



Evaluating multiple technology platforms in the development of large molecule bioanalytical assays

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NovImmune – *Company Profile*

- Focused on advancing targeted medicines that address the causes rather than symptoms of disease
- Proprietary next-generation antibody drug platform
- Bench-to-bedside & pilot manufacturing capabilities
- Pipeline of differentiated antibody-based products
- Scientific excellence
 - Ten patent families
 - 60+ peer-review journal publications
 - 30+ collaborations with academic institutes
 - Roche/Genentech Alliance



Presentation Outline

- Background
 - why compare technology platforms?
 - platforms available at NovImmune
- Case Study 1
 - PK assay development
- Case Study 2
 - ADA assay development
- Conclusions

Why compare technology platforms?

- Number of different platforms available for large molecule bioanalytical assays
 - many claim superior sensitivity and dynamic range
- Publications about technologies comparison
 - one technology is not always the best
 - pros and cons for each of the platforms
 - difficult to predict which technology will be the best for a particular assay
- Perform our own technology platform comparison
 - evaluate which technology meets our assay requirements

Platforms available at NovImmune

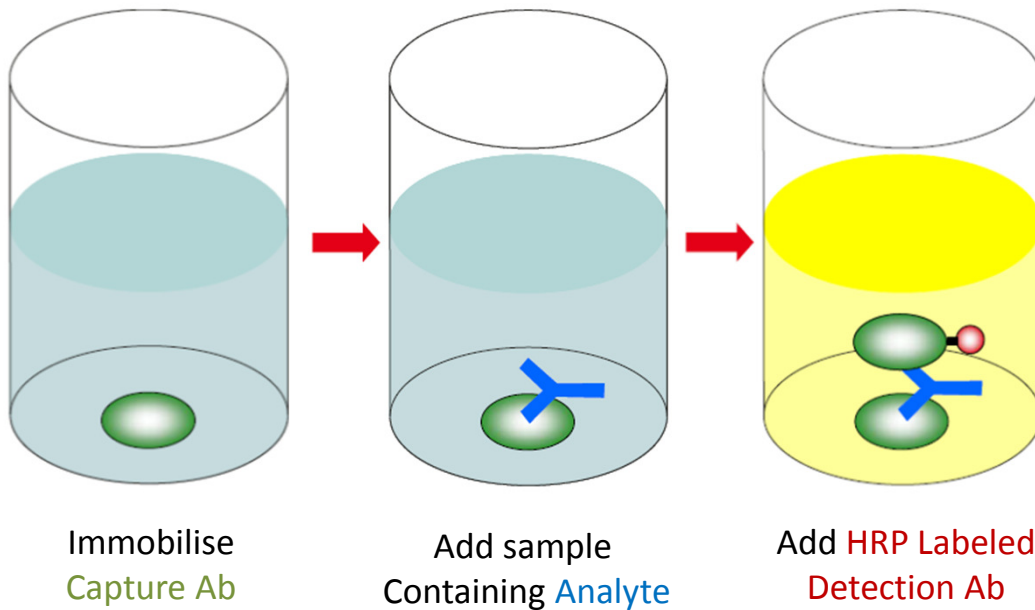
- ELISA
- Gyrolab™
- Meso-Scale Discovery (MSD®)
- AlphaLISA®
- Luminex®



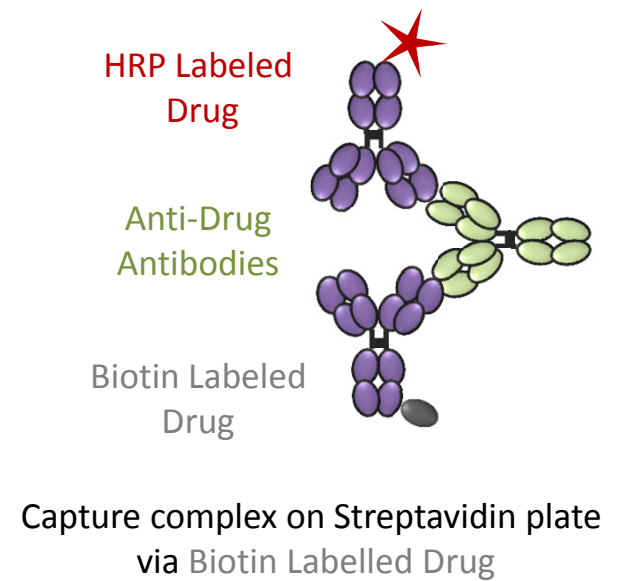
ELISA

- Plate-based assay
- Readout via simple plate reader

Sequential (Sandwich)

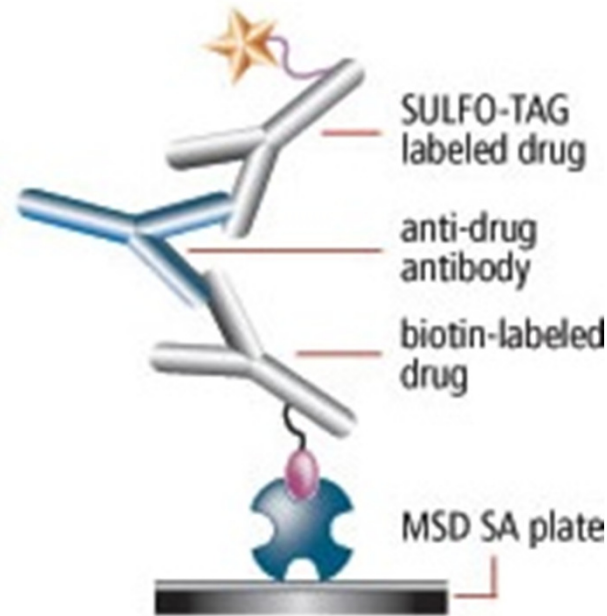


Homogeneous (bridging)



