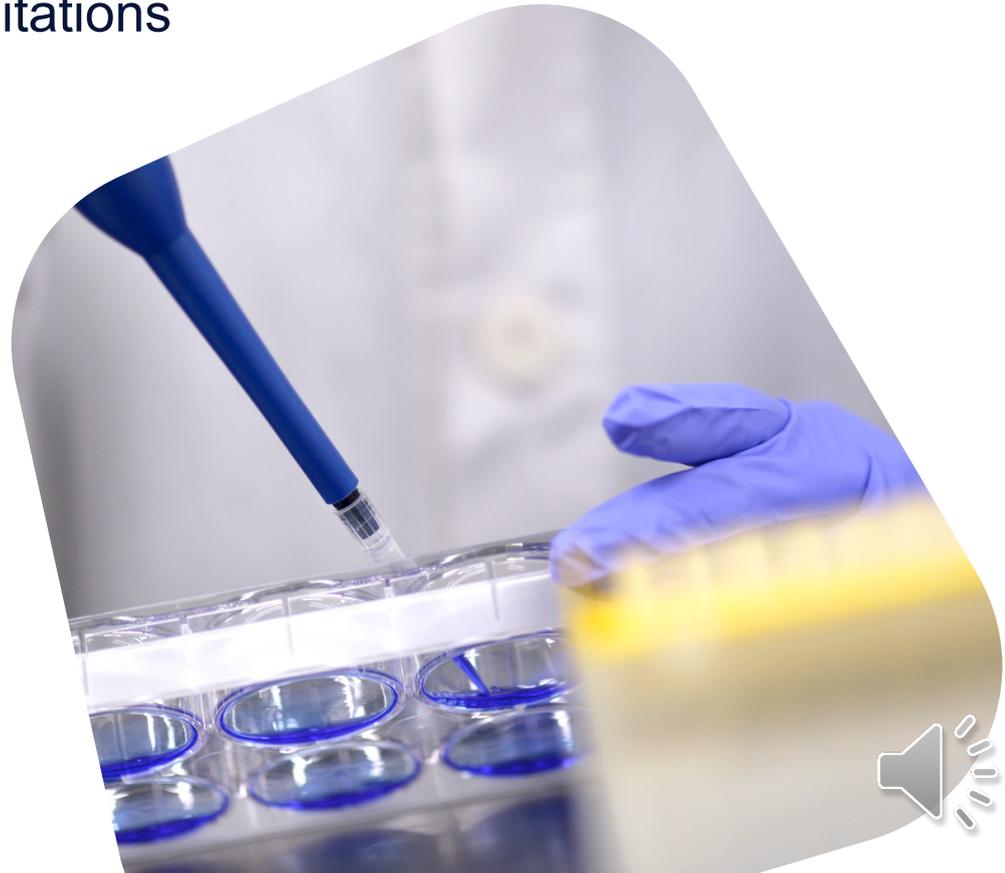


# Development of a high sensitivity, single-molecule based ligand binding assay for the detection of biomolecules in a regulated bioanalysis lab – Opportunities, Challenges and Limitations

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20<sup>th</sup> of May 2021

2021 - EBF Cyberconnect Events:  
Focus Workshop: Spotlight on TECHNOLOGY

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# Disclosure

Christoph Bock is an employee of AbbVie and may own AbbVie stock. AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication.

# References

Merck SMCxPro:

- SMCxPro System Introduction - Merck ([www.merckmillipore.com](http://www.merckmillipore.com))
- Hamren *et al.* (2018) - The power of Single Molecule Counting (Poster); *EMDMillipore*
- Hwang *et al.* (2018) - *Methods*

Quanterix SR-X and HD-X:

- Simoa® Technology - Quanterix ([www.quanterix.com](http://www.quanterix.com))
- Todd *et al.* (2007) - *Clinical Chemistry*
- Rissin *et al.* (2010) - *Nature biotechnology*
- Rissin *et al.* (2011) - *Analytical Chemistry*



# Presentation Overview

## Problem Statement

Why is there a need for a high sensitivity ligand binding assay for PK analysis?

Why in a regulated bioanalytical lab?

## Opportunities

Assay Development -  
SMCxPro & SR-X



Development and Optimization of a high sensitivity homebrew ligand binding assay in the pg/mL range

## Challenges

Points to consider ...

