Optimization of sTREM2 ELISA to understand the role of microglia in Alzheimer's disease

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Human Biology Final Degree Project 2023

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SUMMARY

Triggering receptor expressed on myeloid cells 2 (TREM2), a microglial receptor, plays a crucial role in the innate immune response in Alzheimer's disease (AD), aiding in the clearance of amyloid beta plaques and preventing Tau propagation. As a result of cleavage, soluble TREM2 (sTREM2) can be detected in human cerebrospinal fluid (CSF) and plasma across the AD continuum, serving as a biomarker of microglial activity. This study aimed to optimize a Meso Scale Discovery (MSD) enzyme-linked immunosorbent assay (ELISA) for the quantification of sTREM2 in biofluids. Validation parameters considered for optimization included signal-to-noise (S/N) ratio, background noise and coefficient of variation (CV%). Optimal capture and detection antibodies concentrations were determined to be 15.63 ng/ml and 0.125 µg/ml, respectively, resulting in improved assay performance. Despite higher background noise, storing MSD reagents at 4ºC and the use of MSD Small Spot coated-streptavidin plates improved the S/N ratio. Finally, a concentration of 0.0625 µg/ml of secondary antibody rendered a lower background and higher S/N ratio. These optimizations significantly increased the sensitivity and accuracy of the immunoassay, making it a promising tool for the early detection of pathological alterations linked to AD.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the leading cause of dementia [1]. The neuropathological hallmarks of AD compromise the extracellular aggregation of amyloid beta (Aβ) plaques and intracellular deposits of tau neurofibrillary tangles (NFTs) [1, 2].

The innate immune response and, in particular, the activation of microglia also play an important role. Microglia are the resident macrophages of the central nervous system (CNS) that actively contribute to neuronal plasticity and synaptogenesis [2]. In the early stages of AD, microglia have been suggested to play a neuroprotective role by facilitating the clearance, degradation, and phagocytosis of Aβ, and reducing Tau propagation [3-7]. However, in the late stages of the disease Aβ accumulation, tau propagation and neuroinflammation are promoted by the accumulation of activated microglia [8].

Triggering receptor expressed on myeloid cells 2 (TREM2) is a pattern recognition receptor found on the plasma membrane of myeloid cells, including macrophages, monocytes, osteoclasts, dendritic cells and, in the CNS, is uniquely expressed by microglia [9]. TREM2 ligands include lipopolysaccharide [10], phospholipids [3], and lipoproteins [4]. When activated by these ligands, TREM2 triggers a microglial response consisting of phagocytosis, chemotaxis, and microglia proliferation, increasing survival and migration [3, 11-13]. The canonical TREM2 signal is predominantly mediated by the binding of DAP12 (DNAX-activating protein of 12 kDa), which leads to the activation of Syk tyrosine kinase, triggering the downstream signaling cascade [14-16].

Genetic variations in the TREM2 gene have been correlated with neurodegenerative diseases. In particular, heterozygous missense mutations in the gene encoding TREM2 have been described as a risk factor for late-onset AD [17, 18]. In addition, homozygous mutations associated with loss of TREM2 function can cause early-onset neurodegenerative disorders called Nasu-Hakola disease (NHD) and frontotemporal dementia (FTD)-like syndrome [19].

TREM2 is a transmembrane protein with a single-pass type I structure. It consists of a small C-terminus located on the inner side of the plasma membrane and an N-terminal ectodomain that harbors the ligand binding region. A Disintegrin And Metalloproteases 10 and 17 (ADAM10 and ADAM17) are the proteases responsible for cleaving the ectodomain of TREM2, resulting in the release of its soluble form (sTREM2) into the extracellular medium [11, 20, 21].

sTREM2 can be detected in human cerebrospinal fluid (CSF) from individuals with sporadic AD, suggesting that CSF sTREM2 may serve as an indicator of the microglial TREM2-mediated response in AD [22, 23]. Although several studies have suggested that CSF sTREM2 levels are elevated across the AD continuum, there are inconsistencies regarding the different stages of the disease [24]. Most studies show that CSF sTREM2

levels increase in the early symptomatic and mild cognitive impairment (MCI) stages of AD, but decrease in the earliest asymptomatic stage prior to Alzheimer's diagnosis and significantly decline in the dementia stage of the disease [22, 23, 25-34]. Early-increased CSF sTREM2 levels have been associated with tau pathology and neurodegeneration, whereas Aβ plaque deposition is associated with decreased CSF sTREM2 levels [22, 23, 26, 28, 29]. In patients with NHD and FTD-like syndrome, CSF and blood sTREM2 levels are undetectable because misfolded TREM2 is retained in the endoplasmic reticulum, preventing its maturation and cleavage at the plasma membrane [11].

The function of sTREM2 is unknown, but several recent studies have suggested that sTREM2 may be involved in neuroprotection in AD by interacting with Aβ plaques. Specifically, microglial TREM2 binds to oligomeric Aβ and induces the release of sTREM2, which also preferentially binds to Aβ oligomers and affects their aggregation state. As a result, the aggregates refold into soluble forms, thereby inhibiting the deleterious effects of Aβ on the CNS. [35-38]. Moreover, it is believed that sTREM2 is involved in the regulation of microglial dynamics [1].

The overarching aim of this project is to study the function of the TREM2-mediated microglial response in AD and, in particular, the role of sTREM2. Therefore, the specific goal of this Human Biology Final Degree Project is to optimize an enzyme-linked immunosorbent assay (ELISA) to measure sTREM2 levels in human CSF and plasma. The optimized assay will be then applied for measuring CSF and plasma sTREM2 in human samples from the AD studied at BBRC and Hospital del Mar. The clinical application of this immunoassay has the potential to improve the prognosis of individuals with AD by enabling early detection of the disease.

RESULTS

The aim of this study was to optimize an immunoassay that can accurately and precisely measure sTREM2 levels in CSF and plasma in the range of interest for individuals at different stages of AD. We will use the MSD immunoassay platform, which is based on electrochemiluminescent (ECL) labels conjugated to secondary antibodies (Sulfo-TAG) that generate light when stimulated with electricity. The ECL signal provides a higher level of sensitivity, a wider dynamic range of detection and a lower level of background noise than other quantification techniques such as the standard ELISA assay, thereby making it the method of choice.

Critical optimization steps, such as antibody concentrations, MSD reagent storage and MSD coated or Small Spot coated-streptavidin plates, have been adjusted to improve the performance of the assay and assess the likelihood that this assay can fulfill its intended purpose.

