NUVISAN

Don’t get hooked on homogenous assays
(Avoid the Hook Effect in homogenous MSD assays)

9th YSS (11/12-May-2023)

Dr. Fabian Gaertner

Background Information

- **LBA assay** requested to detect a specific analyte in human plasma utilizing a pair of **anti-idiotypic Fab fragments**

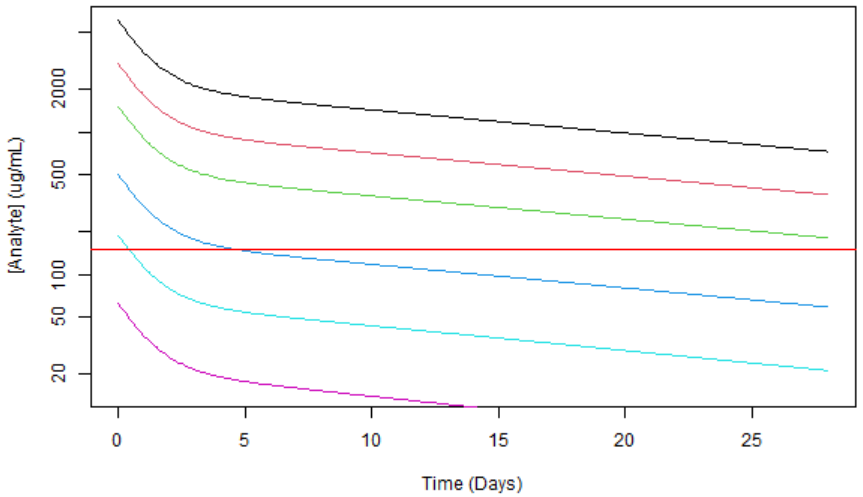
- **Aim:** support pharmacokinetics in a dose escalation clinical trial

→ Quick turnaround time necessary to provide data for safety assessment meetings

Background Information

- Initial tests using a **sequential approach** indicated **poor signals** and the working assay range was not sensitive enough

    - 6 dosing levels are planned, and the assay should be able to at least generate values for the **first time points** for **all dosing levels**