SMCxPro:  
Last transfer step  
→ Integra ViaFlo96



[www.integra-biosciences.com](http://www.integra-biosciences.com)

SR-X:

- (I) Batch variability of critical reagents
- (II) Assay reproducibility
- (III) Regulatory aspect

## Limitations

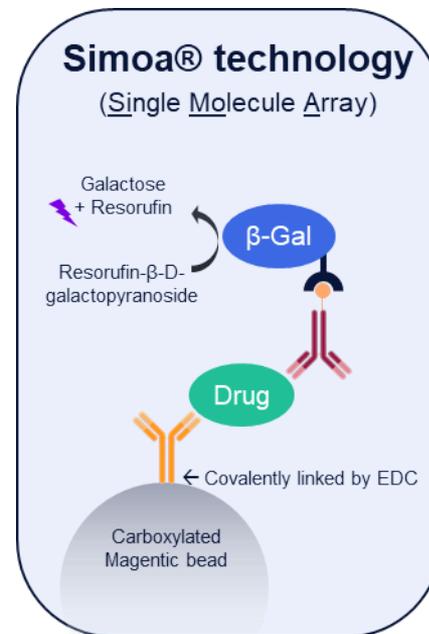
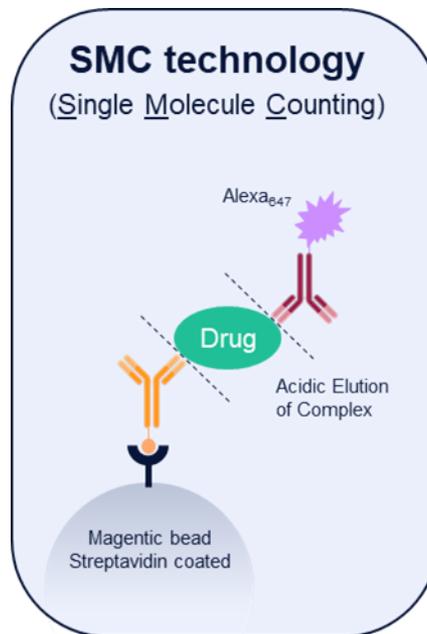
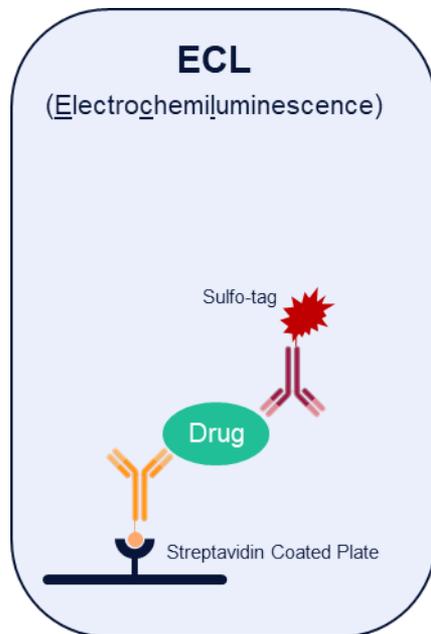
Transfer SR-X → HD-X



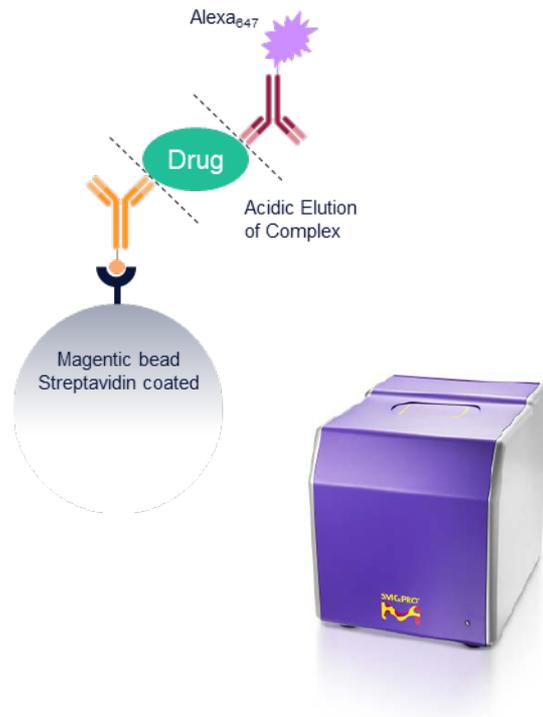
Homebrew assay transfer from the SR-X to the fully automated HD-X



