Immunoassays for Ophthalmology Microsampling Support

PK and Multi-Biomarker Quantification in Aqueous Humour

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Ultra Sensitive Immunoassays

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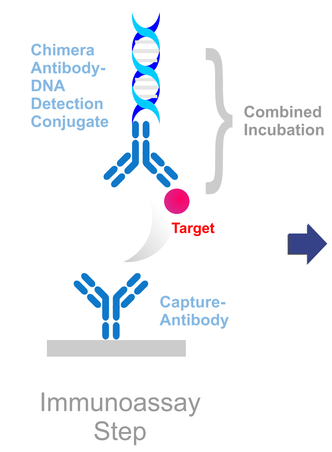
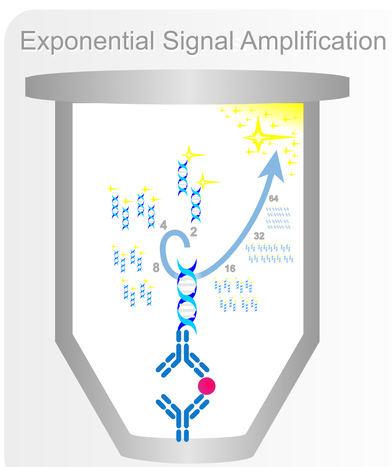
INTRODUCTION