The following optimization steps were followed:

1. Optimization of the capture and detection antibody concentration
2. Comparative evaluation of MSD reagents storage conditions
3. Comparative evaluation of the MSD coated-streptavidin plate types
4. Optimization of secondary antibody concentration

For each of the above steps, the best performing condition was selected based on the following criteria (More detailed information in **Table 1**):

1. Signal-to-noise (S/N) ratio
2. Background noise
3. Coefficient of variation (CV%)

1. Optimization of the capture and detection antibody concentrations

First, several concentrations of both capture and detection antibodies were tested to determine the optimal conditions for the sTREM2 immunoassay. To evaluate the performance of distinct antibody combinations, standard curves with TREM2 recombinant protein were generated encompassing the concentration range expected in human biofluids (8000 to 62.5 pg/ml) and assuming a 1/4 dilution of the samples, as previously described [22]. The data obtained are presented in separate tables (**Tables 2, 3 and 4**).

Different MSD coated-streptavidin plate types were evaluated in the same experimental assay. However, the analysis of these two types is discussed in the *Comparative evaluation of the MSD coated-streptavidin plate types* of the results section.

As shown in **Table 2**, both antibodies were serially diluted (concentration range from 0.25 to 0.0625 µg/ml) in the antibody dilution buffer. Notably, the highest dilution of the capture antibody (0.0625 µg/ml) showed better performance. When analyzing the standard curve at a concentration of 2000 pg/ml and using a fixed detection antibody concentration of 0.0625 µg/ml, a higher S/N ratio was observed with the capture antibody concentration of 0.0625 µg/ml compared to 0.25 µg/ml. Specifically, in the MSD Coated-Streptavidin Plate, the S/N ratios were 25.99 *vs.* 15.09 for 0.0625 µg/ml and 0.25 µg/ml, respectively. Similarly, in the MSD Small Spot Coated-Streptavidin Plate the corresponding ratios were 30.41 *vs.* 15.59 (**Table 2**).

Interestingly, contrasting results were obtained when evaluating the highest dilution of the detection antibody (0.0625 µg/ml) compared to the higher concentrations (0.25 and 0.125 µg/ml). The obtained results can be interpreted by comparing the S/N ratio at a standard curve concentration of 2000 pg/ml, considering the different concentrations of detection antibody together with a fixed concentration of 0.0625 of the capture antibody. For both the MSD coated-streptavidin plate and the MSD small spot coated-streptavidin plate, higher S/N ratios were obtained with the higher concentrations of the detection antibody (26.38 *vs.* 26.92 *vs.* 25.99; 36.15 *vs.* 35.20 *vs.* 30.41, corresponding to the detection antibody concentrations of 0.25, 0.125 and 0.0625 µg/ml respectively) (**Table 2**).

These results suggest that lower concentrations of capture antibody and higher concentrations of detection antibody than those initially tested may provide more reliable results for the quantification of sTREM2 levels. Based on this, further dilutions of capture antibody and higher detection antibody concentrations needed to be tested for improved performance.

Therefore, in the following experimental assay, a higher concentration of detection antibody (0.5 µg/ml) was tested along with two additional diluted concentrations of capture antibody (31.25 and 15.63 ng/ml) (**Table 3**). Contrary to expectations, increasing the concentration of the detection antibody did not improve performance. On the contrary, higher background noises were observed, resulting in lower signal-to-noise ratio values. For example, lower signal discrimination was observed at a detection antibody concentration of 0.5 µg/ml compared to 0.25 µg/ml (0.92 *vs.* 1.49) at a capture concentration of 31.25 ng/ml. In addition, the background noises for these conditions were 1103.44 and 1049.80, respectively (**Table 3**).

On the other hand, decreasing the concentration of the capture antibody resulted in improved assay performance in terms of background noise and S/N ratios. For example, at a

detection antibody concentration of 0.25 µg/ml, the S/N ratio improved (26.94 *vs.* 21.35; standard curve concentration of 2000 pg/ml) and the background noise decreased (1049.80 *vs.* 1897.33) as the concentration of the capture antibody was reduced to 31.25 ng/ml compared to a concentration of 0.0625 µg/ml. Similarly, when the capture antibody concentration was further diluted to 15.63 ng/ml, the S/N ratio increased to 30.38, and the background decreased to 521.50 (**Table 3**).

Based on the results of the previous experimental assay, an optimal concentration of 0.125 µg/ml for the detection antibody was chosen as it gave better S/N and background results. However, two additional dilutions of the capture antibody were evaluated to assess whether better sensitivity parameters could be achieved: 7.81 and 3.91 ng/ml (**Table 4**).

Surprisingly, the results indicated that decreasing the concentration of the capture antibody did not result in improved detection performance, suggesting that a threshold is reached at 31.25 ng/mL, below which no significant increase in the S/N ratio is observed. The validity of this statement is clearly demonstrated in **Table 4**, which shows a comparison of S/N ratios. Within a dynamic range of 500 to 2000 pg/ml, a consistent decrease in S/N ratios is observed when the concentration of the capture antibody is diluted from 15.63 ng/ml to 3.91 ng/ml (conc. 500 pg/ml: 12.67 *vs.* 8.84; conc. 2000 pg/ml: 47.82 *vs.* 28.41, respectively). This indicates that dilution of the capture antibody concentration results in decreased sensitivity of the detection assay.

Capture concentrations of 31.25 ng/ml and 15.63 ng/ml had similar S/N ratios for each concentration of the standard curve. However, a concentration of 15.63 ng/ml was defined as optimal as the background noise was significantly better (733.34 *vs.* 399.87; **Table 4**).

Overall, through a series of consecutive experimental assays evaluating different combinations of capture and detection antibodies, optimal concentrations were determined. Specifically, the optimal concentration for the detection antibody was found to be 0.125 µg/ml, while the concentration of 15.63 ng/ml proved to be optimal for the capture antibody. The following optimization steps were performed based on these concentrations of capture and detection antibodies.

2. Comparative evaluation of MSD reagents storage conditions

In the field of immunoassays, the performance and reliability of the assay is influenced by several factors, including the storage conditions of the reagents. In particular, maintaining the integrity and stability of reagents during transport and storage is critical to achieving accurate and reproducible results. Considering this, we next investigated the effect of storage temperature on the performance of an MSD ELISA assay for the detection of sTREM2. We decided to perform these experiments based on a shipping error. The MSD reagents (secondary antibody, reading buffer 4X and MSD coated-streptavidin plates) had

been shipped in dry ice, contrary to the recommended storage temperature of 4°C. Therefore, it was important to determine whether storage conditions (4ºC *vs.* frozen) could affect assay performance. Different dilutions of the secondary antibody were evaluated in the same experimental assay. However, the analysis of these dilutions is discussed in the *Optimization of secondary antibody concentration* in the results section.

The results showed that storage of MSD reagents at 4ºC resulted in higher background noises for secondary antibody concentrations of 1 µg/ml, 0.5 µg/ml and 0.25 µg/ml (1412.91 *vs.* 964.24 *vs.* 603.30) compared to frozen conditions (873.46 *vs.* 672.14 *vs.* 485.22). However, it is noteworthy that at a dilution of 0.125 µg/ml, similar background noises were obtained between the 4ºC and frozen conditions (384.46 vs. 316.50) (**Table 5**).

