Human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents under an IRB/REC approved protocol

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Challenges in human tear analysis: Development of a fit-for-purpose qualitative immunoassay to detect biopharmaceutical exposure in rare matrices

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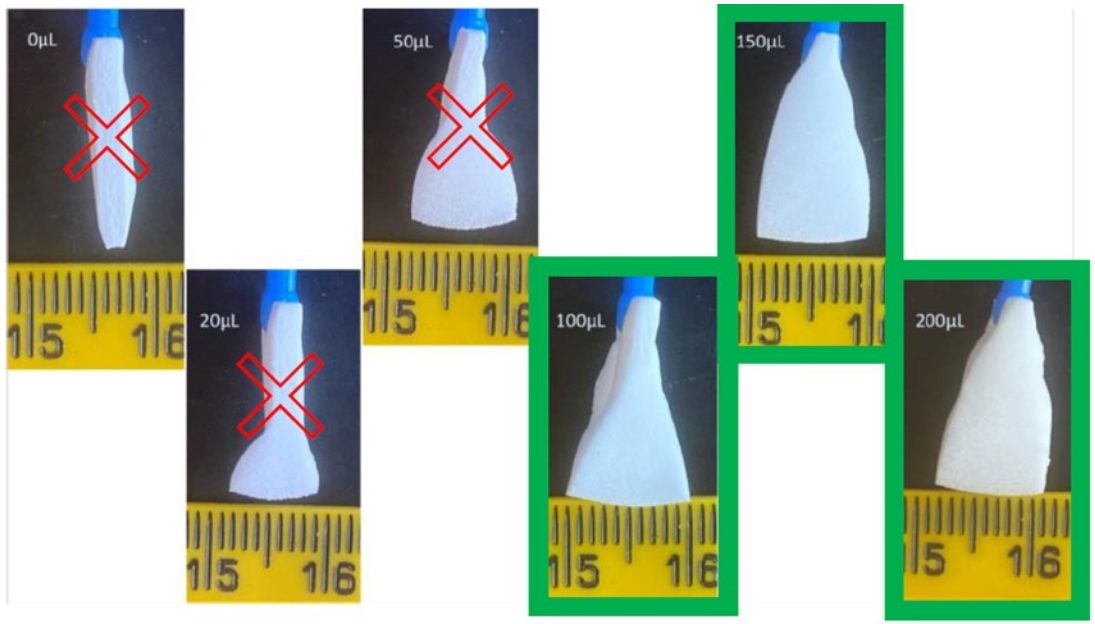
Introduction

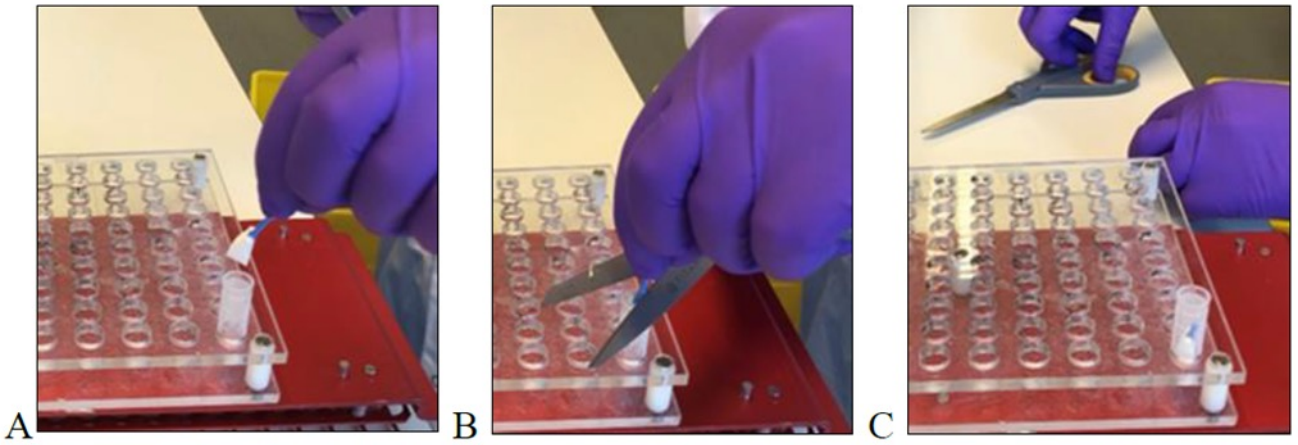
- Evaluating therapeutic antibody exposure at target sites is a critical task in biopharmaceutical development in the clinic
- Clinical study design to understand the pharmacokinetics, safety, tolerability and relationship between exposure in tear fluid and plasma concentration
- Challenges encountered in bioanalytical support of human tear analysis

