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DATASHEET

Methods to Determine the Binding of Trastuzumab to Fc receptors and Complement C1q

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
- Comparability testing
    - Fc Receptors & C1q binding
    - HER2 kinetic binding assay
    - ADCC assay

The purpose of this study was to develop a suite of methods to measure the binding of trastuzumab to recombinant human Fc receptors CD64 (FcγRI), CD32a (FcγRIIA), CD16a (FcγRIIIA) and FcRn by Surface Plasmon Resonance and to complement component C1q by ELISA.

These methods form a suite of assays for comparability assessment of biosimilars. In this evaluation a reference sample and up to 3 test samples are analyzed in triplicate over a concentration range of 7.8 µg/mL to 1000 µg/mL and the dose response curves compared by parallel line analysis for relative potency and parallelism (χ^2 (Chi-squared)).

METHOD

SPR-based methods are based on immobilising an anti-His antibody to a CM5 chip by standard amine chemistry. His-tagged recombinant protein (CD16a or CD64) was then captured to the anti-His antibody. CD32a and FcRn are coupled directly to the CM5 chip directly. The binding of increasing concentrations of trastuzumab was measured using a BIAcore wizard. The binding responses for test and reference samples were compared by parallel line analysis using a four- and five-parameter logistic (4PL and 5PL) fits.

The C1q by ELISA method is based on the drug is adsorbing the drug to a high binding flat-bottom microplate. Human C1q is then added, followed by anti-C1q antibody as the detector. The binding absorbance for test and reference samples were collected using SoftMax Pro software (Molecular Devices) and compared by parallel line analysis using 4PL on PLA and 5PL on StatLIA. A comparison of both analytical methods carried out. Data analysis was carried out using PLA 2.0 (Stegmann Systems) and StatLIA version 3.2 (Brendan Technologies).

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RESULTS

CD16a assay

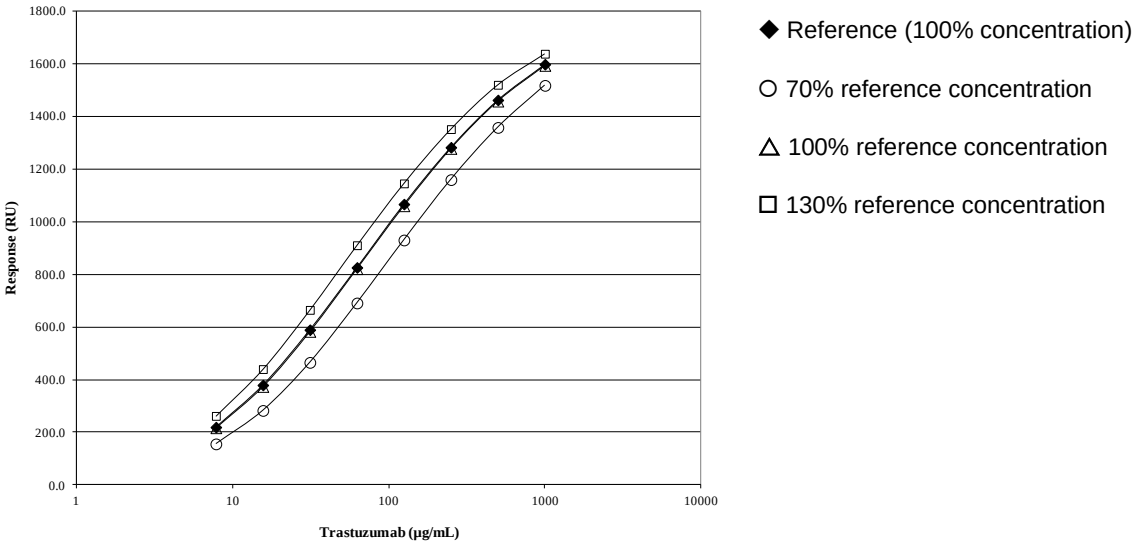

FIGURE 1. Representative analysis of trastuzumab binding to CD16a was performed over eight concentrations of drug (1000 –7.8 µg/mL) between 50 and 150 % (70%, 100% and 130% represented) of the reference concentration. The complete dose-response curves were generated using this concentration range. A five-parameter logistic (5PL) curve fit was used.