→ A homogenous approach was tested

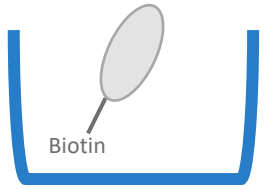
Principles of a homogenous assay

Sequential Approach

Homogenous Approach

Principles of a homogenous assay

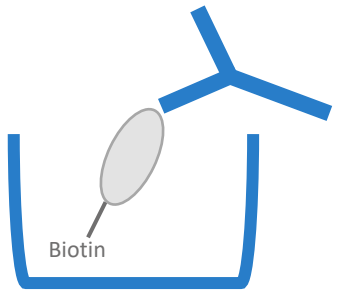
Sequential Approach



Homogenous Approach

Principles of a homogenous assay

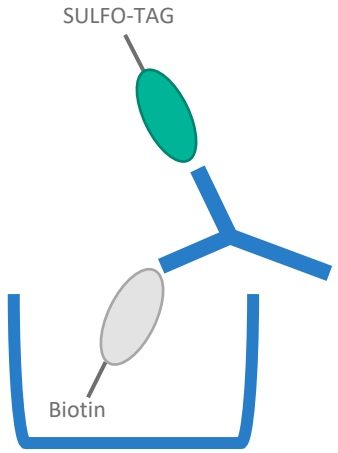
Sequential Approach



Homogenous Approach

Principles of a homogenous assay

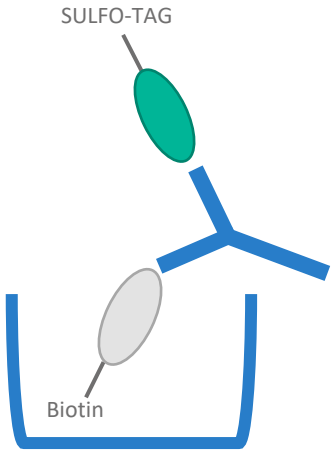
Sequential Approach



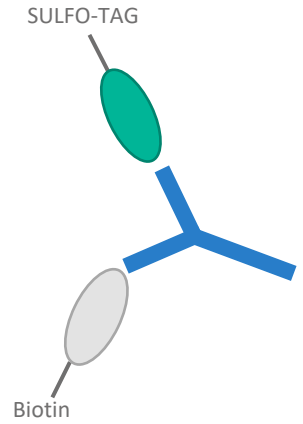
Homogenous Approach

Principles of a homogenous assay

Sequential Approach

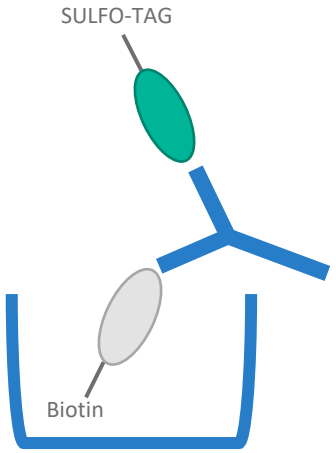


Homogenous Approach

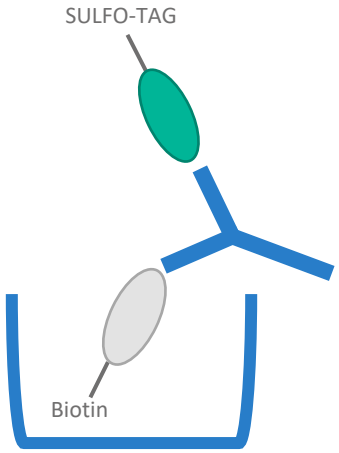


Principles of a homogenous assay

Sequential Approach

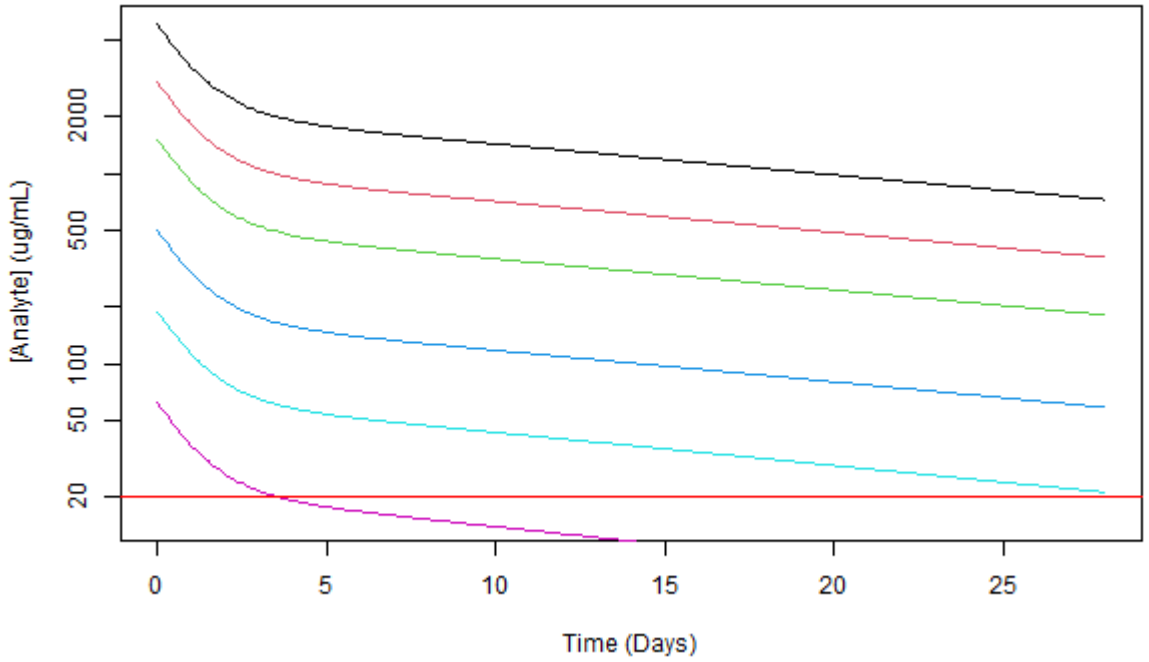


Homogenous Approach



Homogenous assay - Result

- A homogenous approach was tested and generated much higher signals
  → additionally, a better sensitivity was achieved

