REF Catalog No. BC2209

Banyan BTI™
Brain Trauma Indicator

For *in vitro* diagnostic use

℞ only

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1.0 Name

Banyan BTI™

2.0 Intended Use

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.

The Banyan BTI is for prescription use only.

3.0 Summary and Explanation of the Test

Traumatic brain injury (TBI) is an insult to the brain, caused by external physical force, that disrupts normal brain function, resulting in an impairment of cognitive abilities or physical functioning. In 2013, the Centers for Disease Control and Prevention (CDC) estimated approximately 2.8 million emergency department visits, hospitalizations, or deaths in the United States were due to TBI, either as an isolated injury or in combination with other injuries (CDC, 2017). It is a leading cause of death and disability in the United States, accounting for approximately 30% of injury-related deaths (Taylor CA, 2017).

The current medical standard of care for suspected TBI is to conduct a neurological assessment using the 15-point Glasgow Coma Scale (GCS) (American College of Surgeons Committee on Trauma, 1997) to assess the severity of a brain injury followed by structural neuroimaging, most commonly via CT scan of the head, to create a detailed view of the brain to visualize fractures and intracranial lesions.

Currently, the CT scan is the only objective, simple and reliable option widely available to assist clinicians in the evaluation of TBI; however, CT scans may increase the risk for radiation-induced carcinogenesis (Berrington de Gonzalez A, 2009). The severity of a TBI may range from mild to severe with mild TBI (GCS 13-15) accounting for 94.5% of all cases (Korley FK, 2016). Over 90% of patients presenting to the Emergency Department with mild TBI, sometimes described as “concussion”, have a negative CT scan (Toth, 2015). Moreover, no more than 1% of these patients will require neurosurgical intervention (Papa L, 2012). Given there is a very low percentage of positive CT scans in these patients, a simple and objective blood test would be exceedingly useful in ruling out the need for a CT scan of the head thus preventing unnecessary use of neuroimaging and associated radiation exposure to minimal risk patients. Additionally, such testing would effectively enrich the population of higher risk patients sent for CT imaging whom are more likely to have a CT positive lesion.

UCH-L1 and GFAP are two novel protein biomarkers that are brain specific and are detectable in the serum shortly after TBI. UCH-L1 is primarily found in neurons within the brain and is involved in cellular protein regulation (US National Library of Medicine, 2012). GFAP is a member of the intermediate filament family of cytoskeletal proteins which form polymeric networks that provide structural support to glia, which support and nourish cells in the brain and spinal cord (US National Library of Medicine, 2008).

4.0 Principles of the Procedure

The Banyan BTI measures UCH-L1 and GFAP concentrations in human serum using a 96-well microplate immunochemical chemiluminescent assay. The Banyan BTI consists of 2 separate kits, one for measuring UCH-L1 concentration, and one for measuring GFAP concentration. Both the Banyan UCH-L1 and Banyan GFAP kits are based on the same chemiluminescent sandwich immunoassay technique, which uses both capture and detection antibodies that recognize different epitopes on the target analyte.

Samples (clinical specimens, controls, or standards) are pipetted into wells of a microplate that are coated with either a UCH-L1 or GFAP-specific mouse monoclonal antibody that captures the target analyte (UCH-L1 or GFAP), thereby immobilizing the target analyte to the well. After washing away any unbound proteins, a second UCH-L1 or GFAP-specific mouse monoclonal antibody, which has been conjugated to the enzyme horseradish peroxidase (HRP), is added to the well. The HRP-conjugated antibody completes the immunochemical sandwich. After washing away unbound HRP-conjugated antibody, a chemiluminescent substrate is added to the well. The HRP enzyme catalyzes a specific reaction with the chemiluminescent substrate to produce light at 300 to 700 nm, which is detected with the Synergy 2 Multi-mode Reader, a 96-well plate-based luminometer. The amount of light generated is proportional to the amount of conjugated antibody in the well. The results from the wells containing standards are used to create a dose-response curve to quantify the amount of target analyte in the sample.

UCH-L1 and GFAP are measured in separate plates and, thus, Banyan UCH-L1 and Banyan GFAP results are reported separately. The operator uses the Banyan UCH-L1 and Banyan GFAP results to determine the Banyan BTI result.

5.0 Reagents

Banyan BTI

The Banyan BTI consists of 2 kits, one for Banyan UCH-L1 components, and one for Banyan GFAP components. In each kit, sufficient quantities of each component are provided to test samples from up to 30 patients. The contents of each kit are shown in **Table 1** and **Table 2** below.

Note: Components within the same kit are intended to be used together. **Do not mix components from different kit lots.**

Do not reuse. Kit components are single-use only. Unused residual reagents should be discarded.

Table 1. Materials Provided in the Banyan UCH-L1 Kit

Item	Description	Quantity
UCH-L1 Assay Plate	96-well microtiter strip plate, wells coated with mouse UCH-L1 monoclonal antibody (Ab).	1 microtiter plate
UCH-L1 Assay Diluent	Buffer with preservatives, clear colorless liquid.	2 vials, 5 mL per vial
UCH-L1 Detection Ab	HRP-conjugated mouse UCH-L1 monoclonal Ab in buffer, concentrate, amber-colored liquid.	1 vial, 0.23 mL
UCH-L1 Detection Ab Diluent	Buffer containing protein, red dye, and preservatives; red-colored liquid.	2 vials, 6.5 mL per vial
UCH-L1 Calibrator	Human UCH-L1 recombinant protein, lyophilized, green-colored.	1 vial
UCH-L1 Calibrator Diluent	Buffer with carrier protein and preservative (0.5% ProClin 300), clear colorless to light yellow liquid.	1 vial, 4 mL
UCH-L1 Control 1	Low control consisting of human UCH-L1 recombinant protein in buffer with carrier protein, lyophilized, yellow-colored.	1 vial
UCH-L1 Control 2	High control consisting of human UCH-L1 recombinant protein in buffer with carrier protein, lyophilized, yellow-colored.	1 vial
Substrate A	Chemiluminescent substrate solution A, clear colorless liquid.	2 vials, 4.5 mL per vial
Substrate B	Chemiluminescent substrate solution B, clear colorless liquid.	2 vials, 4.5 mL per vial
Wash tablet	PBS (phosphate-buffered-saline)-Tween, solid, white tablet.	1 tablet
Plate seals	Adhesive plate seal, clear.	4 seals

Table 2. Materials Provided in the Banyan GFAP Kit

Item	Description	Quantity
GFAP Assay Plate	96-well microtiter strip plate, wells coated with mouse GFAP monoclonal Ab.	1 microtiter plate
GFAP Assay Diluent	Buffer with protein and preservative (0.1% ProClin 300), clear colorless liquid.	1 vial, 10 mL
GFAP Detection Ab	HRP-conjugated mouse GFAP monoclonal Ab in buffer, concentrate, amber-colored liquid.	1 vial, 0.23 mL
GFAP Detection Ab Diluent	Buffer containing protein, red dye, and preservative (0.1% ProClin 300); red-colored liquid.	1 vial, 14 mL
GFAP Calibrator	Human GFAP native protein in phosphate buffer containing animal serum, dye, and preservative (0.125% ProClin 300); lyophilized, blue-colored.	1 vial
GFAP Calibrator Diluent	Phosphate buffer containing animal serum and preservative (0.125% ProClin 300), clear colorless to amber-colored liquid.	1 vial, 4 mL
GFAP Control 1	Low control consisting of human GFAP native protein in buffer containing animal serum, dye, and preservative (0.125% ProClin 300); lyophilized, orange-colored.	1 vial
GFAP Control 2	High control consisting of human GFAP native protein in buffer containing animal serum, dye, and preservative (0.125% ProClin 300); lyophilized, orange-colored.	1 vial
Substrate A	Chemiluminescent substrate solution A, clear colorless liquid.	2 vials, 4.5 mL per vial
Substrate B	Chemiluminescent substrate solution B, clear colorless liquid.	2 vials, 4.5 mL per vial
Wash tablet	PBS (phosphate-buffered-saline)-Tween, solid, white tablet.	1 tablet
Plate seals	Adhesive plate seal, clear.	4 seals

6.0 Warnings and Precautions

In vitro diagnostic medical device

- For in vitro diagnostic use only
- The Banyan BTI is indicated for use as an adjunct to standard clinical practice to aid in the evaluation of patients who are being considered for a head CT. While a negative assay result is highly associated with the absence of acute intracranial lesions visualized on a head CT scan, a head CT scan is required for a positive diagnosis of acute intracranial lesions.
- The Banyan UCH-L1 and Banyan GFAP procedures are nearly identical except that the clinical sample volume and Assay Diluent volume are different for each assay. See the **Banyan UCH-L1 Procedures** and **Banyan GFAP Procedures** sections for details.
- Pipetting accuracy and consistency when preparing the standard curve is critical to obtaining a valid standard curve.
- Any deviation from recommended test procedures, including modifying the plate read protocols or wash programs, could affect the performance of the Banyan BTI and may invalidate reported results.
- The Banyan BTI is to be used by laboratory professionals in a clinical laboratory setting.
- The assay was validated using the software versions noted in the **Materials Required but Not Provided** section. Contact Banyan Biomarkers Inc before modifying or upgrading beyond these software versions.

Caution. Handle all specimens and kit components containing biological materials (microtiter plates, calibrators, and controls) as though capable of transmitting infectious agents. The GFAP calibrators and controls are made with native protein derived from the human brain; blood from the tissue source was tested and found to be nonreactive for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) antibodies, and syphilis. Because blood screening tests cannot guarantee specimens with nonreactive results are truly negative for a pathogen, all materials derived from human sources should be handled as though capable of transmitting infectious agents.

- Dispose of all specimens, reagents, and other potentially biohazardous or hazardous materials in accordance with local, state, and federal regulations.
- Use good laboratory practices and follow the instructions herein to minimize the risk of contamination.

Consult the instrument manuals for warnings and precautions related to the instruments.

- Turn on automated operating system updates to keep operating system security patches up to date.

- Windows Defender, with automatic virus definition updates, is recommended on Windows 7 as the antivirus software. Other antivirus software can be used if it is specified by the laboratory's information technology policy.
- If plugging in a USB drive, turn off AutoPlay in Windows to reduce the likelihood of a virus spreading using this feature.
- On a regular basis, back up assay results by copying data to a separate, secure location.
- Administrative controls, including limiting physical access to the computer system to authorized personnel and roles based user privileges, are recommended to prevent unauthorized access and modification to the wash program, plate read protocols, and results.

6.1 Safety Precautions

Consult the instrument manuals for safety precautions related to the instruments.

Caution. Some components of the Banyan UCH-L1 and Banyan GFAP kits contain a hazardous chemical, ProClin 300. ProClin 300 is an irritant and contains a 3:1 mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4 isothiazolin-3-one.

The following are appropriate hazard and precautions for ProClin 300:

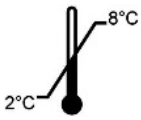
Hazard:	H317: May cause an allergic skin reaction
Precaution:	P261: Avoid breathing mist
	P272: Contaminated work clothing should not be allowed out of the workplace
	P280: Wear protective gloves/protective clothing
	P302+P352: If on skin: Wash with plenty of soap and water
	P333+P313: If skin irritation or rash occurs: get medical attention
	P363: Wash contaminated clothing before reuse

Refer to the safety data sheets available on the Banyan Biomarkers, Inc., website for more specific information.