Nevertheless, improved S/N ratios were observed when stored at 4ºC compared to the frozen conditions at concentrations of 500 pg/ml (6.85 vs. 5.65) and 2000 pg/ml (41.55 vs. 27.04) on the standard curve (data shown in **Table 5**).

Despite the higher background noises observed with storage at 4ºC, this was still considered the optimal condition for the sTREM2 ELISA.

3. Comparative evaluation of the MSD coated-streptavidin plate types

Different MSD coated-streptavidin plates can be used and type can significantly affect the performance of the assay.

In this step, we compared the performance of two different types of MSD coated-streptavidin plates, namely the regular MSD coated-streptavidin plate and the MSD Small Spot coated-streptavidin plate (see *Materials and Methods* section for details). The MSD Small Spot coated-streptavidin plates are known to provide higher assay signals and thereby superior sensitivity. As a result, better immunoassay performance is expected when using this specific plate variant.

Despite the higher background noises observed in the MSD Small Spot coated-streptavidin plate compared to the regular plate, it exhibits higher signal ranges and S/N ratios. Specifically, at a capture antibody concentration of 0.0625 µg/ml and a detection antibody concentration of 0.25 µg/ml, the signal ranges observed on the regular MSD plate range from 437.11 to 7775 over a concentration from 125 to 2000 pg/ml. As expected, the signal range on the MSD Small Spot coated-streptavidin plate is much broader, ranging from 1210.53 to 26978.51 within the same concentration range (data shown in **Table 2**).

Similarly, when considering the S/N ratios, the MSD regular plate shows ratios of 1.48 and 26.38 within the concentration range of 125 to 2000 pg/ml. In contrast, the MSD Small Spot coated-streptavidin plate shows higher S/N ratios of 1.62 and 36.15 over the same concentration range (**Table 2**).

Therefore, considering the superior performance in terms of signal range and S/N ratios, the MSD Small Spot coated-streptavidin plate proves to be the optimal choice to achieve improved performance in the detection of sTREM2 levels, despite the higher background noises.

4. Optimization of secondary antibody concentration

Using the MSD Small Spot plates, we tested the optimal concentration of the secondary antibody. As mentioned above, the secondary antibody is conjugated to a SULFO-TAG that lights up when stimulated with electricity. The light emission allows precise quantification of the signal generated by sTREM2 binding to the antibodies.

Therefore, the following experimental assays are designed to evaluate the performance of different concentrations of secondary antibodies. Standard curves were generated in a concentration range from 8000 to 62.5 pg/ml and serial dilutions of the secondary antibodies from 1 µg/ml to 31.25 ng/ml were evaluated. The data obtained are presented in separate tables (**Tables 5, 6 and 7**).

First, concentrations ranging from 1 µg/ml to 0.125 µg/ml of secondary antibody were tested as detailed in **Table 5**. Remarkably, the more diluted concentrations of the secondary antibody (0.25 µg/ml and 0.125 µg/ml) showed improved performance in terms of background noise and S/N ratio on both MSD reagent conditions (4ºC *vs.* frozen conditions). Specifically, for the MSD reagents at 4ºC, the background noise decreased from 1412.91 to 603.30 for the 1 µg/ml and 0.25 µg/ml concentrations, respectively. Furthermore, when considering the standard curve concentration range of 62.5 to 8000 pg/ml, the S/N ratios for the 1 µg/ml and 0.25 µg/ml concentrations showed a significant improvement from 1.03 to 65.54 and 1.08 to 82.31 respectively, under the same conditions. On the other hand, although the background noises were slightly lower with a concentration of 0.125 µg/ml compared to 0.25 µg/ml, the S/N ratio did not provide definitive results to determine the optimal concentration for the immunoassay. Consequently, the 0.25 µg/ml and 0.125 µg/ml concentrations were further investigated, together with two additional diluted forms (0.0625 µg/ml and 31.25 ng/ml).

The results presented in **Table 6** show that increasing the dilution of the secondary antibody by a factor of two (31.25 ng/ml) did not improve the performance of the immunoassay. Surprisingly, the results indicate the existence of a threshold between concentrations of 0.125 µg/ml and 0.0625 µg/ml. Comparable S/N ratios were observed over the sample concentration range of 500 to 2000 pg/ml when both concentrations were tested. Specifically, the S/N ratios were 13.03 *vs.* 13.13 at 500 pg/mL, and 46.32 *vs.* 46.49 at 2000 pg/mL, for the concentrations of 0.125 µg/ml and 0.0625 µg/ml respectively. However, based

on the significantly improved background noise (476.48 *vs.* 297.10), a concentration of 0.0625 µg/ml was determined to be optimal to ensure the best signal detection.

Finally, to assess the precision of sTREM2 concentration measurements, a comparative analysis was performed using different concentrations of the secondary antibody (0.25 µg/ml and 0.125 µg/ml, 0.0625 µg/ml and 31.25 ng/ml; **Table 7**) in real CSF and plasma human samples.

In this study, we replicated measurements of high plasma, low plasma and CSF samples over the different dilutions of the secondary antibody. By ensuring that sample concentrations remained relatively constant, we aimed to investigate the effect of antibody dilution on the variability of sTREM2 concentration measurements. This analysis was important to ensure the robustness and reproducibility of the MSD ELISA. Diluted samples were measured in duplicate on the same test plate for each standard curve. We found that the observed CV% between replicate measurements was within the predefined intra-plate acceptance range (<20%), indicating consistent sTREM2 across different dilutions of the secondary antibody and sample types (data shown in **Table 7**).

DISCUSSION

In the present study, we aimed to optimize the performance of an immunoassay that could potentially detect sTREM2 levels in both human CSF and plasma samples from individuals with signs of cognitive impairment. To achieve the main objective of this study, several critical steps in the development of the sTREM2 ELISA had to be optimized. The validation parameters considered to determine the most optimal conditions were the S/N ratio, background noise, and CV%.

The main results of our study were the following. Firstly, through our optimization process, we identified that a concentration of 15.63 ng/ml for the capture antibody and 0.125 µg/ml for the detection antibody provided the best results. Secondly, we observed that MSD reagents used in the immunoassay remained well-preserved when stored at 4ºC. Thirdly, MSD Small Spot streptavidin-coated plates notably improved the assay performance and expanded the range of detectable signals for sTREM2. Lastly, although no significant differences were found between the 0.125 µg/ml and 0.0625 µg/ml concentrations, the 0.0625 µg/ml concentration of secondary antibody was chosen as optimal to achieve accurate sTREM2 measurements by reducing background noise.

It is noteworthy that in all experimental assays, the CV% between duplicates was within the acceptable range (< 20%), indicating a high precision of the MSD ELISA. Therefore, as a quality control measure, the acceptable range can be further reduced to 15% in future experiments.