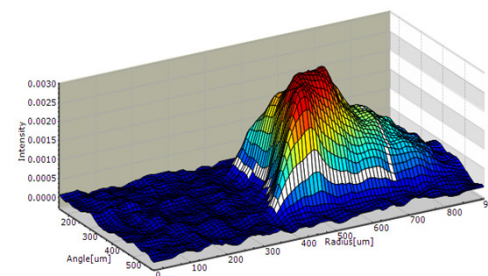
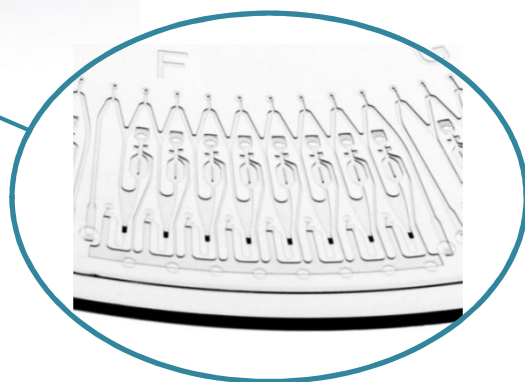
Meso Scale Discovery (MSD[®])

- Plate-based assay
- SulfoTAG label emits light when electrochemically stimulated

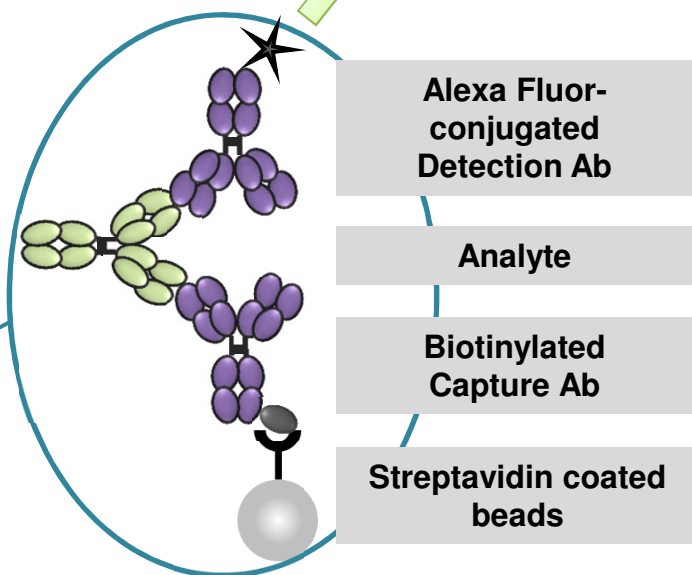


Gyrolab™ xP Workstation (Gyros®)

- Automated nano-liter-scale immunoassay

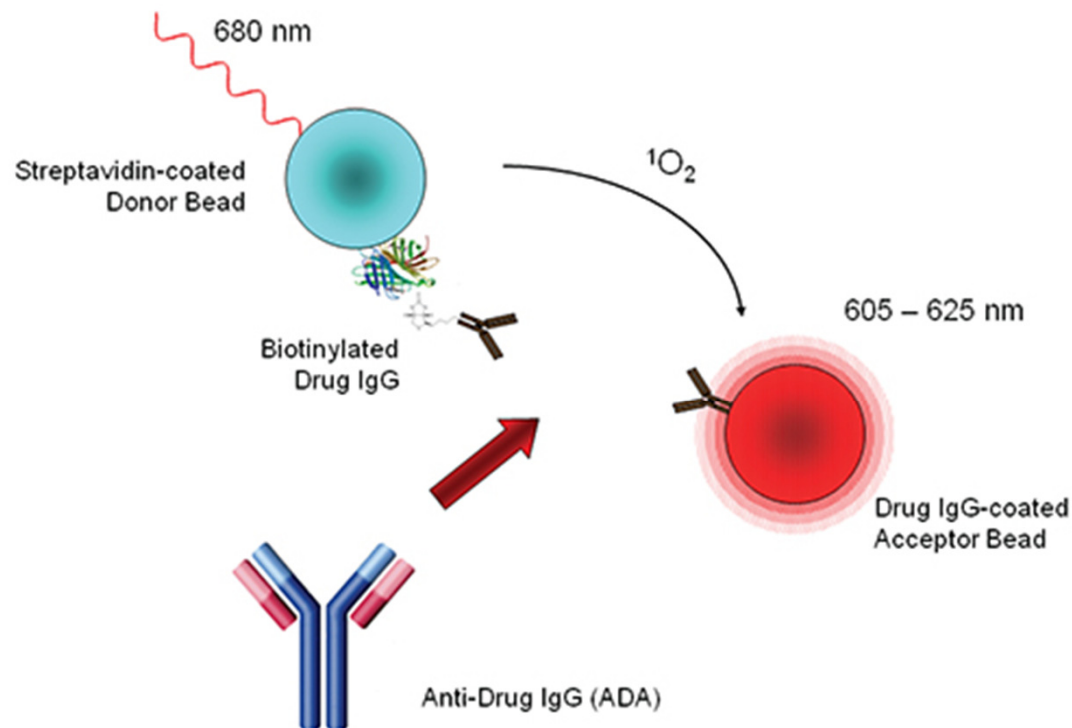


Laser excitation of the AF
Emission measurement –
fluorescent profile



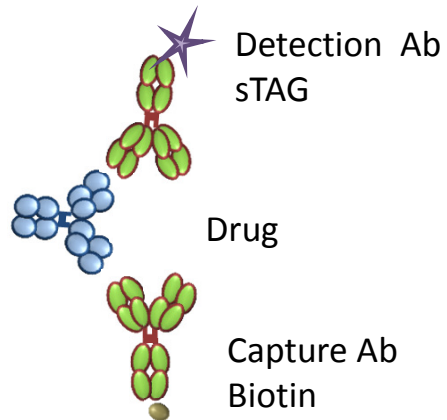
AlphaLISA[®]

