

DATASHEET

A flow cytometry-based antibody-dependent cell-mediated cytotoxicity (ADCC) assay to measure the potency of trastuzumab

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
 - Fc Receptor & C1q binding
 - HER2 kinetic binding assay
 - ADCC assay

A flow cytometry method has been developed and qualified to measure the potency of the humanized therapeutic monoclonal antibody trastuzumab (Herceptin[®]) by antibody-dependent cell-mediated cytotoxicity (ADCC) using a human breast cancer cell line SK-BR-3 as targets and human mononuclear cells as effectors.

METHOD

SK-BR-3 target cells labelled with CFSE were incubated with serial dilutions of trastuzumab followed by effector human PBMCs. After 4 hour incubation at 37oC and 5% CO₂, cells were resuspended with cell count solution containing propidium iodide and control latex particles and analysed using flow cytometer. The number of the live target cells was enumerated using latex beads for normalization.

RESULTS

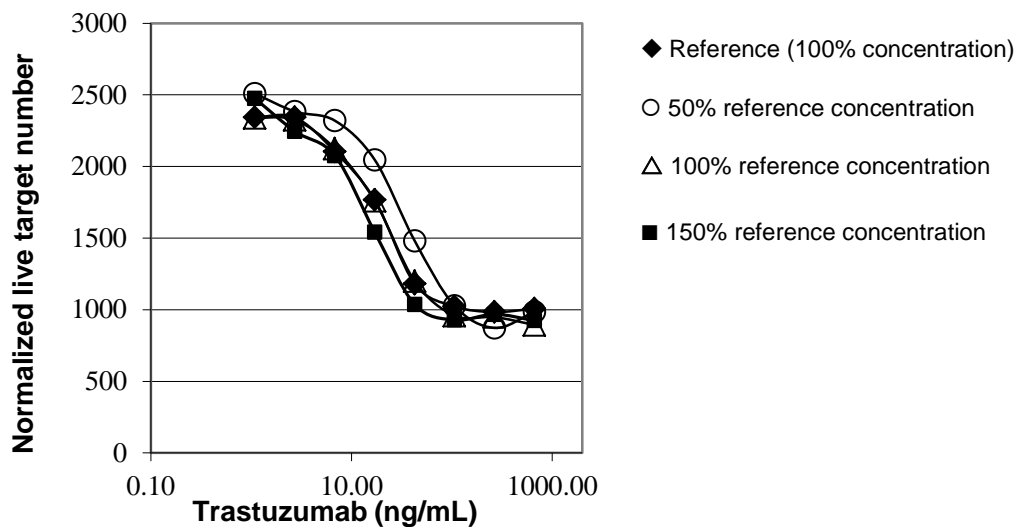


FIGURE 1. Representative (Test Batch TB04) trastuzumab dependent ADCC with SK-BR-3 target cells and PMBC effector cells.

Qualification Principle	Qualification Parameters	Acceptance Criteria	Qualification Results
Assay Linearity and Range	Correlation coefficient	> 0.8	0.963
	Y-intercept	report result	15.0
	Slope	0.8 to 1.2	0.915
	Residual sum of squares	report result	376
Repeatability (Intra-assay precision)	%CV	<20%	≤20.0 with most data points ≤10.0
Intermediate precision (Inter-assay precision)	%CV	<30%	<9.2%
Accuracy	Recovery	80% to 120%	89.6% to 108.4%

TABLE 2. Summary of trastuzumab potency assay performance results

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