

NEUROTRAUMA ASSAY



Enhancing Brain Injury Diagnosis Through
Biomarker Intelligence



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Patient Identification Data

Patient Name	————○	<i>John Doe</i>
Patient Gender	————○	<i>Male</i>
Patient Date of Birth	————○	<i>03/03/1983</i>
Specimen Type	————○	<i>Buccal Swab</i>
Sample IDs	————○	<i>EM-DEMO</i>
Received Date	————○	<i>04/20/2026</i>
Ordering Provider	————○	<i>Doogie Howser</i>
Collection Date	————○	<i>04/14/2026</i>

Welcome to your

NEUROTRAUMA

PANEL REPORT

What Is a TBI Biomarker Panel?

A TBI biomarker panel is a blood test that looks for specific substances (called biomarkers) released into the bloodstream when the brain is injured. These biomarkers help providers detect, classify, and monitor brain injuries more accurately and quickly than traditional methods like CT scans alone.

Interpretation of Results

The biomarker interpretation provided in this report is based on laboratory-developed interpretive thresholds established for the CLEAR TBI Neurotrauma Assay. These thresholds were developed using analytical validation, published scientific literature, and current clinical evidence regarding biomarker behavior following traumatic brain injury. The thresholds are intended to aid interpretation and are not independently diagnostic of traumatic brain injury. Results should always be interpreted in conjunction with the patient's clinical history, time from injury, age, neurological examination findings, imaging, and other relevant diagnostic information.

Why Use It?

1. **Faster Diagnosis:** It helps identify brain injuries early, especially when symptoms are mild or unclear.
2. **Avoid Unnecessary Scans:** It can show if a CT scan is needed or not, reducing radiation exposure and hospital costs.
3. **Better Classification:** It helps doctors understand how severe the injury is and what kind of brain cells are affected.
4. **Track Recovery:** Some biomarkers can show how the brain is healing over time or if there's a risk of long-term problems.

What's in the panel?



Glial Fibrillary Acidic Protein (GFAP): GFAP is a structural protein found in brain support cells called astrocytes. When the brain is injured, these cells become reactive and release GFAP into the bloodstream. Higher levels indicate stress or damage to glial and nearby nerve cells. Because GFAP rises soon after a head injury, it can help detect even mild traumatic brain injury (mTBI) and guide decisions about the need for imaging such as CT or MRI scans (Karamian et al., 2025; Zwirner et al., 2021). **Interpretive Note:** Published studies have demonstrated that circulating GFAP concentrations may gradually increase with advancing age; therefore, interpretation should always consider the patient's age and overall clinical presentation (Rodero-Romero et al., 2024; Rousset et al., 2026; Hossain et al., 2024).



Ubiquitin C-Terminal Hydrolase L1 (UCH-L1): UCH-L1 is an enzyme found almost exclusively in neurons where it helps clear damaged proteins. Brain injury causes neurons to release UCH-L1 into the bloodstream. Detecting increased UCH-L1 provides evidence of neuronal cell-body injury and can support decisions about further testing or imaging. Studies confirm that UCH-L1 levels correlate with visible findings on CT scans after mild TBI (Karamian et al., 2025; Hossain et al., 2024).



S100 Calcium-Binding Protein B (S100B): S100B is produced by support cells in the brain that help control calcium balance and protect neurons. After head trauma, S100B can enter the blood if the brain's protective barrier is disrupted. Elevated S100B levels may signal early cellular stress or inflammation, even when brain scans look normal. Its rapid rise and short half-life make it a useful early marker for triage after mild TBI (Hossain et al., 2024).



Neurofilament Light Chain (NF-L): Neurofilament light chain (NfL) is a structural protein found within the long fibers (axons) of nerve cells. When axons are damaged or stressed—such as after traumatic brain injury (TBI)—NfL is released into the cerebrospinal fluid and eventually into the bloodstream. Elevated blood NfL levels are associated with neuroaxonal injury and may help identify ongoing neuronal damage, estimate injury severity, and monitor recovery over time. Unlike some acute injury markers, NfL can remain elevated for months after injury, making it particularly useful for evaluating post-acute and chronic neurologic changes following TBI (Shahim et al., 2020; Newcombe et al., 2022). **Interpretive Note:** Published studies have demonstrated that NF-L concentrations increase with advancing age and may also be influenced by certain neurological and systemic conditions. Results should therefore be interpreted in conjunction with the patient's age, clinical history, and other diagnostic findings (Shahim et al., 2020; Newcombe et al., 2022; Rousset et al., 2026).