- No-wash immunoassay system
- Bead-based assay with fluorescent detection

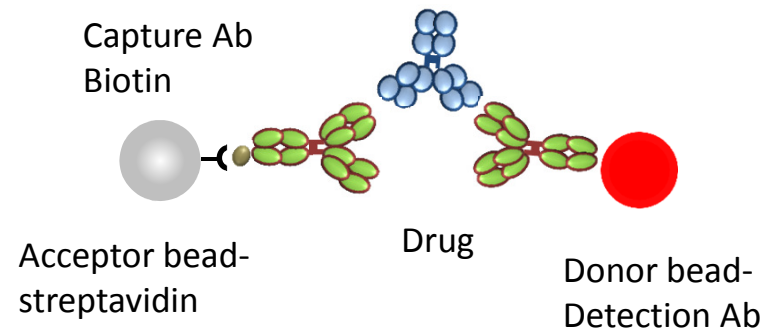


Case Study 1: PK Platform Comparison

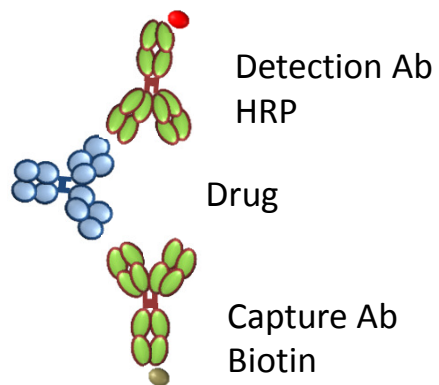
MSD® Homogeneous and Sequential



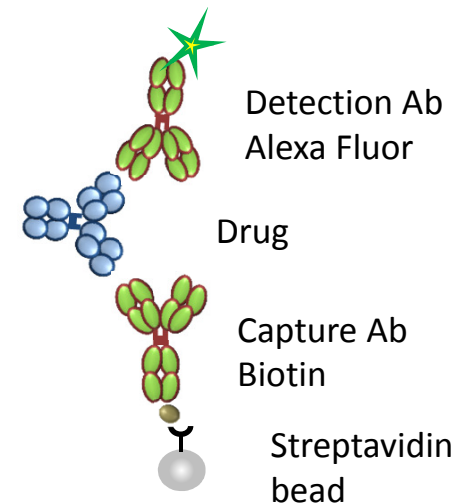
AlphaLISA® Homogeneous



ELISA Homogeneous and Sequential



Gyrolab™ Homogeneous and Sequential



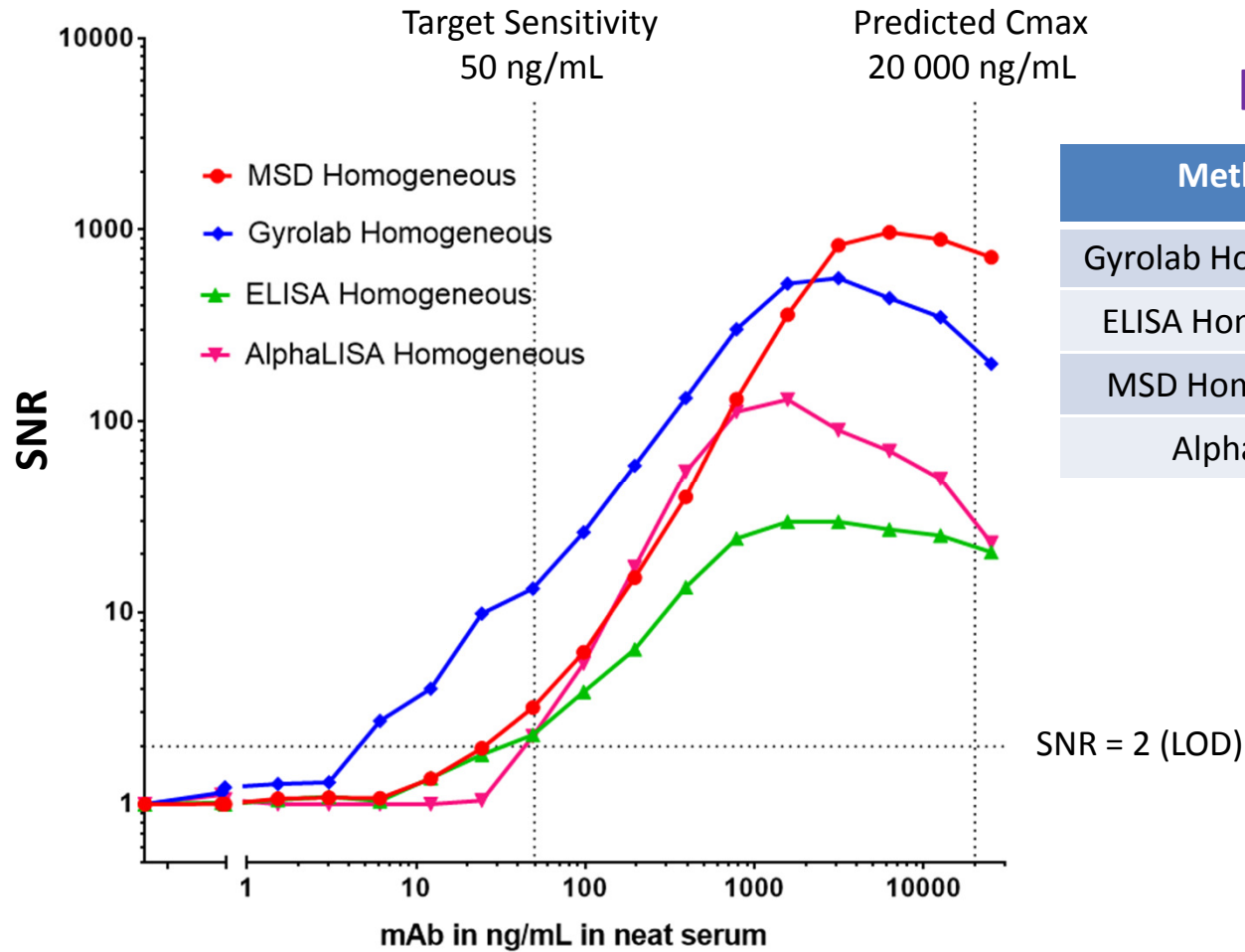
PK Platform Comparison

- 7 assay formats
- 3 runs per assay format

- Intra- and inter-assay precision and accuracy
- Sensitivity & dynamic range
- Hook effect
- Selectivity (inter-donor variability)