Best practices for handling chemicals and potentially infectious substances should be followed.

- Avoid direct contact with the reagents.
- Ensure adequate ventilation.
- Do not eat, drink, chew gum, apply cosmetics, or handle contact lenses in the laboratory.
- Do not pipet by mouth.
- Wear disposable, powder-free protective gloves that are impermeable and resistant to substances handled. Dispose of contaminated gloves in accordance with good laboratory practices. Wash hands often including after removing gloves, before breaks, and before leaving the laboratory.
- Wear a lab coat and shoes that cover exposed skin. Immediately remove all contaminated clothing and wash before reuse.

7.0 Kit Storage Instructions



Store the Banyan UCH-L1 and Banyan GFAP kits as packaged and store at 2°C to 8°C until ready for use. **Do not store frozen.**

Do not reuse. Kit components are single-use only. Unused residual reagents should be discarded.

The kits are stable up until the expiration date printed on the container if stored properly. **Do not use expired kits**.

8.0 Indication of Instability or Deterioration of Reagents

If kit components do not appear as expected, or there is evidence of contamination (such as loose caps, damage of foil seals, loss of vacuum in Calibrator or Control vials), **do not use**.

Invalid results in the assay controls, blanks, or standards may indicate issues with the reagents.

9.0 Specimen Collection and Storage

Human serum processed from whole blood specimens collected within 12 hours of head injury may be used with the Banyan BTI. Whole blood collected in a BD SST blood collection tube must be used. A minimum volume of 250 µL of serum is needed to perform the Banyan BTI procedure. Follow the collection tube manufacturer's instructions for processing into serum.

If not tested immediately, serum can be stored at room temperature for up to 120 minutes. A minimum of 250µL of serum should also be stored immediately at -80°C in the event retesting is desired due to a Not Reportable result. Serum samples should not be subjected to more than 5 freeze/thaw cycles.

Note: High levels of hemoglobin, rheumatoid factor, or HAMA in serum may affect Banyan BTI results. See **Potentially Interfering Substances** section for further information.

10.0 Banyan BTI Procedure

10.1 Materials Provided

Banyan BTI (1 Banyan UCH-L1 Kit, 1 Banyan GFAP Kit), catalog number BC2209

Do not reuse. Single-use only.

10.2 Materials Required but Not Provided

Instruments/Equipment

Consult the instrument manuals for information relating to installation, operation, maintenance, and other details.

- BioTek Instruments, Inc., Synergy 2 Multi-mode Reader, model SL (plate reader)

- Personal computer with Microsoft Windows 7, Microsoft Excel 2013, and Gen5 IVD Microplate Reader and Imager software version 2.09.1
- BioTek Instruments, Inc., 405 TS Microplate Washer, model 405TSRSQ (plate washer)
- Boekel Scientific, Jitterbug 4, model 270440 (plate incubator/shaker)
- BD Vacutainer SST Venous Blood Collection tube
- Single-channel pipettes (nominal capacities of 100 µL, 200 µL, 1000 µL)
- Multichannel repeating pipette (nominal capacity 20 µL to 300 µL)
- Magnetic stir plate and stir bar
- Vortex mixer
- Timer
- Pipet aid
- Graduated cylinder, 1 L
- 1 L container

Consumables

- Serological pipettes, 10 mL
- Filtered pipette tips
- Microcentrifuge tubes, 1.7 mL, polypropylene
- Deionized (DI) water, 18 MΩ
- Conical tubes, clear and amber, 50 mL, polypropylene
- Reagent troughs
- Kimwipes
- Disposable gloves, powder-free

Note: Performance characteristics of pipettes used must comply with ISO 8655-2.

Other

- Banyan UCH-L1 plate read protocol (PN 200011) for the BioTek Gen5 plate reader software
- Banyan GFAP plate read protocol (PN 200012) for the BioTek Gen5 plate reader software
- Banyan BTI plate wash program (PN 200003) for the BioTek 405 TS plate washer

10.3 Optional Materials Not Provided

- Wedge-type barcode scanner

10.4 Procedural Notes

- Initiation of the Banyan UCH-L1 and Banyan GFAP procedures should be staggered 15 to 30 minutes apart since the plate reader can read only 1 plate at a time.
- For each kit, prepare the Detection Ab Solution while the reconstituted Calibrator and Controls are incubating.
- For each kit, the run must be completed within the time and temperature limits specified herein.
- Sample IDs can be manually entered or imported into the Gen5 plate reader software. From the Gen-5 Experiment File Menu, select File > Sample IDs to reach the Sample ID window. Sample IDs can be imported from a text file, or manually entered from this window. If imported from a text file, only Sample IDs should be listed in the txt file (list ID once for every 2 wells plated), starting with the first sample tested in the plate, and ending with the last sample in the plate.

- Deviations from the instructions provided herein may lead to invalid or erroneous results.
- Room temperature is 15°C to 30°C.
- Kit components must be from the same master kit lot.
- Before testing, kit components and specimen(s) should be at room temperature and mixed (if appropriate).
- Do not use distilled water.
- Avoid contamination of reagents and equipment.
- Use properly calibrated instruments/equipment and maintain instruments/equipment in accordance with the manufacturer's recommendations.
- Unless otherwise indicated, volumetric measurements are to be made with a single channel pipette.
- To avoid invalid or erroneous results and reduce variability, use good laboratory practices and the following precautions:
    - Wear disposable, powder-free gloves. Handle the assay plate by touching the sides of the plate only. Avoid touching the wells.
    - Use filtered pipette tips
    - Clean bench area and pipettes prior to each run.
    - Avoid contamination. Pipet carefully. After pipetting into a well that contains sample (e.g., Standard, Control, or clinical sample), eject the tip and acquire a new tip for the next dispense.
    - The plate read is performed with a plate seal covering the wells. Use a new plate seal if glove contact with the adhesive side of the seal occurs, or if the seal is not placed correctly the first time.

10.5 Banyan UCH-L1 Procedures

Procedures for the Banyan UCH-L1 kit are described starting on step 1. The Banyan GFAP procedures are described starting on step 49. The Banyan GFAP procedure is identical to the Banyan UCH-L1 procedure except that components from the Banyan GFAP kit are used, and the sample volume and Assay Diluent volume are different.

Consult the instrument manuals for details on instrument operation.

Before Starting the Banyan UCH-L1 Procedure

1. If needed, contact Banyan Biomarkers, Inc to have the Banyan UCH-L1 plate read protocol (PN 200011) installed in the Gen5 application.
2. If needed, contact Banyan Biomarkers, Inc to have the Banyan BTI wash program (PN 200003) installed on the microplate washer.
3. Remove the Banyan UCH-L1 Kit from storage and place at room temperature for 45 ± 15 minutes.
4. Turn on the incubator/shaker. Set the temperature to 37°C and the speed to 500 rpm.
5. Prepare the wash buffer. In a graduated cylinder, measure 1 L of 18 MΩ deionized (DI) water. Add the measured DI water, Wash Tablet, and a magnetic stir bar to a 1 L container.

Stir on magnetic stir plate for at least 15 minutes with enough speed to create a vortex. Prepare fresh wash buffer as needed, no less than once per testing day.

6. Turn on the plate washer. Fill the supply bottle with the prepared wash buffer. If a system flush has not been performed on the day of testing, conduct a system prime of the washer per the microplate washer operator's manual.
7. Turn on the reader and the associated personal computer.
8. Clean the bench area and pipettes per standard lab procedures.

Banyan UCH-L1 Procedure

Clinical Sample, UCH-L1 Calibrator, and UCH-L1 Control Preparation

9. Ensure a minimum sample volume of 100µL is available. **Note**: Up to 30 clinical samples can be tested in duplicate per run.
10. To release the vacuum from the UCH-L1 Calibrator vial, slowly pull up the stopper. Reconstitute the lyophilized UCH-L1 Calibrator by adding DI water to the UCH-L1 Calibrator vial using the reconstitution volume indicated on vial label. Cap the vial with the stopper. **Do not vortex.** Swirl the vial by hand for 10 to 15 seconds. Invert the vial to wash off material from the stopper. Incubate for 25 ± 5 minutes at room temperature. **Note:** The reconstituted UCH-L1 Calibrator must be plated within 60 minutes following the room temperature incubation period.
11. To release the vacuum from the UCH-L1 Control vials (UCH-L1 Control 1 and UCH-L1 Control 2), slowly pull up the stoppers. Reconstitute the lyophilized UCH-L1 Controls by adding DI water to each of the UCH-L1 Control vials using the reconstitution volume indicated on the respective vial label. Cap the vials with the original stoppers. **Do not vortex.** Swirl the vials by hand for 10 to 15 seconds. Invert the vials to wash off material from the stoppers. Incubate for 25 ± 5 minutes at room temperature. **Note:** The reconstituted UCH-L1 Controls must be used within 60 minutes following the room temperature incubation period.

Preparation of UCH-L1 Detection Ab Solution

12. Vortex the UCH-L1 Detection Ab Diluent and UCH-L1 Detection Ab vials at the highest setting for 1 second.
13. In a 50-mL clear conical tube, combine the UCH-L1 Detection Ab Diluent and UCH-L1 Detection Ab as follows:

    13.1. Using a serological pipette, add 12.8 mL of UCH-L1 Detection Ab Diluent to the conical tube.

    13.2. Using a micropipette, add 200 µL of UCH-L1 Detection Ab to the same tube.

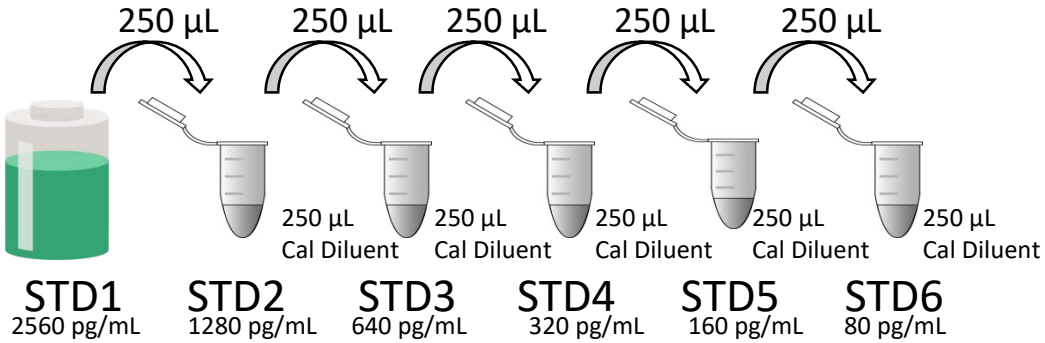
14. Vortex the mixture at the highest setting for 1 second. Store at room temperature until use.

Preparation of UCH-L1 Assay Standards

15. Label 5 microcentrifuge tubes "STD2" through "STD6". **Note:** STD1 will be the reconstituted glass vial.
16. Vortex the UCH-L1 Calibrator Diluent at the highest setting for 1 second.
17. Add UCH-L1 Calibrator Diluent to STD2 through STD6 tubes as indicated in **Figure 1**.