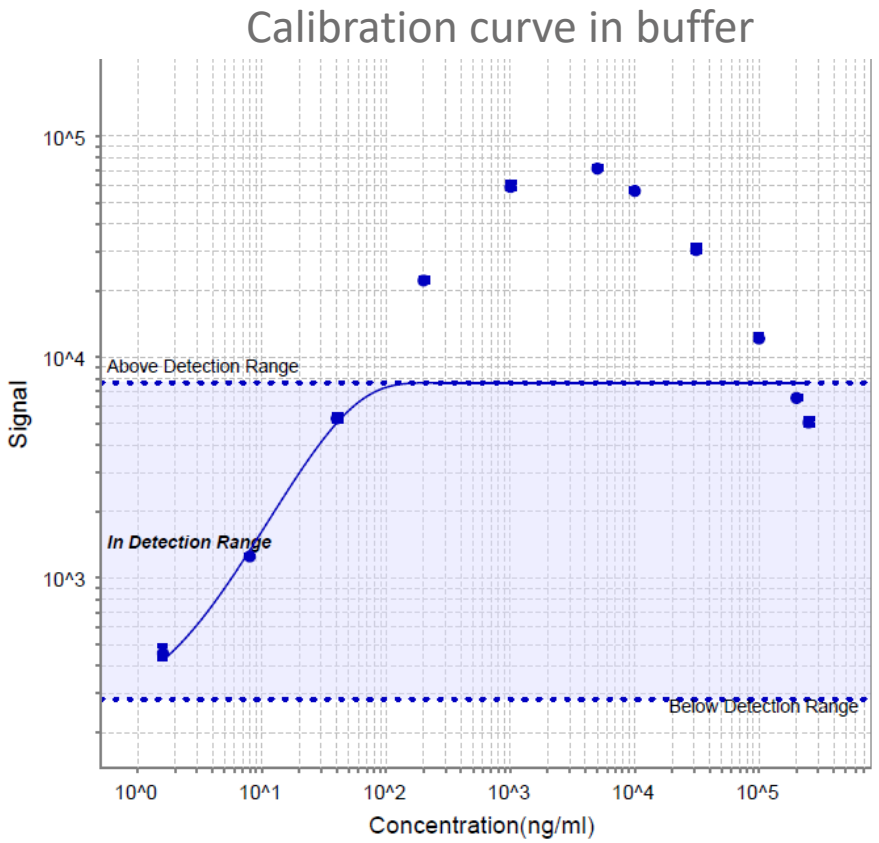


- However, during assay range investigations …

The Hook Effect

- … a prominent hook effect was present
- Since various approaches to develop a sequential assay failed during method development (sensitivity)

→ solution driven changes of the assay procedure while maintaining the homogenous approach



The Hook Effect: Solutions

- Measure all samples twice (with different dilutions) to pin-point on which „side“ the sample was measured

→ Only deals with the symptoms

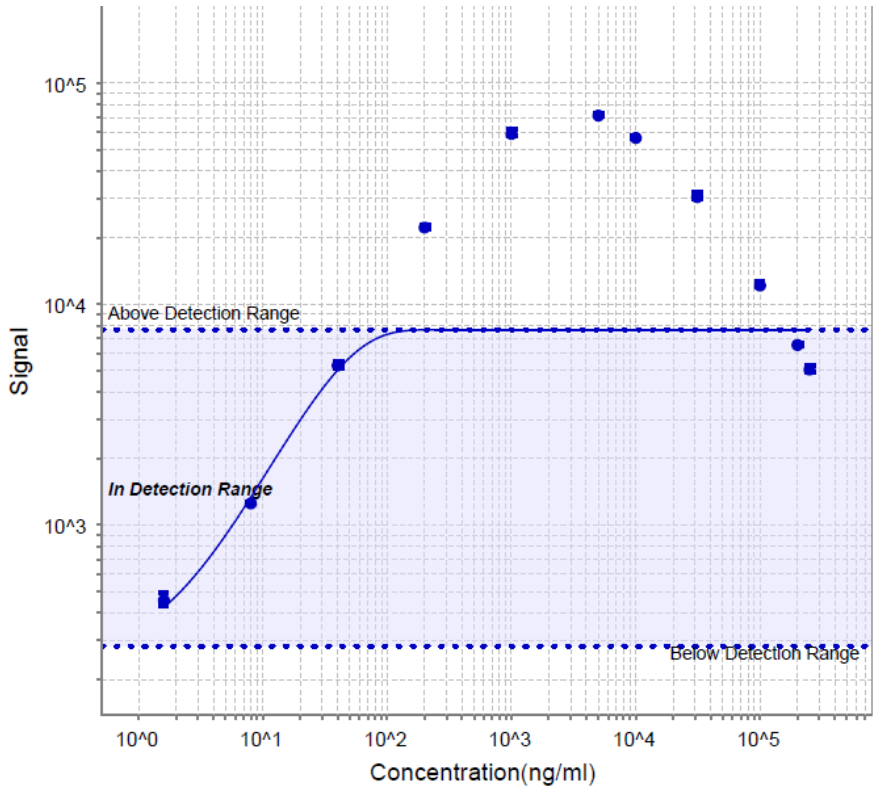
- Can it be solved utilizing dilutions / MRD approaches?

