A Highly Sensitive Point of Care Test for GFAP, A Brain Biomarker in Serum

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Abstract

Gial fibrillary acidic protein (GFAP) in serum has been proposed as a biomarker for traumatic brain injury (TBI). Many immunochromel method have been developed for quantitating human GFAP in serum, including ELSIA. However, a sensitive and quantitative assay which could be used as a point-of-care diagnostic test is not commercially available. Here we report the development of a sensitive, quantitative, and highly specific lateral flow assay for measuring GFAP in human serum. The assay utilizes a high affinity monoclonal antibody against human GFAP for capture and an europium (Eu, III) doped polystyrene nanoparticle conjugated to the Fab' fragment of a high affinity second monoclonal antibody against human GFAP for detection. The fluorescence of Eu signal on the lateral flow membrane was measured using a custom made lateral flow cassette capable of time-resolved fluorescence measurement. The background fluorescence from the matrix and associated material (membrane and plastic) is greatly diminished in the time-resolved mode which significantly increased the signal window as well as detection limit to below pg/ml concentration of GFAP. The assay generates a linear calibration curve from 0 pg/ml to 250 pg/ml using human serum spiked with recombinant human GFAP. The assay performs equally well with the break-down product of GFAP (GFAP-BDP). The sensitivity of the assay is about 15 fold higher in the Time Resolved Fluorescence (TRF) mode when compared to the prompt fluorescence. The assay is highly reproducible, sensitive and fast, with an LOQ of 0.125 pg/ml.

Introduction

Traumatic brain injury (TBI) constitutes a major health and socioeconomic problem throughout the world. TBI is a leading cause of death and disability in children and young adults. It is currently diagnosed by neurological examination and radiographic imaging (CT, MRI, etc.). As a result, proper and timely diagnosis of the TBI patient is limited. Basic science research has greatly advanced the knowledge and the mechanisms involved in the damage, and have led to the discovery of new biomarkers. Gial Fibrillary Acidic Protein (GFAP), which is a filament protein found in the cytoskeleton of astro-gial cells, or its breakdown product GFAP-BDP, is one of the biomarkers released into the blood after a traumatic brain injury. Several studies using clinical blood and CSF samples have independently confirmed GFAP and GFAP-BDP as potential biomarkers for TBI. Currently there is no sensitive point of care test for quantitating the levels of GFAP. Here we report a lateral flow assay (LFA) format for measuring GFAP at sub-picogram levels in human serum and CSF samples.

Materials and Methods

Mouse monoclonal antibodies: mAb-1 and mAb-2 were developed in-house using full length human GFAP as antigen. Biotinylated Fab'2 fragment of mAb-2 was prepared and conjugated to the Streptavidin-Europium labeled latex nanoparticles. Recombinant human GFAP or GFAP-BDP were used as calibrators.

Preparation of test cassettes: Test line (mAb-1) and control line (Ag) were striped on the nitrocellulose membrane (3μm/cm) using Bio-dot dispenser. A 8mm wide glass fiber and a 19mm wide cellulose were used as sample pad and absorption pads, respectively. The nitrocellulose membrane was cut into 3.7mm wide bands and assembled along with sample, conjugate and absorbent pads into lateral flow cassettes. The membrane along with the cassette was dried at 37°C and sealed individually in a desiccant containing pouch and stored at room temperature.

Preparation of calibrator solutions: Purified recombinant human GFAP-BDP was diluted in human serum to a final concentration of 100 ng/ml stock solution. The stock solution was serially diluted from 250 pg/ml to 0.5 pg/ml. Neat serum was used as background (0 pg/ml) control.

Performing the assay: A 10 μl of 10% Triton X-100 was added to the sample port, followed by 100 μl of the samples containing indicated concentrations of GFAP-BDP. After 5 minutes, 50 μl of the 1X PBS containing 0.1% Tween 20 was added and the cassettes were incubated for 30-60 minutes at room temperature.

Signal detection: The amount of fluorescence signal on the test and the control lines were measured using a lateral flow time resolved fluorescence reader. For a comparative study, the cassettes were read in a prompt lateral flow fluorescence reader.

Results

1. Assay sensitivity: The sensitivity of the assay was evaluated by generating dose response curves (DRC) using GFAP-BDP. The results are shown in Table 1 and Figure 2 below.

2. Time for maximum signal development: Time required for maximum signal development was evaluated by reading lateral flow cassette at 30, 60 and 90 minutes after sample application. The results are shown below.

Conclusions

- We have developed an ultra sensitive lanthanide based point of care lateral flow immunodiagnostic test for detecting sub-picogram per ml of GFAP, a potential biomarker for TBI.
- The same technology can be used for detecting other potential brain biomarkers such as UCH-L1, S100B, Myelin basic protein. SBDP, Tau protein, etc.
- Because of its high sensitivity, this POC technology will be very valuable for measuring any biomarker where high sensitivity is required and for sample volume is limited.

References


Table 1: Concentration of GFAP in serum/GFAP of normal and suspected TBI patients.

Table 2: Normal subjects & TBI patients

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<tr>
<td>H</td>
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** = Concentration extrapolated from standard curve

Figure 1A: Schematic illustration of lateral flow assay (LFA). 1B: Principle of the Sandwich LFA. 1C: Prompt fluorescence reader (Glopper) and Time resolved fluorescence reader (Dx-Sys).

Figure 2: Dose response curves for GFAP-BDP using Time resolved fluorescence. A: RFU values for different concentrations of GFAP-BDP after 20, 40 and 80 minutes incubation. B: DRC of GFAP-BDP from 0 to 200 pg/ml after 30, 60 and 90 minutes incubation. The RFU values for all the concentrations indicated by 30 minutes incubation.