/mL. The measured values for both biomarkers were measured at the highest calibration point suggesting no hook effect (Table 9).

Table 9: Hook effect studied with high concentrations of antigen.

UCH-L1 Kit		
Sample	**Obs.Conc (pg/mL)**	**Mean RLU**
Blank	<LoD	2030
UCH-L1 at 2.56 ng/mL	2560	76231
UCH-L1 at 500 ng/mL	>2560	6801909

GFAP Kit		
Sample	**Obs.Conc (pg/mL)**	**Mean RLU**
Blank	<LoD	678
GFAP at 0.32ng/mL	320	48999
GFAP at 32ng/mL	>320	3035919

J. Cross Talk

A study was performed to evaluate if cross-light contamination occurs between two adjunct cartridges. Cartridges; 1, 3, 5, and 7 were not spiked while adjunct cartridges; 2, 4, 6 and 8 were spiked with the highest calibration concentration. We observe no increase in cartridges 3, 5, and 7 RLUs that were read immediately after cartridges containing the antigen suggesting no RLU leakage (Table 10).

Table 10: Light cross talk between cartridges RLU cross talk.

GFAP		
Conc. (pg/mL)	**Obs.Conc (pg/mL)**	**RLU**
0	<LoD	420
320	≥320	48334
0	<LoD	858
320	≥320	49666
0	<LoD	502
320	≥320	48282
0	<LoD	410
320	≥320	44455

UCH-L1		
Conc. (pg/mL)	**Obs.Conc (pg/mL)**	**RLU**
0	<LoD	1520
2560	≥2560	75093
0	<LoD	2047
2560	≥2560	75906
0	<LoD	1908
2560	≥2560	77694
0	<LoD	1883
2560	≥2560	71394

K. Method Comparison with Predicate Device

Serum samples from normal volunteers and TBI patients were measured by DxDATA TBI™ and FDA DeNovo approved Banyan ELISA kits. DxDATA GFAP cutoff was set at equal or above 20 pg/mL and UCH-1 cutoff at equal or above 294 pg/mL. Banyan cutoff values for GFAP and UCH-L1 were equal or above 22 for GFAP and equal or above 327 pg/ml for UCH-L1. Samples with equal or above the cut-off were assigned "Positive" and below the cutoff were assigned "Negative".

Using CLSI EP12 protocol for evaluation of qualitative test performance we find a 100% Positive percent agreement (PPA) and 89% Negative percent agreement (NPA) with Banyan ELISA determinant of Above/Bellow, suggesting excellent agreement between the two tests. Critically, all samples identified by the Banyan ELISA as positive were identified by the DRG DxDATA™ resulting in 100% PPA (Table 12).

Table 12: The PPA and the NPA were calculated GFAP (A) and UCH-L1 (B) serum samples measured concentration by Banyan ELISA and DRG DxDATA with assigned "Positive" or "Negative" determinants.

		Banyan Determination: GFAP and UCH-L1		
		Positive (40)	**Negative (54)**	**Total (94)**
DxDATA™ Test Result	**Positive**	40	7	7
	Negative	0	47	47
	Total	40	54	94

Positive percent agreement	**PPA**	40/40	100%
Negative percent agreement	**NPA**	47/54	89%

Discussion

Consistent with FDA's stated priority to deliver innovative testing technologies that minimize radiation to mTBI patients while still providing accurate and reliable results we have developed a TBI test using DxDATA, an automated CLIA reader utilizing a single disposable cartridge.

Here we describe our analytical studies as well as a method comparison with predicate Banyan ELISA. The analytical studies followed Banyan design approved by the FDA de Novo decision while using CLSI guidance. These studies included linearity in serum, precision over 20 days using a total of 1000 samples as well as five days repeatability using several reagents lots and instruments with a total of 1200 samples. The precision and repeatability results were analyzed for %CV using Analyze-It, a commercial software approved by CLSI. Qualitative agreement for each panel member was calculated based on the number of positive TBI results. For example, a panel with a mean value above the cutoff and with all samples measured above the cutoff will be a 100% qualitative call. We find that of the 10 panels tested for repeatability nine were at 100% correct and one GFAP panel at a mean value of 26 pg/mL had a 97.5% correct call while Banyan for comparison at 23 pg/mL had 65% correct call. A similar trend was observed for the reproducibility study with UCH-L1, all had a 100% agreement except for panel 378 pg/mL which had 94% correct call while Banyan panel 282 pg/mL had 95% call. These results point to the excellent repeatability and reproducibility of the DxDATA™ TBI assay.