→ only dilutes the effect out of the assay range

- Increase the amount capture antibody

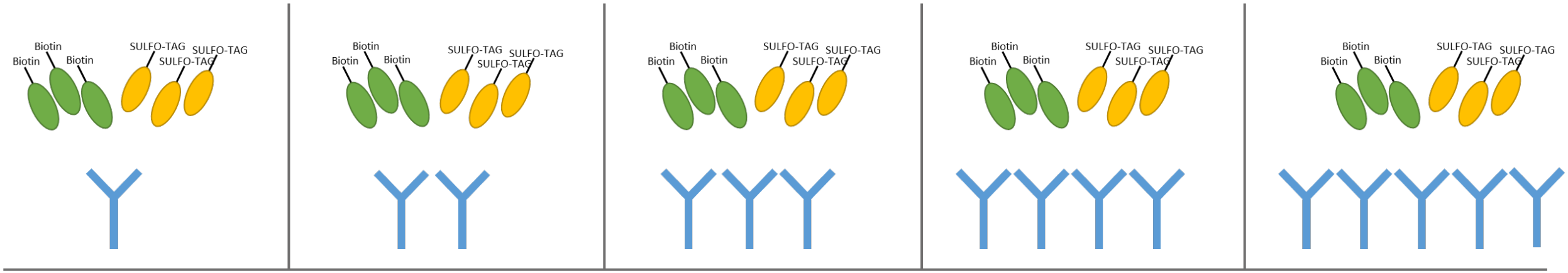
→ No effect

Calibration curve in buffer



ECL Signal	ULOQ [200 µg/mL]	ALOQ_1 [1620 µg/mL]	ALOQ_2 [3240 µg/mL]
Homogen	40693	20058	10990
Homogen + increased Capture	35159	19108	11022

Working hypothesis: Reason for hook effect



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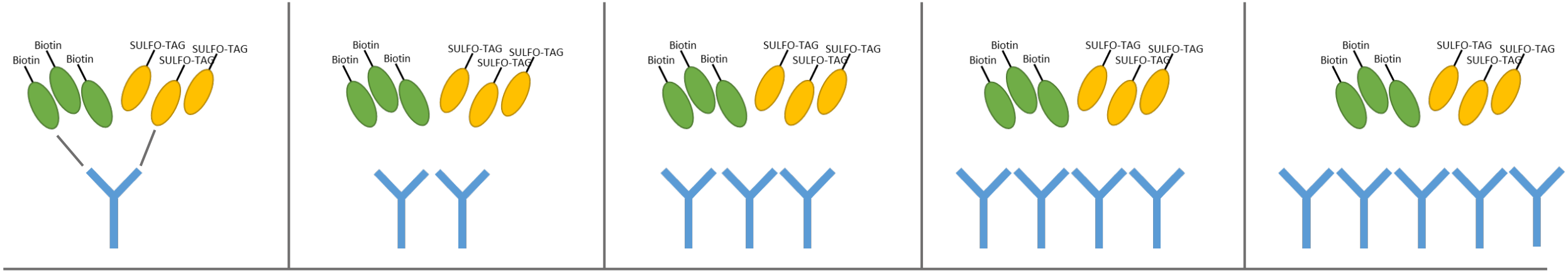
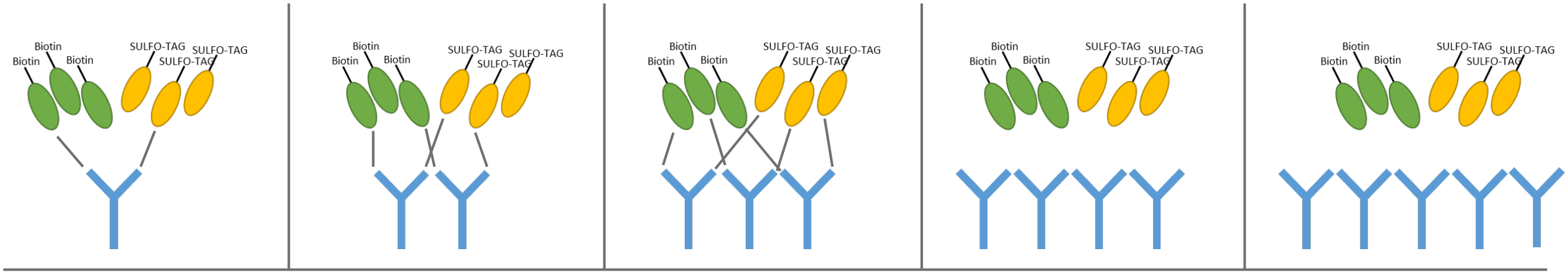


