Development and Validation of the Anti-Trastuzumab Neutralizing Antibody Detection Methods for Trastuzumab Biosimilar Program
R. Meng\textsuperscript{1}, Y. Ma\textsuperscript{1}, R. Weaver\textsuperscript{1}, X. Wang\textsuperscript{1}, C-H. Cai\textsuperscript{2}, J. Kolman\textsuperscript{1}
\textsuperscript{1}QPS, LLC, \textsuperscript{2}Pfizer Inc.

**Purpose**
Trastuzumab (Herceptin\textsuperscript{®}) is a monoclonal antibody specifically interacts with Human Epidermal Growth Factor Receptor 2 (HER2). Trastuzumab target overexpressed HER2 on tumor cells, preventing HER2 shedding and blocking downstream signaling pathways. Trastuzumab-tagged tumor cells are then destroyed by immune cells in which the mechanism is known as antibody-dependent cell-mediated cytotoxicity. It has been shown that trastuzumab treatment regimens can effectively improve overall survival and disease-free survival of both early and late-stage HER2+ breast cancers. Due to its therapeutic value, potential biosimilars to trastuzumab are currently being developed. Antibody drug treatments could generate Anti-Drug Antibodies (ADA) including Neutralizing anti-drug antibodies (Nab). Presence of Nab in cancer patients could potentially affect drug efficacy. Therefore, it is critical to develop immunoassays to evaluate Nab generation in both trastuzumab and trastuzumab biosimilar treated patient populations. Here we report that 2 immunoassays were developed and validated for the detection of anti-trastuzumab or anti-trastuzumab biosimilar Nab in human HER2+ breast cancer serum samples. The assay was specifically optimized to reduce the interference of drug, HER2 and HER2/drug complexes in human serum samples.

**Methods**
The assay format for the detection of Nab is MSD ECL technology. Both trastuzumab marketed products and potential biosimilar drugs were labeled with either Biotin or Ruthenium. They were compared side by side during the method development to ensure similar responses with either the negative control (NC) or the positive control (PC) antibody. The assay incorporates two acid dissociation steps in order to minimize the HER2/drug interference and maximize assay sensitivity. Briefly, the samples undergo first acid dissociation to release neutralizing antibodies, drug and target HER2 from all the complexes, followed by an incubation of samples with Biotin-labeled drugs on a streptavidin-coated high binding plate. After neutralizing antibodies are immuno-captured, free drugs and HER2 are washed away from the streptavidin-coated high binding plate. Following this, a second acid dissociation is applied to the plate to liberate captured neutralizing antibodies. After an over-night incubation of samples with Ruthenium-labeled drug, the samples are then transferred to a HER2 pre-coated MSD high binding plate. Presence of neutralizing antibodies competes with the binding of Ruthenium-labeled drug to HER2. Therefore, the generated signal is reversely proportional to the amount of neutralizing antibodies in the sample.

**Results**
With this strategy, neutralizing antibodies can be greatly enriched, and the free drug and HER2 are effectively depleted from the samples. It significantly enhanced the assay drug tolerance and reduced interference from HER2, drug and HER2/drug complex. We have concluded that both validated assays showed a minimum interference up to 500 ng/mL HER2 in pooled human serum. With a spiked PC concentration at 500 ng/mL, the drug alone can be tolerated for at least 25\textmu g/mL, and the drug/HER2 complex interference can be tolerated with at least 500ng/mL for HER2 and 50 \textmu g/mL for the drug. The assay sensitivity was also established based on two different anti-trastuzumab antibodies. The sensitivities for two assays are comparable and less than 500 ng/mL. Based on the evaluation of 42 HER2+ individual breast cancer serum lots, the assay screening cut point factors were determined for both assays and they showed a high similarity. In addition, both assays demonstrated high precision with a less than 5\% inter- and intra-batch %CV.

**Conclusion**
Two independent immunoassays for the detection of anti-trastuzumab originator (marketed product) and potential biosimilar neutralizing antibodies have been developed and validated to support ongoing clinical studies. Our study demonstrated that both assays are comparable in multiple assay parameters, including assay screening cut point factors, assay sensitivity, HER2 target interference and drug or drug/HER2 complex tolerance.