PK Platform Comparison

Sensitivity & Dynamic Range: Homogeneous Assay

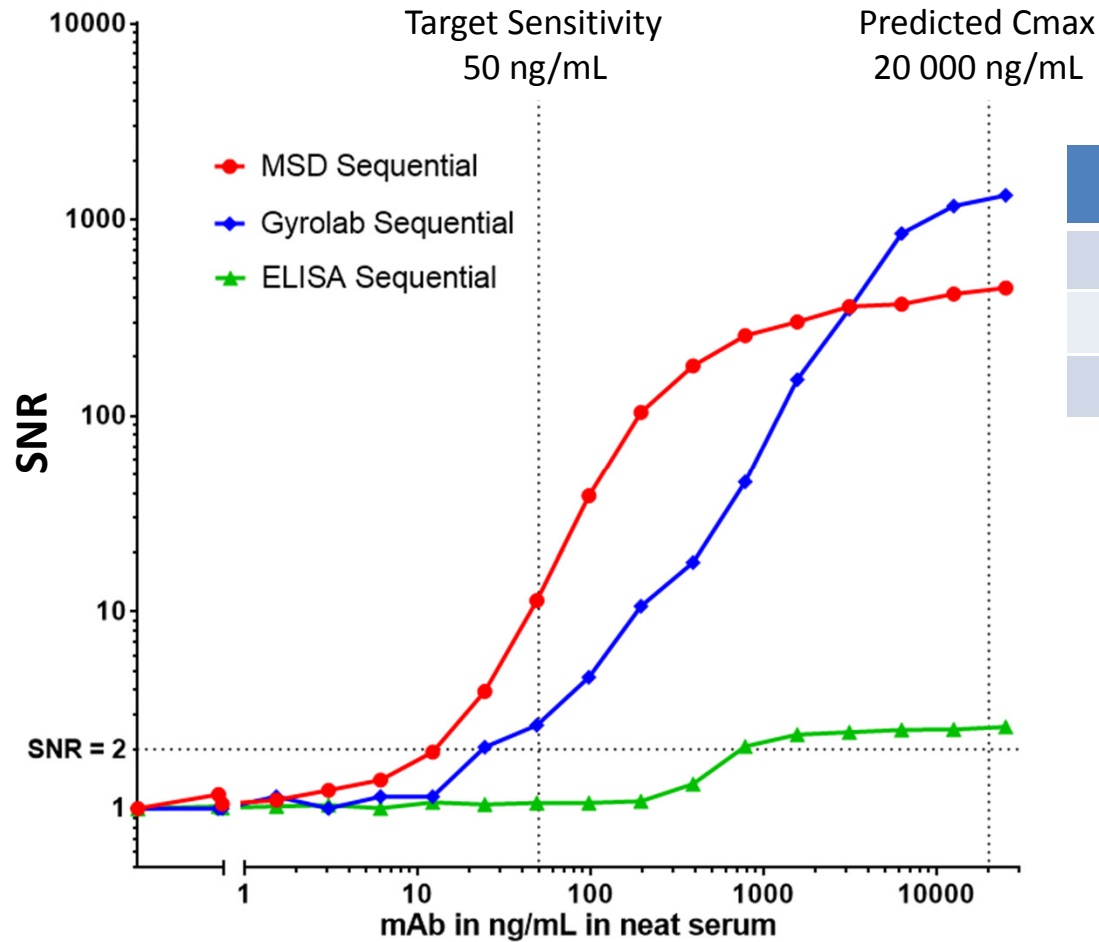


Hook Effect

Methods	Hook effect
Gyrolab Homogenous	Yes – 3125 ng/mL
ELISA Homogenous	Yes – 3125 ng/mL
MSD Homogenous	Yes – 6250 ng/mL
AlphaLISA	Yes – 1562 ng/mL