Sampling Device

- PVA eye sponges were selected as tear fluid sample collection devices due to patient comfort, compliance and ease of use

- Request was for a quantitative assay:
  - PVA spear tips stored in CryoTubes and transferred to BIB laboratory
  - Spears centrifuged to collect liquid tear eluate
  - Eluate analysed to provide quantitative data





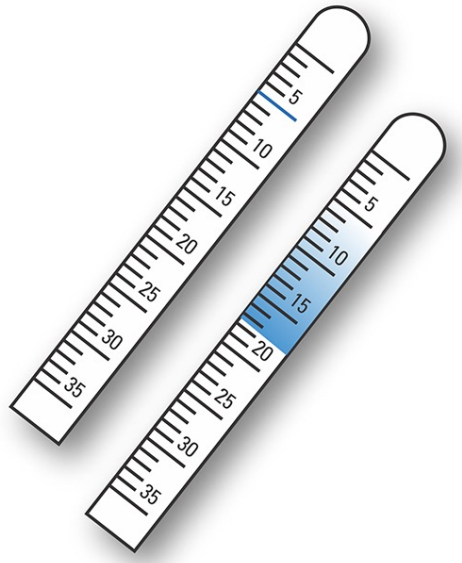
Quantitative Immunoassay

- A quantitative analyte specific immunoassay on both the Gyrolab and MSD platform had already been developed, validated and used extensively both in-house and externally

- Species:
  - Mouse, Rat, Rabbit, Cyno and Human

- Matrices:
  - Plasma, Serum, Blood:Water

  - **Tears** (Schirmer Tear Test Strip)
    - Analyte extracted directly into assay buffer diluent
    - Quantitative result given per volume of tear



- Validation to support these clinical studies
  - Assessment of analyte (QC) recovery from PVA sample collection device