Bound to Plate
1

Detect
1

Working hypothesis: Reason for hook effect



Bound to Plate 1

Detect 1

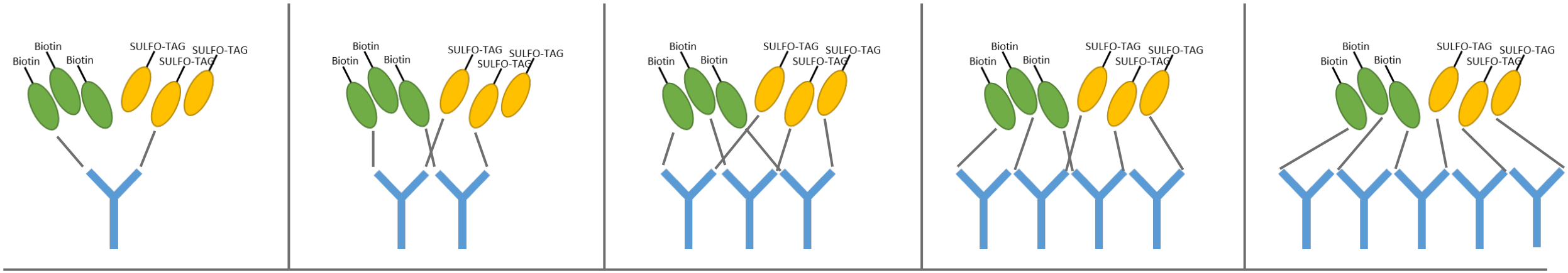
Bound to Plate 2

Detect 2

Bound to Plate 3

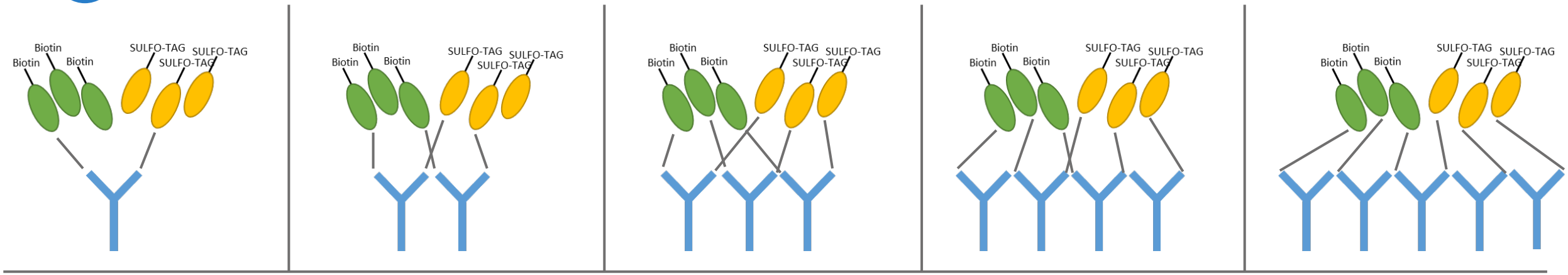
Detect 3

Working hypothesis: Reason for hook effect

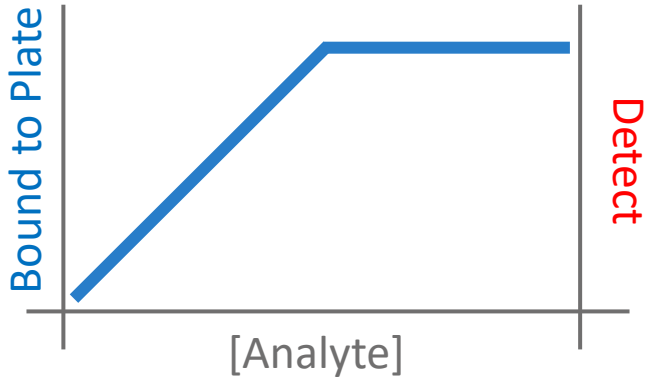


Bound to Plate 1	Bound to Plate 2	Bound to Plate 3	Bound to Plate 3	Bound to Plate 3
Detect 1	Detect 2	Detect 3	Detect 2	Detect 1