PK Platform Comparison

Sensitivity & Dynamic Range: Sequential Assay



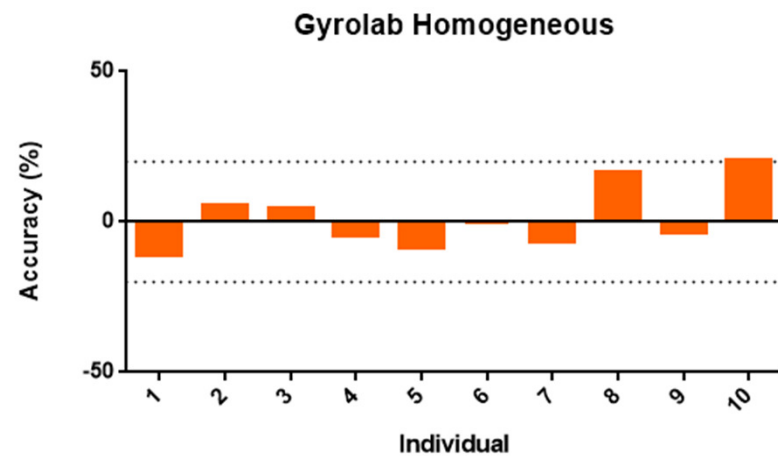
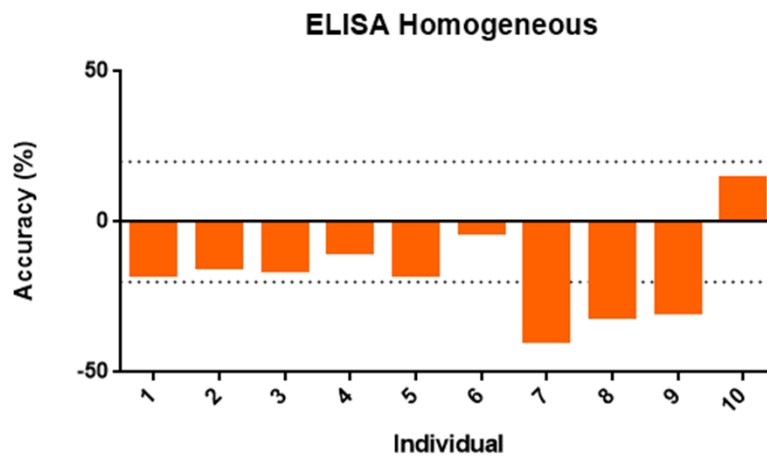
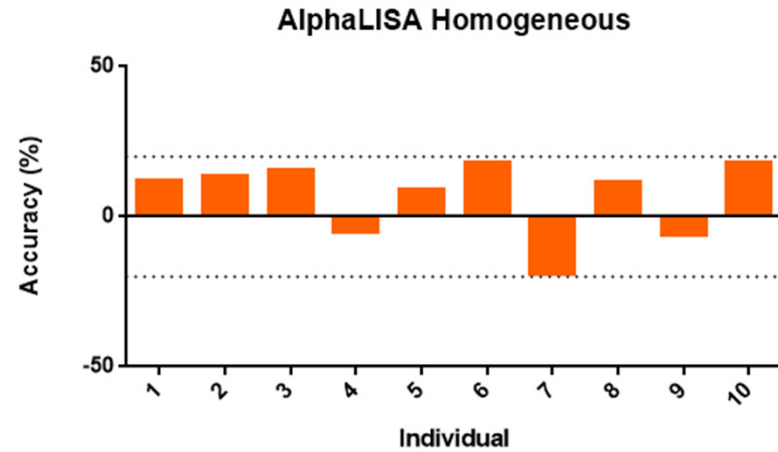
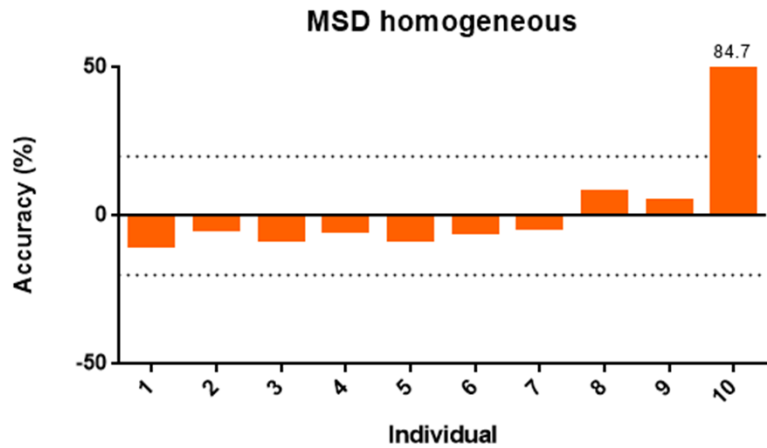
Hook Effect

Methods	Hook effect
Gyrolab Sequential	No
ELISA Sequential	No
MSD Sequential	No

High background observed with ELISA sequential format

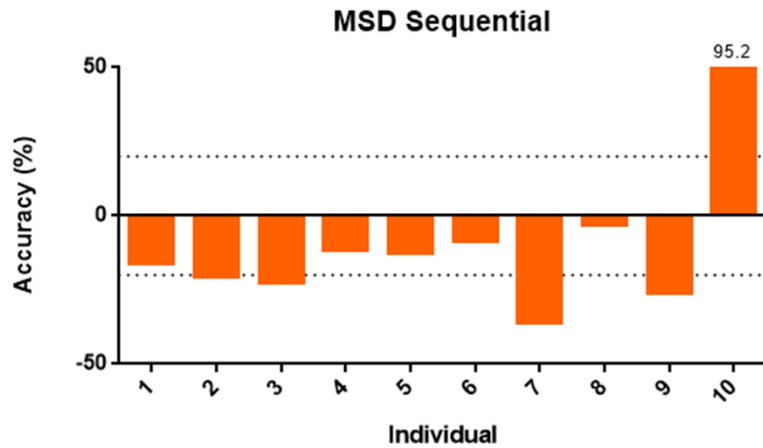
PK Platform Comparison

Selectivity: Homogeneous Assay Formats

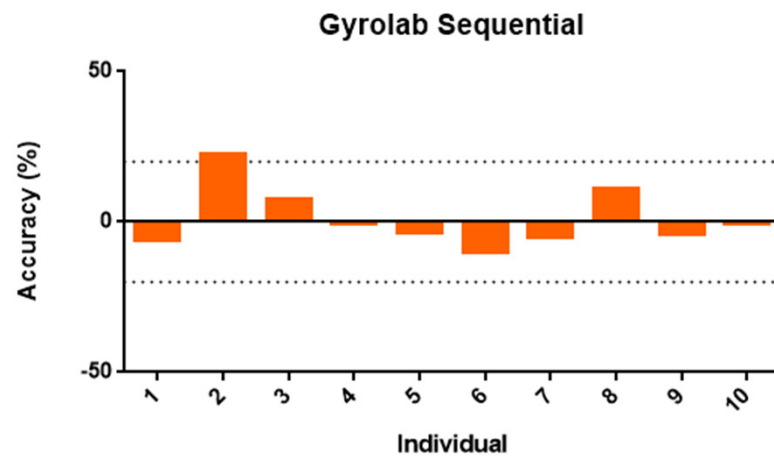


PK Platform Comparison

Selectivity: Sequential Assay Formats



ELISA Sequential
Not determined due to high background



PK Platform Comparison: Summary

	Sensitivity	Dynamic Range	Selectivity	Hook Effect
MSD Homogenous	++	++	+++	Yes
MSD Sequential	+++	+++	+	No
ELISA Homogenous	++	++	+	Yes
ELISA Sequential	-	-	ND	No
Gyrolab Homogenous	+++	+++	+++	Yes
Gyrolab Sequential	+	+++	+++	No
AlphaLISA	+++	++	++	Yes