Qualification Principle	Qualification Parameters	Acceptance Criteria	Qualification Results
Assay Linearity and Range	Correlation coefficient Y-intercept Slope Residual sum of squares Range	>0.95 Report result 0.8 to 1.2 Report result Report result	0.99 -4.90 1.0 54.9 7.8 µg/mL to 1000 µg/mL
Repeatability (Intra-assay precision)	%CV	Mean Range	1.5% 0.1 – 10.6%
Intermediate precision (Inter-assay precision)	%CV	Mean Range	5.0% 1.1 – 13.1%
Accuracy	Recovery	80% to 120%	93.7 – 102.6%

CD32a Assay

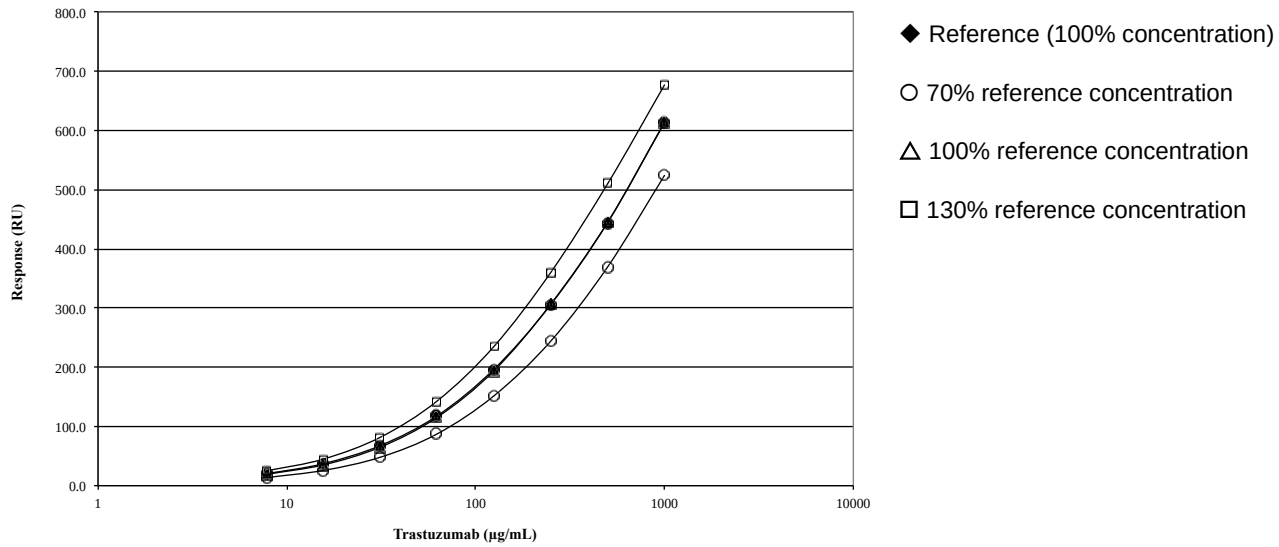

FIGURE 2. Representative analysis of trastuzumab binding to CD32a was performed over eight concentrations of drug (1000 –7.8 µg/mL) between 50 and 150 % (70%, 100% and 130% represented) of the reference concentration. The complete dose-response curves were generated using this concentration range. A five-parameter logistic (5PL) curve fit was used.

Qualification Principle	Qualification Parameters	Acceptance Criteria	Qualification Results
Assay Linearity and Range	Correlation coefficient Y-intercept Slope Residual sum of squares Range	>0.95 Report result 0.8 to 1.2 Report result Report result	0.99 -0.81 1.0 47.0 7.8 µg/mL to 1000 µg/mL
Repeatability (Intra-assay precision)	%CV	Mean Range	2.4% 0.1 – 23.8%
Intermediate precision (Inter-assay precision)	%CV	Mean Range	3.2% 0.1 – 6.7%
Accuracy	Recovery	80% to 120%	97.1– 107.3%