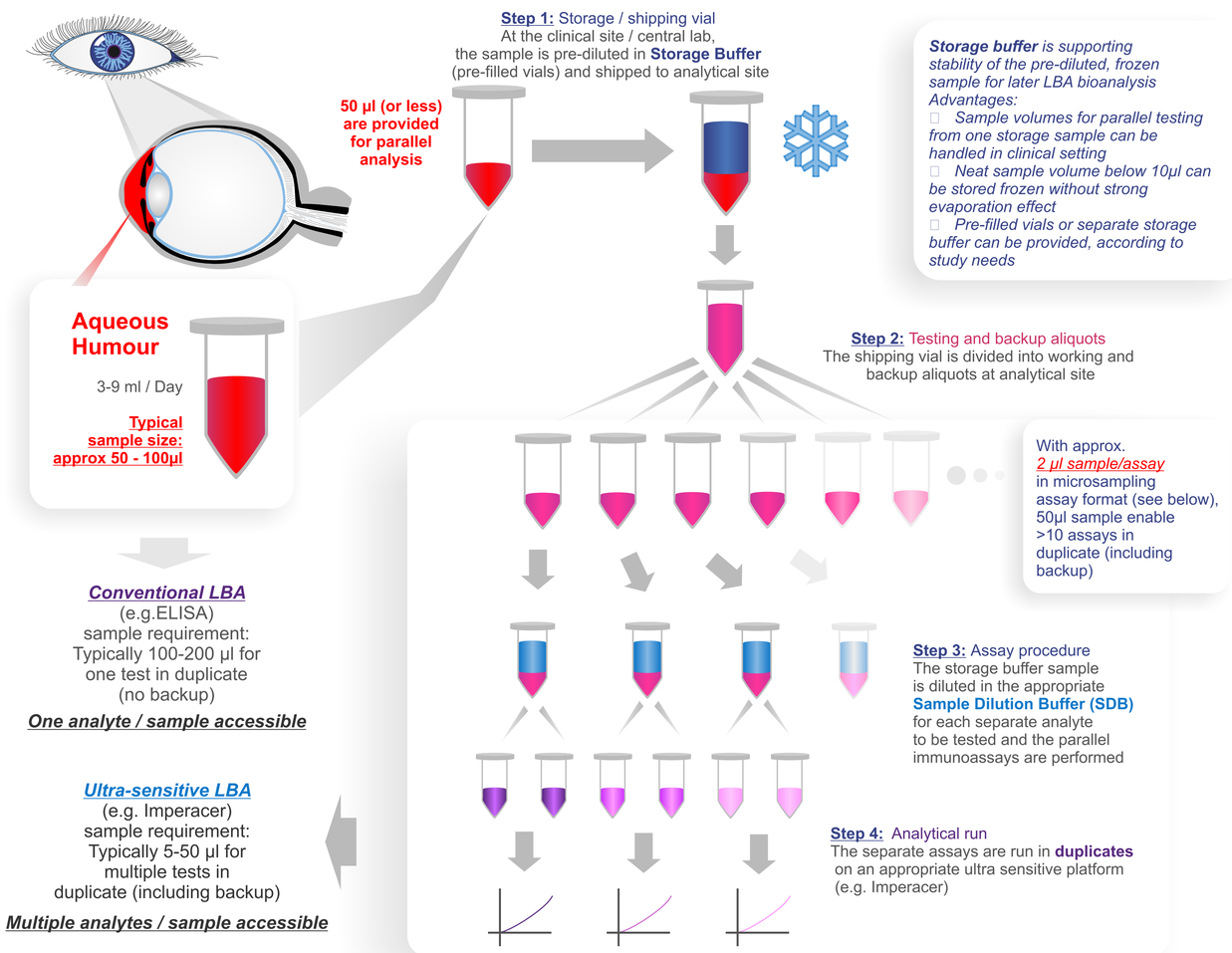




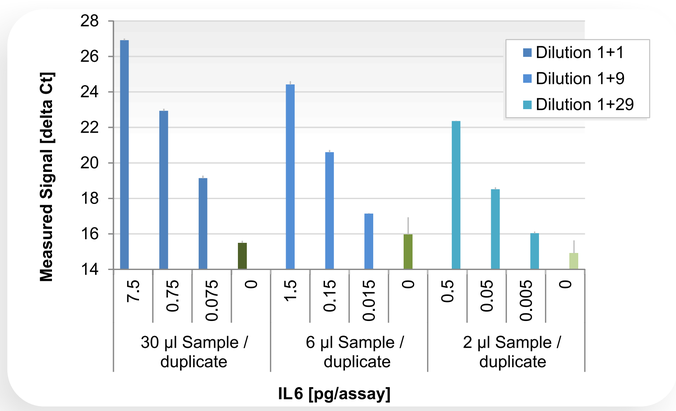
Purpose: Ligand-binding assay (LBA) quantification of biotherapeutics or endogenous biomarkers in ophthalmology support, or other therapeutic areas involving sampling of rare matrices, is limited due to sample volume availability. A major hurdle is the correlation of required sample volume vs. assay sensitivity on most immunoassay platforms. Here, we report an easy-to-use sample handling to allow parallel PK / multi biomarker sample testing support from low nominal volume of aqueous humour. Exemplarily, multi-, parallel biomarker quantification with low pg/ml sensitivities and good (+3log) assay range for IL6, VEGF and GM-CSF is presented from a few µl neat aqueous humour.

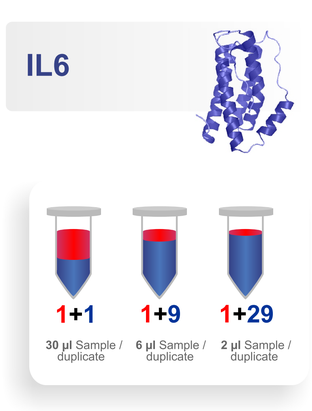
Methods: Samples were pre-diluted in a custom developed buffer, allowing handling of sample volume in the clinic and frozen long-term storage. Subsequent dilution of the thawed storage sample in assay buffer for quantification on an ultra sensitive LBA platform, e.g. Imperacer (IPCR) or HD-1 analyzer (Simoa). We confirmed cross reactivity of assay reagents between human and porcine system for IL6 and VEGF, thus individual porcine samples were used for proof of concept evaluation.

