Hes·so
Haute Ecole Spécialisée de Suisse occidentale
Fachhochschule Westschweiz
University of Applied Sciences and Arts Western Switzerland

Master of Science HES-SO in Life Sciences

Conceptualization and development of a multiplex, high-sensitivity immunodiagnostic assay for neuropathologies

Edis Saini

Applied Biosciences
HES-SO Valais

Advisor: Dr. Marc E. Pfeifer

DESCRIPTION

Among the most commonly observed neuropathologies in humans there is traumatic brain injury (TBI), with an estimated 46 millions new cases reported annually in Canada, USA and Europe combined. These cases include different types of TBI according to their severity. Mild TBI (mTBI) more commonly referred as concussion, has been the most common type (80 - 90 % of all TBI). Currently, it is well known that mTBI patients can suffer from symptoms and/or disability that persist for more than a year after the injury. Moreover, in mTBI cases, traditional neuroimaging method (computed tomography (CT) scan, magnetic resonance imaging (MRI)) are unnecessary because in most of case unremarkable lesions occurred. Therefore, the development of a rapid point-of-care (POC) mTBI-related biomarker-based test could significantly improve patient management, especially for emergency department.

OBJECTIFS

The main objective of this work was to develop immunoassays toward a sandwich format for the detection of some mTBI-relevant biomarkers (i.e., protein 1, 2 and 3) as illustrated in **Figure 1A**. Ultimately it would be desirable to have an assay in multiplex configuration.

During the preliminary stages development of the assay different methods such as chemiluminescence (CL) have been used. Electrochemiluminescence (ECL) which is a highly sensitive method that has already shown to be successful for the detection of these biomarkers on centralized laboratory device such as the Meso Scale Discovery (MSD) QuickPlex SQ 120, has been used as read-out method for the development of these immunoassays. In order to have a POC version, a small device called demonstrator has been used as read-out device with linear sweep voltammetry (LSV) for the sandwich immunoassay on commercial screen-printed electrodes (SPEs). Noted that throughout the development, the MSD device was used as a basis of comparison for the evaluation and interpretation of the results, since the sandwich immunoassay were also conducted in parallel in carbon-based 96-well plates dedicated for the measurement. For detection, the detection antibody was labelled with a commercial ruthenium complex (SULFO-TAG™ from MSD) as described in the **Figure 1** below.

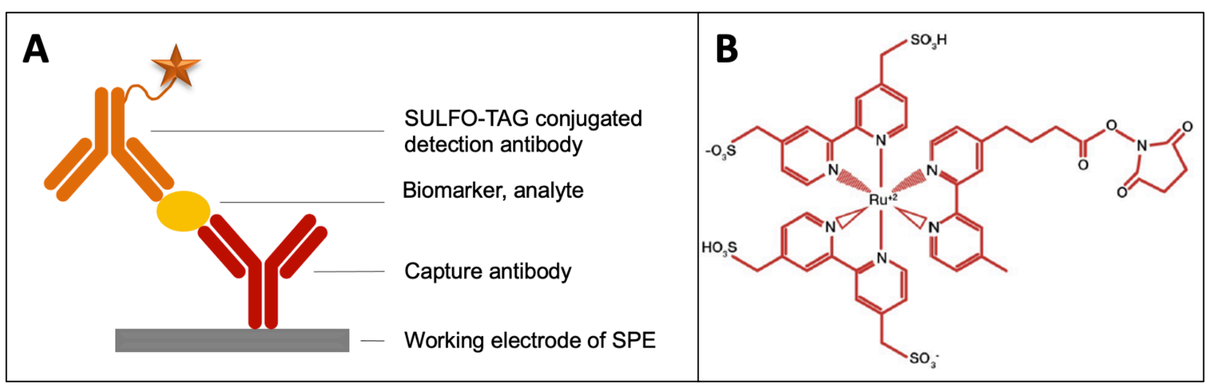

Figure 1: A) *Sandwich type immunoassay used during this project where MSD SULFO-TAG™ label is directly conjugated to dAb.* ***B)*** *The MSD GOLD SULFO-TAG™ NHS-Ester label chemical structure used for the conjugation of the detection antibody (illustration taken from MSD website).*

RESULTS

In a first step, different solid phases of SPEs were characterized and studied in order to select the most appropriate one in terms of ECL signal intensity. The results of these preliminary experiments dedicated to the choice of the solid phase will not be discussed here but can be found in the section 3 of the Master Thesis. Nevertheless, it turned out that carbon SPEs (SPCEs) showed the best performance and were chosen during the optimization of the sandwich immunoassay on mTBI biomarkers.

During the protein 1 immunoassay optimization the optimum conditions were: a scan rate of 200 mV/s and a reading buffer (from MSD) concentration of 2X. The results of the measurements on the portable demonstrator device showed a correlation between the ECL signals and protein 1 concentrations tested. After performing a linear regression (R^2=0.9167) on these data, an approximative LOD and LOQ of 11 and 31 ng/mL respectively are found by using these values (linear regression shown in Appendix section of the Master Thesis). However, no correlation has been established for protein 2 and protein 3 due to poor ECL signals.

In addition, after comparison with the MSD device, it was found that the protein 1 immunoassay has a major problem potentially related to non-specific binding (NSB) of the detection antibody (dAb) due to its extremely higher background value (~300'000 a.u.) than those observed for protein 2 and protein 3 (~500 a.u.) on MSD QuickPlex SQ120 device. Therefore, the signals measured for protein 1 immunoassay on SPCEs are predominantly from NSBs.

CONCLUSION

To conclude, different experiments have allowed the development of three different preliminary sandwich immunoassays for three biomarkers (protein 1, 2 and 3) related to mTBI diagnostics. The sandwich immunoassay with protein 1 was first developed on the demonstrator instrument with an ECL signal response correlating with the concentrations tested. A linear regression between average ECL maximum intensity and the concentrations tested. However, after comparison with the MSD QuickPlex SQ120, it was found that all measured values (especially for the blanks with at 0 ng/mL of protein 1) were extremely higher (~300'000 a.u.) than those observed for the protein 2 and 3 immunoassays (~500 a.u.). Indeed, the protein 2 and protein 3 immunoassays demonstrated their functionality on the MSD QuickPlex SQ120 (with an estimated LOD up to 3 ng/mL and 4 ng/mL respectively) with significantly lower signals than those observed for protein 1. However, these intensities are unfortunately not sufficiently high to be differentiated among the concentrations tested (0 - 50 - 100 - 200 ng/mL) through the small detector of the demonstrator device contrary to cooled-CCD camera of the MSD device. Consequently both of these two immunoassay require a signal amplification strategy to be detectable on the portable demonstrator. In contrast, for the protein 1 immunosensor, an excessively stronger signal (on both devices) is measured due to an NSB of dAb on the solid surface of the immunoassay or on the cAb used. To mitigate this problem, two strategies should be investigated in priority. The first one of is to change the blocking agent used (i.e., casein) to another conventionally used one, such as BSA. Moreover, addition of non-ionic detergent such as Tween® 20 can be of great interest.

SWISS UNIVERSITIES APPLIED SCIENCES AND ARTS

MLS | MASTER OF SCIENCE IN LIFE SCIENCES