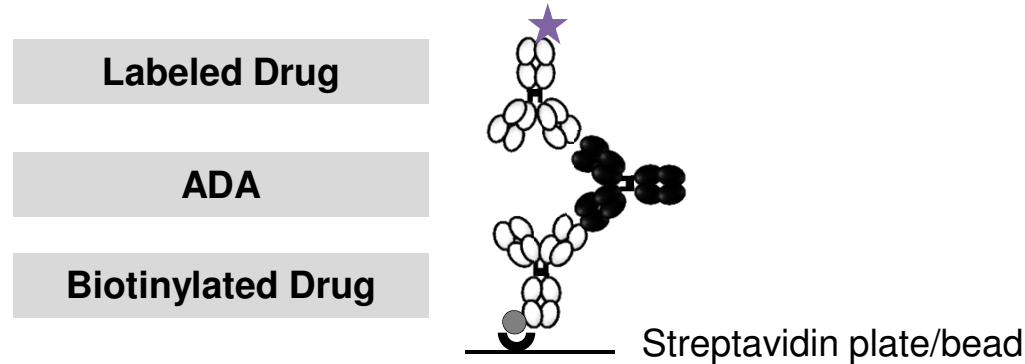
ND = Not determined

PK Platform Comparison: Conclusions

- Hook effect seen with all homogeneous assay formats
- Choice of **technology platform** and **assay format** can influence:
 - dynamic range, sensitivity and selectivity
- In this case study:
 - the **Gyrolab™** and the **MSD®** platforms in the **sequential** assay format met our PK assay development goals

Case Study 2: ADA Platform Comparison

1 ASSAY (bridging)



4 TECHNOLOGIES

ELISA

MSD[®]

Gyrolab[™]

AlphaLISA[®]

2 FORMATS

- Homogeneous bridging
 - capture, detection and sample are mixed together
- Acid Dissociation
 - samples are acidified
 - neutralized with a buffer containing the capture and detection antibodies

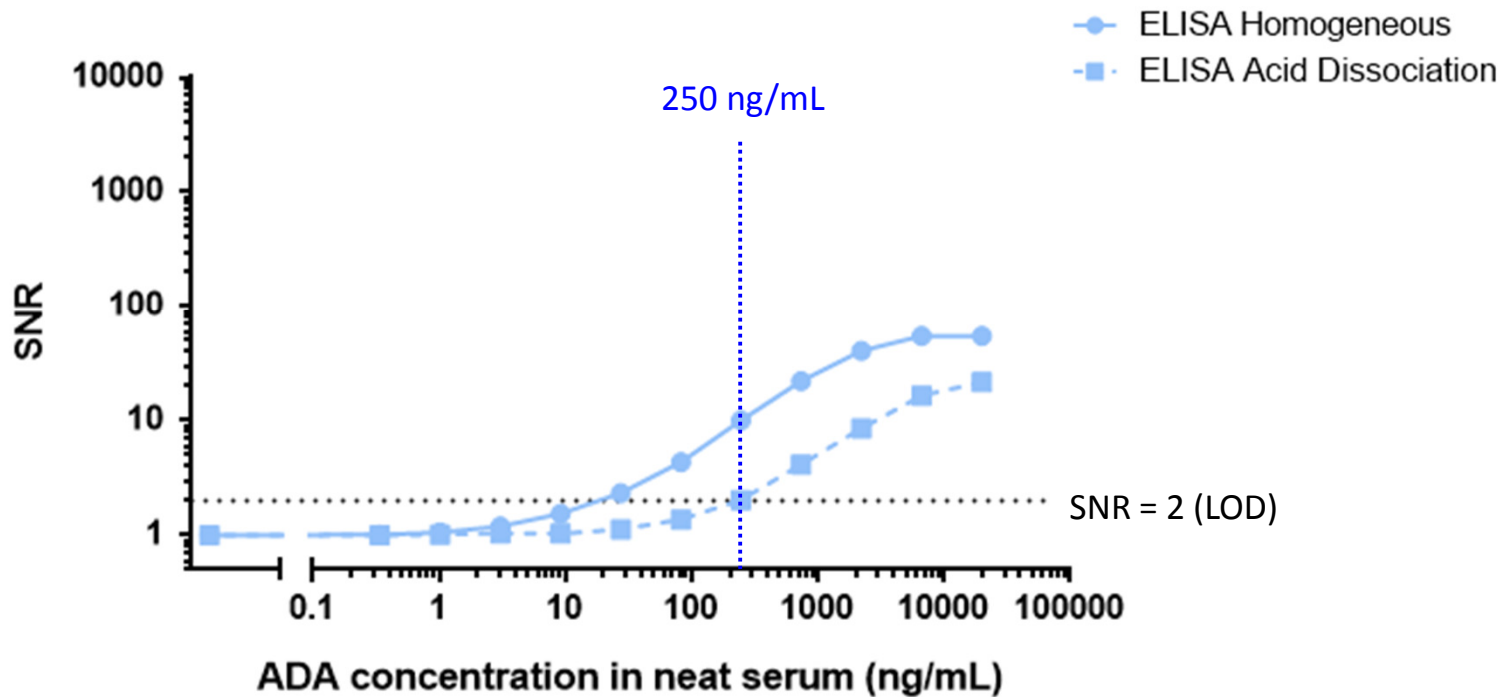
ADA Platform Comparison

- 8 assay formats
- 3 runs per assay format

- Intra- and inter-assay precision
- Sensitivity
- Target interference
- Drug interference