SAMPLE HANDLING

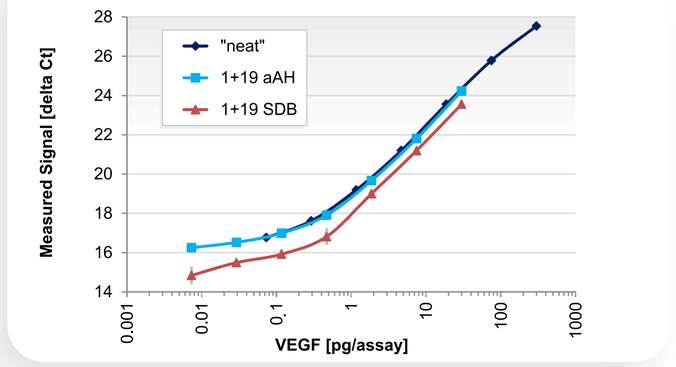


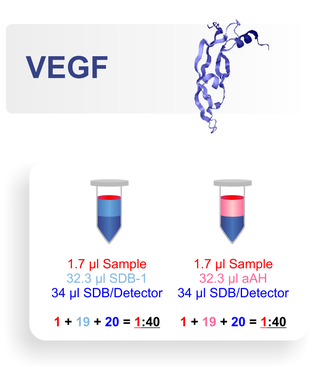
BIOMARKER ANALYSIS



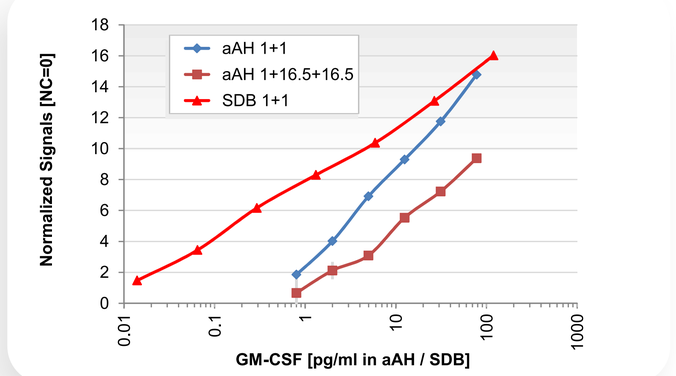


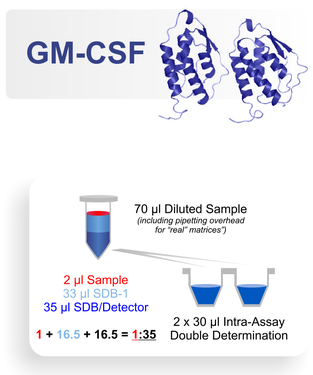
The ability of an ultra sensitive IL6 Imperacer assay to tolerate higher sample dilution to minimize sample volume requirement at a given sensitivity limit (e.g. 5 pg/ml) is demonstrated by serial dilution of **500, 50 & 5 pg/ml IL6 spiked in artificial aqueous humour (aAH).** In 1+1 sample dilution, an absolute sensitivity of 75 fg/assay is required to detect 5 pg/ml target; in 1+29 dilution, an absolute sensitivity of 5 fg/well is required, respectively. Imperacer provides the appropriate sensitivity. At 1+29 dilution, 2 µl sample is sufficient for testing in duplicate.





At the example of **VEGF spiked in aAH (5 pg/ml - 20 ng/ml),** the influence of diluents in bioanalytical microsampling support is demonstrated: While a dilution in identical matrix (aAH) revealed comparable signal response, dilution in a sample dilution buffer ("SDB") converts signal response for better sensitivity. Identical processing of samples and standards is key for quantitative bioanalysis: In 1+19 dilution, 1.7 µl neat sample is sufficient for VEGF testing in duplicate at a sensitivity level of approx. 5pg/ml.