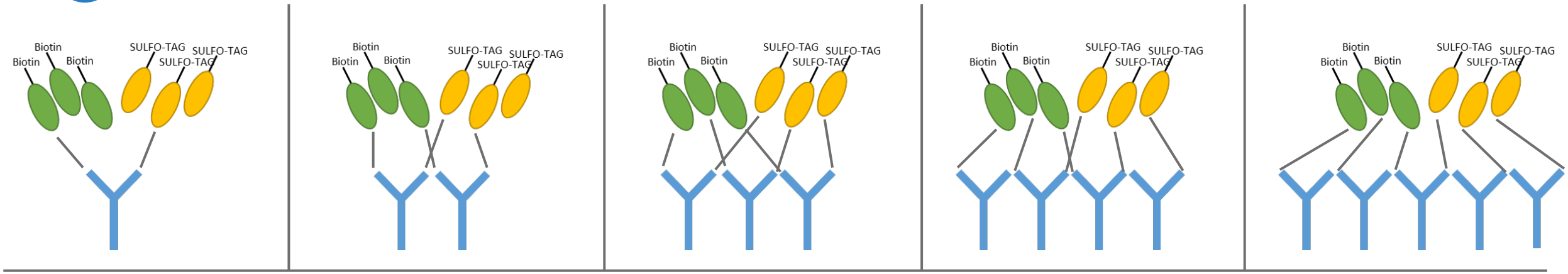
Working hypothesis: Reason for hook effect



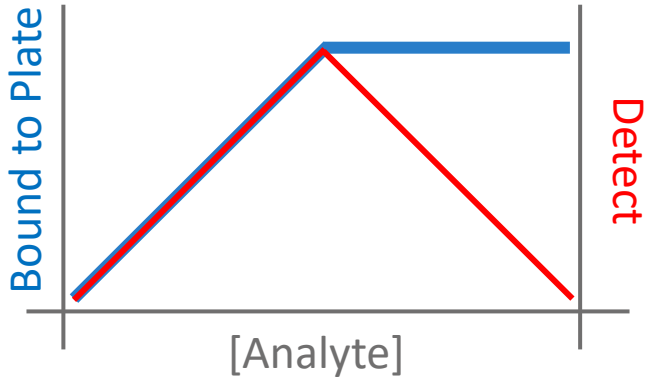
Bound to Plate	Bound to Plate	Bound to Plate	Bound to Plate	Bound to Plate
1	2	3	3	3
Detect	Detect	Detect	Detect	Detect
1	2	3	2	1



Working hypothesis: Reason for hook effect



Bound to Plate 1	Bound to Plate 2	Bound to Plate 3	Bound to Plate 3	Bound to Plate 3
Detect 1	Detect 2	Detect 3	Detect 2	Detect 1