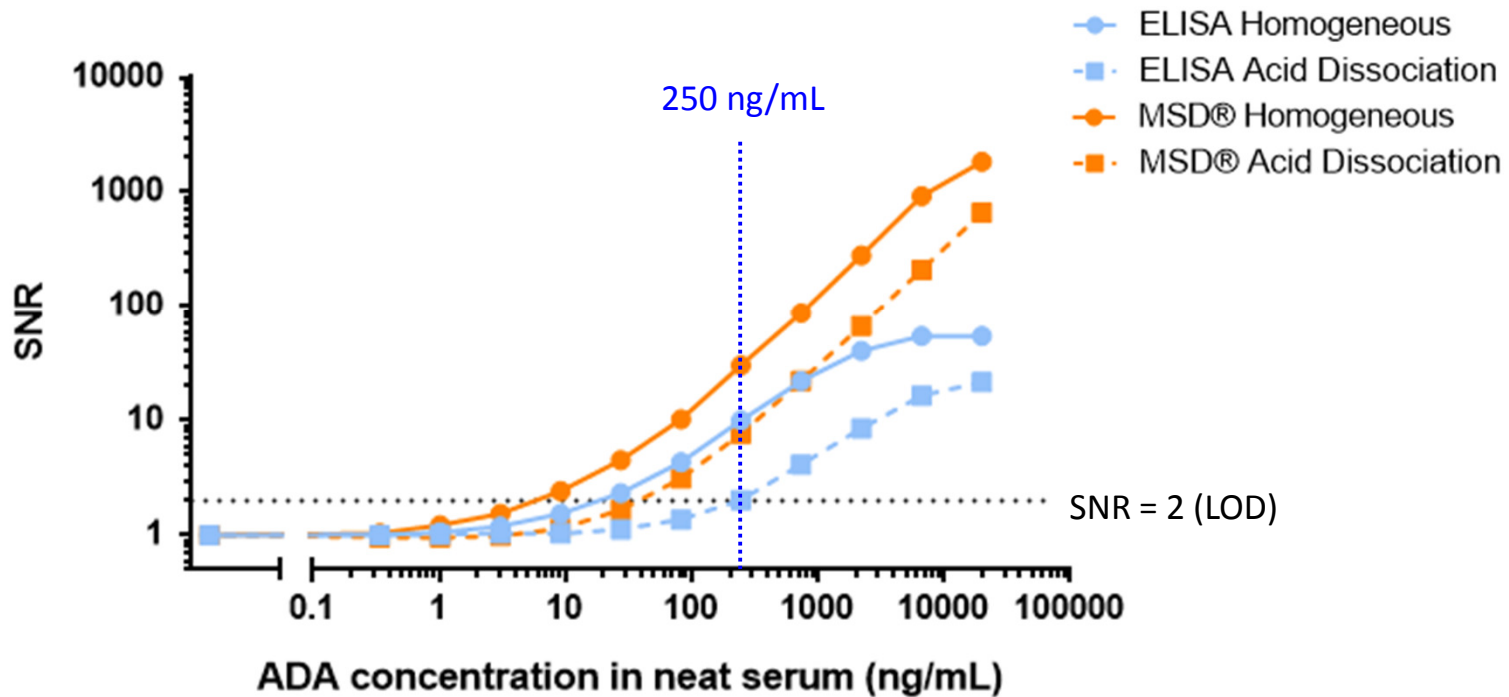
ADA Platform Comparison

Sensitivity



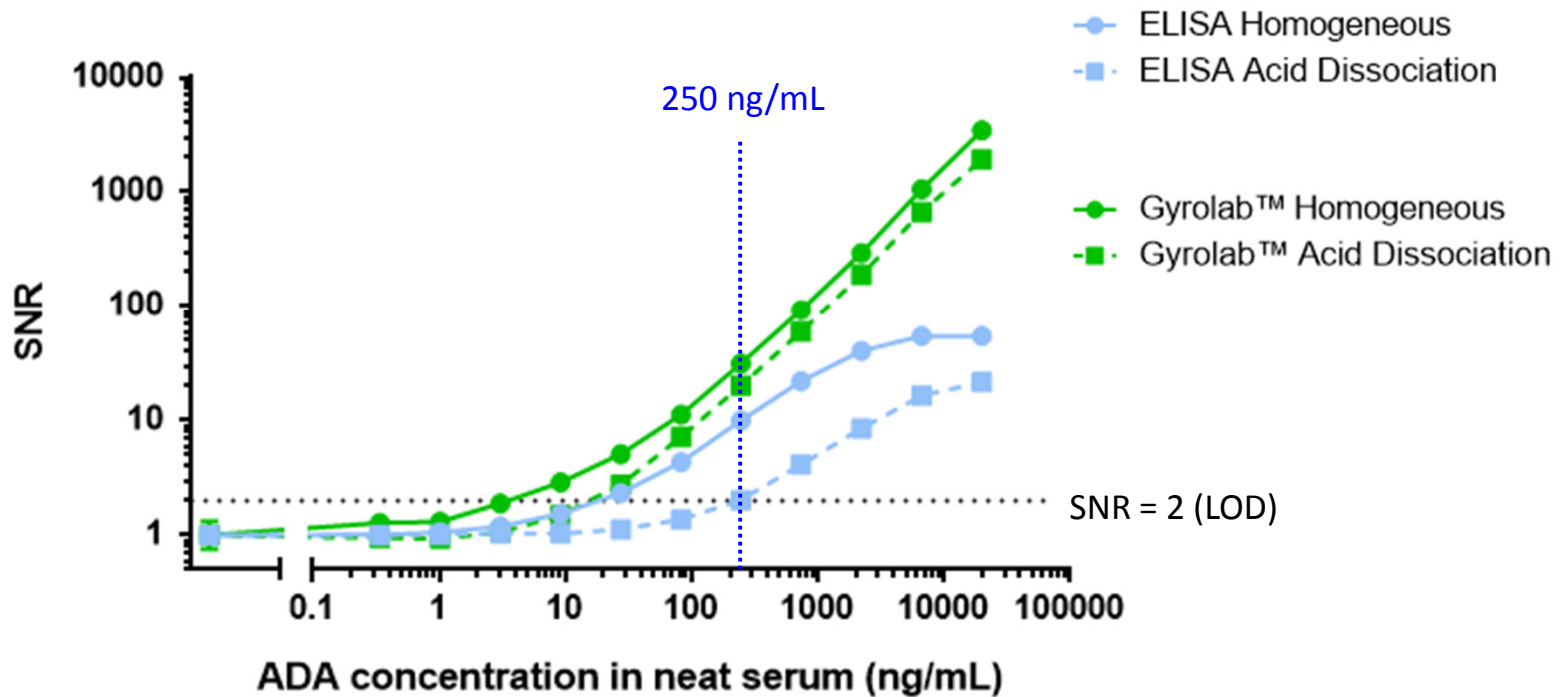
ADA Platform Comparison

Sensitivity



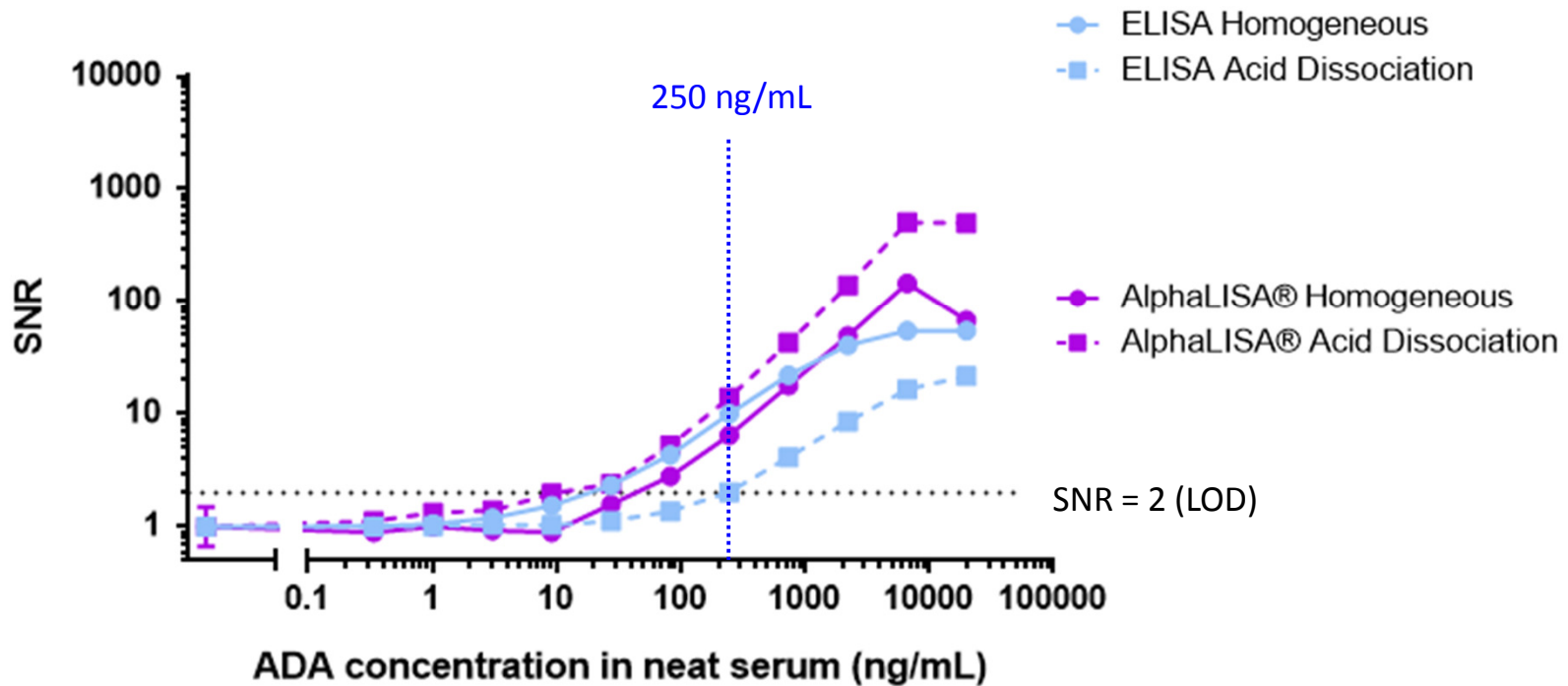
