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DATASHEET

An ELISA for the Detection of Antibodies to Adalimumab in Human Serum

BACKGROUND

Adalimumab (Humira®) is a humanized recombinant human IgG1 monoclonal antibody specific for tumor necrosis factor-alpha (TNF-α) that has been clinically applied for treatment of rheumatoid arthritis (RA), psoriatic arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, chronic psoriasis, Crohn's disease, and ulcerative colitis.

Adalimumab mean steady-state trough concentrations of approximately 5 µg/mL to 12 µg/mL were observed at doses of 40 mg, 80 mg, and 160 mg of adalimumab. Among adalimumab monotherapy recipients, 3—26% had reported development of antibodies to adalimumab.

Evaluation of the comparability of adalimumab biosimilars to the innovator drug should follow the guidelines laid out by the FDA and EMA. The analysis should be multifactorial, taking into account both the physicochemical characteristics and clinical performance of the biosimilar compared to the innovator. Eurofins Bioanalytical Services offers an off-the-shelf ADA assay for comparability testing of biosimilar adalimumab.

The purpose of this study was to develop a sensitive and precise drug tolerant assay for the detection of antibodies to adalimumab in human serum. An Affinity Capture Elution (ACE) ELISA method was developed that is able to detect anti-adalimumab antibodies tolerant up to 20 µg/mL adalimumab in human serum for supporting comparative analysis of biosimilar product clinical development, pharmacovigilance and therapeutic drug monitoring.

METHOD

The analytical method is an Affinity Capture Elution (ACE) ELISA where adalimumab is used for capture of the adalimumab anti-drug antibodies (ADA) present in human serum. Unbound material is washed away and following acid dissociation and neutralization, the captured ADA is transferred to a second plate and detected with biotinylated adalimumab, followed by streptavidin-HRP, washed and visualized using TMB.

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RESULTS

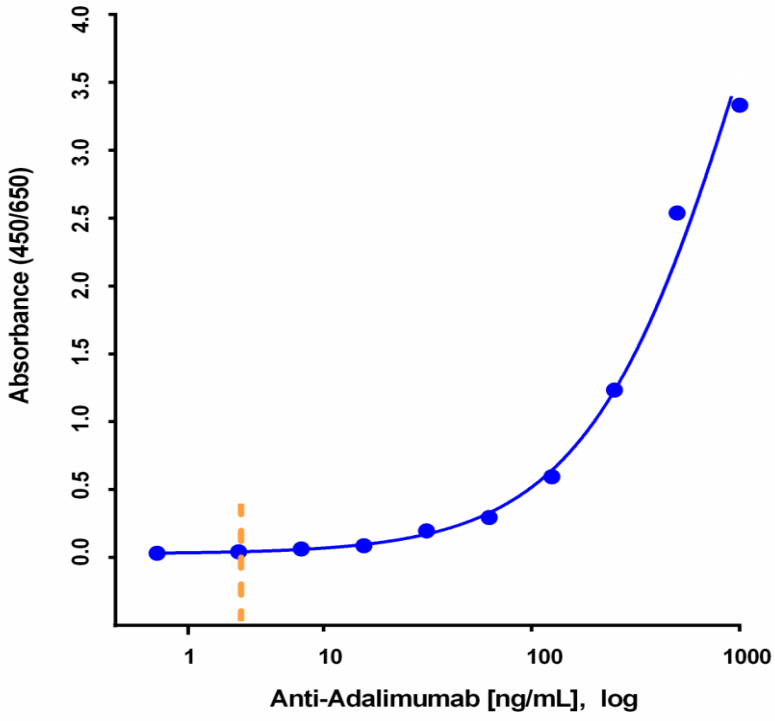

FIGURE I. Representative curve for sensitivity of anti-adalimumab antibody in human serum.

TABLE I. Summary of assay performance results

Performance characteristic	Results
Sensitivity	4 ng/mL
Cut Point Assessment	30 individual normal male & female sera samples Floating screening cut point factor was established at 1.45
Selectivity (Matrix recovery)	9 out of 10 lots of normal human serum spiked with 250 ng/mL and 1250 ng/mL of positive control were within ±25% of reference
Precision Intra-assay Inter-assay	 ≤13.6 %CV ≤14.8 %CV
Drug Tolerance	250 ng/mL ADA is detectable in the presence of 20 µg/mL of adalimumab

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