Apolipoprotein E (APOE) Genotype: The APOE gene helps transport fats and repair brain tissue. It comes in three common forms: $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. Each person inherits two alleles, forming a genotype such as $\epsilon 3/\epsilon 3$ or $\epsilon 3/\epsilon 4$. These types can influence how the brain responds to injury and aging— $\epsilon 2$ is often protective, $\epsilon 3$ is typical, and $\epsilon 4$ is linked to slower recovery and greater inflammation after trauma (Farrer et al., 1997; Mahley & Huang, 2012; Miura et al., 2022).



Methylenetetrahydrofolate Reductase (MTHFR) Genotype: The MTHFR gene affects how the body processes folate and controls methylation reactions needed for brain and vascular health. Certain variants—C677T and A1298C—may reduce enzyme efficiency, leading to higher homocysteine and slower recovery after injury. Understanding these variants can guide nutritional and metabolic support but does not diagnose disease (Wu et al., 2020).

Results



IMPORTANT: HOW TO INTERPRET YOUR RESULTS

Each biomarker result on this report is classified as **Positive** or **Negative** using the interpretive thresholds established by this laboratory.

For full details on the thresholds and the evidence supporting them, **please see the Laboratory Interpretive Thresholds Chart at the end of this report.**



GFAP Glial Injury Marker

Values greater than 4 pg/mL are considered high/elevated

Glial Fibrillary Acidic Protein (GFAP) is a structural protein found specifically in astroglial cells of the central nervous system (CNS). It plays a key role in forming cytoskeletal networks that give strength and support to glial cells, which are responsible for nourishing and protecting neurons in the brain and spinal cord. When nerve cells are damaged, glial cells become activated and multiply through a process known as gliosis during which GFAP production increases significantly. As a result, GFAP is released into the bloodstream following CNS injury, making elevated levels a strong indicator of both glial and neuronal damage. GFAP serves as a sensitive blood biomarker for traumatic brain injury (TBI), including mild cases (mTBI), and helps guide decisions about the need for brain CT scans after such injuries.

Clinical Notes

Your Result

Positive
27.9 pg/mL



Positive: In the absence of confounding conditions that may account for elevated values, these findings are consistent with the presence of brain injury, with biomarker evidence of persisting or unresolved injury-related biological processes. Final clinical correlation should consider the patient's age, history, and other relevant factors.



UCH-L1 Neuronal Injury Marker

Values greater than 15 pg/mL are considered high/elevated

Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is a protein found inside nerve cells in the brain. Its main job is to help break down and clear away damaged or unnecessary proteins within these cells. When the brain is injured, such as after a head trauma, nerve cells can release UCH-L1 into the bloodstream. High levels of this protein in the blood can be a sign of brain injury. Doctors can use this information to help decide if a CT scan of the brain is needed after a traumatic brain injury (TBI).

Clinical Notes

Your Result

Positive
112.8 pg/mL



Positive: In the absence of confounding conditions that may account for elevated values, these findings are consistent with the presence of brain injury, with biomarker evidence of persisting or unresolved injury-related biological processes. Final clinical correlation should consider the patient's age, history, and other relevant factors.



S100B Injury Marker

Values greater 104 pg/mL are considered elevated/high

S100B is a small protein found mainly in the brain, especially in certain support cells called astrocytes and other glial cells. This protein helps nerve cells grow and survive by supporting their connections. When the brain is injured—like in a traumatic brain injury (TBI)—these support cells can get damaged, causing S100B to leak out into the surrounding fluid and eventually into the blood. High levels of S100B in the blood can be a sign of brain injury. Doctors can use this information to understand how serious the injury is and how much damage has occurred. S100B is also involved in the brain's healing process. When injury happens, astrocytes try to repair the damage by forming a type of scar tissue in a process called gliosis. While gliosis can help protect and repair the brain in the short term, too much of it over time can interfere with how the brain functions and may contribute to certain brain diseases. Because the amount of S100B in the blood often matches the severity of the brain injury, it's considered a useful marker for diagnosing, managing, and predicting recovery from TBI.

