The development and validation of an ultra-sensitive ELISA method for the determination of Trastuzumab (Herceptin®) in human serum

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**Methods**

The microtiter plate was coated with recombinant human HER2/ErbB2/CD340 ligands. Standards, quality controls, and samples were then added (100 µL) and incubated for 2 hours. Upon aspiration of well contents, the plate was incubated with an anti-human HER2-conjugated IgG1 antibody specific to trastuzumab. All incubations were performed at 25°C. Next, TMB substrate solution was added where a change in color was exhibited. The enzyme-substrate reaction was terminated by the addition of sulphuric acid and solution and the color change was measured spectrophotometrically at a wavelength of 450nm.

**Results (continued)**

The validated assay had a detection limit of 0.20 ng/mL. The linear calibration curve ranged from 0.20 to 10.00 ng/mL with bias ranging from -19.10% to 15.00%, using a 5PL regression. For precision and accuracy, QCs were at 0.400, 4.00 and 8.00 ng/mL, had CVs ranging from 9.1 to 4.4%, and a bias ranging from -15.30 to 18.29%. Dilution linearity QCs had CVs ranging from 9.1 to 6.4% and a bias ranging from -18.73 to 16.58%. Selectivity samples (10 independent human sera lots) spiked at 0.200 ng/mL showed a bias ranging from -15.50 to 10.00% with all ten lot recovering at within ±20.00% of nominal concentration.

**Conclusion**

The validated assay showed that it was suitable for intended use to support clinical trials pharmacokinetics sample analysis.

**References**