# LBA Platforms for PK Assays



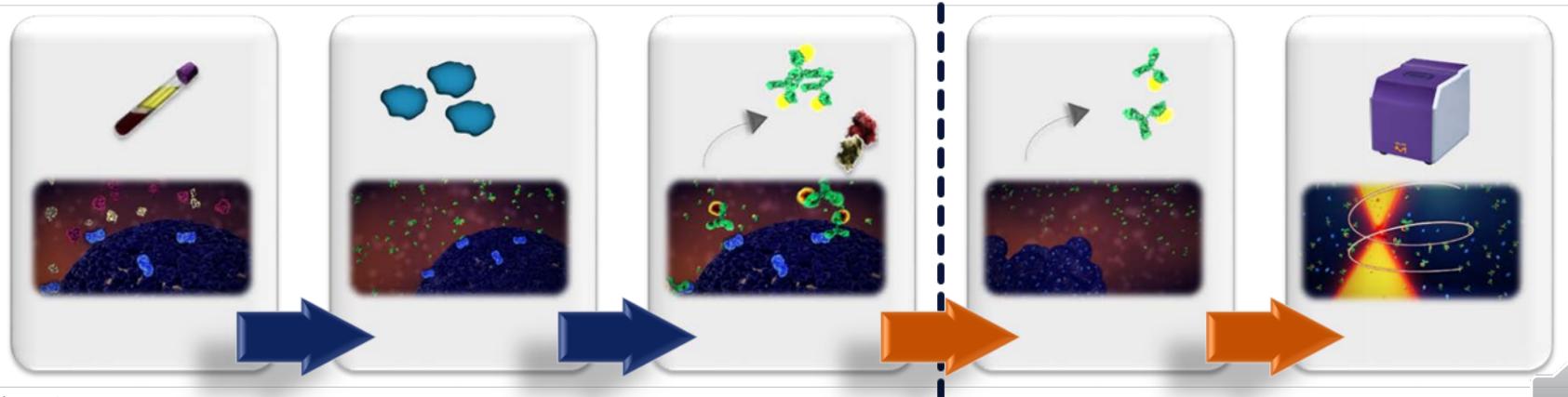
# SMCxPro: Setup Overview



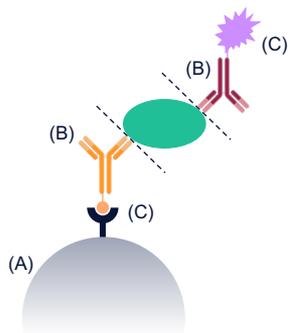
	SMC - Merck
plate vs. bead	bead or plate
signal read-out	fluorescence
data processing	digital & analog signals
sensitivity	pg/mL

The SMCxPro is the 2<sup>nd</sup> generation after the Erenna Singulex instrument and promises detection limits in the pg/mL range.

