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DATASHEET

A Bridging ELISA for the Quantitative Determination of Trastuzumab (Herceptin®) in Human and Mouse 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, which is 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.

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 bridging ELISA for the quantitative determination of trastuzumab in human and mouse serum. The assay format is designed to detect both trastuzumab and biosimilar drug materials. Therefore, biosimilar assay comparability can be quickly validated and a single pharmacokinetic assay may be used to support biosimilar nonclinical and/or clinical studies.

METHOD

The analytical method is a bridging ELISA with acid dissociation where serum samples containing trastuzumab are acidified to separate immune complexes. Immune complexes include trastuzumab bound to either anti-drug antibodies (ADA) and/or soluble target (sHER2). Acidified serum samples are then neutralized on an ELISA plate coated with rHER2 protein allowing trastuzumab to bind to the plate. Unbound material is then washed away. The drug is detected by a biotin labeled anti-trastuzumab monoclonal antibody and subsequent development with HRP-labeled streptavidin and enzyme substrate. Trastuzumab concentrations are interpolated from the 5-parameter regressed standard curve ranging from a Lower Limit of Quantitation of 1.5 µg/mL to the Upper Limit of 80 µg/mL.

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RESULTS

FIGURE I. Representative standard curve for detection of trastuzumab in human serum.

TABLE I. Summary of assay performance results

Performance characteristic	Results
Validated Range (LLOQ/ULOQ)	1.5 µg/mL to 80 µg/mL
Accuracy	**Human Serum** Range 3.3% to 9.3% **Mouse Serum** Range 3.8% to 9.1%
Precision Intra-assay Inter-assay Intra-assay Inter-assay	**Human Serum** Range 5.0 % to 16.1 % Range 7.3 % to 16.6 % **Mouse Serum** Range 2.6% to 9.0 % Range 6.8 % to 13.2 %
Specificity / Selectivity	10 out of 10 lots of human serum within ±20% of nominal (levels tested for each lot: unspiked, 10 and 50 µg/mL)
Dilutional Linearity	%RE Range: -8.5 % to -22.1 %; Overall %CV: 7.5 % Maximum dilution performed 1:50 (exclusive of MRD)
Soluble Target Interference (sHER2)	No interference observed up to 4000 ng/mL sHER2

REFERENCES

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