18. Swirl the reconstituted UCH-L1 Calibrator vial by hand to mix the solution. **Do not vortex.** Prepare the Standards using serial dilutions as described in **Figure 1**, starting with the highest concentration (STD1; the reconstituted UCH-L1 Calibrator). Before preparation of the subsequent dilution, vortex each Standard, except STD1, at the highest setting for 1 second.

Figure 1. Preparation of UCH-L1 Assay Standards



UCH-L1 Assay Plate Setup

19. Vortex the UCH-L1 Assay Diluent at the highest setting for 1 second.
20. Use a multi-channel pipette to add 75 µL of UCH-L1 Assay Diluent to all wells of the microplate that will contain samples (Standards, UCH-L1 Controls, blanks, clinical samples), **except row A**. See **Figure 2** below. When adding UCH-L1 Assay Diluent to the plate, start with column 1, and move left to right. The left to right order is necessary to match the scanning direction of the reader, ensuring consistent performance.

UCH-L1 Assay Standards, Blank, and Control Plating

21. Vortex the prepared Standard tubes (**except STD1**) and UCH-L1 Calibrator Diluent at the highest setting for 1 second.
22. Swirl the UCH-L1 Control 1 and UCH-L1 Control 2 vials by hand to mix the solutions. **Do not vortex**.
23. Using a single-channel micropipette with a new tip and being careful not to pipette onto the wall of the well, add 25 µL of each Standard, UCH-L1 Control 1, UCH-L1 Control 2, or UCH-L1 Calibrator Diluent (as blank) to the wells of the plate according to the plate layout in **Figure 2** below. Each item is tested in duplicate. Pipette straight down. **Do not mix the sample in the well.**

Figure 2. UCH-L1 Assay Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
B	STD1	STD1	STD2	STD2	STD3	STD3	STD4	STD4	STD5	STD5	STD6	STD6
C	CTL1	CTL1	CTL2	CTL2	BLK	BLK	BLK	BLK	BLK	BLK	BLK	BLK
D	SPL1	SPL1	SPL2	SPL2	SPL3	SPL3	SPL4	SPL4	SPL5	SPL5	SPL6	SPL6
E	SPL7	SPL7	SPL8	SPL8	SPL9	SPL9	SPL10	SPL10	SPL11	SPL11	SPL12	SPL12
F	SPL13	SPL13	SPL14	SPL14	SPL15	SPL15	SPL16	SPL16	SPL17	SPL17	SPL18	SPL18
G	SPL19	SPL19	SPL20	SPL20	SPL21	SPL21	SPL22	SPL22	SPL23	SPL23	SPL24	SPL24
H	SPL25	SPL25	SPL26	SPL26	SPL27	SPL27	SPL28	SPL28	SPL29	SPL29	SPL30	SPL30

BLK = blank (UCH-L1 Calibrator Diluent), CTL= UCH-L1 Control, SPL = clinical sample

Specimen Plating

24. Vortex each specimen at the highest setting for 1 second prior to plating.
25. Using a single-channel pipette with a new tip and being careful not to pipette onto the wall of the well, add 25 µL of each specimen according to the plate layout in **Figure 2**, starting with well D1, moving left to right across the row, and then down the plate. Each clinical sample is tested in duplicate. Pipette straight down. **Do not mix the sample in the well.**
26. Seal the plate with an adhesive plate seal ensuring all wells are covered.

Incubation 1

27. Load the plate into the shaker/incubator.
28. Incubate for 60 ± 5 minutes at 37°C and shake at a speed of 500 rpm.

Wash 1

29. Remove the plate seal carefully to avoid cross-contamination across wells. **Do not spin the plate**.
30. Wash the plate on the washer using the preprogrammed Banyan BTI wash program.
31. After washing, invert the plate and tap it on a clean Kimwipe to remove excess liquid from the assay wells.

UCH-L1 Detection Ab Solution Plating

32. Vortex the UCH-L1 Detection Ab Solution at the highest setting for 1 second.
33. Pour the UCH-L1 Detection Ab Solution into a reagent trough.
34. Use a multichannel pipette to add 100 µL of UCH-L1 Detection Ab Solution to all wells containing a sample (Standards, UCH-L1 Controls, blanks, and clinical samples). When adding Detection Ab Solution to the plate, start with column 1, and move left to right. The left to right order is necessary to match the scanning direction of the reader, ensuring consistent performance.
35. Seal the plate with a new adhesive plate seal ensuring all wells are covered.

Incubation 2

36. Load the plate into the shaker/incubator.
37. Incubate for 60 ± 5 minutes at 37°C and shake at a speed of 500 rpm.
38. During the last 10 minutes of the UCH-L1 Detection Ab Solution incubation, prepare the Substrate Solution (see next steps).

Substrate Solution Preparation

39. Vortex Substrate Part A and Substrate Part B at the highest setting for 1 second.
40. Using a serological pipette, add 8 mL of Substrate Part A and 8 mL of Substrate Part B into a 50-mL amber conical tube. **Note:** Assay substrates are photosensitive; protect Substrates and Substrate Solution from exposure to light.
41. Vortex the Substrate Solution at the highest setting for 1 second.

Wash 2

42. Remove the plate seal carefully to avoid cross-contamination across wells. **Do not spin the plate**.
43. Wash the plate using the preprogrammed Banyan BTI wash program.
44. After washing, invert the plate and tap it on a clean Kimwipe to remove excess liquid from the assay wells.

Substrate Solution Addition and Plate Incubation/Read

45. Pour the Substrate Solution into a reagent trough. Using a multichannel pipette, immediately add 150 µL of the Substrate Solution to all wells containing a sample (Standard, UCH-L1 Controls, blanks, or clinical samples). When adding Substrate Solution to the plate, start with column 1, and move left to right. The left to right order is necessary to match the scanning direction of the reader, ensuring consistent performance. **Note:** The Banyan UCH-L1 plate read protocol must be started within 5 minutes of plating of the Substrate Solution.
46. Seal the plate with a new adhesive plate seal ensuring all wells are covered and no wrinkles or bubbles are present.
47. Load the plate into the plate reader. Note: If the temperature of the plate reader is over 25.5°C, the operator must manually override to read the plate. To do this, when prompted, select override and accept.
48. Begin the preprogrammed Banyan UCH-L1 plate read protocol.

10.6 Banyan GFAP Procedures

The Banyan GFAP procedure is identical to the Banyan UCH-L1 procedure except that components from the Banyan GFAP kit are used, and the sample volume and Assay Diluent volume are different.

Consult the instrument manuals for details on operating instruments.

Before Starting the Banyan GFAP Procedure

49. If needed, contact Banyan Biomarkers, Inc to have the Banyan GFAP plate read protocol (PN 200012) installed in the Gen5 application.
50. If needed, contact Banyan Biomarkers, Inc to have the Banyan BTI wash program (PN 200003) installed on the microplate washer.
51. Remove the Banyan GFAP Kit from storage and place at room temperature for 45 ± 15 minutes.
52. As needed, turn on the incubator/shaker. Set the temperature to 37°C and the speed to 500 rpm.
53. Prepare the wash buffer. In a graduated cylinder, measure 1 L of 18 MΩ deionized (DI) water. Add the measured DI water, Wash Tablet, and a magnetic stir bar to a 1 L container. Stir on magnetic stir plate for at least 15 minutes with enough speed to create a vortex. Prepare fresh wash buffer as needed, no less than once per testing day.
54. As needed, turn on the plate washer. Fill the supply bottle with the prepared wash buffer. If a system flush has not been performed on the day of testing, conduct a system prime of the washer per the microplate washer operator's manual.
55. As needed, turn on the reader and the associated personal computer.
56. Clean the bench area and pipettes per standard lab procedures.

Banyan GFAP Procedure

Specimen, GFAP Calibrator, and GFAP Control Preparation

57. Ensure a minimum sample volume of 150 µL is available. **Note**: Up to 30 clinical samples can be tested per run.
58. To release the vacuum from the GFAP Calibrator vial, slowly pull up the stopper. Reconstitute the lyophilized GFAP Calibrator by adding DI water to the GFAP Calibrator vial using the reconstitution volume indicated on vial label. Cap the vial with the stopper. **Do not vortex.** Swirl the vial by hand for 10 to 15 seconds. Invert the vial to wash off material from the stopper. Incubate for 25 ± 5 minutes at room temperature. **Note**: The reconstituted GFAP Calibrator must be plated within 60 minutes following the room temperature incubation period.
59. To release the vacuum from the GFAP Control vials (GFAP Control 1 and GFAP Control 2), slowly pull up the stoppers. Reconstitute the lyophilized GFAP Controls by adding DI water to each of the GFAP Control vials using the reconstitution volume indicated on the respective vial label. Cap the vials with the original stoppers. **Do not vortex.** Swirl the vials by hand for 10 to 15 seconds. Invert the vials to wash off material from the stoppers. Incubate for 25 ± 5 minutes at room temperature. **Note**: The reconstituted GFAP Controls must be used within 60 minutes following the room temperature incubation period.

Preparation of GFAP Detection Ab Solution

60. Vortex the GFAP Detection Ab Diluent and GFAP Detection Ab vials at the highest setting for 1 second.
61. In a 50-mL clear conical tube, combine the GFAP Detection Ab Diluent and GFAP Detection Ab as follows:

61.1. Using a serological pipette, add 12.8 mL of GFAP Detection Ab Diluent to the conical tube.

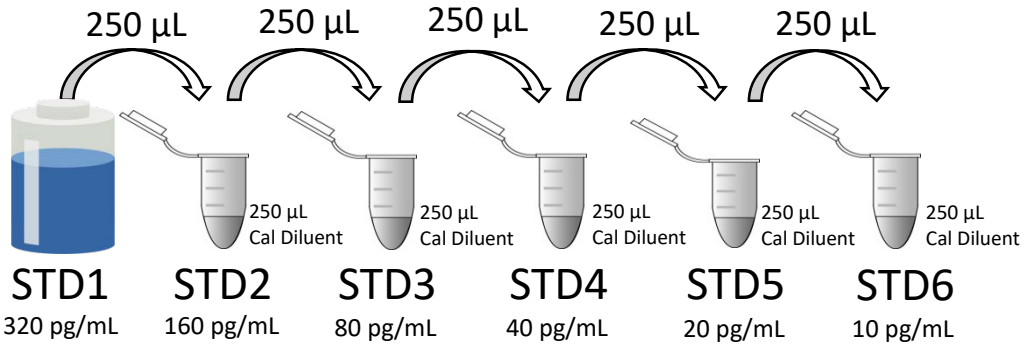
61.2. Using a micropipette, add 200 µL of GFAP Detection Ab to the same tube.

62. Vortex the mixture at the highest setting for 1 second. Store at room temperature until use.

Preparation of GFAP Assay Standards

63. Label 5 microcentrifuge tubes "STD2" through "STD6". **Note:** STD1 will be the reconstituted glass vial.
64. Vortex the GFAP Calibrator Diluent at the highest setting for 1 second.
65. Add GFAP Calibrator Diluent to STD2 through STD6 tubes as indicated in **Figure 3**.
66. Swirl the reconstituted GFAP Calibrator vial by hand to mix the solution. **Do not vortex**.
67. Prepare the Standards using serial dilutions as described in **Figure 3**, starting with the highest concentration (STD1; the reconstituted GFAP Calibrator). Before preparation of the subsequent dilution, vortex each Standard, **except STD1**, at the highest setting for 1 second.