# SMCxPro: Assay Procedure



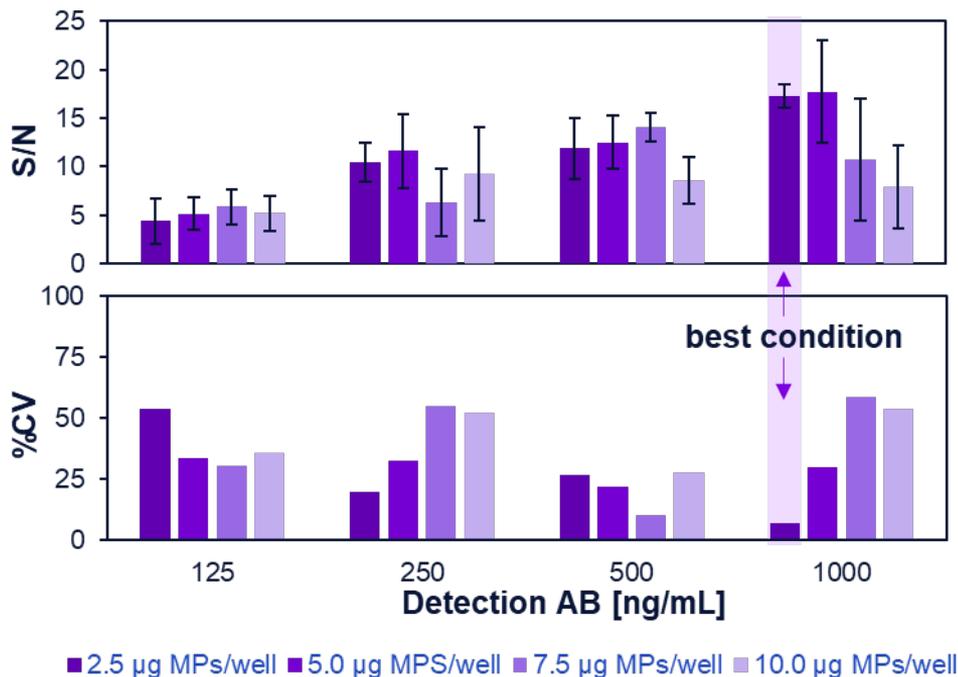
# SMCxPro: Assay Optimization



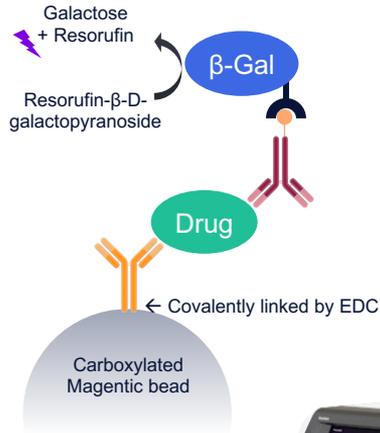
Screening for optimized condition for the detection of the POI:

- (A) Amount of microparticles
- (B) Reagent concentrations
- (C) Labeling strategies

Assay optimization clearly improved the sensitivity. Under best assay conditions the LLOQ was improved by a factor of 40.



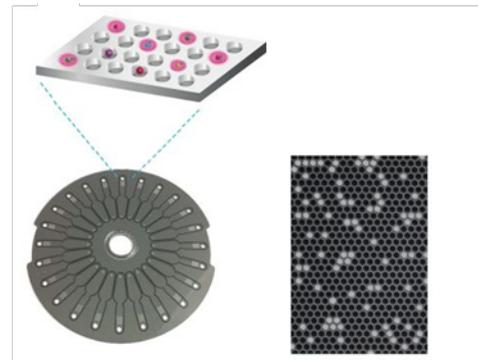
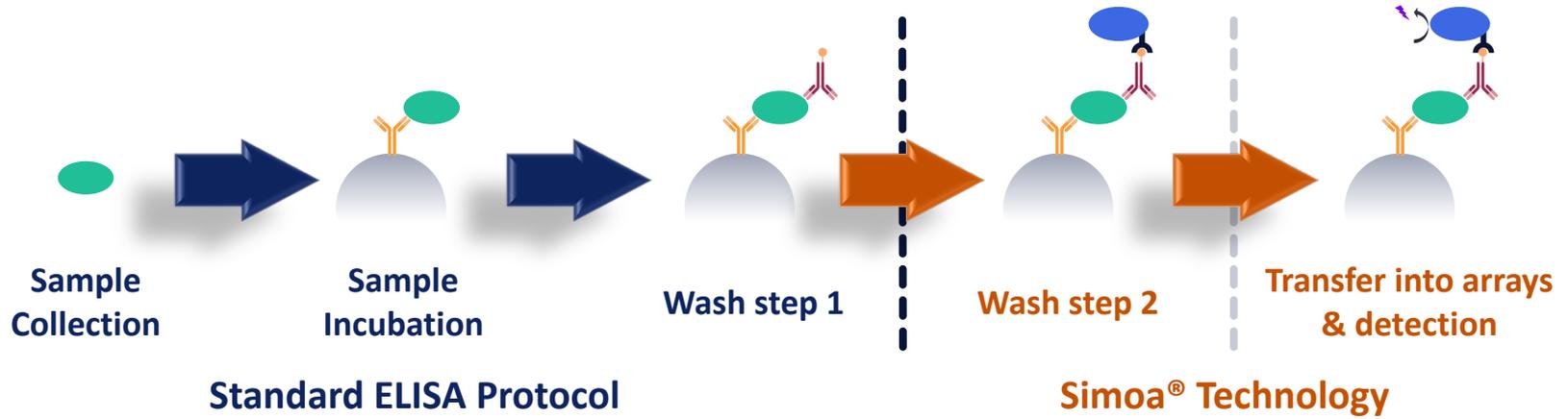
# SR-X: Setup Overview