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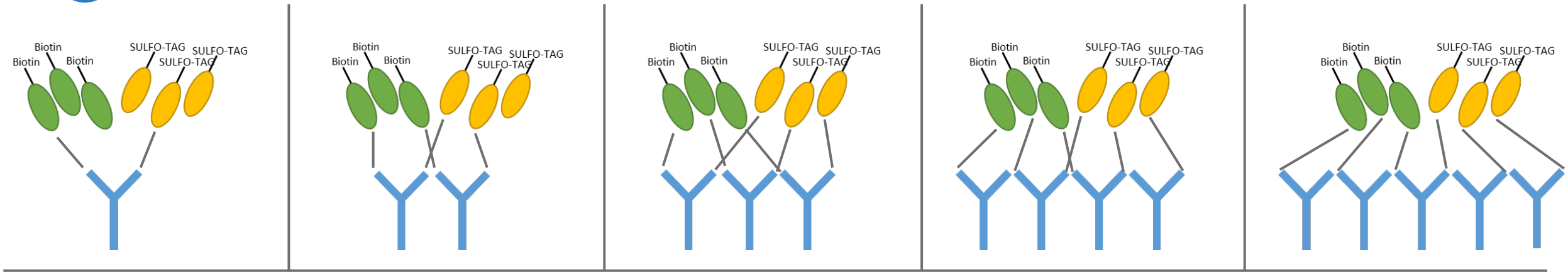
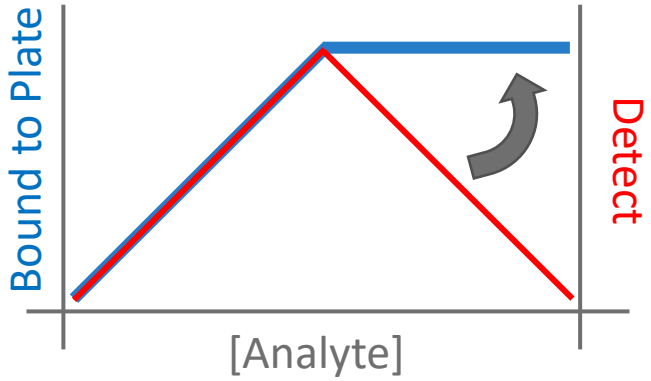
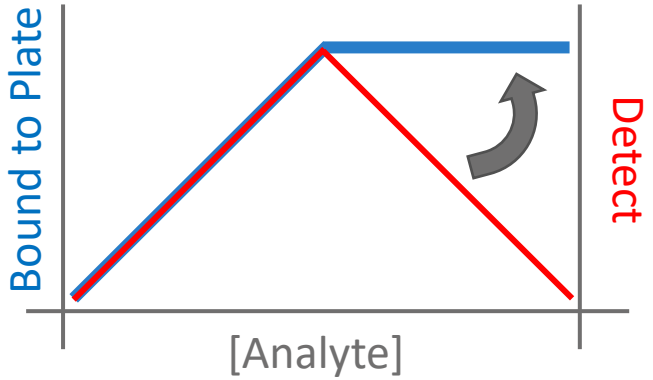


Bound to Plate 1	Bound to Plate 2	Bound to Plate 3	Bound to Plate 3	Bound to Plate 3
Detect 1	Detect 2	Detect 3	Detect 2	Detect 1



Can the signal be rescued with a secondary detection step ?

Results



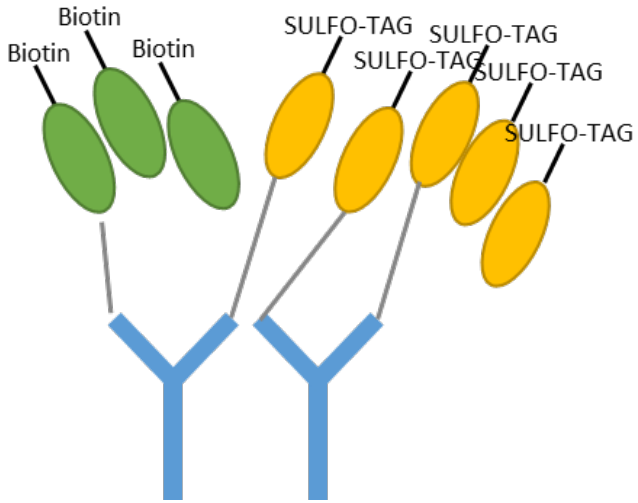
Can the signal be rescued with a secondary detection step ?

- Calibration curve was prepared at MRD, two samples were tested at concentrations that were above the ULOQ of the assay

ECL Signal	ULOQ [200 µg/mL]	ALOQ_1 [1620 µg/mL]	ALOQ_2 [3240 µg/mL]
Homogen	40693	20058	10990
Homogen+2nd Detection step	85773	91191	100410

Final Thoughts – maintain homogenous approach ?

