Fab Based High Sensitivity PK ELISA for the Detection of a Therapeutic Antibody Administered in Neurodegenerative Disease Treatment

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Introduction

A PK assay using Fab fragments for capture and detection was set up for a therapeutic mAb targeting a neurodegenerative disease. The main challenges encountered were the targeted sensitivities (50 and 1 ng/ml in plasma and CSF respectively) as well as the adaptation to small sample volume (<40 µL). The use of Fab fragments in Ligand Binding Assays comprises benefits and drawbacks.

Benefits:
- Closest access to epitopes, which are difficult to reach
- No interaction with rheumatoid factors [unspecific signal]
- Suitable to detect the Fc part of mAb
- Easier production

Drawbacks:
- Only affinity effects (no avidity)
- Reduced labelling efficiency

Assay Development

The assay format was a sandwich ELISA using Fab fragments for capture and detection (Figure 1).

Twelve Fab fragments were tested either for coating or directly conjugated to HRP for detection in an initial screening (Figure 2).

Taking into consideration signal over background ratios, two pairs of fragments were selected for further assay development: Fab 3 and 9; Fab 7 and 8, for capture and detection respectively (Figure 3).

Figure 1: Principle of the assay

Figure 2: Initial screening of Fab fragments

Capture fragments were tested for coating at 0.5 and 5.0 µg/mL. Detection fragments were labelled with 3:1 and 10:1 biotin/Fab molar excess and tested at 0.05 and 0.25 µg/mL. SA-HRP was tested 1:10'000 dilution. Red circles indicate optimal levels of each factor identified as significant in the half-normal plots.

Figure 3: Results from initial screening of Fab for capture and detection signal over background ratios are depicted.

Figure 4: Development approach

Based on these results, the following parameters were chosen for further development and assay qualification:

Coating: Fab fragment for capture: Fab 3; Biotin excess for labelling: 3:1 Concentration of Fab fragment for capture in coating buffer: 0.5 µg/mL
Detection: Fab fragment for detection: Fab 8; Concentration of Fab fragment for detection: 0.25 µg/mL

MRD: MRD100

Further improvement was attained by comparing two different substrates of HRP: a classical chromogenic reagent (TMB) and a fluorometric reagent (QuantaBlu™). The fluorometric assay showed a significant impact on sensitivity (x4 improvement, LOD from 15 to 7 ng/mL, as well as in its dynamic range (Figure 10).

Figure 5: Comparison QuantaBlu™ vs TMB. Signal over background ratios values are shown.

The assay developed in plasma was further adapted to CSF to improve the sensitivity. The method adapted to each biological matrix is summarized in Figure 8.

Discussion and Conclusion

In the present work:
- Biophysical challenges defined during the feasibility phase were assessed
- The method tuning applied throughout development lead to improved reliability, minimal reproducibility and robustness
- State-of-the-art platforms were compared to fulfill sponsors expectations
- Customized assays were successfully qualified

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