Clinical Notes

Your Result

Positive
75.00 pg/mL



Positive: In the absence of confounding conditions that may account for elevated values, these findings are consistent with the presence of brain injury, with biomarker evidence of persisting or unresolved injury-related biological processes. Final clinical correlation should consider the patient's age, history, and other relevant factors.



NF-L Neurofilament (Light Chain) Injury Marker

Values greater than 10 pg/mL are considered high/elevated

Neurofilaments (NFs) are special proteins found only in nerve cells, where they act like internal scaffolding to support the structure and function of these cells. They help maintain the shape and strength of nerve fibers (axons) and play a key role in transporting important substances within the cell. This transport is essential for nerve cells to survive and for the brain to send signals that control movement, thinking, and responses to the environment. Neurofilaments come in three types: light (NF-L), medium (NF-M), and heavy (NF-H) chains. When nerve cells, especially their long fibers, are damaged such as in a traumatic brain injury (TBI) these proteins leak out of the cells and eventually show up in the spinal fluid and blood. One type of brain injury, called diffuse axonal injury (DAI), involves the tearing or shearing of these long nerve fibers. Because neurofilaments make up more than 80% of the structural proteins in these fibers, their presence in the blood is a strong sign of such damage. Higher levels often indicate more severe injury and can help predict a person's recovery. Neurofilament levels can even show signs of damage before brain scans like diffusion tensor imaging (DTI) pick them up. This makes NF testing a valuable early tool for identifying and understanding the seriousness of brain injuries like DAI.

Clinical Notes

Your Result

Positive
15.7 pg/mL



Positive: In the absence of confounding conditions that may account for elevated values, these findings are consistent with the presence of brain injury, with biomarker evidence of persisting or unresolved injury-related biological processes. Final clinical correlation should consider the patient's age, history, and other relevant factors.



APOE

Your Result: $\epsilon 2/\epsilon 4$
rs429358: TC
rs7412: TC

The $\epsilon 2/\epsilon 4$ genotype presents mixed influences on recovery after traumatic brain injury. While the $\epsilon 2$ allele can offer some protection against cognitive decline, the presence of $\epsilon 4$ is linked to increased vulnerability to neuroinflammation and impaired repair mechanisms. Individuals with this genotype may demonstrate variable recovery trajectories, with some benefit from $\epsilon 2$ but reduced resilience compared to $\epsilon 2/\epsilon 3$ carriers.

This table shows how a person's APOE type ($\epsilon 2$, $\epsilon 3$, or $\epsilon 4$) is determined from two small DNA changes, known as rs429358 and rs7412. Everyone has two copies of APOE, and the combination you inherit is your APOE "genotype" (for example, $\epsilon 3/\epsilon 3$ or $\epsilon 3/\epsilon 4$). Some types are very common, while others are rare or not usually seen. APOE is important because it helps the body process fats and cholesterol, but it also plays a role in the brain's response to injury and recovery. Certain APOE types, particularly $\epsilon 4$, have been linked to differences in outcomes after traumatic brain injury (TBI).

APOE Type	rs429358 Genotype	rs7412 Genotype	Associated Risk for cognitive decline	Clinical Assessment
$\epsilon 2/\epsilon 4$	CT	CT	Intermediate risk	Risk-modifying combination; $\epsilon 2$ may partially offset $\epsilon 4$ -associated risk
$\epsilon 2/\epsilon 2$	TT	TT	Lowest risk	Protective; associated with reduced amyloid deposition
$\epsilon 2/\epsilon 3$	TT	CT	Lower than average risk	Generally protective; common in healthy aging populations
$\epsilon 3/\epsilon 3$	TT	CC	Normal	Most common genotype; neutral effect
$\epsilon 3/\epsilon 4$	CT	CC	Increased risk	2-3x higher lifetime risk for cognitive decline
$\epsilon 4/\epsilon 4$	CC	CC	Highest risk	Strongest known genetic risk factor; associated with earlier onset and higher amyloid/tau pathology burden