Overall, these findings contribute to the improved accuracy and reliability of the immunoassay for the detection of sTREM2 levels in human biofluids. The results presented herein are the first step of a more ambitious project that eventually aims at investigating the function of the TREM2-mediated microglial response in AD. Now that we delivered an optimized sTREM2 assay, the next step will be to measure CSF and plasma samples of the BIODEGMAR [39, 40] and ALFA cohorts [41].

There are two main controversies in the field that this study will address. First, it is not yet clear how CSF and plasma sTREM2 levels change in the whole continuum of AD (from preclinical to dementia stages). In both MCI and AD, several studies have reported significant increases in CSF sTREM2 levels compared with cognitively normal controls [11, 22, 28, 29], but these changes may differ depending on the stage. However, non-significant differences in plasma sTREM2 levels have been reported in AD patients [30]. Second, it is not clear whether TREM2-mediated microglial response has a protective or a detrimental effect on AD and when in the continuum this effect occurs. This is a key question to develop treatments for AD that target TREM2.

By optimizing the MSD ELISA assay, the performance of sTREM2 quantification has been improved, allowing a more comprehensive investigation of sTREM2 dynamics throughout

the AD continuum and its potential as a prognostic biomarker in AD. However, rigorous validation of this assay is required before its practical implementation.

Such advances are promising to facilitate the early detection of AD, as well as the development of novel disease-modifying treatments.

MATERIALS & METHODS

1. Sample collection and storage

At the Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), lumbar CSF and plasma samples were collected using standard procedures. CSF samples were obtained by lumbar puncture and both biofluid samples were collected in polyethylene tubes. After centrifugation at 2000g for 10 minutes at 4ºC, the supernatant was transferred to new tubes and stored at -80°C until thawed for analysis. All CSF and plasma samples were aliquoted, stored and analyzed at the BBRC for sTREM2 measurements.

Samples are collected from patients in the BIODEGMAR cohort. The BIODEGMAR cohort is a longitudinal observational study conducted at the Hospital del Mar in Barcelona, Spain. It includes participants with cognitive decline and/or neurodegenerative disorders treated at the Cognitive Decline and Movement Disorders Unit.

Patients enrolled in the cohort underwent several procedures as part of the study protocol. These procedures included a comprehensive neuropsychological assessment, magnetic resonance imaging (MRI), apolipoprotein E (APOE) ε4 genotyping, blood sampling and lumbar puncture for CSF collection. Neuropsychological assessment, performed by a neuropsychologist, included serial standardized cognitive tests and functional scales. In addition, clinical assessment, including medical history, physical examination and clinical diagnosis, was performed by a neurologist.

A detailed description of the BIODEGMAR cohort, together with more specific information on inclusion and exclusion criteria, has been published previously [39, 40].

2. sTREM2 ELISA

An ELISA assay for the quantification of sTREM2 in human CSF and plasma samples was previously developed by Kleinberger G, Suárez-Calvet M, Haass C *et al* [11]. In this study, we optimized the ELISA previously described. The detailed protocol used in this study is as follows:

MSD GOLD 96-well Small Spot Streptavidin SECTOR plates (MSD, cat. nº L45SA-1) were blocked O/N at 4ºC on a shaker with blocking buffer [3% bovine serum albumin (BSA) and 0.05% Tween 20 in PBS (pH 7.4); 250 µl/well]. To detect human sTREM2, the plates were incubated for 1.5 hours at room temperature (RT) on a shaker (ca. 700 rpm) with biotinylated polyclonal goat IgG anti-human TREM2 capture antibody (R&D Systems, cat. nº BAF1828; 25 µl/well) diluted in antibody dilution buffer [1% BSA and 0.05% Tween 20 in PBS (pH 7.4)]. Following four washes with washing buffer [0.05% Tween 20 in PBS (pH 7.4)] samples diluted 1:4 in sample dilution buffer [1% BSA, 0.05% Tween 20 in PBS (pH 7.4) and supplemented with protease inhibitors (Roche; cat nº 04693116001)] were added and

incubated for 2 hours at RT on a shaker (ca. 700 rpm). A recombinant human TREM2 protein (Abyntek, cat. nº 11084-H08H-50) was also diluted in sample dilution buffer in a two-fold serial dilution and used for the standard curve (concentration range, 8000 to 62.5 pg/ml). Plates were washed twice, each of them with 3 cycles, and incubated with monoclonal mouse IgG anti-human TREM2 detection antibody (SantaCruzBiotech, cat. nº sc-373828; 50 µl/well) diluted in antibody dilution buffer for 1 hour at RT on a shaker (ca. 700 rpm). After 2 additional washing steps, each of them with 3 cycles, plates were incubated with a SULFO-TAG-labeled anti-mouse (goat) secondary antibody (MSD, cat. nº R32AC; 25 µl/well) for 1 hour at RT on a shaker (ca. 700 rpm). Last, plates were washed 2 times, each of them with 3 cycles, followed by two final washes in PBS before being developed by adding 1X Meso Scale Discovery Read buffer (cat nº R-92TC-1; 150 µl/well). The Meso Scale Discovery SECTOR Imager 2400 reader was used to measure the light emission at 620 nm after electrochemical stimulation. Raw values are provided as pg/ml.

2.1. Recombinant protein preparation

Recombinant human TREM2 protein was purchased in a 0.1 mg lyophilized format. This amount was diluted in 500µl of dH2O to achieve a pre-stock concentration of 0.2 mg/ml, and aliquots were stored at -80ºC. The pre-stock was diluted 1:1000 to achieve a working stock of 200 ng/ml in two steps: 10µl of the pre-stock solution in 990 µl of sample dilution buffer, and subsequently 200µl of the previous dilution in 1800µl of sample dilution buffer. The working stock solution was aliquoted in different volumes to use the amount needed for each analysis: 10µl, 50µl and 100µl.

2.2. Antibody concentration optimization

The concentrations of the capturing and detecting antibodies were optimized before being established in the sTREM2 ELISA method described above. Two-fold serial dilutions were used for both capture and detection antibodies, and the concentrations of both were varied from each other. Dilutions giving the highest S/N ratio and a CV% in the optimal range were selected. Specifically, the concentration of the detection antibody ranged from 0.5 µg/ml to 0.0625 µg/ml. On the other hand, a concentration range of 0.25 µg/ml to 3.91 ng/ml was tested for the capture antibody.

The optimal concentration of the secondary antibody was also determined. Concentrations ranging from 1 µg/ml to 31.25 ng/ml were tested.

2.3. MSD GOLD 96-well plates

Before implementing the aforementioned optimization of the sTREM2 ELISA method, we evaluated two different types of MSD GOLD 96-well plates: MSD Coated Streptavidin and

MSD Small Spot Coated Streptavidin plates. The regular MSD plates have streptavidin-coated across the whole well surface. In contrast, the Small Spot Coated-Streptavidin Plates are functionalized with a small streptavidin spot in the center of each well, resulting in enhanced assay signals, improved sensitivity and reduced non-specific binding. These plates are widely used for a variety of applications, including the development of biomarker assays. Our selection of the optimal plate type was based on specific criteria such as signal-to-noise ratio (S/N), background noise levels and coefficient of variation (CV%).

