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DATASHEET

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

BACKGROUND

Trastuzumab (Herceptin®) is a humanized recombinant monoclonal antibody that selectively binds to the extracellular domain of the human epidermal growth factor receptor 2, HER2, a transmembrane protein overexpressed in 25-30% of breast cancers. Trastuzumab is used for the treatment of primary breast cancers which overexpress HER2. Herceptin binds with high affinity (kDa 5nM) to tumor cells over expressing HER2 resulting in loss of malignant growth and metastasis.

Evaluation of the comparability of trastuzumab 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 a full range of off-the-shelf trastuzumab assays for comparability testing of biosimilars including:

- PK assay
- ADA assay
- Nab assay
- Comparability testing
    - FcR & C1q binding
    - HER2 kinetic binding assay
    - ADCC assay

Trastuzumab has a mean half-life of 5.8 days in studies using a loading dose of 4 mg/kg dose followed by a weekly maintenance dose of 2 mg/kg. Mean trough and peak concentrations are approximately 79 µg/mL and 123 µg/mL respectively between weeks 16 and 32.

The purpose of this study was to develop a sensitive, specific and precise ELISA for the detection of antibodies to trastuzumab in human serum to support immunogenicity testing for clinical studies.

METHOD

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

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RESULTS

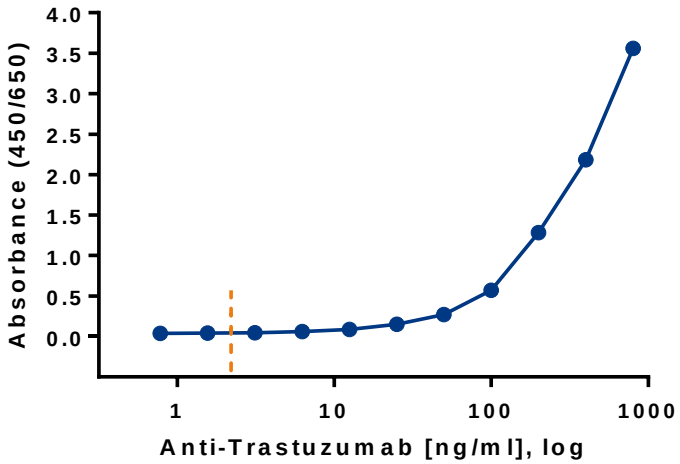

FIGURE I. Representative curve for sensitivity of trastuzumab ADA in human serum. Trastuzumab was tested in a range starting at 800 ng/mL and serially diluted, in two-fold increments, to 0.78 ng/mL. Dotted orange line represents assay cut point.

TABLE I. Summary of assay performance results

Performance characteristic	Results
Sensitivity	5 ng/mL
Cut Point Assessment	30 individual female sera samples Floating cut point factor was established at 1.32
Selectivity (Matrix recovery)	12 out of 12 individual lots of human serum spiked with 50 ng/mL positive control anti-trastuzumab were within ±25% of reference
Precision	
Intra-assay	3.4%
Inter-assay	19.6%
Drug Tolerance	500 ng/mL anti-trastuzumab antibodies are detectable in the presence of 62.5 µg/mL trastuzumab

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