Validation - Assessment of Analyte Recovery

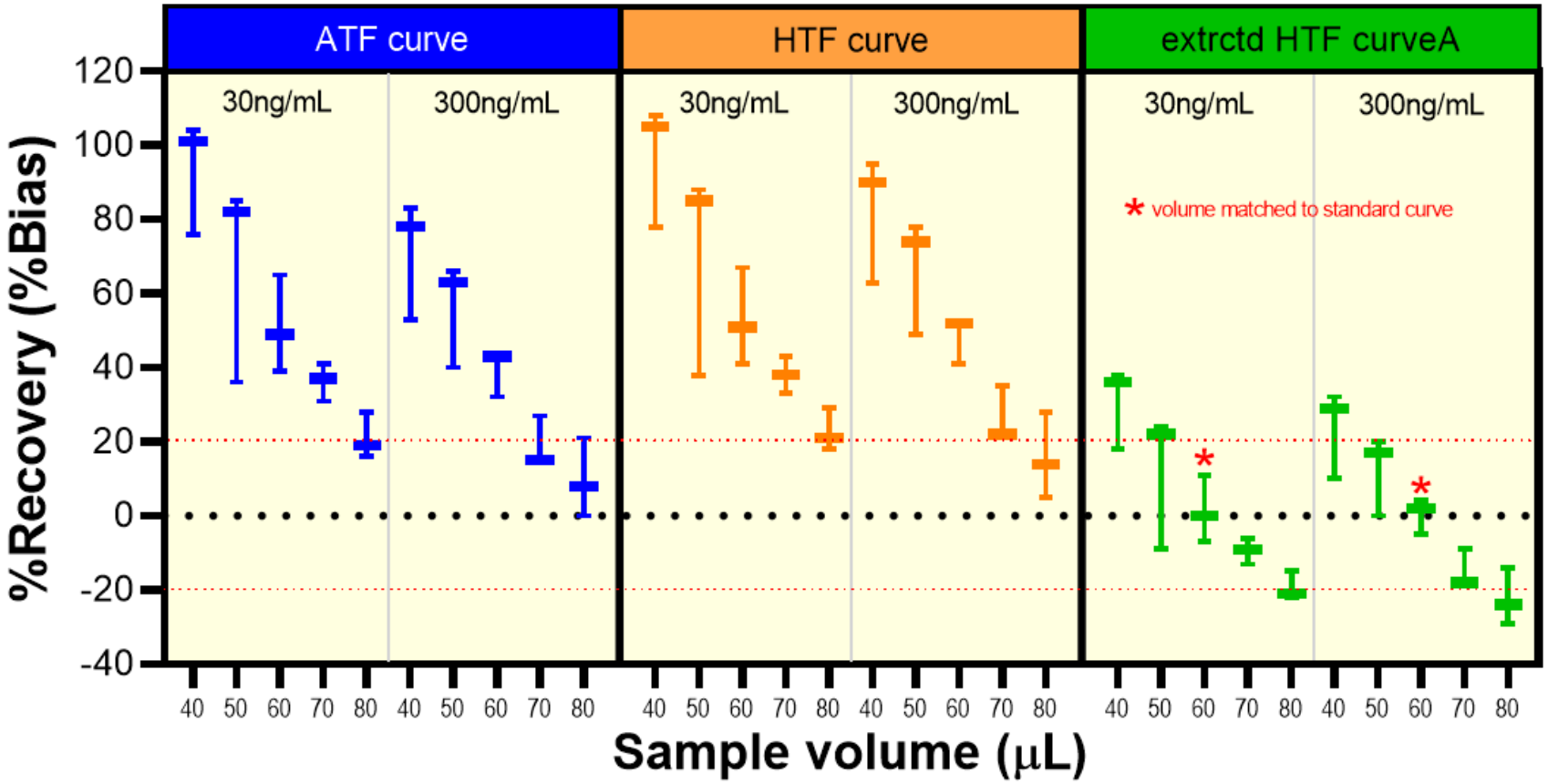
- Challenges once sample analysis began

- Study samples received at BIB laboratory demonstrated a poor and variable recovery of volume
  - Sample range 0 – 100 uL
  - Variable viscosity

- ICH guideline M10 on bioanalytical method validation and study sample analysis:

  “The QCs are intended to mimic study samples and should be prepared by spiking matrix with a known quantity of analyte, stored under the conditions anticipated for study samples and analysed to assess the validity of the analytical method.”

Validation - Assessment of Analyte Recovery



- Assessment of analyte recovery across sample volume ranges showed a non-proportional increase in analyte recovery

Validation – What Now?

