SimplePlex:
A new high-sensitivity platform in the immunoassay area

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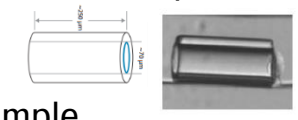
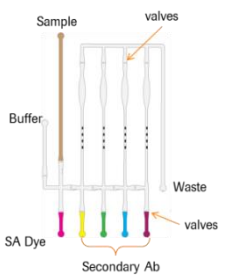
TTB – Proteomics – Immunoassays and Metabolites

Technology

- **Core of technology**
  - Glass Nano-Reactors (GNRs) coated with capture antibody



- **Assay principle**
  - capture antibody ↔ antigen in sample
  - antigen ↔ biotinylated secondary ab
  - biotin ↔ fluorescent streptavidin dye
  - excitation of fluorescence dye
  - detection of emission



- **Characteristics**
  - simultaneous and parallel quantitation of 4 analytes in a sample
  - low volume consumption (≤ 25 ul)
  - channels are physically separated -> elimination of cross-reactions compared to typical multiplexing principle
  - high sensitivity (LLoQ ≈1 – 10 pg/ml)

Procedure, through-put and costs

- **Ease of handling**
  - fully automated system
  - run preparation < 10 min
    - pipet samples into wells of cartridge
    - insert cartridge into analyzer
  - results are obtained after 70 min

- **Through-put**
  - 7 samples in duplicate (plus 1 QC low and 1 QC high) per cartridge
  - 4 analytes per cartridge
  - 196 sample results per day (49 samples per day)

- **Costs**
  - 700 CHF per cartridge
  - 25 CHF per sample result (100 CHF per sample) comparable to ELISA kit costs

Assay menu

validated at TTB			not validated yet	
ANG1	IL17A	PDGF-BB	BMP4	HGF
ANG2	IL18	PIGF	CCL1	IL1α
BMP9	IL1β	TIE2	MIP1a	IL7
CCL2	IL2	TNFα	IP10	MMP1
GM-CSF	IL34	VEGF R2	IL8	MMP8
IFNγ	IL4	VEGFA	MIG	OPN
IL10	IL5		EGF	KIM-1
IL12 p70	IL6		G-CSF	TNFβ

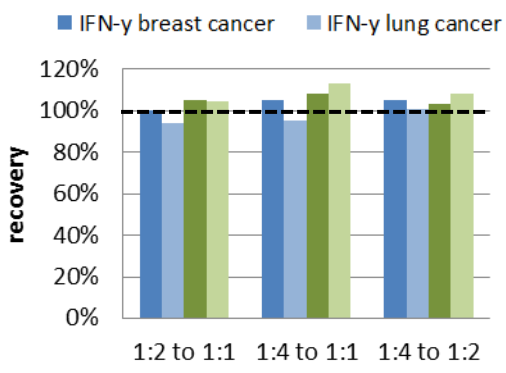
Assays can be combined in 4-plex panel without further adjustment, if sample dilution conditions are identical.

Application in Oncology

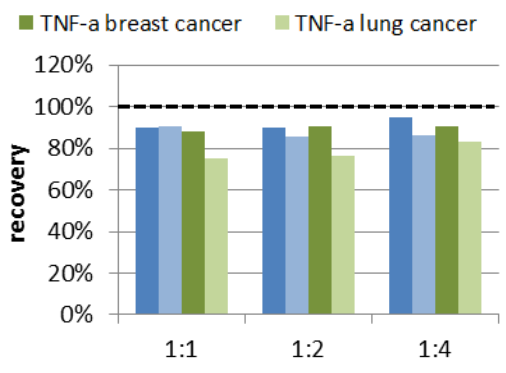
- Quantitation of cytokine release as a response to immunotherapies in phase I studies
- Assessment of assay performance prior to study start: Validation of IL1β, IL10, IL12 p70, IL17 A, IL6, IL8, TNFα, IFNγ…
- Some validation results

lowest detectable standard	IFNγ	TNFα
CyPlex	0.78 pg/mL	1.56 pg/mL
R&D Systems ELISA	15.6 pg/mL	15.6 pg/mL

Dilution linearity in plasma



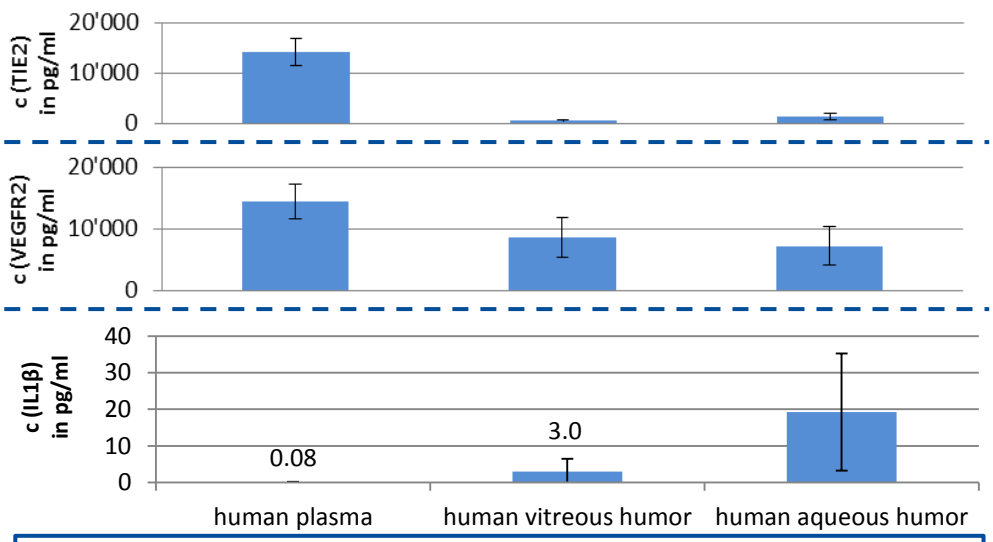
Spike recovery in plasma



Application in Ophthalmology

Are concentrations of circulating biomarkers correlating to intraocular level? Is **plasma a surrogate** of intraocular fluids?

- Analysis of PlGF, PDGF-BB, VEGFA, ANG1, ANG2, TIE2, VEGFR2, CCL2, GM-CSF, IL1α, IL1β, IL6, IL17, IL34 in plasma samples of Lucentis trial planned in Q1/2015.
- Preliminary feasibility results:



Are **physiological and pathological conditions** of the retina and choroid reflected in the composition of the **ocular humor**?

- Analysis of PlGF, PDGF-BB, IL1β, VEGFA in vitreous humor provided by Doheny Eye Institute, USA planned in Q1/2015
- Preliminary feasibility results: highest concentrations in **neurological**, lowest in **cardiovascular**, medium in **oncological** diseases

IL1β concentrations in aqueous humor from organ donors