3. Assay validation

3.1. Signal-to-noise (S/N) ratio

The S/N ratio was determined for each experimental assay by comparing the intensity of the desired signal to the background noise level using the following formula:

$$S/N\ ratio\ =\ \frac{Average\ Measured\ Signal}{Average\ Measured\ Signal\ Noise\ (blank)}$$

A low S/N ratio is an indication that the background noise is higher than optimal, which reduces the sensitivity of the immunoassay. Therefore, higher S/N ratios have been considered to define optimal conditions.

3.2. Coefficient of variation (CV%)

As an indication of assay precision, the CV% between the standard curve and the sample duplicates was calculated to assess the intra-plate variation using the following equation:

$$CV\%\ =\ \frac{Standard\ Deviation\ (SD)}{Average\ Measured\ Signal}\times 100$$

Samples and/or calibration duplicates with a CV% above the predefined intra-plate acceptance range (<20%) were discarded.

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BIBLIOGRAPHY

[1] Zhong L, Xu Y, Zhuo R, Wang T, Wang K, Huang R, Wang D, Gao Y, Zhu Y, Sheng X *et al*. Soluble TREM2 ameliorates pathological phenotypes by modulating microglial functions in an Alzheimer's disease model. Nat Commun. 2019 Mar 25; 10(1): 1365.

[2] Tiwari S, Atluri V, Kaushik A, Yndart A, Nair M. Alzheimer's disease: pathogenesis, diagnostics, and therapeutics. Int J Nanomedicine. 2019 Jul 19; 4: 5541-5554.

[3] Wang Y, Cella M, Mallinson K, Ulrich JD, Young KL, Robinette ML, Gilfillan S, Krishnan GM, Sudhakar S, Zinselmeyer BH et al. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. Cell. 2015 Mar 12; 160(6): 1061-71.

[4] Wang Y, Ulland TK, Ulrich JD, Song W, Tzaferis JA, Hole JT, Yuan P, Mahan TE, Shi Y, Gilfillan S et al. TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. J Exp Med. 2016 May 2; 213(5): 667-75.

[5] Yuan P, Condello C, Keene CD, Wang Y, Bird TD, Paul SM, Luo W, Colonna M, Baddeley D, Grutzendler J. TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. Neuron. 2016 May 18; 90(4): 724-39.

[6] Jay TR, Hirsch AM, Broihier ML, Miller CM, Neilson LE, Ransohoff RM, Lamb BT, Landreth GE. Disease Progression-Dependent Effects of TREM2 Deficiency in a Mouse Model of Alzheimer's Disease. J Neurosci. 2017 Jan 18; 37(3): 637-647.

[7] Bemiller SM, McCray TJ, Allan K et al. TREM2 deficiency exacerbates tau pathology through dysregulated kinase signaling in a mouse model of tauopathy. Mol Neurodegeneration. 2017; 12: 74.

[8] Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. J Cell Biol. 2018; 217(2): 459-472.

[9] Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang LC, Means TK, El Khoury J. The microglial sensome revealed by direct RNA sequencing. Nat Neurosci. 2013 Dec; 16(12): 1896-905.

[10] Daws MR, Sullam PM, Niemi EC, Chen TT, Tchao NK, Seaman WE. Pattern recognition by TREM-2: binding of anionic ligands. J Immunol. 2003 Jul 15; 171(2): 594-9.

[11] Kleinberger G, Yamanishi Y, Suárez-Calvet M, Czirr E, Lohmann E, Cuyvers E, Struyfs H, Pettkus N, Wenninger-Weinzierl A, Mazaheri F et al. TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. Sci Transl Med. 2014 Jul 2; 6(243): 243ra86.

[12] Colonna M, Wang Y. TREM2 variants: new keys to decipher Alzheimer disease pathogenesis. Nat Rev Neurosci. 2016 Apr; 17(4): 201-7.

[13] Mazaheri F, Snaidero N, Kleinberger G, Madore C, Daria A, Werner G, Krasemann S, Capell A, Trümbach D, Wurst W et al. TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury. EMBO Rep. 2017 Jul; 18(7): 1186-1198.

[14] Takaki R, Watson SR, Lanier LL. DAP12: an adapter protein with dual functionality. Immunol Rev. 2006 Dec; 214: 118-29.

[15] N'Diaye EN, Branda CS, Branda SS, Nevarez L, Colonna M, Lowell C, Hamerman JA, Seaman WE. TREM-2 (triggering receptor expressed on myeloid cells 2) is a phagocytic receptor for bacteria. J Cell Biol. 2009 Jan 26; 184(2): 215-23.

[16] Peng Q, Malhotra S, Torchia JA, Kerr WG, Coggeshall KM, Humphrey MB. TREM2- and DAP12-dependent activation of PI3K requires DAP10 and is inhibited by SHIP1. Sci Signal. 2010 May 18; 3(122): ra38.

[17] Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, Cruchaga C, Sassi C, Kauwe JS, Younkin S et al. TREM2 variants in Alzheimer's disease. N Engl J Med. 2013 Jan 10; 368(2): 117-27.

[18] Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, Bjornsson S, Huttenlocher J, Levey AI, Lah JJ et al. Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med. 2013 Jan 10; 368(2): 107-16.

[19] Paloneva J, Manninen T, Christman G, Hovanes K, Mandelin J, Adolfsson R, Bianchin M, Bird T, Miranda R, Salmaggi A, Tranebjaerg L et al. Mutations in two genes encoding

different subunits of a receptor signaling complex result in an identical disease phenotype. Am J Hum Genet. 2002 Sep; 71(3): 656-62.

[20] Piccio L, Buonsanti C, Cella M, et al. Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. Brain. 2008; 131(Pt 11): 3081-3091.

[21] Schlepckow K, Kleinberger G, Fukumori A, Feederle R, Lichtenthaler SF, Steiner H, Haass C. An Alzheimer-associated TREM2 variant occurs at the ADAM cleavage site and affects shedding and phagocytic function. EMBO Mol Med. 2017 Oct; 9(10): 1356-1365.

[22] Suárez-Calvet M, Kleinberger G, Araque Caballero MÁ, Brendel M, Rominger A, Alcolea D, Fortea J, Lleó A, Blesa R, Gispert JD et al. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. EMBO Mol Med. 2016 May 2; 8(5): 466-76.

[23] Suárez-Calvet M, Morenas-Rodríguez E, Kleinberger G, Schlepckow K, Araque Caballero MÁ, Franzmeier N, Capell A, Fellerer K, Nuscher B, Eren E et al. Early increase of CSF sTREM2 in Alzheimer's disease is associated with tau related-neurodegeneration but not with amyloid-β pathology. Mol Neurodegener. 2019 Jan 10; 14(1): 1.