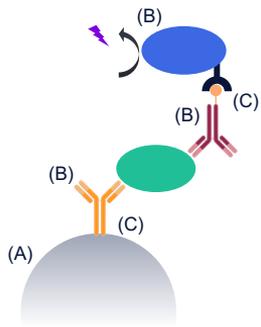
	Simoa® – Quanterix
plate vs. bead	bead
signal read-out	enzyme/fluorescent substrate
data processing	digital & analog signals
sensitivity	pg/mL

The SiMoA SR-X is the 2<sup>nd</sup> generation after the SP-X and promises detection limits in the pg/mL range.

# SR-X: Assay Procedure



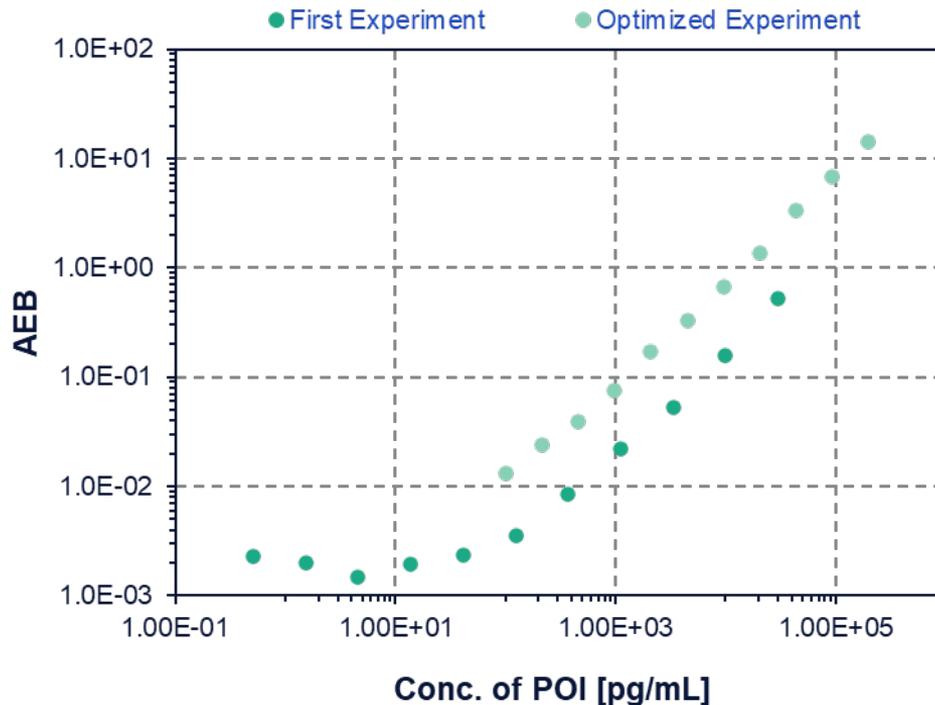
# SR-X: Assay Optimization



Screening for optimized condition for the detection of the POI:

- (A) Amount of microparticles
- (B) Reagent concentrations
- (C) Labeling strategies

Assay optimization clearly improved the sensitivity. Under best assay conditions the LLOQ was improved by a factor of 200.