Figure 3. Preparation of GFAP Assay Standards



GFAP Assay Plate Setup

68. Vortex the GFAP Assay Diluent at the highest setting for 1 second.
69. Use a multi-channel pipette to add 50 µL of GFAP Assay Diluent to all wells of the microplate containing samples (Standards, GFAP Controls, blanks, clinical samples), **except row A**. See **Figure 4** below. When adding GFAP Assay Diluent to the plate, start with column 1, and move left to right. The left to right order is necessary to match the scanning direction of the reader, ensuring consistent performance.

GFAP Assay Standards, Blank, and Control Plating

70. Vortex the prepared Standard tubes (**except STD1**) and GFAP Calibrator Diluent at the highest setting for 1 second.
71. Swirl the GFAP Control 1 and GFAP Control 2 vials by hand to mix the solutions. **Do not vortex**.

72. Using a single-channel micropipette with a new tip and being careful not to pipette onto the wall of the well, add 50 µL of each Standard, GFAP Control 1, GFAP Control 2, or GFAP Calibrator Diluent (as blank) to the wells of the plate according to the plate layout in **Figure 4** below. Each item is tested in duplicate. Pipette straight down. **Do not mix the sample in the well**.

Figure 4. GFAP Assay Plate Layout

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
B	STD1	STD1	STD2	STD2	STD3	STD3	STD4	STD4	STD5	STD5	STD6	STD6
C	CTL1	CTL1	CTL2	CTL2	BLK	BLK	BLK	BLK	BLK	BLK	BLK	BLK
D	SPL1	SPL1	SPL2	SPL2	SPL3	SPL3	SPL4	SPL4	SPL5	SPL5	SPL6	SPL6
E	SPL7	SPL7	SPL8	SPL8	SPL9	SPL9	SPL10	SPL10	SPL11	SPL11	SPL12	SPL12
F	SPL13	SPL13	SPL14	SPL14	SPL15	SPL15	SPL16	SPL16	SPL17	SPL17	SPL18	SPL18
G	SPL19	SPL19	SPL20	SPL20	SPL21	SPL21	SPL22	SPL22	SPL23	SPL23	SPL24	SPL24
H	SPL25	SPL25	SPL26	SPL26	SPL27	SPL27	SPL28	SPL28	SPL29	SPL29	SPL30	SPL30

BLK = blank (GFAP Calibrator Diluent), CTL= GFAP Control, SPL = clinical sample

Specimen Plating

73. Vortex each specimen at the highest setting for 1 second prior to plating.
74. Using a single-channel pipette with a new tip and being careful not to pipette onto the wall of the well, add 50 µL of each specimen according to the plate layout in **Figure 4**, starting with well D1, moving left to right across the row, then down the plate, and ending with well H12. Each clinical sample is tested in duplicate. Pipette straight down. **Do not mix the sample in the well**.
75. Seal the plate with an adhesive plate seal ensuring all wells are covered.

Incubation 1

76. Load the plate into the shaker/incubator.
77. Incubate for 60 ± 5 minutes at 37°C and shake at a speed of 500 rpm.

Wash 1

78. Remove the plate seal carefully to avoid cross-contamination across wells. **Do not spin the plate**.
79. Wash the plate on the washer using the preprogrammed Banyan BTI wash program.
80. After washing, invert the plate and tap it on a clean Kimwipe to remove excess liquid from the assay wells.

GFAP Detection Ab Solution Plating

81. Vortex the GFAP Detection Ab Solution at the highest setting for 1 second.
82. Pour the GFAP Detection Ab Solution into a reagent trough.

83. Use a multichannel pipette to add 100 µL of GFAP Detection Ab Solution to all wells containing a sample (Standards, GFAP Controls, blanks, and clinical samples). When adding Detection Ab Solution to the plate, start with column 1, and move left to right. The left to right order is necessary to match the scanning direction of the reader, ensuring consistent performance.
84. Seal the plate with a new adhesive plate seal ensuring all wells are covered.

Incubation 2

85. Load the plate into the shaker/incubator.
86. Incubate for 60 ± 5 minutes at 37°C and shake at a speed of 500 rpm.
87. During the last 10 minutes of the GFAP Detection Ab Solution incubation, prepare the Substrate Solution (see next steps).

Substrate Solution Preparation

88. Vortex Substrate Part A and Substrate Part B at the highest setting for 1 second.
89. Using a serological pipette, add 8 mL of Substrate Part A and 8 mL of Substrate Part B into a 50-mL amber conical tube. **Note**: Assay substrates are photosensitive; protect substrates and Substrate Solution from exposure to light.
90. Vortex the Substrate Solution at the highest setting for 1 second.

Wash 2

91. Remove the plate seal carefully to avoid cross-contamination across wells. **Do not spin the plate**.
92. Wash the plate using the preprogrammed Banyan BTI wash program.
93. After washing, invert the plate and tap it on a clean Kimwipe to remove excess liquid from the assay wells.

Substrate Solution Addition and Plate Incubation/Read

94. Pour the Substrate Solution into a reagent trough. Using a multichannel pipette, immediately add 150 µL of the Substrate Solution to all wells containing a sample (Standards, GFAP Controls, blanks, or clinical samples). When adding Substrate Solution to the plate, start with column 1, and move left to right. The left to right order is necessary to match the scanning direction of the reader, ensuring consistent performance. **Note**: The Banyan GFAP plate read protocol must be started within 5 minutes of plating of the Substrate Solution.
95. Seal the plate with a new adhesive plate seal ensuring all wells are covered and no wrinkles or bubbles are present. If there are wrinkles or bubbles, carefully remove the seal and apply a new seal.
96. Load the plate into the plate reader. **Note**: If the temperature of the plate reader is over 25.5°C, the operator must manually override to read the plate. To do this, when prompted, select override and accept.
97. Begin the preprogrammed Banyan GFAP plate read protocol.

11.0 Clean-up and Disposal

After testing is completed for the day, clean up and dispose of all waste according to standard practices/procedures.

Consult the instrument manual. Microplate washers require daily maintenance after use. Refer to the microplate washer operator's manual for details.

12.0 Quality Control Procedures

Run validity is automatically determined by the reader software. Valid Standard curve parameters, assay Controls, and blank samples are required in each run as shown in **Figure 2** and **Figure 4**. If any of the run validity parameters do not meet pre-determined requirements, the run is not valid. All clinical samples tested in an invalid run have Invalid results, thus, concentrations are not reportable. Clinical samples with Invalid results that lead to a Not Reportable result may be retested once to obtain a Positive or Negative Banyan BTI result (**Table 3**).

13.0 Results

The Banyan BTI is a semi-quantitative assay.

After samples are tested with the Banyan UCH-L1 or Banyan GFAP kits, the reader software processes the data generated. For each well containing sample, the concentration of the target analyte in pg/mL is determined. Then, results from duplicate samples are averaged. A report is automatically generated for each kit by the plate reader software.

The validity of results for each clinical sample tested is automatically determined by the reader software. If the results for a clinical sample meet predetermined validity criteria, the validity status is valid, and the concentration of the analyte is reported along with the categorization (Above or Below) of the concentration relative to the cutoff value (327 pg/mL for the Banyan UCH-L1 Kit and 22 pg/mL for the Banyan GFAP Kit[1]), if the concentration is between the pre-established lower and upper limits of quantitation. If the concentration is above the reportable range for UCH-L1 or GFAP, the concentration will be reported as >2560 pg/mL or >320 pg/mL, respectively, and the result will be reported as above the cutoff value (i.e., Above). If the concentration is below the reportable range for UCH-L1 or GFAP, the concentration will be reported as <80 pg/mL or <10 pg/mL, respectively, and the result will be reported as below the cutoff value (i.e., Below). The Banyan UCH-L1 and Banyan GFAP results are combined to determine the Banyan BTI result (see **Interpretation of Results**).

If the results for a clinical sample do not meet predetermined validity criteria, the validity is invalid, and the result is Invalid. If a clinical sample causes a run to abort, the validity for all samples located in the microtiter plate row containing the sample that caused the abort and all samples in subsequent rows on the microtiter plate is invalid, and the result is No Result. Clinical samples with Invalid results or No Result (e.g., from an aborted run) that lead to a Not Reportable Banyan BTI result may be retested once to obtain a Positive or Negative Banyan BTI result (**Table 3**).

[1] "Above" means the target analyte concentration is equal to or above the cutoff; "Below" means the target analyte concentration is below the cutoff.

14.0 Interpretation of Results

The Banyan BTI is a semi-quantitative assay. Banyan UCH-L1 and Banyan GFAP results are reported separately. The reader software reports the calculated concentration of the target analyte and whether that concentration is below or above the cutoff value for that analyte. The reader does not report a Banyan BTI result. The Banyan BTI result must be interpreted according to **Table 3**.

Table 3. Interpretation of Banyan UCH-L1 and Banyan GFAP Results

Banyan UCH-L1 Assay Result (relative to cutoff)[A]	**Banyan GFAP Assay Result (relative to cutoff)[B]**	**Banyan BTI Result**
Below	Below	Negative
Below	Above	Positive
Above	Below	Positive
Above	Above	Positive
Invalid or No Result	Below	Not Reportable[C]
Invalid or No Result	Above	Positive
Below	Invalid or No Result	Not Reportable[C]
Above	Invalid or No Result	Positive
Invalid or No Result	Invalid or No Result	Not Reportable[C]

[A] **Above** means the UCH-L1 concentration is equal to or above 327 pg/mL; **Below** means the UCH-L1 concentration is below 327 pg/mL.

[B] **Above** means the GFAP concentration is equal to or above 22 pg/mL; **Below** means the GFAP concentration is below 22 pg/mL.

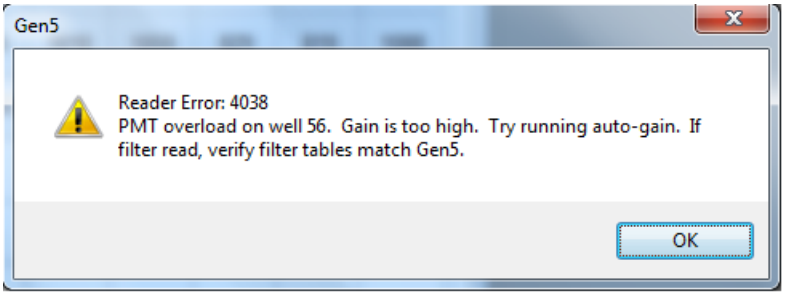
[C] Clinical samples with **Invalid Results** that yield a **Not Reportable** Banyan BTI result may be retested once to obtain a **Negative** or **Positive** result. For clinical samples with **No Result** that yield a **Not Reportable** Banyan BTI result, see **Troubleshooting**.

