QPS, LLC
Delaware Technology Park
3 Innovation Way, Suite 240
Newark, DE 19711

QPS™
QUALITY • PERFORMANCE • SERVICE

WEB www.qps-usa.com
TEL (302) 369-5601
FAX (302) 369-5602

CONFERENCE

2010 AAPS Annual Meeting and Exposition

TITLE (QPS 2010-006)

# Evolving ELISA Methods to Detect a Monoclonal Antibody Therapeutic Agent

AUTHORS

Chad Asher[1], Lindsay E. King[2], Shawn Connelly[1], Michael Mays[1], Katie Grieco[1], Caroline Becker[1], Jeannine M Lovering[2], Huifen F Wang[2], LingSing Chen[1], Chun-Hua Cai[3], Hui Zhang[1]
QPS, LLC, DE, USA, [2] Pfizer-Groton Research and Development/PDM, Groton, CT
[3] Pfizer-Primary Care Business Unit/Clinical Pharmocology, New London, CT

ABSTRACT

**Introduction**

To validate and apply ELISAs for a monoclonal antibody drug through multiple development stages.

**Methods**

Drug X is a fully humanized monoclonal antibody. ELISAs were constructed to measure drug X in monkey and human serum. In the initial assay, monkey serum samples containing drug X were captured by the target coated on Maxisorp plates. After washing away unbound material, HRP conjugated to anti-human IgG Fc was added to bind drug X, which was detected with TMB substrate. Color development was proportional to the concentration of Drug X present in the monkey serum sample.

The assay was further developed and validated as the development of Drug X moved to clinical stage. The basic assay format in human serum remained the same. But to sustain the balance between specificity and sensitivity, biotinylated isotype specific detection antibody and Chemiluminescence detection were used along with changes in the assay conditions for measuring Drug X in human serum.

**Results**

Both ELISA assays were validated. The established assay ranges (incorporating validated dilution) were 50-500,000 and 100-640,000 ng/mL in monkey and human serum, respectively. The inter- and intra-batch precision and accuracies were comparable in both matrices, with inter-batch total error at 7.0-29.1% and 10.4-22.3% in monkey and human serum, respectively. The therapeutic target at up to 1000x (50 ng/mL) above normal level in human serum did not interfere with the assay (Within ±10.7% of nominal concentration). Assay selectivity was acceptable in normal monkey and human serum (>80% samples tested were within 100±20% recovery when spiked with drug X), but showed differentiation in samples from patients, in contrast to healthy volunteers.

Incurred sample reanalysis for the monkey TK study, in about 5% of total samples, showed 100% reproducibility (10.0 ± 6.4% different from original measurements). About 10% reanalysis in the ongoing human clinical trials showed 98% reproducibility (-0.5 ± 13.0% different from the original measurements).

**Conclusions**

ELISA assays for monoclonal antibody Drug X were developed and validated in both monkey and human serum to successfully support the preclinical and clinical drug development.