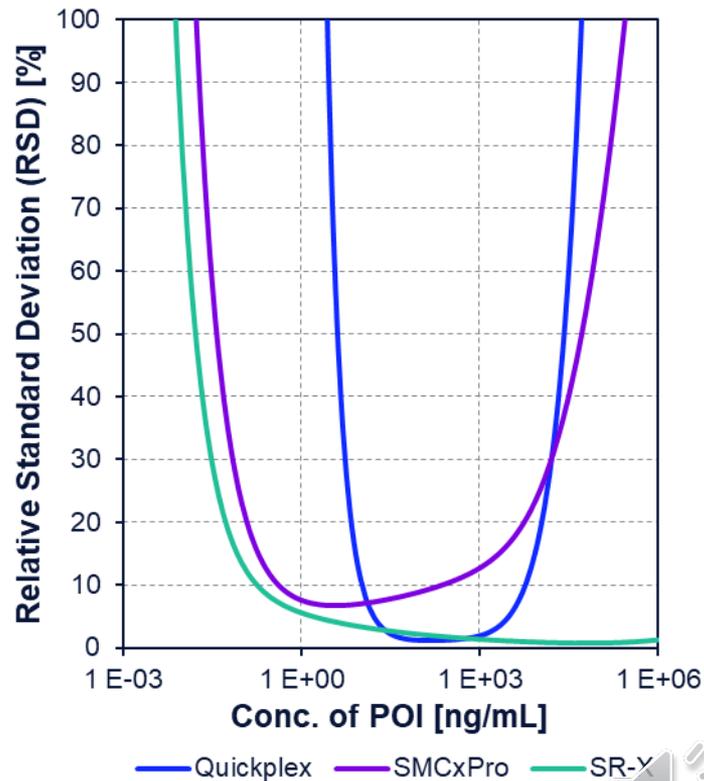


# ECL vs. Single Molecule Counting

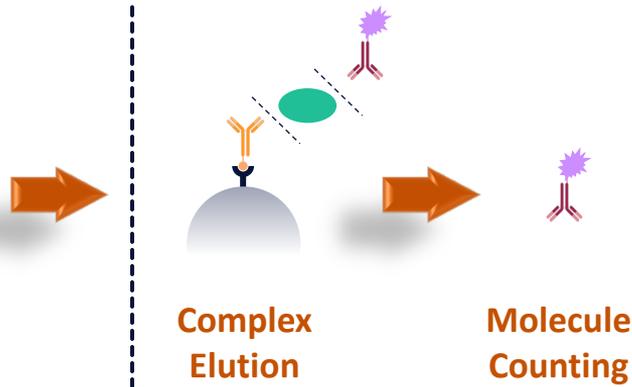
- Comparison of Sensitivity (LLOQ), Accuracy (%Bias) and Precision (%CV)

	Quickplex	SMCxPro	SR-X
LLOQ (pg/mL)	27,800	634	144
Mean %Bias	2.3 ± 2.8	8.3 ± 6.6	5.5 ± 5.0
Mean %CV	2.2 ± 0.8	9.8 ± 8.6	4.6 ± 3.6

40- and 200-fold sensitivity increase compared to ECL format, but reduced accuracy and precision.



# SMCxPro: Transfer Step



Assay precision and accuracy is strongly dependent on the last transfer step after acidic elution of the complex and neutralization.

→ Transfer of 10  $\mu$ L from 96-well assay-plate into 384-well readout-plate.

**ViaFlo96:** freely programmable and automated pipetting robot  
One program for the (I) addition of neutralization buffer after acidic elution and (II) transfer into 384-well plate.

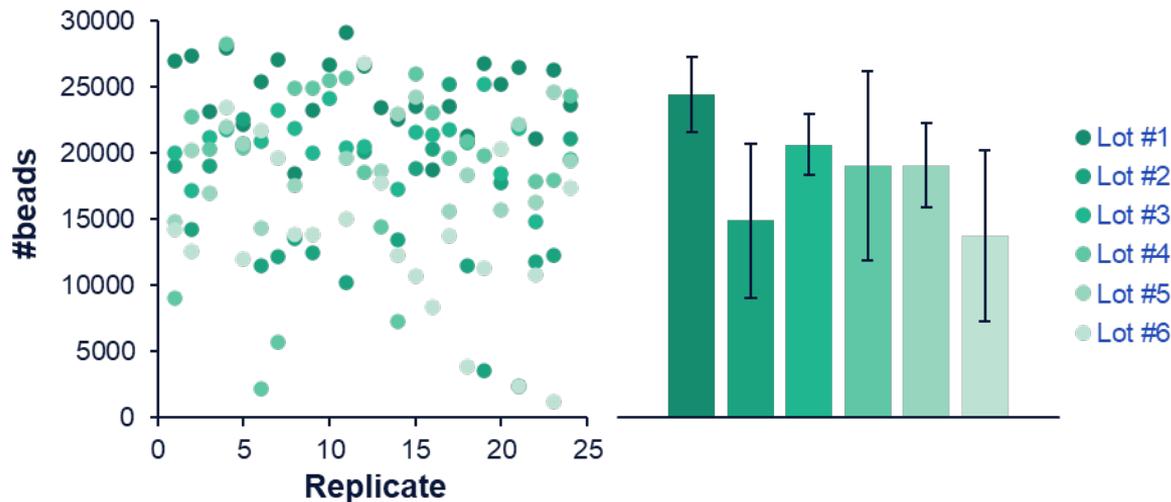


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# SR-X: Bead Lot Analysis

Reproducibility of labeling procedures and thereby assay performance is dependent on quality of bead lots.