[24] Carmona S, Zahs K, Wu E, Dakin K, Bras J, Guerreiro R. The role of TREM2 in Alzheimer's disease and other neurodegenerative disorders. Lancet Neurol. 2018 Aug; 17(8): 721-730.

[25] Suárez-Calvet M, Araque Caballero MÁ, Kleinberger G, Bateman RJ, Fagan AM, Morris JC, Levin J, Danek A, Ewers M & Haass C. Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. Sci Transl Med. 2016 Dec 14; 8(369): 369ra178.

[26] Gispert JD, Suárez-Calvet M, Monté GC, Tucholka A, Falcon C, Rojas S, Rami L, Sánchez-Valle R, Lladó A, Kleinberger G et al. Cerebrospinal fluid sTREM2 levels are associated with gray matter volume increases and reduced diffusivity in early Alzheimer's disease. Alzheimers Dement. 2016 Dec; 12(12): 1259-1272.

[27] Henjum K, Almdahl IS, Årskog V et al. Cerebrospinal fluid soluble TREM2 in aging and Alzheimer’s disease. Alz Res Therapy. 2016; 8: 17.

[28] Heslegrave A, Heywood W, Paterson R, Magdalinou N, Svensson J, Johansson P, Öhrfelt A, Blennow K, Hardy J, Schott J et al. Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. Mol Neurodegener. 2016 Jan 12; 11: 3.

[29] Piccio L, Deming Y, Del-Águila JL, Ghezzi L, Holtzman DM, Fagan AM, Fenoglio C, Galimberti D, Borroni B, Cruchaga C. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. Acta Neuropathol. 2016 Jun; 131(6): 925-33.

[30] Bekris LM, Khrestian M, Dyne E, Shao Y, Pillai JA, Rao SM, Bemiller SM, Lamb B, Fernandez HH, Leverenz JB. Soluble TREM2 and biomarkers of central and peripheral inflammation in neurodegenerative disease. J Neuroimmunol. 2018 Jun 15; 319: 19-27.

[31] Liu D, Cao B, Zhao Y, Huang H, McIntyre RS, Rosenblat JD, Zhou H. Soluble TREM2 changes during the clinical course of Alzheimer's disease: A meta-analysis. Neurosci Lett. 2018 Nov 1; 686:10-16.

[32] Nordengen K, Kirsebom BE, Henjum K, Selnes P, Gísladóttir B, Wettergreen M, Torsetnes SB, Grøntvedt GR, Waterloo KK, Aarsland D, Nilsson LNG, Fladby T. Glial activation and inflammation along the Alzheimer's disease continuum. J Neuroinflammation. 2019 Feb 21; 16(1): 46.

[33] Rauchmann BS, Schneider-Axmann T, Alexopoulos P, Perneczky R. CSF soluble TREM2 as a measure of immune response along the Alzheimer's disease continuum. Neurobiol Aging. 2019 Feb; 74: 182-190.

[34] Ma LZ, Tan L, Bi YL, Shen XN, Xu W, Ma YH, Li HQ, Dong Q, Yu JT. Dynamic changes of CSF sTREM2 in preclinical Alzheimer's disease: the CABLE study. Mol Neurodegener. 2020 Apr 10; 15(1) :25.

[35] Zhao Y, Wu X, Li X, Jiang LL, Gui X, Liu Y, Sun Y, Zhu B, Piña-Crespo JC, Zhang M, Zhang N, Chen X, Bu G, An Z, Huang TY, Xu H. TREM2 Is a Receptor for β-Amyloid that Mediates Microglial Function. Neuron. 2018 Mar 7; 97(5): 1023-1031.e7.

[36] Zhong L, Wang Z, Wang D, Wang Z, Martens YA, Wu L, Xu Y, Wang K, Li J, Huang R et al. Amyloid-beta modulates microglial responses by binding to the triggering receptor expressed on myeloid cells 2 (TREM2). Mol Neurodegener. 2018 Mar 27; 13(1): 15.

[37] Lessard CB, Malnik SL, Zhou Y, Ladd TB, Cruz PE, Ran Y, Mahan TE, Chakrabaty P, Holtzman DM, Ulrich JD et al. High-affinity interactions and signal transduction between Aβ oligomers and TREM2. EMBO Mol Med. 2018 Nov; 10(11): e9027.

[38] Vilalta A, Zhou Y, Sevalle J, Griffin JK, Satoh K, Allendorf DH, De S, Puigdellívol M, Bruzas A, Burguillos MA et al. Wild-type sTREM2 blocks Aβ aggregation and neurotoxicity, but the Alzheimer's R47H mutant increases Aβ aggregation. J Biol Chem. 2021 Jan-Jun; 296: 100631.

[39] Ashton NJ, Puig-Pijoan A, Milà-Alomà M, Fernández-Lebrero A, García-Escobar G, González-Ortiz F, Kac PR, Brum WS, Benedet AL, Lantero-Rodriguez J et al. Plasma and CSF biomarkers in a memory clinic: Head-to-head comparison of phosphorylated tau immunoassays. Alzheimers Dement. 2023 May; 19(5):1913-1924.

[40] Lantero-Rodriguez J, Vrillon A, Fernández-Lebrero A, et al. Clinical performance and head-to-head comparison of CSF p-tau235 with p-tau181, p-tau217 and p-tau231 in two memory clinic cohorts. Alzheimers Res Ther. 2023 Mar 10; 15(1): 48.

[41] Molinuevo JL, Gramunt N, Gispert JD, et al. The ALFA project: A research platform to identify early pathophysiological features of Alzheimer's disease. Alzheimers Dement (N Y). 2016 Mar 3; 2(2): 82-92.

FIGURES AND TABLES

Table 1. Validation parameters. Overview of the criteria used to determine the optimal conditions for optimizing the MSD ELISA. The table outlines the definitions of the validation parameters, namely signal-to-noise ratio, background noise and coefficient of variation. It also highlights the factors that can influence each parameter and emphasizes the importance of different parameter values in selecting the optimal assay conditions.

Parameter	Validation criteria
Signal-to-noise ratio (S/N)	The sensitivity of an immunoassay can be defined as the ratio of the specific signal generated to the background noise of the system. The S/N ratio can be affected by several factors such as blocking buffer, washing steps, antibody concentrations, incubation times and temperatures, and sample dilution. Therefore, when evaluating the performance of different conditions, higher S/N ratios were considered in order to select the optimal condition.
Background noise	Background noise can be defined as the non-specific signals or interferences that can affect the sensitivity and accuracy of the assay. It can be caused by several factors such as contaminants, non-specific binding of antibodies and/or streptavidin, incomplete washing, high sample matrix interference, cross-reactivity, substrate instability and instrument noise. Therefore, several strategies and optimizations can be used to reduce the background noise. When evaluating the performance of different conditions, lower background noise was taken into account to select the optimal condition.
Coefficient of variation (CV%)	The precision or reproducibility of measurement in an ELISA can be quantified by the CV%. It provides an indication of the precision of the assay and the variation between replicates. This parameter can be affected by several factors such as sample distribution, pipetting errors, inconsistent incubation times or temperatures and changes in reagent batches which can lead to intra-assay, inter-assay, sample or plate-to-plate variability. Therefore, when evaluating the performance of different conditions, a lower CV% (<20%) was considered in order to select the optimal condition.