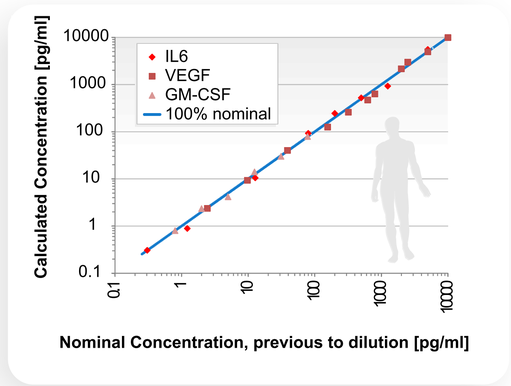




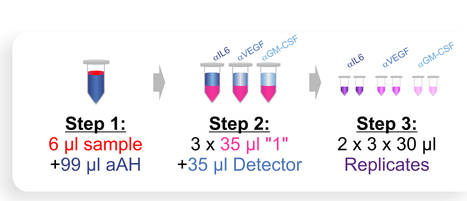
GM-CSF signal response in buffer vs. artificial aqueous humour (aAH): Dilution of GM-CSF spiked in sample dilution buffer (SDB) revealed an overall sensitivity massively below 0.1 pg/ml. In contrast, analysis from spiked artificial aqueous humour (aAH) revealed a very different signal response and sensitivities below 1 pg/ml. Microsampling support compatible additional 1+16.5 dilution in SDB converts signal response gradient to that of pure buffer with identical sensitivity compared to 1+1 diluted aAH (approx. 0.8 pg/ml).

RESULTS

1. Human Standards in aAH: Recovery Plot



3. Sample Processing



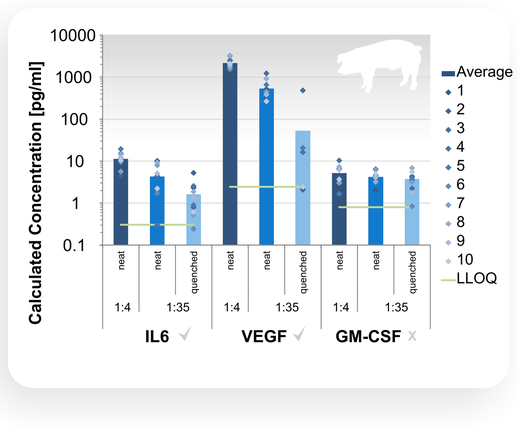
10 porcine aqueous humour samples were tested as a model system for 3 human biomarkers (IL6, VEGF, GM-CSF) with microsampling compatible Imperacer. The assays were developed for human cytokines and revealed good recovery for recombinant (see reccovery plot) and endogenous (data not shown) human targets.

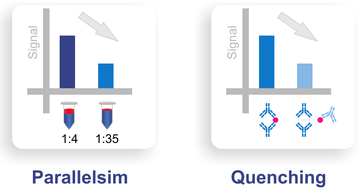
Pig AH samples were uniformly pre-diluted **1+16.5** in artificial aqueous humour (aAH) as **storage buffer** and subsequently incubated **1+1 in sample dilution buffer (SDB)** containing biomarker specific antibody-DNA detection conjugate. 3 parallel assays were run in duplicate, starting from the identical pre-diluted storage aliquot.

Total dilution factor: **1:35**

Total sample consumption for 3 assays in duplicate:
6 µl of aqueous humour.

2. Quantification of Individual Porcine Samples





All 1:35 diluted microsampling porcine samples revealed signals within assay range. To evaluate if these results represent endogenous biomarker in the pig model system, the assays were tested for:

(I) Parallelism: Analysis of neat samples at different dilutions (1:4 & 1:35) and

(II) Specificity: Spiking of 5 µg/ml of a biomarker-binding quenching antibody. This represents a typical drug-target interaction model experiment.

For IL6 and VEGF, parallelism and specificity were confirmed for the pig biomarker.

In contrast, GM-CSF assay revealed no specific response for pig GM-CSF, indicating that the signal response is not specific for the biomarker target, most probably due to differences in GM-CSF isoforms between human and

CONCLUSIONS

Approx. 10 analytes (e.g. drug and 9 PD biomarkers) can be quantified in duplicate (incl. backup sample for potential re-analysis) from a 50µl aqueous humour sample without potential antibody crosstalk, improving data generation in clinical trials with limited sample availability.

- Polyplex concept (parallel testing) is feasible for rare matrices with limited volume availability
- Assay range vs. sample requirement can be adapted for each analytical target
- PK / multiple PD testing (polyplex) accessible from identical storage sample
- Free choice of targets within the polyplex assay
- Increased quality of study data due to parallel testing free of antibody cross talk
- Porcine aqueous humour is a model system for VEGF & IL6 assays, reducing the need for human reference material. In contrast, measured concentrations for GM-CSF in pig AH do not represent actual porcine GM-CSF (most probably due to insufficient cross-species compatibility of the human specific antibody reagents)