→ Analysis of bead lot quality prior to EDC coupling.



The bead number is critical for AEB calculations, especially at low concentrations (digital readout).

→ Bead clumping and aggregation increase the variance and affect assay sensitivity (up to a factor of 10).

# SR-X: Assay Reproducibility & Regulatory Aspects

➤ Reproducible assay during and after method development

➤ However: Impact of bead lot variability on assay sensitivity and performance

Lot	Assay range [pg/mL]	ANOVA	Selectivity	Dilution linearity
#1	45.7 to 300,000	Yes	Yes	Yes
#3	300 to 300,000	Yes	No	N/P

→ Need for optimization after bead lot change

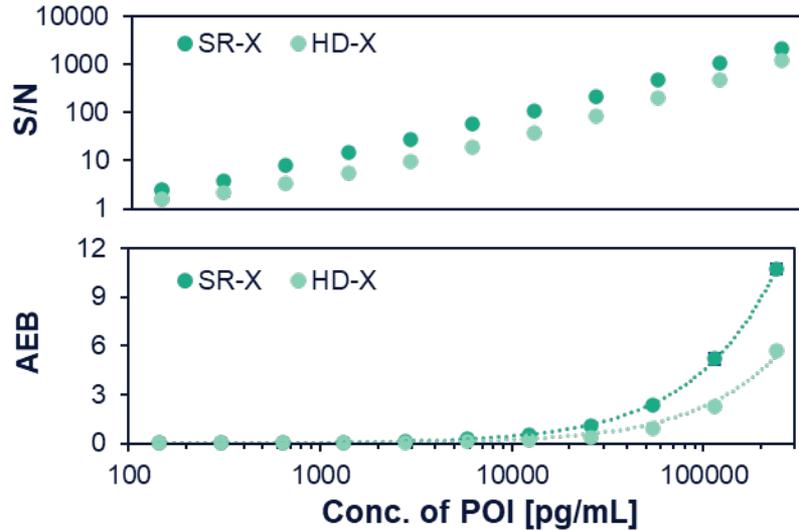
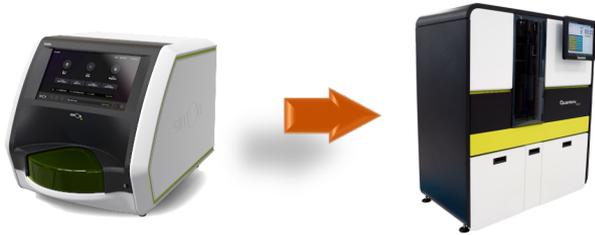
Points to consider when using the SR-X in a regulatory bioanalysis lab:

- All-in-one instrument
  - Local accounts for users in the software
  - Incorporation in company network
- Event Log/Audit Trail
- IQ/OQ documentation from Quanterix
- Data analysis
  - Data analysis included in the software
  - Raw data export as .csv file for analysis
  - Validation of Excel Makro for analysis (e.g. in a LIMS system)

# Simoa®: Assay Transfer Limitations

Assay procedure on the SR-X is time consuming and limited to ~ 2 plates per day.

→ Assay transfer from the SR-X to the automated HD-X platform.



S/N ratio and overall signal on the HD-X are reduced by a factor of ~ 2.  
→ differences based on different sample handling and technical limitations on the HD-X; additional assay optimization for automated sample analysis.

# Presentation Summary

- Both SMCxPro and SR-X instruments can be used to develop and validate a high sensitivity ligand binding assay for pharmacokinetic data readout.
- SMCxPro and SR-X increased sensitivity for the detection of the POI by a factor of 40 and 200, respectively, compared to a standard ECL PK assay.
- Use of the ViaFlo96 in the last transfer step improved SMCxPro assay performance (accuracy and precision).
- Bead lot change during assay development and validation on the SR-X is a critical point concerning reproducibility and variability.
- For automated sample analysis, perform assay development directly on the HD-X.



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