CD64 Assay

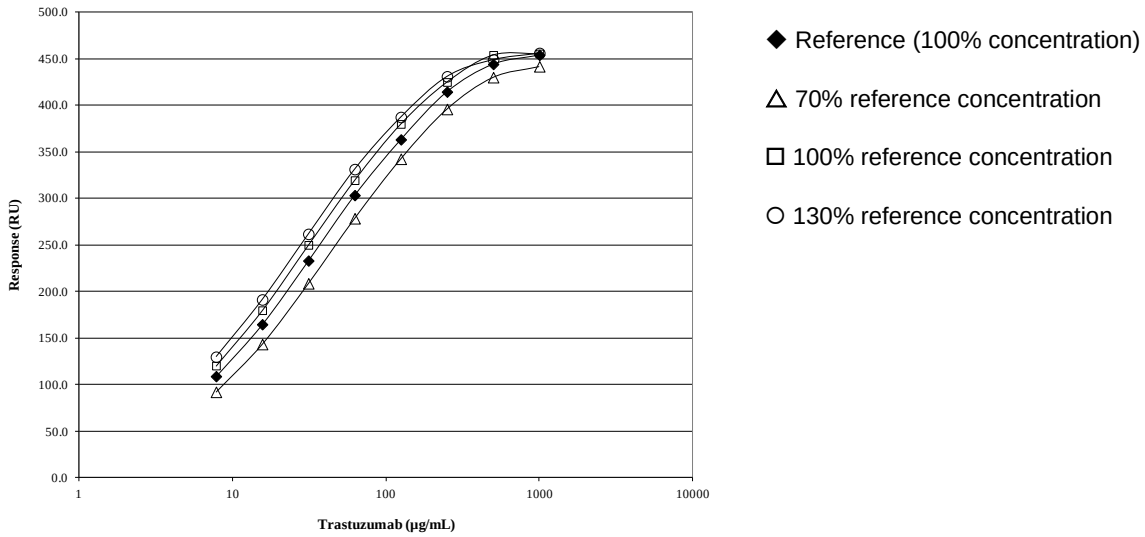

FIGURE 3. Representative analysis of trastuzumab binding to CD64 was performed over eight concentrations of drug (1000 –7.8 µg/mL) between 50 and 150 % (70%, 100% and 130% represented) of the reference concentration. The complete dose-response curves were generated using this concentration range. A five-parameter logistic (5PL) curve fit was used.

Qualification Principle	Qualification Parameters	Acceptance Criteria	Qualification Results
Assay Linearity and Range	Correlation coefficient Y-intercept Slope Residual sum of squares Range	>0.95 Report result 0.8 to 1.2 Report result Report result	1.00 -5.26 1.0 28.5 7.8 µg/mL to 1000 µg/mL
Repeatability (Intra-assay precision)	%CV	Mean Range	3.6% 0.1 – 6.0%
Intermediate precision (Inter-assay precision)	%CV	Mean Range	1.7% 0.3 – 3.5%
Accuracy	Recovery	80% to 120%	96.1 – 102.4%

FcRn Assay

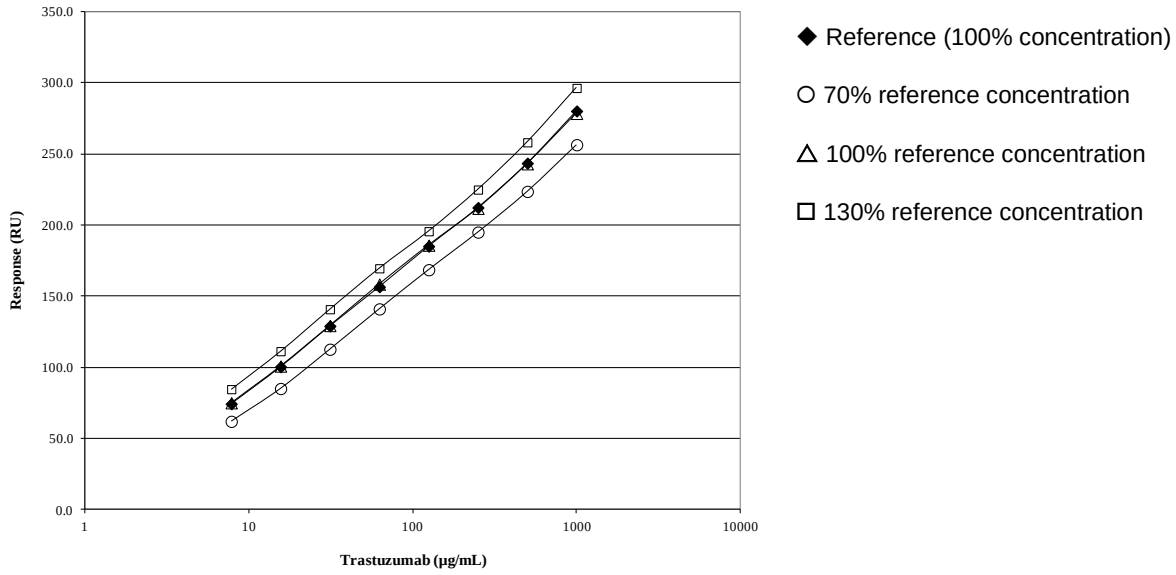

FIGURE 4. Representative analysis of trastuzumab binding to FcRn was performed on reference over eight concentrations of drug (1000 –7.8 µg/mL) between 50 and 150 % (70%, 100% and 130% represented) of the reference concentration. The complete dose-response curves were generated using this concentration range. A four parameter logistic (4PL) curve fit was used.