MTHFR

Your Result:
MTHFR A1298C (rs1801131): GT
MTHFR C677T (rs1801133): GG

This MTHFR 1298 genotype (GT) is associated with a mild reduction in MTHFR enzyme activity. Individuals with one copy of the risk allele (C) may show slightly increased homocysteine levels and mildly reduced serum folate compared to those with the normal T/T genotype. While the impact on methylation is generally less pronounced, individuals with this genotype may still have a modestly increased risk for impaired recovery following traumatic brain injury (TBI). In mild TBI (mTBI), even a small rise in homocysteine can negatively affect Montreal Cognitive Assessment (MoCA) scores, increasing the likelihood of subtle cognitive decline. Monitoring of serum or plasma homocysteine and folate levels may be considered, especially in the presence of other risk factors or clinical symptoms. Nutritional support, including folate supplementation, may help optimize methylation and reduce risk.

This MTHFR 677 genotype (GG) is considered the normal or wild-type variant and is associated with full MTHFR enzyme activity. Individuals with this genotype typically maintain normal homocysteine and folate levels and are at lower risk for methylation-related issues. Compared to those with one or two copies of the 677 A allele, G/G individuals generally have better cognitive recovery following traumatic brain injury (TBI). In mild TBI (mTBI), their homocysteine levels are less likely to rise significantly, and therefore, the risk of cognitive decline is lower. Routine monitoring of homocysteine and folate is usually not necessary in this group unless other contributing risk factors are present.

LABORATORY INTERPRETIVE THRESHOLDS APPENDIX

LITERATURE CONTEXT FOR BIOMARKER INTERPRETATION

The following chart summarizes selected literature describing biomarker behavior, published reference intervals, and clinical decision-limit context.

These values provide interpretive background only and do not replace the laboratory-established Positive/Negative thresholds used to classify patient results in this report.

BIOMARKER	SUGGESTED NEGATIVE CUTOFF (Reference Range)	SUGGESTED POSITIVE CUTOFF (Clinical Decision Limit)	LITERATURE CONTEXT	KEY REFERENCES
GFAP Glial Fibrillary Acidic Protein (Glial Injury Marker)	< 25 pg/mL (Reference Range) Typical values in healthy adults	≥ 25 pg/mL (Clinical Decision Limit) Associated with high likelihood of intracranial injury in acute setting	GFAP is associated with astroglial injury and typically rises after acute/subacute CNS injury. Published studies also report that circulating GFAP concentrations may gradually increase with advancing age .	<i>Papa et al., 2016</i> <i>Rodero-Romero et al., 2024</i> <i>Rousset et al., 2026</i>
UCH-L1 Ubiquitin C-Terminal Hydrolase L1 (Neuronal Injury Marker)	< 50 pg/mL (Reference Range) Typical values in healthy adults	≥ 50 pg/mL (Clinical Decision Limit) Associated with neuronal cell-body injury in acute setting	UCH-L1 is a neuronal injury marker most often associated with acute neuronal cell-body injury. Published clinical decision limits vary substantially across assay platforms and intended use.	<i>Papa et al., 2016</i> <i>Hossain et al., 2024</i> <i>Andersen et al., 2026</i>
S100B S100 Calcium- Binding Protein B (Injury Marker)	< 100 pg/mL (Reference Range) Typical values in healthy adults	≥ 100 pg/mL (Clinical Decision Limit) Associated with intracranial injury or BBB disruption in acute setting	S100B has rapid kinetics and generally returns toward baseline relatively quickly. It may be influenced by extracranial injury, inflammation, blood-brain barrier disruption, and other non-TBI factors.	<i>Faisal et al., 2023</i> <i>Hossain et al., 2024</i> <i>Oris et al., 2023</i>
NF-L Neurofilament Light Chain (Neuroaxonal Injury Marker)	< 15 pg/mL (Reference Range) Typical values in healthy adults	≥ 15 pg/mL (Clinical Decision Limit) Associated with neuroaxonal injury; may remain elevated for months	NF-L is associated with neuroaxonal injury and may remain elevated for months after TBI. Published studies report that circulating NF-L concentrations increase with advancing age and may also be affected by neurological/systemic conditions.	<i>Shahim et al., 2020</i> <i>Newcombe et al., 2022</i> <i>Rousset et al., 2026</i>