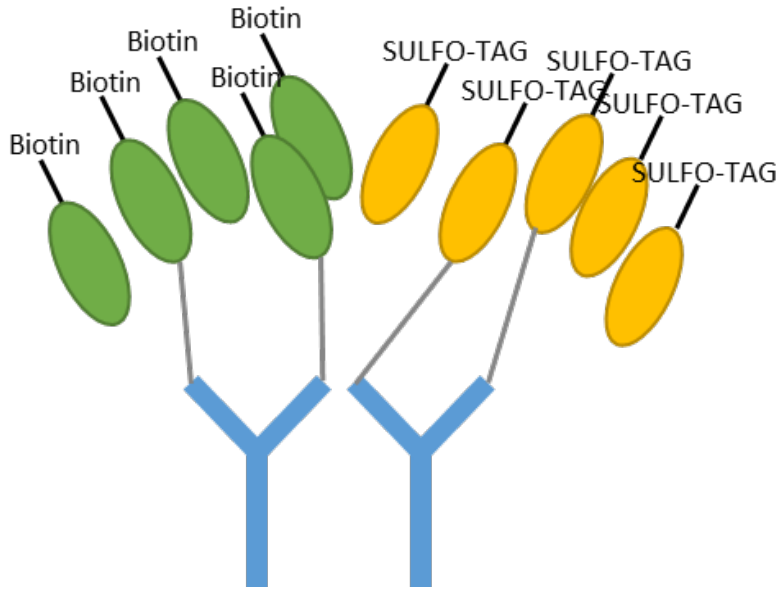
- Would detection antibody in excess during the incubation of analyte, detection antibody and capture antibody have solved the issue as well ?



- Increases the probability, that two Sulfo-Tagged anti-ID Fab fragments bind to one analyte → no binding to the plate

Final Thoughts – more reagents?

- Would increasing amount of capture AND detection reagents during the incubation of analyte, detection antibody and capture antibody have solved the issue as well ?

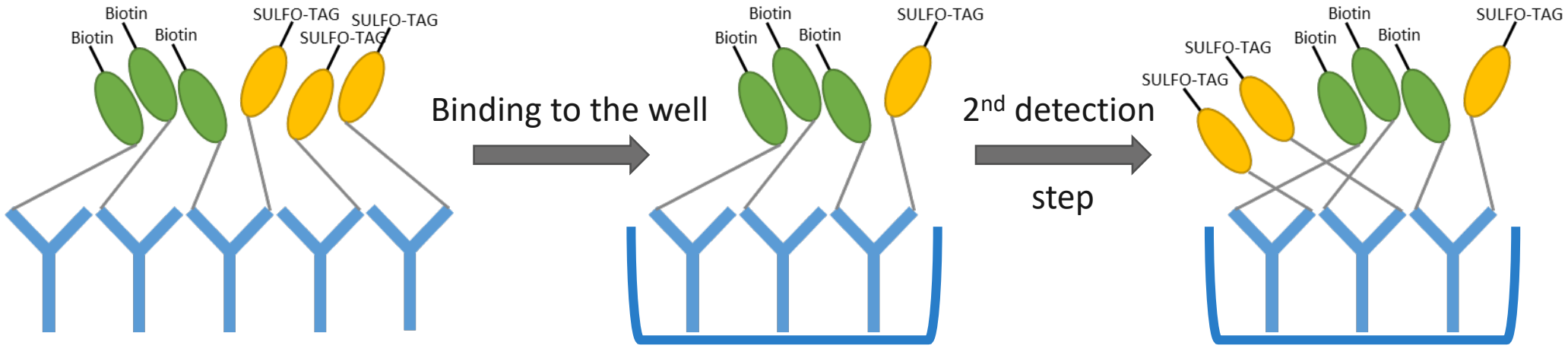


- Shifts the hook effect to higher concentrations and looses sensitivity
- Increases the probability, that two Biotin or two Sulfo-Tagged anti-ID Fab fragments bind to one analyte → binding to the plate, but no detection or no binding to the plate

Final Assay Setup

- Adaption of the homogenous approach to a semi-homogenous assay with a secondary detection antibody solved the prominent hook effect

- Sensitivity of the assay was still maintained → Assay was validated



THANK YOU FOR YOUR ATTENTION!

Any questions?

Feel free to ask!

Acknowledgement

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