Furthermore, we find no cross reactivity with all the nine biomarkers tested by Banyan. To further investigate cross-reactivity to UCH-L1 we also tested UCH-L5 which did not cross react. We find no interferences with external drug substances. Albumin had interference for both biomarkers at 5g/dL which is at the top range of concentration found in the healthy human population, which will require further investigation and potential optimization. Rheumatoid factor interfered with UCH-L1 at 25 IU/mL and GFAP at 400 IU/mL which are above the 0-20 IU/mL observed in the healthy population but might interfere with Rheumatoid arthritis patients. Triglycerides at 1500mg/dL, and HAMA at 20X (the activity of a known negative as characterized by Sun Diagnostics (New Gloucester, ME, USA)) interfered with GFAP. For healthy adults, normal physiological concentrations are generally below 150 mg/dL for triglycerides and approximately 1X for HAMA.

We find no hook-effect and no chemiluminescence cross talk between cartridges. Finaly, we demonstrated kit stability for two years at 4°C.

Method comparison was done using serum samples from patients with TBI and from healthy volunteers. GFAP and UCH-L1 values were measured by Banyan ELISA and DxDATA™ TBI. Of the 43 normal serum samples three were detected by DxDATA GFAP as positive while none by UCH-L1 which is in line with Banyan report of positive results in 18% of the normal population. For the 51 TBI samples four of the negative Banyan TBI samples were detected as positive by DxDATA, yielding 89% negative agreement. However, 40 TBI patients identified by Banyan as positive were assigned positive by DxDATA™ TBI demonstrating 100% positive agreement.

While GFAP and UCH-L1 have been proven to be important in the clinical management of mTBI several studies have demonstrated their potential use in several brain neurological diseases [7]. Briefly, in multiple sclerosis (MS) patient serum levels of GFAP increase from low 59 pg/mL to 246 pg/mL with the clinical severity scores and greater magnetic resonance imaging (MRI) lesion count suggesting its use as a disease progression marker (8,9). Also, levels of GFAP have shown to increase in the serum of intracerebral hemorrhage (ICH) patients but not in serum of ischemic stroke (IS) patients [10]. Finaly, high GFAP concentration measured at baseline of Alzheimer's disease (AD) was relatively strong indicator of AD pathology and accurately predicted future development of AD dementia. Significantly baseline levels were associated with the Aβ status; higher concentrations at baseline were observed in every Aβ-positive subgroup compared to Aβ-negative ones. Thus, suggesting that plasma GFAP is an accurate biomarker for AD diagnosis and progression. Mean GFAP detection range in plasma ranged from 36 to 94 pg/mL (11). UCH-L1 concentration has increased from 201.2 to 422.5 pg/mL in CSF of Amyloid Lateral Sclerosis (ALS) patients [12]. Also, several clinical studies have demonstrated that UCH-L1 concentration increases in blood within 4.5 hours of symptoms onset of ICH, IS and subarachnoid hemorrhage (SAH) patients from 20-50 pg/ml to 170, 130 and 210 pg/mL, respectively. Unlike GFAP, UCHL-1 did not show higher levels in ICH compared to IS. Furthermore, UCH-L1 concentration is increased from 241.1 to 1132.2 pg/mL in patients after cardiac arrest providing crucial prediction of neurological outcome after cardiac arrest [13]. Recently it has been observed that UCHL1 plasma levels in neuroendocrine carcinomas, such as small-cell lung carcinoma (SCLC) and neuroendocrine prostate cancer (NEPC) patients were significantly higher than those in patients with non-SCLC (NSCLC) and localized prostate adenocarcinoma, respectively. This observation is consistent with the high levels of UCH-L1 protein observed in these cancers. Taken together, these results indicate that UCH-L1 has the potential to be a diagnostic marker for these cancers [14].