- Unable to validate quantitative assay which was reflective of study samples
  - Variable sample volume
  - Non-proportional increase in analyte recovery

- Understanding what your data will be used for – generating scientifically meaningful data

Clinical Study Design

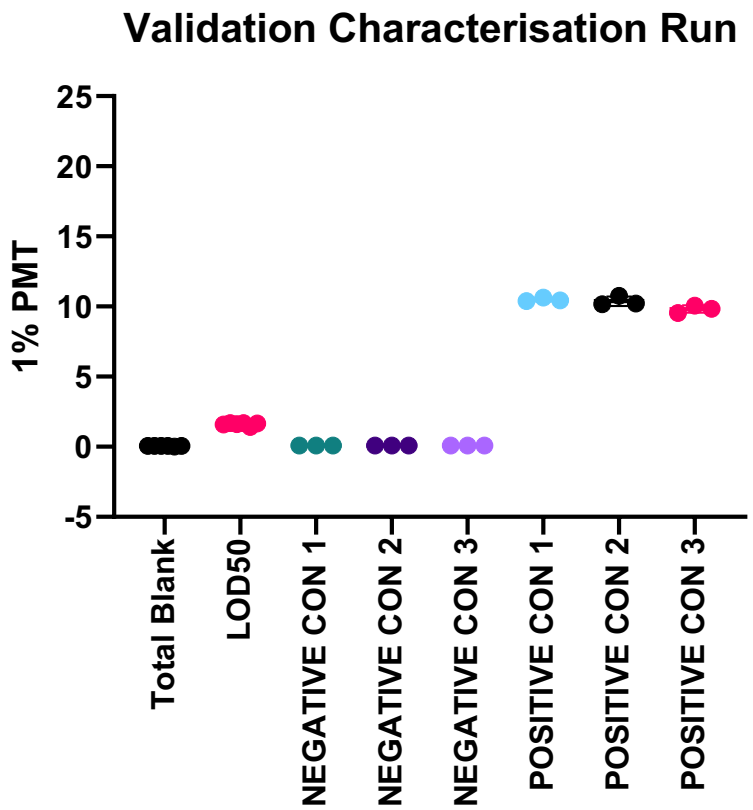
Objectives	Endpoint
Primary	
• To describe the effect of renal impairment on the analyte PK	• Analyte concentration in plasma, PK parameters
Secondary	
• To evaluate safety and tolerability using clinical parameters, including adverse events, vital signs and clinical laboratory assessments	• Change from baseline in vital signs (blood pressure and heart rate), monitoring and incidence of adverse events, toxicity grading of clinical laboratory tests, and physical examinations
Exploratory	
• To evaluate the presence/concentrations of analyte in tear fluid in participants and explore relationship between tear fluid and plasma concentration	• Presence/concentrations of analyte in tear fluid at baseline and on treatment, as data permits • Relationship between tear fluid and plasma concentrations of analyte, as data permits

Taking a different approach – Qualitative Assay

- Designed to minimise the false positive rate for drug naïve individuals by using a floating cut point
- Cut-point based on a Limit of Detection (LoD) calibrator
  - LoD calibrator level established by screening using naïve individual human tears
- Negative and positive control samples were prepared in human tears, stored on the PVA sample collection device and extracted in the same manner as the study samples
- Acceptance criteria set on validation performance

Taking a different approach – Qualitative Assay

- **Characterisation:**



- **Validation:**

Validation Experiment	On-Spear				Extract			
	NC1	NC2	PC1	PC2	NC1	NC2	PC1	PC2
Selectivity	✗	✗	✓	✓	✗	✗	✓	✓
Combined Short Term Stability with Freeze-Thaw	✗	✗	✓	✓	✗	✗	✓	✓
Long Term Stability	✗	✗	✓	✓	✗	✗	✓	✓