Qualification Principle	Qualification Parameters	Acceptance Criteria	Qualification Results
Assay Linearity & Range	Correlation coefficient Y-intercept Slope Residual sum of squares Range	>0.95 Report result 0.8 to 1.2 Report result Report result	1.00 -24.80 1.2 31.20 7.8 µg/mL to 1000 µg/mL
Repeatability (Intra-assay precision)	%CV	Mean Range	1.1% 0.1 – 4.0%
Intermediate precision (Inter-assay precision)	%CV	Mean Range	3.2% 0.1 – 2.7%
Accuracy	Recovery	80% to 120%	83.0 – 103.5%

C1q Assay

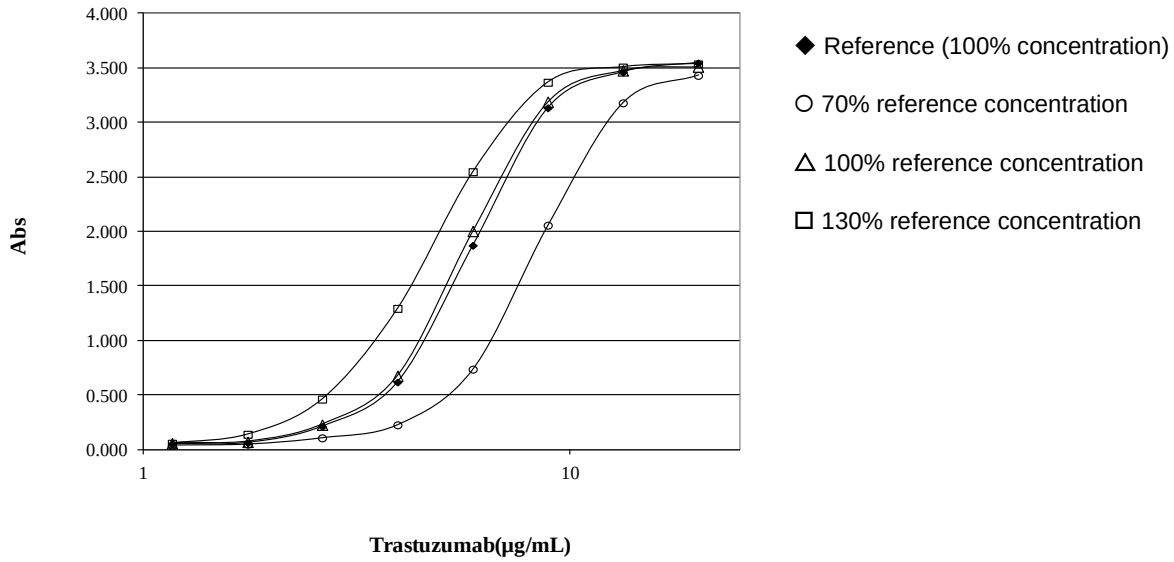

FIGURE 5. Representative analysis of trastuzumab binding to C1q was performed on reference over eight concentrations of drug (1000 –7.8 µg/mL) between 50 and 150 % (70%, 100% and 130% represented) of the reference concentration. The complete dose-response curves were generated using this concentration range. One-in-two dilution format was utilized. A four parameter logistic (4PL) curve fit was used.

Qualification Principle	Qualification Parameters	Acceptance Criteria	PLA Qualification Results	StatLIA Qualification Results
Assay Linearity & Range	Correlation coefficient Y-intercept Slope Residual sum of squares	> 0.95 -15% to +15% 0.8 to 1.2 Report result	1.00 -0.62 0.9 31.0	1.00 3.57 1.0 31.0
Range	Range	Report result	7.8 µg/mL to 1000 µg/mL	
Repeatability (Intra-assay precision)	%CV	Mean Range	1.7% 0 – 3.7%	
Intermediate precision (Inter-assay precision)	%CV	Mean Range	2.5% 0.2 – 3.2%	1.5% 1.2 – 1.8%
Accuracy	Recovery	80% to 120%	98.1 – 104.4%	98.8 – 103.8%

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