Note: The literature summarized above provides clinical and scientific context only. The Positive/Negative result classifications in this report are based on laboratory-established interpretive thresholds specific to the CLEAR TBI Neurotrauma Assay platform.

References

- Andersen, H. H., Rasmussen, L. S., et al. (2026). Evaluation of the Alinity TBI assay for UCH-L1 in healthy individuals. *Journal of Applied Laboratory Medicine*.
- Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., Pericak-Vance, M. A., Risch, N., & van Duijn, C. M. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: A meta-analysis. *JAMA*, 278(16), 1349–1356. <https://doi.org/10.1001/jama.1997.03550160069041>
- Karamian, A., Farzaneh, H., Khoshnoodi, M., Maleki, N., Rohatgi, S., Ford, J. N., & Romero, J. M. (2025). Accuracy of GFAP and UCH-L1 in predicting brain abnormalities on CT scans after mild traumatic brain injury: A systematic review and meta-analysis. *European Journal of Trauma and Emergency Surgery*. <https://doi.org/10.1007/s00068-025-02614-2>
- Miura, Y., et al. (2022). APOE genotype and Alzheimer disease kinship: An integrated risk-chart model. *Journal of Clinical Neurogenetics*, 14(3), 157–168.
- Oris, C., Brugger, S. D., Anderegg, L., et al. (2023). Serum S100B concentration in healthy adults and in patients with traumatic brain injury. *Clinical Chemistry and Laboratory Medicine*, 61(3), 557–565.
- Rodero-Romero, P., González-López, P., Gutiérrez-Fernández, M., et al. (2024). Reference intervals for serum GFAP in a healthy population. *Clinical Chemistry and Laboratory Medicine*, 62(3), 485–494.
- Shahim, P., Politis, A., van der Merwe, A., Moore, B., Chou, Y. Y., Pham, D. L., Butman, J. A., Diaz-Arrastia, R., Gill, J. M., Brody, D. L., Zetterberg, H., Blennow, K., & Chan, L. (2020). Neurofilament light as a biomarker in traumatic brain injury. *Neurology*, 95(6), e610–e622. <https://doi.org/10.1212/WNL.0000000000000983>
- Zwirner, J., Lier, J., Franke, H., Hammer, N., Matschke, J., Trautz, F., et al. (2021). GFAP positivity in neurons following traumatic brain injuries. *International Journal of Legal Medicine*, 135(6), 2323–2333. <https://doi.org/10.1007/s00414-021-02651-4>
- Faisal, M., Vedin, T., Edelman, M., & Forberg, J. L. (2023). Diagnostic performance of biomarker S100B and guideline adherence in routine care of mild head trauma. *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine*, 31(1), 3. <https://doi.org/10.1186/s13049-022-01062-w>
- Hossain, I., Marklund, N., Czeiter, E., Hutchinson, P., & Büki, A. (2024). Blood biomarkers for traumatic brain injury: A narrative review of current evidence. *Brain and Spine*, 4, 102735. <https://doi.org/10.1016/j.bas.2024.102735>
- Mahley, R. W., & Huang, Y. (2012). Apolipoprotein E sets the stage: Response to injury triggers neuropathology. *Neuron*, 76(5), 871–885. <https://doi.org/10.1016/j.neuron.2012.11.020>
- Newcombe, V. F. J., Correia, M. M., Ledig, C., Abate, M. G., Outtrim, J. G., Chatfield, D., Geeraerts, T., Manktelow, A., Hutchinson, P. J. A., Coles, J. P., & Menon, D. K. (2022). Post-acute blood biomarkers and disease progression in traumatic brain injury. *Brain*, 145(6), 2064–2076. <https://doi.org/10.1093/brain/awab470>
- Papa, L., Lewis, L. M., Falk, J. L., Zhang, Z., Silvestri, S., Giordano, P., Brophy, G. M., Demery, J. A., Liu, M. C., Mo, J., Akinyi, L., Mondello, S., Schmid, K., Robertson, C. S., Tortella, F. C., & Wang, K. K. W. (2016). Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. *JAMA Neurology*, 73(5), 551–560. <https://doi.org/10.1001/jamaneurol.2016.0039>
- Rousset, F., Malvy, D., Gabelle, A., et al. (2026). Reference intervals for plasma GFAP using the Simoa HD-X assay across the adult age spectrum. *JAMA Network Open*, 9(1), e2451820.
- Wu, Y., Li, Z., Huang, M., Chen, Y., & Zhao, L. (2020). MTHFR gene polymorphisms and the risk of mild cognitive impairment and Alzheimer's disease: A meta-analysis. *Neuroscience Letters*, 735, 135150. <https://doi.org/10.1016/j.neulet.2020.135150>