Table 2. Optimization of capture and detection antibody concentrations and MSD plate types. Standard curves were generated using recombinant TREM2 protein covering the concentration range expected in human biofluids (2000 to 125 pg/ml) and assuming a 1/4 dilution of samples. Three different detection antibody concentrations (0.25 µg/ml, 0.125 µg/ml and 0.0625 µg/ml) were tested in combination with three different capture antibody concentrations (0.25 µg/ml, 0.125 µg/ml and 0.0625 µg/ml). In addition, two types of MSD plates were compared: an MSD Coated-Streptavidin Plate *vs.* an MSD Small Spot Coated-Streptavidin Plate. The table provides information on background noise, TREM2 recombinant protein concentration (pg/ml), adjusted signal mean, coefficient of variation (CV%) and signal-to-noise ratio (S/N ratio). The validation parameters were used to determine the optimal conditions for the assay.

Experimental assay 1					
MSD coated-streptavidin plate					
Antibody concentration	**Background noise**	**Concentration (pg/ml)**	**Adj. signal mean**	**CV %**	**S/N**
Capture 0.25 µg/ml					
Detection 0.25 µg/ml	812.35	125	941.79	3.00	1.16
		2000	13615.25	2.26	16.76
Detection 0.125 µg/ml	832.39	125	920.42	1.92	1.11
		2000	12543.53	3.56	15.07
Detection 0.0625 µg/ml	760.89	125	833.9850119	0.85	1.10
		2000	11485.48815	0.20	15.09
Capture 0.125 µg/ml					
Detection 0.25 µg/ml	455.47	125	599.99	0.71	0.74
		2000	11368.57	1.81	13.99
Detection 0.125 µg/ml	432.43	125	535.89	2.90	1.24
		2000	10183.67	8.01	23.55
Detection 0.0625 µg/ml	422.99	125	522.49	0.68	1.24
		2000	9523.17	3.45	22.51
Capture 0.0625 µg/ml					
Detection 0.25 µg/ml	294.71	125	437.11	5.99	1.48
		2000	7775	0.07	26.38
Detection 0.125 µg/ml	275.46	125	401.98	1.41	1.46
		2000	7414.48	0.31	26.92

Detection 0.0625 µg/ml	273	125	374.99	1.13	1.37
		2000	7093.88	1.86	25.99
MSD Small Spot coated-streptavidin plate					
Antibody concentration	**Background noise**	**Concentration (pg/ml)**	**Adj. signal mean**	**CV %**	**Signal-to-noise ratio**
Capture 0.25 µg/ml					
Detection 0.25 µg/ml	2804.69	125	3110.43	3.71	1.11
		2000	47638.78	0.78	16.99
Detection 0.125 µg/ml	2681.95	125	2931.97	2.68	1.09
		2000	43857.96	0.19	16.35
Detection 0.0625 µg/ml	2480.95	125	2747.96	2.80	1.11
		2000	38677.29	1.12	15.59
Capture 0.125 µg/ml					
Detection 0.25 µg/ml	1357.01	125	1728.63	2.95	0.62
		2000	35481.58	4.68	12.65
Detection 0.125 µg/ml	1302.87	125	1626.32	4.09	1.25
		2000	36377.97	0.19	27.92
Detection 0.0625 µg/ml	1295.35	125	1560.51	3.53	1.20
		2000	32006.49	2.74	24.71
Capture 0.0625 µg/ml					
Detection 0.25 µg/ml	746.34	125	1210.53	5.67	1.62
		2000	26978.51	2.10	36.15
Detection 0.125 µg/ml	713.77	125	1095.86	4.84	1.54
		2000	25127.00	4.09	35.20
Detection 0.0625 µg/ml	756.34	125	1083.45	1.37	1.43
		2000	22999.03	0.90	30.41

Table 3. Optimization of capture and detection antibody concentrations. Standard curves were generated using recombinant TREM2 protein covering the concentration range expected in human biofluids (2000 to 125 pg/ml) and assuming a 1/4 dilution of samples. Three different detection antibody concentrations (0.5 µg/ml, 0.25 µg/ml and 0.125 µg/ml) were tested in combination with three different capture antibody concentrations (0.0625 µg/ml, 31.25 ng/ml and 15.63 ng/ml). The table provides information on background noise, TREM2 recombinant protein concentration (pg/ml), adjusted signal mean, coefficient of variation (CV%) and signal-to-noise ratio (S/N ratio). The validation parameters were used to determine the optimal conditions for the assay.

Experimental assay 2					
Antibody concentration	**Background noise**	**Concentration (pg/ml)**	**Adj. signal mean**	**CV %**	**Signal-to-noise ratio**
Capture 0.0625 µg/ml					
Detection 0.5 µg/ml	1892.90	125	2622.76	3.37	1.39
		2000	42913.41	1.01	22.67
Detection 0.25 µg/ml	1897.33	125	2430.84	1.63	1.28
		2000	40510.99	0.10	21.35
Detection 0.125 µg/ml	1815.45	125	2139.07	4.17	1.18
		2000	36821.93	0.28	20.28
Capture 31.25 ng/ml					
Detection 0.5 µg/ml	1103.44	125	1736.46	1.02	0.92
		2000	28785.14	4.14	15.21
Detection 0.25 µg/ml	1049.80	125	1568.92	1.44	1.49
		2000	28278.59	4.36	26.94
Detection 0.125 µg/ml	874.99	125	1437.06	3.49	1.64
		2000	26508.66	3.09	30.30
Capture 15.63 ng/ml					
Detection 0.5 µg/ml	584.81	125	933.92	1.82	1.60
		2000	16176.37	3.20	27.66
Detection 0.25 µg/ml	521.50	125	938.44	1.58	1.80
		2000	15841.77	8.14	30.38
Detection 0.125 µg/ml	533.46	125	905.14	3.98	1.70
		2000	15448.21	3.13	28.96

Table 4. Optimization of the capture antibody concentration. Standard curves were generated using recombinant TREM2 protein covering the concentration range expected in human biofluids (8000 to 62.5 pg/ml) and assuming a 1/4 dilution of samples. Four different concentrations of capture antibody (31.25 ng/ml, 15.63 ng/ml, 7.81 ng/ml and 3.91 ng/ml) were tested in combination with a fixed concentration of detection antibody (0.125 µg/ml). The table provides information on background noise, TREM2 recombinant protein concentration (pg/ml), adjusted signal mean, coefficient of variation (CV%) and signal-to-noise ratio (S/N ratio). The validation parameters were used to determine the optimal conditions for the assay.