15.0 Troubleshooting

15.1 Reader Error: 4XXX

Reader Error: 4XXX occurs when a run is aborted. The reader will abort the run to prevent sensor damage from light generated from a sample that contains a very high concentration of target analyte. In this situation, the resulting report lists “No Result” for all samples located in the microtiter plate row containing the sample that caused the abort, and for all samples in subsequent rows on the microtiter plate. When this occurs, the reader software generates an error message that includes an error code and the number of the well that triggered the run abort (**Figure 5**).

Figure 5. Example Reader Error: 4XXX



There is no need to interpret the reader error code. The sample associated with the well that triggered the run abort can be determined by using the well number provided in the error message as follows:

1. Identify the well number provided in the error message, which is well 56 in the example in **Figure 5**.
2. Identify the Run Abort Well/Sample using the plate map in **Figure 6**. Using the example in **Figure 5**, sample 10 (SPL10), which was plated in well 56, triggered the run abort.

Figure 6. Reader Error Code Sample Identification

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	--	--	--	--	--	--	--	--	--	--	--	--
B	--	--	--	--	--	--	--	--	--	--	--	--
C	--	--	--	--	--	--	--	--	--	--	--	--
D	37	38	39	40	41	42	43	44	45	46	47	48
	SPL1		SPL2		SPL3		SPL4		SPL5		SPL6	
E	49	50	51	52	53	54	55	56	57	58	59	60
	SPL7		SPL8		SPL9		SPL10		SPL11		SPL12	
F	61	62	63	64	65	66	67	68	69	70	71	72
	SPL13		SPL14		SPL15		SPL16		SPL17		SPL18	
G	73	74	75	76	77	78	79	80	81	82	83	84
	SPL19		SPL20		SPL21		SPL22		SPL23		SPL24	
H	85	86	87	88	89	90	91	92	93	94	95	96
	SPL25		SPL26		SPL27		SPL28		SPL29		SPL30	

3. The result (relative to cutoff) for the clinical sample that triggered the run abort shall be considered Above. The Banyan BTI result for the applicable sample is then re-interpreted using the rules in **Table 3**.
4. All other clinical samples with No Result may be retested once using a new kit to obtain a Negative or Positive Banyan BTI result. The Banyan BTI results for the retested samples are re-interpreted using the rules in **Table 3**.

15.2 Other Reader Error Codes

Please refer to the Synergy 2 manual for information on all other reader error codes.

16.0 Limitations of the Procedure

- The Banyan BTI is not intended to be used as a stand-alone device but as an adjunct to other clinical information to aid in the evaluation of patients who are being considered for standard of care neuroimaging.
- Due to cross-reactivity of neurofilament light with the antibodies in the Banyan GFAP Kit, patients with neurodegenerative disease states such as Guillain-Barré syndrome, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, or Creutzfeldt-Jakob disease may have erroneously high Banyan GFAP results, potentially leading to a false-positive Banyan BTI result.
- Levels of hemoglobin, rheumatoid factor, or HAMA exceeding the normal physiological concentration or activity in serum may have erroneously high Banyan UCH-L1 or Banyan GFAP results, potentially leading to a false positive Banyan BTI result.
- A negative result is generally associated with the absence of acute intracranial lesions. An appropriate neuroimaging method is required for diagnosis of acute intracranial lesions.
- Customers must independently validate a data transfer process.
- Washers other than the 405 TS Microplate Washer (BioTek Instruments, Inc.) were not evaluated. Incomplete washing may adversely affect the test outcome.
- Incubators/shakers other than the Jitterbug 4 plate incubator/shaker (Boekel Scientific) were not evaluated. Incubation/shaking under conditions deviating from those specified herein may adversely affect the test outcome.
- Inappropriate specimen collection, handling, preparation, storage, and transport may negatively impact assay performance.
- Human serum specimens only are indicated for use. The use of plasma has not been validated and may adversely affect the test outcome.
- This device is for use by laboratory professionals in a clinical laboratory setting.

17.0 Traceability and Value Assignment

There is no recognized standard or reference material for UCH-L1 and GFAP. The calibrator and control values are traceable to in-house Reference Standards prepared from commercially available antigens for GFAP (HyTest GFAP antigen) and UCH-L1 (Origene UCH-L1 antigen).

The calibrators and positive controls are prepared from antigen that has been value assigned against the Reference Standard. The GFAP calibrators are prepared at six different levels with native protein purified from the human brain and assigned values ranging from 10−320 pg/mL. The UCH-L1 calibrators are prepared at six different levels from human UCH-L1 recombinant protein and assigned values ranging from 80−2560 pg/mL. The nominal values for the controls are assigned using the released calibrators. Lot-to-lot consistency is achieved by adjusting the reconstitution volume of the calibrator so that the reported results for QC Reference Panel members are within the predefined acceptable tolerance of the nominal assigned values. QC Reference Panel members are prepared from GFAP or UCH-L1-positive sera whose concentration is directly traceable to the Reference Standards.

18.0 Expected Values/Reference Range

A reference range study was conducted in the general population. Serum samples were tested using the Banyan BTI. The study demonstrated that 82% of the general population would be expected to have a negative Banyan BTI result, with no significant differences observed across race or gender. No substantial difference was observed in Banyan UCH-L1 or Banyan GFAP levels between genders. A slight trend of increasing biomarker levels was observed with age, especially over the age of 60 years. Although 18% of the general population was found to have a positive Banyan BTI result, it is important to note Banyan UCH-L1 and Banyan GFAP cut-off levels for the Banyan BTI were optimized in a population of head injured patients, not the general population.

It is recommended that each laboratory establish its own reference range, which may be unique to the population it serves depending on geographical or patient factors.

Table 4. Expected Values/Reference Range for Gender and Age

All Subjects		Age group (years)						
		<=30	**31−40**	**41−50**	**51−60**	**>=61**	**Age Unknown**	**All Subjects**
		(N=250)	(N=126)	(N=130)	(N=112)	(N=66)	(N=11)	(N=695)
Banyan UCH-L1 Result (pg/mL)								
Male	N	110	44	55	42	31	7	289
	Mean (SD)	174.9 (271.7)	113.9 (156.4)	139.0 (208.4)	141.7 (169.1)	120.6 (47.5)	163.8 (175.9)	147.9 (212.7)
	Median	82.2	80[A]	80[A]	89.3	105.7	88.4	83.9
	Min, Max	80[A], 2239	80[A], 1119	80[A], 1491	80[A], 1137	80[A], 252	80[A], 557	80[A], 2239
	2.5[th], 97.5th Percentile	80[A], 888.0	80[A], 160.3	80[A], 683.7	80[A], 320.1	80[A], 252.1	80[A], 556.9	80[A], 782.0
Female	N	139	82	75	70	35	4	405
	Mean (SD)	136.1 (186.6)	103.1 (69.4)	117.4 (84.5)	110.1 (57.0)	172.7 (245.3)	107.8 (57.6)	124.4 (142.1)
	Median	80[A]	80[A]	81.2	89.8	120.9	80[A]	80[A]
	Min, Max	80[A], 1377	80[A], 622	80[A], 485	80[A], 355	80[A], 1534	80[A], 194	80[A],1534
	2.5th, 97.5th Percentile	80[A], 774.1	80[A], 226.7	80[A], 461.7	80[A], 342.7	80[A], 1534.3	80[A], 194.2	80[A], 431.6
Unknown	N	1	0	0	0	0	0	1
	Mean (SD)	80[A] (-)	--	--	--	--	--	80[A] (-)
	Median	80[A]	--	--	--	--	--	80[A]
	Min, Max	80[A], 80[A]	--	--	--	--	--	80[A], 80[A]
	2.5th, 97.5th Percentile	80[A], 80[A]	--	--	--	--	--	80[A], 80[A]
Banyan GFAP Result (pg/mL)								
Male	N	110	44	55	42	31	7	289
	Mean (SD)	32.5 (56.7)	15.6 (24.6)	21.5 (42.0)	23.1 (47.8)	15.7 (8.1)	17.7 (12.2)	24.3 (45.0)

	Median	10[A]	10[A]	10[A]	10[A]	13.4	12.7	10[A]
	Min, Max	10[A], 320[A]	10[A], 161	10[A], 258	10[A], 316	10[A], 46	10[A], 43	10[A], 320[A]
	2.5th, 97.5th Percentile	10[A], 284.9	10[A], 60.6	10[A], 175.6	10[A], 57.6	10[A], 45.8	10[A], 42.7	10[A], 173.9
Female	N	139	82	75	70	35	4	405
	Mean (SD)	20.8 (41.1)	14.7 (15.9)	18.9 (24.3)	17.1 (16.4)	27.6 (36.3)	10[A] (0.0)	19.0 (30.1)
	Median	10[A]	10[A]	10[A]	10[A]	18.3	10[A]	10[A]
	Min, Max	10[A], 320[A]	10[A], 119	10[A], 141	10[A], 99	10[A], 219	10[A], 10[A]	10[A], 320[A]
	2.5th, 97.5th Percentile	10[A], 136.1	10[A], 56.5	10[A], 128.6	10[A], 71.0	10[A], 219.2	10[A], 10[A]	10[A], 98.8
Unknown	N	1	0	0	0	0	0	1
	Mean (SD)	10[A] (-)	--	--	--	--	--	10[A] (-)
	Median	10[A]	--	--	--	--	--	10[A]
	Min, Max	10[A], 10[A]	--	--	--	--	--	10[A], 10[A]
	2.5th, 97.5th Percentile	10[A], 10[A]	--	--	--	--	--	10[A], 10[A]
Banyan BTI Result								
Positive		50 (20.0%)	12 (9.5%)	20 (15.4%)	24 (21.4%)	17 (25.8%)	2 (18.2%)	125 (18.0%)
Negative		200 (80.0%)	114 (90.5%)	110 (84.6%)	88 (78.6%)	49 (74.2%)	9 (81.8%)	570 (82.0%)

[A] These values are artificially constrained by the reportable ranges of the Banyan UCH-L1 and GFAP Kits

Table 5. Expected Values/Reference Range for Race and Age

All Subjects		**Age group (years)**						
		<=30	**31−40**	**41−50**	**51−60**	**>=61**	**Age Unknown**	**All Subjects**
		(N=250)	(N=126)	(N=130)	(N=112)	(N=66)	(N=11)	(N=695)
Banyan UCH-L1 Result (pg/mL)								
Caucasian	N	167	82	99	83	59	9	499
	Mean (SD)	147.8 (232.3)	113.1 (131.4)	136.1 (169.8)	129.4 (128.8)	147.5 (192.2)	103.7 (43.7)	135.9 (183.6)
	Median	80[A]	80[A]	80[A]	92.4	105.1	80[A]	81.1
	Min, Max	80[A], 2239	80[A], 1119	80[A],1491	80[A], 1137	80[A], 1534	80[A], 194	80[A], 2239
	2.5th, 97.5th Percentile	80[A], 634.7	80[A], 278.9	80[A], 485.4	80[A], 342.7	80[A], 311.6	80[A], 194.2	80[A], 485.4
Black or African American	N	23	26	19	24	7	0	99
	Mean (SD)	137.6 (167.4)	100.2 (35.4)	99.3 (28.3)	92.6 (21.8)	154.3 (50.3)	--	110.7 (86.4)
	Median	80[A]	80[A]	80[A]	80[A]	151	--	80[A]
	Min, Max	80[A], 888	80[A], 196	80[A], 160	80[A], 164	89, 252	--	80[A], 888
	2.5th, 97.5th Percentile	80[A], 888.0	80[A], 196.3	80[A], 159.8	80[A], 163.8	88.6, 252.1	--	80[A], 208.3
Other[B]	N	60	18	12	5	0	2	97