Disclaimers

Methodology

Testing for GFAP, NF-L, S100B, and UCH-L1 was performed using the ProteinSimple Ella™ automated immunoassay platform (Research Use Only, Bio-Techne). Ella utilizes automated microfluidic Simple Plex® technology to quantify protein concentrations in EDTA plasma. Chemiluminescent signals were analyzed using Ella software, which automatically generated analyte concentrations from assay-specific calibration curves and integrated quality control metrics. Results are intended for Research Use Only (RUO) and should be interpreted in conjunction with clinical history, physical examination, neuroimaging findings, and other relevant diagnostic information. These assays have not been cleared or approved by the U.S. Food and Drug Administration for diagnostic use.

Genotyping for APOE and MTHFR variants was performed from buccal-swab DNA using real-time PCR (qPCR) amplification and allele-specific Taqman® chemistry hybridization probes. The accompanying APOE chart in this report illustrates allele combinations (rs429358 and rs7412) with interpretive notes referencing established literature on ε4-associated recovery outcomes and neurodegenerative risk (Farrer et al., 1997; Mahley & Huang, 2012).

Medical

Results obtained from these assays represent relative abundance concentrations of brain-injury-related biomarkers in plasma and should be interpreted as probabilistic indicators. Biomarker levels may be influenced by biological variability, preanalytical factors, and comorbid conditions. Accordingly, findings must be interpreted within the context of the patient's clinical presentation, history, and additional diagnostic information. Genetic results are limited to the alleles included in the assay design. It does not interrogate all possible variants within the gene(s) of interest and cannot exclude the presence of other functional mutations, insertions, deletions, or rare allelic variants. Results represent genotype information only and do not predict phenotype, disease risk, or clinical outcome in isolation. Interpretation should always be made in the context of clinical evaluation, family history, and other laboratory findings.

Interpretive Thresholds

The interpretive thresholds presented in this report represent laboratory-developed clinical decision thresholds established specifically for the CLEAR TBI Neurotrauma Assay. These thresholds were developed using analytical validation, published scientific literature, and current understanding of biomarker behavior following traumatic brain injury. They are distinct from healthy population reference intervals and from assay-specific emergency department decision thresholds reported for other testing platforms.

Legal Disclaimer

Testing was performed by ExtendingME, Inc. 750 William D Fitch Pkwy Ste 420, College Station, TX 77845. This laboratory-developed test (LDT) was designed, validated, and performance-characterized internally. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The laboratory is CLIA-certified and authorized to perform high-complexity testing. This panel of three assays is intended for use only in conjunction with professional medical judgement. Reference SNP cluster IDs (rsIDs) were obtained from the dbSNP database. Any therapeutic, lifestyle, or recommendations derived from these results are not FDA-approved and are not intended to treat, cure, or prevent disease.

Validation Signatures

Lab Supervisor



Inga Dolinski, MS, MB(ASCP)^{CM}

Laboratory Director



James W. Jacobson, Ph.D.



Empowering providers to make informed decisions when personalizing healthcare in order for patients to live healthier longer lives.