Conclusion

In this white paper we show that the DxDATA™ TBI test meets the analytical performance required for aiding in the evaluation of mTBI patients. The detection range meets and outperforms some of the commercial mTBI diagnostic tests. We observe 100% positive agreement for TBI patients between DxDATA and Banyan ELISA suggesting equivalency. The kit test is stable for two years at 2-8°C, it detects at pg/mL levels and results for both biomarkers are obtained in 30 min. These characteristics suggest that the DxDATA platform is sensitive and specific enough to meet the regulatory requirements for the IVD market. Finaly, we discussed the potential use of this test for studying other neurological diseases.

References

1. Dewan CM, Rattani A, Gupta S, et al. Estimating the global incidence of traumatic brain injury. J Neurosurgery 2018 Apr 27;130(4):1080-1097.
2. Centers for Disease Control and Prevention. National Center for Health Statistics: Mortality data on CDC WONDER. Available at: https://wonder.cdc.gov/mcd.html.
3. Roozenbeek B, Maas AIR, and Menon DK. Changing patterns in the epidemiology of traumatic brain injury. Nat Rev Neurol. 2013; 9(4):231–6.
4. Maas RA, Menon KD, Manley TG, et al. Traumatic brain injury: progress and challenges in prevention, clinical care, and research. Lancet Neurol. 2022 Nov; 21(11): 1004–1060.
5. Papa L, Lewis ML, Silvestri S, et al. Serum levels of Ubiquitin C-terminal Hydrolase (UCH-L1) distinguish mild traumatic brain injury (TBI) from trauma controls and are elevated in mild and moderate TBI patients with intracranial lesions and neurosurgical intervention. J Trauma Acute Care Surg. 2012 May; 72(5): 1335–1344.
6. Bazarian JJ, Biberthaler P, Welch RD, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicenter observational study. Lancet Neurol. 2018 Sep;17(9):782-789.
7. Abdelhak A, Foschi M, Abu-Rumeileh S, Yue JK, D'Anna L, Huss A, et al. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. Nature Reviews. Neurology. 2022 March; 18 (3): 158–172.
8. Ayrignac X, Le Bars E, Duflos C, et al. Serum GFAP in multiple sclerosis: correlation with disease type and MRI markers of disease severity. Sci Rep. 2020 Jul 2;10(1):10923.
9. Abdelhak A, Huss A, Kassubek J, Tumani H, Otto M. Serum GFAP as a biomarker for disease severity in multiple sclerosis. Sci Rep. 2018 Oct 4;8(1):14798.
10. Foerch C, Niessner M, Back T, et al. Diagnostic accuracy of plasma glial fibrillary acidic protein for differentiating intracerebral hemorrhage and cerebral ischemia in patients with symptoms of acute stroke. Clin Chem. 2012 Jan;58(1):237-45.
11. Cicognola C, Janelidze S, Hertze J, et al. Plasma glial fibrillary acidic protein detects Alzheimer pathology and predicts future conversion to Alzheimer dementia in patients with mild cognitive impairment. Alzheimer's Res Ther. 2021 Mar 27;13(1):68.
12. Li R, Wang J, Xie W, Liu J, Wang C. UCHL1 from serum and CSF is a candidate biomarker for amyotrophic lateral sclerosis. Ann Clin Transl Neurol. 2020 Aug;7(8):1420-1428.
13. Ren C, Kobeissy F, Alawieh A, Li N, Li N, Zibara K, Zoltewicz S, Guingab-Cagmat J, Larner SF, Ding Y, Hayes RL, Ji X, Mondello S. Assessment of Serum UCH-L1 and GFAP in Acute Stroke Patients. Sci Rep. 2016 Apr 14;6:24588.
14. Liu S, Chai T, Garcia-Marques et al. UCHL1 is a potential molecular indicator and therapeutic target for neuroendocrine carcinomas. Cell Rep Med 2024 Feb 20;5(2):101381.

Product information:

DxDATA Automated Chemiluminescent Immunoassay Analyzing System	DXD-0001
Glial Fibrillary Acidic Protein (GFAP) CLIA Kit, 10 strips/kit	DXDA-1003-10
Protein Gene Product 9.5 (PGP 9.5) CLIA Kit, 10 strips/kit	DXDA-1004-10