	Mean (SD)	173.2 (238.0)	87.9 (18.2)	90.6 (31.7)	139.2 (78.1)	--	322.2 (331.8)	148.4 (196.2)
	Median	80.3	80[A]	80[A]	99.9	--	322.3	80[A]
	Min, Max	80[A], 1327	80[A], 140	80[A], 189	80[A], 261	--	88, 557	80[A], 1327
	2.5th, 97.5th Percentile	80[A], 992.5	80[A], 140.0	80[A], 189.3	80[A], 261.2	--	87.6, 556.9	80[A], 882.0
Banyan GFAP Result (pg/mL)								
Caucasian	N	167	82	99	83	59	9	499
	Mean (SD)	24.0 (43.5)	17.1 (23.2)	22.3 (37.2)	21.8 (36.6)	21.8 (28.2)	14.3 (11.2)	21.7 (36.2)
	Median	10[A]	10[A]	10[A]	10[A]	16.4	10[A]	10[A]
	Min, Max	10[A], 320[A]	10[A], 161	10[A], 258	10[A], 316	10[A], 219	10[A], 43	10[A], 320[A]
	2.5th, 97.5th Percentile	10[A], 142.2	10[A], 68.8	10[A], 140.5	10[A], 71.0	10[A], 65.5	10[A], 42.7	10[A], 123.5
Black or African American	N	23	26	19	24	7	0	99
	Mean (SD)	22.1 (40.8)	11.5 (7.5)	11.9 (7.3)	12.6 (8.1)	24.0 (21.1)	--	15.2 (21.6)
	Median	10[A]	10[A]	10[A]	10[A]	10.9	--	10[A]
	Min, Max	10[A], 155	10[A], 43	10[A], 37	10[A], 46	10[A], 61	--	10[A], 155
	2.5th, 97.5th Percentile	10[A], 155.4	10[A], 43.3	10[A], 37.1	10[A], 45.7	10[A], 61.2	--	10[A], 61.2
Other[B]	N	60	18	12	5	0	2	97
	Mean (SD)	32.7 (63.5)	10.2 (2.7)	14.1 (8.2)	10.8 (2.3)	--	15.5 (9.2)	24.7 (50.9)
	Median	10[A]	10[A]	10[A]	10.5	--	15.5	10[A]
	Min, Max	10[A], 320[A]	10[A], 17	10[A], 30	10[A], 15	--	10[A], 22	10[A], 320[A]
	2.5th, 97.5th Percentile	10[A], 284.9	10[A], 16.9	10[A], 29.8	10[A], 14.7	--	10[A], 22.0	10[A], 203.4
Banyan BTI Result								
Positive		50 (20.0%)	12 (9.5%)	20 (15.4%)	24 (21.4%)	17 (25.8%)	2 (18.2%)	125 (18.0%)
Negative		200 (80.0%)	114 (90.5%)	110 (84.6%)	88 (78.6%)	49 (74.2%)	9 (81.8%)	570 (82.0%)

[A] These values are artificially constrained by the reportable ranges of the Banyan UCH-L1 and GFAP Kits
[B] Includes subjects who indicated more than one race.

19.0 Clinical Performance

19.1 Clinical Utility Study

A prospective, multi-center clinical study was conducted to evaluate the clinical performance of the Banyan BTI. Specimens were collected from consented subjects enrolled from 22 geographically and ethnically diverse emergency departments within (n=16) and outside (n=6) the United States. Subjects were men and women (at least 18 years of age) who presented with suspected traumatic brain injury (TBI) with an initial Glasgow Coma Scale (GCS) score of 13 to 15 and who had a computed tomography (CT) scan performed per the clinical site's standard of care.

CT scans were performed in accordance with the clinical sites' normal procedures. Images were transmitted to a central neuroimaging processing center. Images were interpreted by at least 2 neuroradiologists who were masked to other clinical and laboratory data; procedures for scoring images were established before conducting image review. The clinical outcome was based on the consensus interpretation between the 2 neuroradiologists. Outcomes were positive or negative as defined by the presence or absence of acute intracranial lesions, respectively. Acute intracranial lesion was defined as any trauma induced or related finding visualized upon head CT scan.

Whole blood was collected into BD Vacutainer SST Venous Blood Collection tubes from each subject using standard venipuncture techniques and processed to obtain serum. Serum was aliquoted into cryovials and the samples were frozen before samples were provided to testing sites. A specimen stability study was conducted to demonstrate integrity of archival clinical samples.

Clinical sample testing with the Banyan BTI was conducted at 3 external US testing sites in accordance with the package insert instructions. The software-reported Banyan UCH-L1 and Banyan GFAP results were interpreted by operators in accordance with package insert instructions to obtain the Banyan BTI result and who were blinded to TBI-status and neurological status of the subject. The Banyan BTI results were compared to the consensus CT scan results to estimate clinical performance characteristics.

Of the 1994 subjects enrolled, 47 (2.4%) subjects were excluded from the performance analyses due to study discontinuation, inconclusive or unreadable CT scan results, and/or missing or invalid assay results. The remaining 1947 of 1994 (97.6%) subjects were evaluable.

Of the 1947 subjects, 1312 (67.4%) of the subjects were enrolled in the United States and 635 (32.6%) were enrolled in Germany or Hungary. **Table 6** summarizes demographic characteristics for the evaluable subjects.

Table 6. Demographic Characteristics

Characteristic	**Positive (N=120)**	**Negative (N=1827)**	**All (N=1947)**
Age			
Mean (SD)	58.8 (18.29)	48.3 (20.94)	48.9 (20.94)
Median	58.5	48.0	49.0
Min, max	20, 95	18, 98	18, 98
Sex			
Male	70 (58.3%)	1033 (56.5%)	1103 (56.7%)
Female	50 (41.7%)	794 (43.5%)	844 (43.3%)
Ethnicity			
Hispanic or Latino	1 (0.8%)	89 (4.9%)	90 (4.6%)
Not Hispanic or Latino	118 (98.3%)	1737 (95.1%)	1855 (95.3%)
Not Reported	1 (0.8%)	1 (0.1%)	2 (0.1%)
Race[A]			
Black or African American	16 (13.3%)	513 (28.1%)	529 (27.2%)

American Indian or Alaska Native	1 (0.8%)	12 (0.7%)	13 (0.7%)
Asian	5 (4.2%)	24 (1.3%)	29 (1.5%)
Native Hawaiian or other Pacific Islander	1 (0.8%)	2 (0.1%)	3 (0.2%)
White	98 (81.7%)	1259 (68.9%)	1357 (69.7%)
Unknown	1 (0.8%)	27 (1.5%)	28 (1.4%)

max = maximum, min = minimum, SD = standard deviation
[A] Subjects may indicate more than one race.

Table 7 summarizes head injury characteristics of the evaluable subjects. The mean time from head injury to blood draw was 3.50 hours. Most subjects had a GCS score of 15 (94/120 [78.3%] in CT scan positive subjects and 1738/1827 [95.1%] in CT scan negative subjects). The percentage of subjects with GCS scores of 13 and 14 were higher in the CT scan positive subjects compared to the CT scan negative subjects.

Table 7. Head Injury Characteristics

Characteristic	**CT Results**		**Total (N=1947)**
	CT positive (N=120)	**CT negative (N=1827)**	
Time from head injury to blood draw (hours)			
n	120	1824 [A]	1944 [A]
Mean (SD)	3.75 (1.918)	3.49 (2.064)	3.50 (2.056)
Median	3.26	3.13	3.17
Min, max	0.3, 9.3	0.3, 35.3	0.3, 35.3
GCS Score			
13	7 (5.8%)	15 (0.8%)	22 (1.1%)
14	19 (15.8%)	74 (4.1%)	93 (4.8%)
15	94 (78.3%)	1738 (95.1%)	1832 (94.1%)

GCS = Glasgow Coma Scale, max = maximum, min = minimum, SD = standard deviation
[A] 3 subjects did not have time from head injury to blood draw recorded.

Table 8 shows the performance estimates for the Banyan BTI. Of the 1947 evaluable subjects, 120 (6.2%) had positive CT scan results. Of the 120 subjects with positive CT scan results, 117 had a positive Banyan BTI result (sensitivity = 97.5%). The remaining 3 of 120 CT scan positive subjects had negative results (i.e., false negative results). Of the 1827 subjects with negative CT scan results, 666 had a negative Banyan BTI result (specificity = 36.5%).

Overall, there were 669 subjects with negative Banyan BTI results. Of these, 666 had negative CT scan results (NPV = 99.6%). In other words, approximately 99 of 100 subjects with negative assay results will likely have negative CT scan results.

The results showed that the Banyan BTI is characterized by high sensitivity and high NPV, which supports clinical utility for ruling out the need for a CT scan in subjects presenting with a GCS score of 13 to 15 with a negative Banyan BTI result.

Table 8. Clinical Performance

	CT Result		
Banyan BTI Result	**Positive**	**Negative**	**Total**
Positive	117	1161	1278
Negative	3	666	669
Total	120	1827	1947
Prevalence = 6.2%			
Sensitivity = 97.5% (95% CI[A]: 92.9-99.5%)			
Specificity = 36.5% (95% CI[A]: 34.2-38.7%)			
Negative Predictive Value (NPV) = 99.6% (95% CI[A]: 98.7-99.9%)			
Positive Predictive Value (PPV) = 9.2% (95% CI[A]: 7.6-10.9%)			
Likelihood Ratio Negative (LRN) = 0.069 (95% CL[B]: 0.170)			
Likelihood Ratio Positive (LRP) = 1.534 (95% CL[B]: 1.468)			

CI = confidence interval, CL = confidence limit, CT = computed tomography scan
[A] Two-sided, exact 95% binomial confidence interval using the Clopper-Pearson method.
[B] One-sided, lower, exact 95% binomial confidence limit using the Clopper-Pearson method.

19.2 Reproducibility

The UCH-L1 and GFAP panels each consisted of 5 panel members made of pooled normal human serum (from healthy volunteers). Higher level panel members were spiked with positive clinical specimens (i.e., serum with endogenous UCH-L1 and GFAP resulting from a TBI) and/or recombinant protein. Panel member concentrations spanned the measuring range for the Banyan UCH-L1 Kit and the Banyan GFAP Kit.

Reproducibility was evaluated at 3 external US sites. The study was designed using CLSI Guidance EP05-A3 (CLSI, 2014). At each site, 1 operator performed testing using 1 Synergy 2 Multi-mode Reader and 1 reagent lot. Each operator performed 1 Banyan UCH-L1 run and 1 Banyan GFAP run on each of 5 nonconsecutive days. For each run, the operator tested panel member replicates to obtain 5 measurements per panel member. Combining sites, 75 measurements were obtained per panel member.

Table 9 and **Table 10** show the variance component estimates.

