

## Case Study

# A Clinical Sample Receptor Occupancy Assay Using Flow Cytometry

**Number of Clinical Sites:** 5

**Disease Indication:** Type 1 Diabetes Mellitus

**Therapeutic Area:** Endocrinology

**Drug:** Monoclonal antibody

**Molecular Target:** T cell surface ligand

**Study Phase:** Clinical Phase I/IIa

**Bioanalytical Services:** Cellular biomarker endpoint

**Data Delivery:** Cohort-based data transfers  
Final full bioanalytical report

## PROJECT AT A GLANCE

A global pharmaceutical company was conducting a European multi-site Phase I/IIa clinical study across several countries. One of the endpoints was assessment of changes in the amount of therapeutic drug bound to the target cell populations. Clinical protocol required assessment of changes from baseline; to allow correlation with the PK/PD endpoints. The project used a flow cytometry based receptor occupancy (RO) assay.

- As a secondary endpoint, it required a formal assay validation and the ability to monitor assay performance during the conduct of the clinical study.
- The dynamic nature of the RO endpoint required fresh whole blood samples were either be analyzed with 8 hours of collection, or stabilized to allow reliable analysis.
- The clinical protocol required study subjects to be enrolled individually and analyses conducted over several years.
- QA audited data were required on completion of each cohort as part of the dose escalation decision making process.

Sample Analysis at the  
**RIGHT TIME & RIGHT PLACE**  
to satisfy data delivery requirements

## CHALLENGES

- Development and validation of a multi-color flow cytometry assay able to robustly measure changes in the amount of bound therapeutic and the total available cellular target on several cell types.
- RO endpoint samples needed had to be processed to isolate the peripheral blood mononuclear cells (PBMC) and frozen within 8 hours of draw to stabilize the endpoint in multi-site clinical study.
- Sample stability required local site processing of blood for PBMC isolation and cryopreservation.
- The enrolment criteria meant a slow rate of recruitment and required longitudinal assay comparability for robust data analysis.
- RO changes were required to allow comparison of all time points to the baseline for each subject.
- QCs and criteria to monitor assay performance and sample quality, allowing client review of anomalous data.

## SOLUTIONS

- Collaboration with the client to meet the needs of the study by staying within the pre-defined performance acceptance criteria.
- The assay was developed and validated utilizing frozen PBMC samples and commercially sourced PBMCs. Utilizing commercially sourced PBMC's as QC controls to monitor performance against acceptance criteria resulted in overall reduced cost and assay variability.
- Assay variability was reduced by streamlining PBMC processes and conducting training at the clinical sites to include PBMC isolation and cryopreservation procedures. All clinical sites were supplied with sample collection kits and worksheets linked to the training materials that included practical sessions, collection and processing guides, proforma worksheets, formal operator certification, a training video and ongoing WebEx conference support.
- Sample data were normalized to allow cross-cohort and longitudinal data analysis using Molecules of Equivalent Soluble Fluorochrome (MESF) conversion.
- Sample quality was monitored against prior criteria that was based on viability and cell number at analysis, to enable the client to review anomalous data.

## OUTCOMES

- Good quality PBMC samples were received over a >2 year period.
- Reported reliable and robust data, which was successfully used to correlate PK/PD and study dosing escalation models from the client.
- Eurofins Bioanalytical Services was selected as preferred supplier for these assays based on the results for safety and tolerability.



Bioanalytical Services

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### About Eurofins Bioanalytical Services

Over 20 years of industry-leading global **Scientific Expertise** supporting the widest breadth of Biologics' clinical trials with PK/TK, ADA, NAb and Biomarker assays and sample analyses.

**Versatile Performance and Project Management Excellence** to adapt to a client's specific needs. Clinical or preclinical, regulated or non-regulated, assay development, qualification or validation; we custom design our support to match the client's program.

State-of-the art laboratory facilities in St. Charles, MO, USA providing **Global Reach and Capacity** to address clients' needs while simultaneously offering regionally-based solutions.

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