Experimental assay 3					
Detection 0.125 µg/ml					
Antibody concentration	**Background noise**	**Concentration (pg/ml)**	**Adj. signal mean**	**CV %**	**Signal-to-noise ratio**
Capture 31.25 ng/ml	733.34	62.5	879.00	0.00	1.20
		500	8126.30	1.00	11.08
		2000	34650.97	2.95	47.25
		8000	69999.22	0.40	95.45
Capture 15.63 ng/ml	399.87	62.5	510.49	0.97	1.28
		500	5067.86	1.03	12.67
		2000	19122.34	1.18	47.82
		8000	35974.78	4.62	89.97
Capture 7.81 ng/ml	249.49	62.5	318.21	6.00	1.28
		500	2759.45	2.82	11.06
		2000	8673.95	9.74	34.77
		8000	16986.51	3.60	68.09
Capture 3.91 ng/ml	165.33	62.5	202.47	2.44	1.22
		500	1461.18	2.95	8.84
		2000	4697.07	1.91	28.41
		8000	8264.35	0.85	49.99

Table 5. Optimization of secondary antibody concentration and MSD reagents storage conditions. Standard curves were generated using recombinant TREM2 protein covering the concentration range expected in human biofluids (8000 to 62.5 pg/ml) and assuming a 1/4 dilution of samples. Four different secondary antibody concentrations (1 µg/ml, 0.5 µg/ml, 0.25 µg/ml and 0.125 µg/ml) were tested. In addition, two storage conditions of the MSD reagents were analyzed: 4ºC and frozen. The table provides information on background noise, TREM2 recombinant protein concentration (pg/ml), adjusted signal mean, coefficient of variation (CV%) and signal-to-noise ratio (S/N ratio). The validation parameters were used to determine the optimal conditions for the assay.

Experimental assay 4					
4ºC MSD reagents					
Antibody concentration	**Background noise**	**Concentration (pg/ml)**	**Adj. signal mean**	**CV %**	**Signal-to-noise ratio**
Secondary 1 µg/ml	1412.91	62.5	1458.51	3.68	1.03
		500	5277.65	5.40	3.74
		2000	39112.11	0.95	27.68
		8000	92604.5	2.71	65.54
Secondary 0.5 µg/ml	964.24	62.5	1032.92	1.78	1.07
		500	5570.24	3.01	5.78
		2000	32682.96	0.23	33.90
		8000	73708.69	1.53	76.44
Secondary 0.25 µg/ml	603.30	62.5	651.81	15.08	1.08
		500	3828.49	0.35	6.35
		2000	23099.82	1.71	38.29
		8000	49660.63	0.55	82.31
Secondary 0.125 µg/ml	384.46	62.5	467.95	2.12	1.22
		500	2634.92	1.07	6.85
		2000	15972.81	0.69	41.55
		8000	30997.45	0.24	80.63
Frozen MSD reagents					
Dilutions	**Background noise**	**Concentration (pg/ml)**	**Adj. signal mean**	**CV %**	**Signal-to-noise ratio**

Secondary 1 µg/ml	873.46	62.5	1027.96	1.24	1.18
		500	3741.09	10.04	4.28
		2000	28718.01	1.18	32.88
		8000	71450.88	0.26	81.80
Secondary 0.5 µg/ml	672.14	62.5	762.76	3.52	1.13
		500	3809.50	0.09	5.67
		2000	21863.38	1.97	32.53
		8000	50562.43	4.67	75.23
Secondary 0.25 µg/ml	485.22	62.5	526.76	4.30	1.09
		500	2669.15	2.30	5.50
		2000	15673.18	1.84	32.30
		8000	32871.22	0.98	67.75
Secondary 0.125 µg/ml	316.50	62.5	343.88	3.70	1.09
		500	1788.83	1.98	5.65
		2000	8558.96	16.92	27.04
		8000	19716.48	0.20	62.30

Table 6. Optimization of secondary antibody dilution. Standard curves were generated using recombinant TREM2 protein covering the concentration range expected in human biofluids (8000 to 62.5 pg/ml) and assuming a 1/4 dilution of samples. Four different secondary antibody concentrations (0.25 µg/ml, 0.125 µg/ml, 0.0625 µg/ml and 31.25 ng/ml) were tested. The table provides information on background noise, TREM2 recombinant protein concentration (pg/ml), adjusted signal mean, coefficient of variation (CV%) and signal-to-noise ratio (S/N ratio). The validation parameters were used to determine the optimal conditions for the assay.

Experimental assay 5					
Dilutions	**Background noise**	**Concentration (pg/ml)**	**Adj. signal mean**	**CV %**	**Signal-to-noise ratio**
Secondary 0.25 µg/ml	774.69	62.5	1017.90	1.95	1.31
		500	8927.21	2.40	11.52
		2000	32688.91	1.78	42.20
		8000	60013.96	0.16	77.47
Secondary 0.125 µg/ml	476.48	62.5	628.00	0.00	1.32
		500	6208.76	1.25	13.03
		2000	22072.32	2.20	46.32
		8000	41376.85	0.79	86.84
Secondary 0.0625 µg/ml	297.10	62.5	394.92	2.86	1.33
		500	3902.13	3.75	13.13
		2000	13810.84	1.39	46.49
		8000	24456.31	1.40	82.32
Secondary 31.25 ng/ml	179.30	62.5	243.97	2.32	1.36
		500	2287.80	1.85	12.76
		2000	7977.38	0.77	44.49
		8000	14294.88	2.13	79.73

Table 7. Measurement of sTREM2 levels in human CSF and plasma samples. Quantification of sTREM2 levels in high plasma, low plasma and CSF samples. Concentrations were determined using different concentrations of secondary antibody (0.25 µg/ml, 0.125 µg/ml, 0.0625 µg/ml and 31.25 ng/ml). The table shows the calculated mean concentration (pg/ml) obtained from duplicate measurements and the coefficient of variation (CV%).

Experimental assay 5								
	Secondary 0.25 µg/ml		Secondary 0.125 µg/ml		Secondary 0.0625 µg/ml		Secondary 31.25 ng/ml	
Samples	**Calc. [ ] mean (pg/ml)**	**CV %**	**Calc. [ ] mean (pg/ml)**	**CV %**	**Calc. [ ] mean (pg/ml)**	**CV %**	**Calc. [ ] mean (pg/ml)**	**CV %**
Plasma high	2616	0.12	2628	4.76	2529	6.03	2519	7.91
Plasma low	1035	3.61	1067	3.12	1143	0.98	1093	2.87
CSF	3055	1.13	2968	1.44	3042	0.41	3119	2.14