✗ - Not Detected

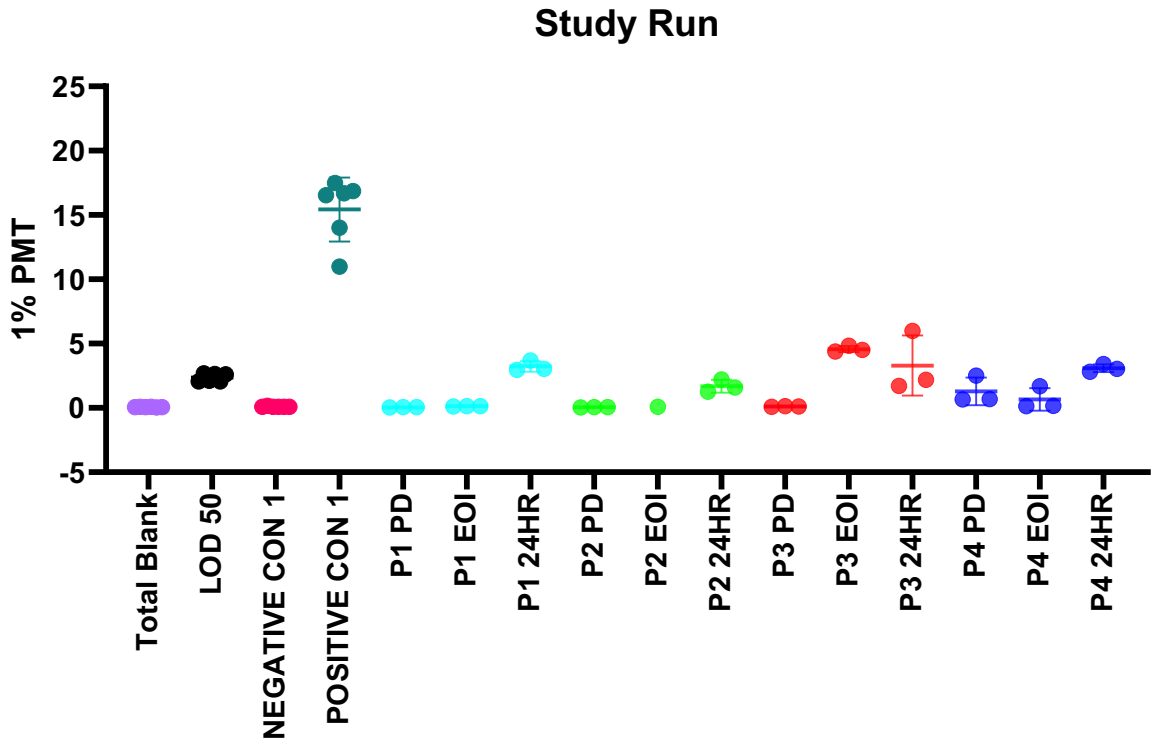
✓ - Detected

Taking a different approach – Qualitative Assay

- **Run Acceptance Criteria:**

Controls (n=6 replicates)	Samples (n=3 replicates)
PC: 4/6 replicates positive; response greater than mean LoD response	**Positive:** 2/3 replicates positive; response greater than mean LoD response "**Detected**"
NC: 4/6 replicates negative; response less than mean LoD response	**Negative:** 2/3 replicates negative; response less than mean LoD response "**Not Detected**"

- **In Study Data:**



Challenges Working With Unusual Matrices and Sampling Devices

- Artificial Tear Fluid (ATF), evaluated during method development
  - Good concordance with human tears
  - Issue with recovery from sampling device led us away from using as a true surrogate matrix
    - ATF used for initial working stock preparation
- Sample preparation on Echo 525 liquid handler was not compatible with the sponge eluate
- Tears are complex
  - Three layers containing enzymes, lipids, metabolites and electrolytes
    - Inner mucus layer, watery middle layer, outer oily layer
  - Different types: Basal, Reflex and Emotional
- Unknown how the PVA sampling device changes this composition

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Summary

- Challenges in-study with sample volume recovery from collection device led us to take a different approach
  - Validation samples must mimic study samples
- Qualitative assay developed which was able to address the question set out in the clinical study design
  - To evaluate the presence/concentrations of analyte in tear fluid in participants and explore relationship between tear fluid and plasma concentration
- Able to demonstrate control in a qualitative assay by using a strategy that minimised the false positive rate and used a floating cut point
- Challenges with unusual and difficult to obtain matrices and sample collection device

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