Table 9. Banyan UCH-L1 Variance Component Estimates

Mean Conc	N	Site		Day		Within-Run		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
2245.9	75	85.6	2.6%	54.9	2.4%	58.6	3.8%	117.3	5.2%
1216.6	75	37.6	2.5%	27.8	2.3%	30.4	3.1%	55.8	4.6%
413.8	75	16.3	2.8%	12.3	3.0%	11.7	3.9%	23.5	5.7%
305.1	75	13.7	2.6%	10.6	3.5%	7.9	4.5%	19.1	6.2%
203.8	75	8.6	2.9%	7.2	3.5%	5.9	4.2%	12.7	6.2%

Conc = concentration (measured in pg/mL), CV = coefficient of variation, SD = standard deviation

Table 10. Banyan GFAP Variance Component Estimates

Mean Conc	N	Site		Day		Within-Run		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
258.7	75	4.6	1.8%	1.1	0.4%	8.0	3.1%	9.3	3.6%
146.8	74[A]	2.0	1.4%	3.7	2.5%	3.0	2.1%	5.2	3.5%
37.9	75	0.5	1.4%	0.8	2.2%	1.1	2.9%	1.5	3.9%
25.4	75	0.1	0.6%	0.8	3.0%	1.0	3.8%	1.3	4.9%
8.1	73[A]	0.5	6.1%	0.5	5.9%	0.4	5.2%	0.8	9.9%

Conc = concentration (measured in pg/mL), CV = coefficient of variation, SD = standard deviation
[A] A total of 3 measurements were invalid and excluded from analysis

20.0 Nonclinical Performance

20.1 Limit of Quantitation

The lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ) were verified by testing 7 panel members made with pooled human serum. Panel member concentrations ranged from below the measurable range to above the measurable range. Some panel members were spiked with positive clinical specimen, and/or recombinant UCH-L1 protein or native GFAP protein to achieve targeted concentrations.

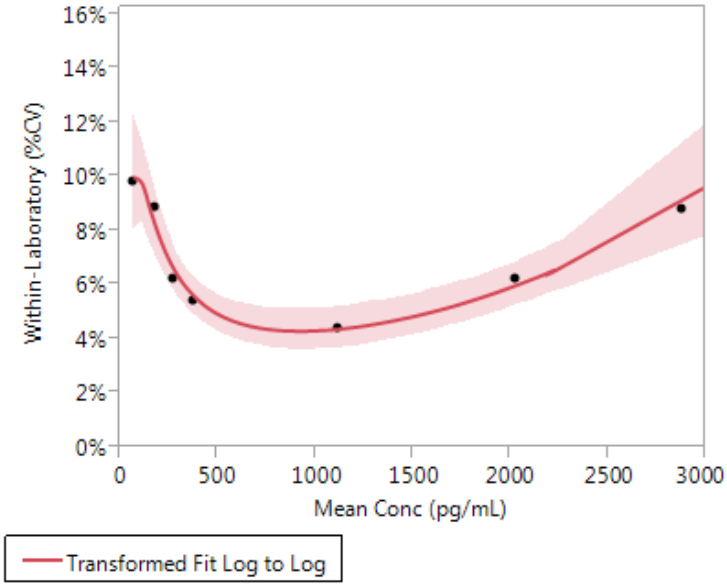
The study was designed using CLSI guidance EP17-A2 (CLSI, 2012). At a single site, 3 operators performed testing. Each operator performed 1 Banyan UCH-L1 run and 1 Banyan GFAP run on each of 5 days for each of 3 reagent lots. Each run contained 4 measurements of each panel member. Three (3) incubators and 3 readers were used randomly across operators.

The LLoQ was defined as the minimum concentration at which the within-laboratory precision CV was 15% (bounded by the concentration of the lowest standard). The ULoQ was defined as

the maximum concentration at which the within-laboratory precision CV was 15% (bounded by the concentration of the highest standard).

For the Banyan UCH-L1 Kit, the precision profile with the least precision at the lower and higher ends of the measuring range was lot 3 (**Figure 7**). The LLoQ of the Banyan UCH-L1 Kit was verified at 80.0 pg/mL and the ULoQ was verified at 2560.0 pg/mL.

Figure 7. Banyan UCH-L1 Precision Profile for Pilot Lot 3



For the Banyan GFAP, precision profiles using within-laboratory precision as CV and mean measured concentrations were generated and fit with numerous regression models, however, none of the models resulted in an acceptable fit for the data (significance of regression coefficients [>0.05]). As suggested in the CLSI guidance EP17-A2 (CLSI, 2012), precision profiles with precision expressed as SD were modeled as an alternative to CV. The precision profile with the least precision at the lower and higher ends of the measuring range was lot 2 (**Figure 8**) and lot 1 (**Figure 9**), respectively. The LLoQ of the Banyan GFAP was verified at 10.0 pg/mL and the ULoQ was verified at 320.0 pg/mL.

Figure 8. Banyan GFAP Precision Profile for Lot 2

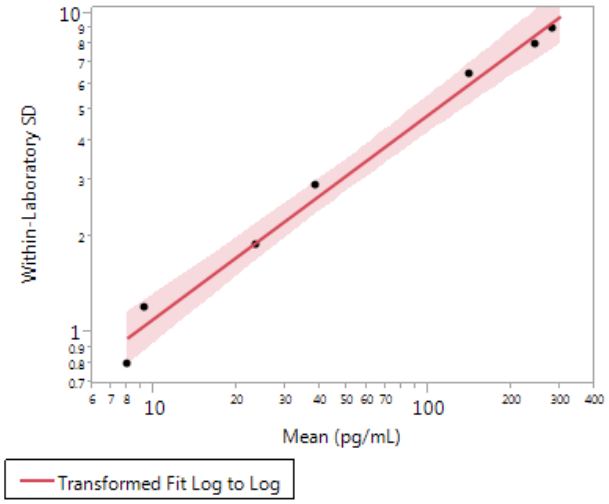
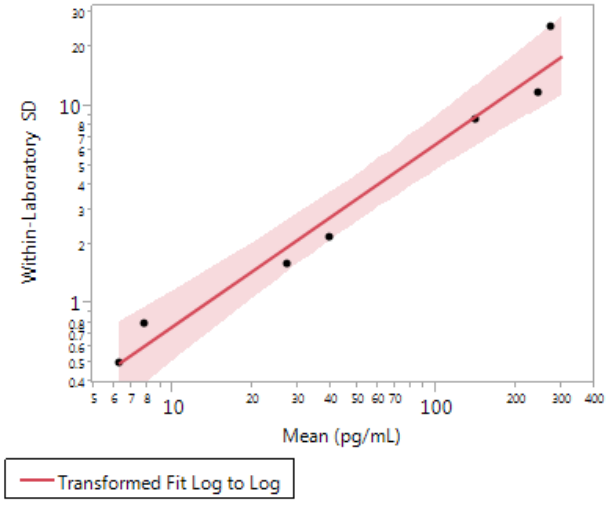


Figure 9. Banyan GFAP Precision Profile for Lot 1



20.2 Cross-Reactivity

Cross-reactivity of the proteins listed in **Table 11** with the antibodies used in the Banyan UCH-L1 Kit or Banyan GFAP Kit was evaluated. The proteins were individually spiked into UCH-L1 or GFAP Calibrator Diluent. Four (4) measurements of each sample type were tested to determine if cross-reactivity exists in the absence of target. One Banyan UCH-L1 and 1 Banyan GFAP reagent lot was used.

No cross-reactivity was observed with the Banyan GFAP Kit except for neurofilament light. Neurofilament light was tested at 68 pg/mL and was quantitated with the Banyan GFAP Kit at a mean of 10 pg/mL. A concentration of ≥ 68 pg/mL is seen in neurodegenerative disease states such as Guillain-Barré syndrome, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, or Creutzfeldt-Jakob disease. Therefore, patients with these disorders may

have a false-positive Banyan BTI result due to GFAP being quantitated above the cutoff for the Banyan GFAP Kit. One study reported healthy controls and neurology patients without any CNS structure damage had < 5 pg/mL of neurofilament light detected in serum (Gaiottino J, 2013). At these lower levels of neurofilament light, it is expected there would be no measurable cross-reactivity with the Banyan GFAP Kit in patients without neurodegenerative disease states such as Guillain-Barré syndrome, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, or Creutzfeldt-Jakob disease.

Table 11. Potential Cross-Reactants Tested With the Banyan UCH-L1 Kit or Banyan GFAP Kit

Kit	Protein	Testing Concentration
Banyan UCH-L1	UCH-L3	354 ng/mL
Banyan GFAP	Vimentin	354 ng/mL
	Desmin	127 ng/mL
	Peripherin	5 ng/mL
	Internexin	77 ng/mL
	Neurofilament light	68 pg/mL[A]
	Neurofilament medium	8.6 ng/mL
	Neurofilament heavy	77 ng/mL
	Keratin type II	10 ng/mL

[A] Neurofilament light was quantitated with the Banyan GFAP Kit at a mean of 11 pg/mL.

20.3 Linear Range

The linear range was established by testing panels of native clinical specimens diluted in pooled human serum in accordance with the CLSI EP06-A guidance (CLSI, 2003). Concentrations of UCH-L1 or GFAP in the panels ranged from below the measurable range to above the measurable range. Linearity was demonstrated throughout the measurable range for Banyan UCH-L1 (80 pg/mL to 2560 pg/mL; **Figure 10**). Banyan GFAP was linear from 10.0 pg/mL to 277.7 pg/mL (**Figure 11**).

Figure 10. Banyan UCH-L1 Linearity

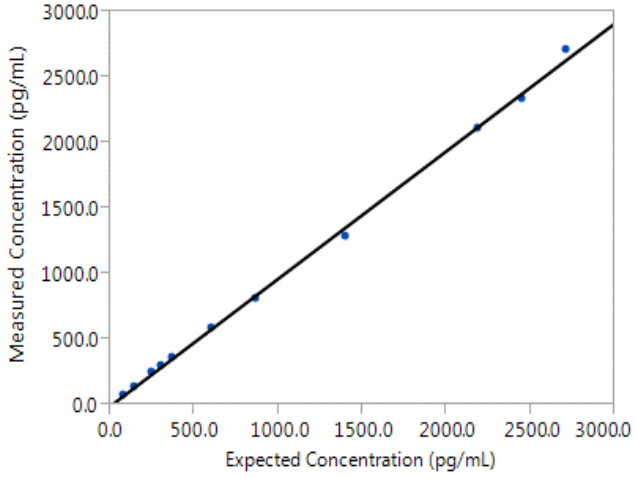
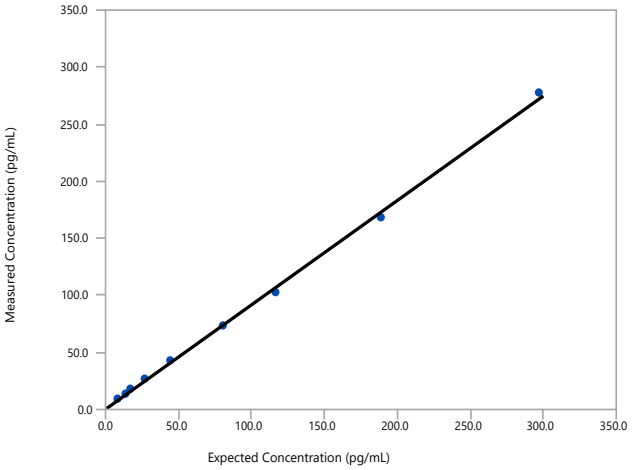


Figure 11. Banyan GFAP Linearity



20.4 Within-Laboratory Precision and Between-Lot Variation

The UCH-L1 and GFAP panels each consisted of 5 panel members made of pooled normal human serum (from healthy volunteers). Higher level panel members were spiked with positive clinical specimens (i.e., serum with endogenous UCH-L1 and GFAP resulting from a TBI) and/or recombinant protein. Panel member concentrations spanned the measuring range for the Banyan UCH-L1 Kit or Banyan GFAP Kit.

The study was designed using CLSI Guidance EP05-A3 (CLSI, 2014). At a single site, 3 operators performed testing. Each operator performed 1 Banyan UCH-L1 run and 1 Banyan GFAP run on each of 5 days for each of 3 reagent lots. Each run contained 4 measurements of each panel member for a total of 180 measurements per panel member for each assay. Three (3) incubators and 3 readers were used randomly across operators.

Table 12 and **Table 13** show the variance component estimates.

Table 12. Banyan UCH-L1 Variance Component Estimates

Mean Conc	Repeatability		Between-Day		Between-Operator		Between-Lot		Within-Laboratory	
	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
2086.8	70.5	3.4%	36.4	1.7%	76.7	3.7%	55.3	2.6%	123.5	5.9%
1122.3	36.1	3.2%	20.4	1.8%	22.3	2.0%	25.8	2.3%	53.8	4.8%
385.5	12.8	3.3%	14.8	3.8%	3.2	0.8%	9.1	2.4%	21.8	5.7%
289.3	10.0	3.5%	13.2	4.6%	0.0	0.0%	15.1	5.2%	22.4	7.7%
189.2	7.7	4.1%	11.2	5.9%	0.0	0.0%	7.7	4.1%	15.6	8.2%

Conc = concentration (measured in pg/mL), CV = coefficient of variation, SD = standard deviation

Table 13. Banyan GFAP Variance Component Estimates

Mean Conc	Repeatability		Between-Day		Between-Operator		Between-Lot		Within-Laboratory	
	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
247.4	6.2	2.5%	6.8	2.7%	3.5	1.4%	5.4	2.2%	11.3	4.6%
141.6	5.5	3.9%	3.7	2.6%	0.0	0.0%	2.1	1.5%	6.9	4.9%
38.6	1.4	3.6%	1.7	4.4%	0.6	1.6%	1.1	2.8%	2.5	6.5%
25.3	1.0	4.0%	1.3	5.1%	0.3	1.2%	1.9	7.5%	2.5	9.9%
8.2	0.6	7.3%	0.8	9.8%	0.0	0.0%	0.8	9.8%	1.3	15.9%

Conc = concentration (measured in pg/mL), CV = coefficient of variation, SD = standard deviation

20.5 Carryover Contamination

Carryover contamination was assessed using contrived samples made with GFAP Calibrator Diluent. High titer samples (33 times the concentration of the GFAP Calibrator) were created by spiking GFAP protein into the GFAP Calibrator Diluent matrix. High titer samples were interspersed between blank samples in a checkerboard pattern on 2 plates and plates were tested. A control plate (consisting of all blank samples) was also tested. Testing was conducted using 1 lot of Banyan GFAP reagents and 1 plate reader.

All wells with blanks had reported concentrations of < 10 pg/mL (below the lowest calibrator). When analyzing the RLU values generated, wells with blanks next to wells with high titer sample had RLU readings of up to 2483 RLU. Wells on the control plate had RLU readings of up to 225 RLU. For reference, the RLU readings for the second-lowest calibrator from the 2 plates with high titer samples were 44,088 RLU and 53,983 RLU which gave reportable concentrations of 19 pg/mL and 21.5 pg/mL, respectively. Thus, clinically significant carryover does not occur with the Banyan GFAP. Because the Banyan UCH-L1 Kit and Banyan GFAP Kit are performed, plated, and read in an identical manner, and the Banyan GFAP Kit is more sensitive to changes in RLU due to the clinical cutoff being close to the lower end of the dynamic range, it is inferred that there is also no clinically significant carryover with the Banyan UCH-L1 Kit.

20.6 Potentially Interfering Substances

The susceptibility of the Banyan UCH-L1 Kit and Banyan GFAP Kit to interference by elevated levels of exogenous and endogenous substances was evaluated. The study was designed using CLSI EP07-A2 (CLSI, 2005).

Pooled human serum was spiked with recombinant UCH-L1 protein or purified human GFAP protein to achieve concentrations of 400 pg/mL and 1500 pg/mL for UCH-L1 and 25 pg/mL and 70 pg/mL for GFAP. Test samples were created by spiking with potentially interfering substances and an appropriate solvent. Control samples were spiked with the appropriate solvent only.

Potentially interfering substances for the Banyan UCH-L1 Kit, Banyan GFAP Kit, and Banyan BTI are provided in **Table 14**.

Table 14. Interfering Substances

	Substance	Normal Physiological Concentration or Activity	Highest Concentration Tested that Did Not Interfere with Assay Performance	Notes
Banyan UCH-L1 Kit	Hemoglobin	5 mg/dL	62.5 mg/dL	12.5X Normal
	Rheumatoid Factor	20 IU	250 IU	12.5X Normal
	HAMA	1X	40X*	40X Normal
Banyan GFAP Kit	Hemoglobin	5 mg/dL	62.5 mg/dL	12.5X Normal
	HAMA	1X	40X*	40X Normal
Banyan BTI	Hemoglobin	5 mg/dL	62.5 mg/dL	12.5X Normal
	Rheumatoid Factor	20 IU	250 IU	12.5X Normal
	HAMA	1X	40X*	40X Normal

* The activity of the specimen tested ranged from 5 to 80 times the reactivity of a negative specimen (denoted as 1X) as determined by Sun Diagnostics (New Gloucester, ME, www.sundiagnostics.us).

No interference was observed in the presence of the substances listed in **Table 15**.

Table 15. Banyan BTI Non-Interfering Substances

Banyan BTI	
Substance Tested	**Test Concentration**
Albumin	12 g/dL
Triglycerides	3,000 mg/dL
Bilirubin	20 mg/dL
Acetaminophen	1324 µmol/L
Aspirin	3.62 mmol/L
Cardene	400 ng/mL
Plavix	9 µg/mL
Ibuprofen	2,425 µmol/L
Ethanol	5% (w/v)
Benzoylecgonine	37.5 ng/mL
EDDP[A]	125 ng/mL
d-Methamphetamine	125 ng/mL
Methadone	37.5 ng/mL
Methaqualone	37.5 ng/mL
Morphine	250 ng/mL
Oxazepam	25 ng/mL
Phencyclidine	3.1 ng/mL
Propoxyphene	37.5 ng/mL
Secobarbital	25 ng/mL
Coumadin	32.5 µmol/L
Lopressor	18.7 µmol/L

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

No interference was observed in the presence of rheumatoid factor (at ≤ 250 IU), hemoglobin (at ≤ 62.5 mg/dL), and HAMA (at ≤ 40X the activity of a known negative as characterized by Sun Diagnostics (New Gloucester, ME, www.sundiagnostics.us)). Normal physiological concentration or activity for these substances is as follows: 5 mg/dL for hemoglobin, 20 IU for rheumatoid factor, and 1X for HAMA.

21.0 Bibliography

1. American College of Surgeons Committee on Trauma. Advanced trauma life support manual. 6th ed. Chicago: American College of Surgeons, 1997.
2. Berrington de Gonzalez A, Mahesh M, Kim KP, et al. "Projected cancer risks from computed tomographic scans performed in the United States in 2007." Arch Intern Med 169 (2009): 2071-7.
3. CDC. Injury prevention and control: Traumatic brain injury and concussion. 27 April 2017. 16 August 2017. <http://www.cdc.gov/traumaticbraininjury/get_the_facts.html>.
4. CLSI. Evaluation of detection capability for clinical laboratory measurement procedures; approved guideline-second edition. CLSI document EP17-A2. Wayne: Clinical and Laboratory Standards Institute, 2012.
5. CLSI. Evaluation of linearity of quantitative measurement procedures: a statistical approach. CLSI document EP06-A. Wayne: Clinical and Laboratory Standards Institute, 2003.
6. CLSI. Evaluation of precision quantitative measurement procedures; approved guideline - third edition. CLSI document EP05-A3. Wayne: Clinical and Laboratory Standards Institute, 2014.
7. CLSI. Interference testing in clinical chemistry; approved guideline--second edition. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
8. Gaiottino J, Norgren N, Dobson R, et al. "Increased neurofilament light chain blood levels in neurodegenerative neurological diseases." PLoS ONE 8 (2013): e75091.
9. Korley FK, Kelen GD, Jones CM, Diaz-Arrastia R. "Emergency department evaluation of traumatic brain injury in the United States, 2009-2010." J Head Trauma Rehabil 31 (2016): 379-387.
10. Papa L, Stiell IG, Clement CM, et al. "Performance of the Canadian CT Head Rule and the New Orleans Criteria for predicting any traumatic intracranial injury on computed tomography in a United States Level I trauma center." Acad Emerg Med (2012): 2-10.
11. Taylor CA, Bell JM, Breiding MJ, Xu L. "Traumatic brain injury-related emergency department visits, hospitalizations, and deaths - United States, 2007 and 2013." MMWR Surveill Summ 66 (2017): 1-16.
12. Toth, A. "Magnetic resonance imaging application in the area of mild and acute traumatic brain injury: implications for diagnostic markers?" FH, Keobeissy. Brain neurotrauma: molecular, neurophysical, and rehabilitation aspects. Boca Raton: CRC Press/Taylor & Francis, 2015.

13. US National Library of Medicine. Genes: GFAP. November 2008. <https://ghr.nlm.nih.gov/gene/GFAP>.

14. US National Library of Medicine. Genes: UCHL1. May 2012. <https://ghr.nlm.nih.gov/gene/UCHL1>.

22.0 Trademarks and Patents

Patents
This notice is intended to serve as notice under 35 U.S.C. § 287(a). Patents covering this product are provided at www.banyanbio.com/patents.

23.0 Technical Assistance

For technical assistance, call Banyan Biomarkers, Inc., at (760) 710-0460.
Manufacturer:

Banyan Biomarkers, Inc.
16470 West Bernardo Drive, Suite 100
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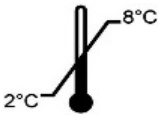
24.0 Glossary of ISO 15223-1:2016 Symbols Used in Labeling

Symbol	Definition	Symbol Reference Number
	Do not reuse	5.4.2
	Consult instructions for use	5.4.3

Symbol	Definition	Symbol Reference Number
	Caution	5.4.4
	Batch code	5.1.5
	Manufacturer	5.1.1
	In vitro diagnostic medical device	5.5.1
	Temperature limitation 2°C to 8°C	5.3.7
	Catalog number	5.1.6
	Use by date	5.1.4