ADA Platform Comparison

Sensitivity



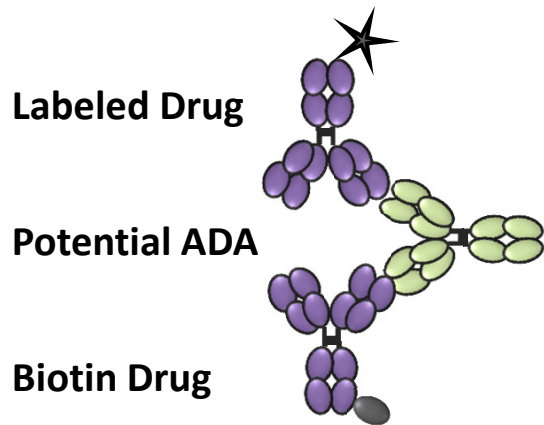
ADA Platform Comparison

Sensitivity

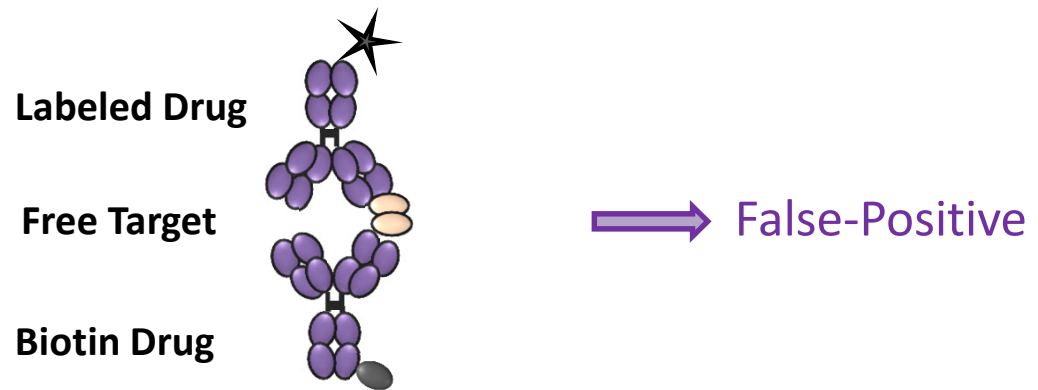


Concept of Target Interference

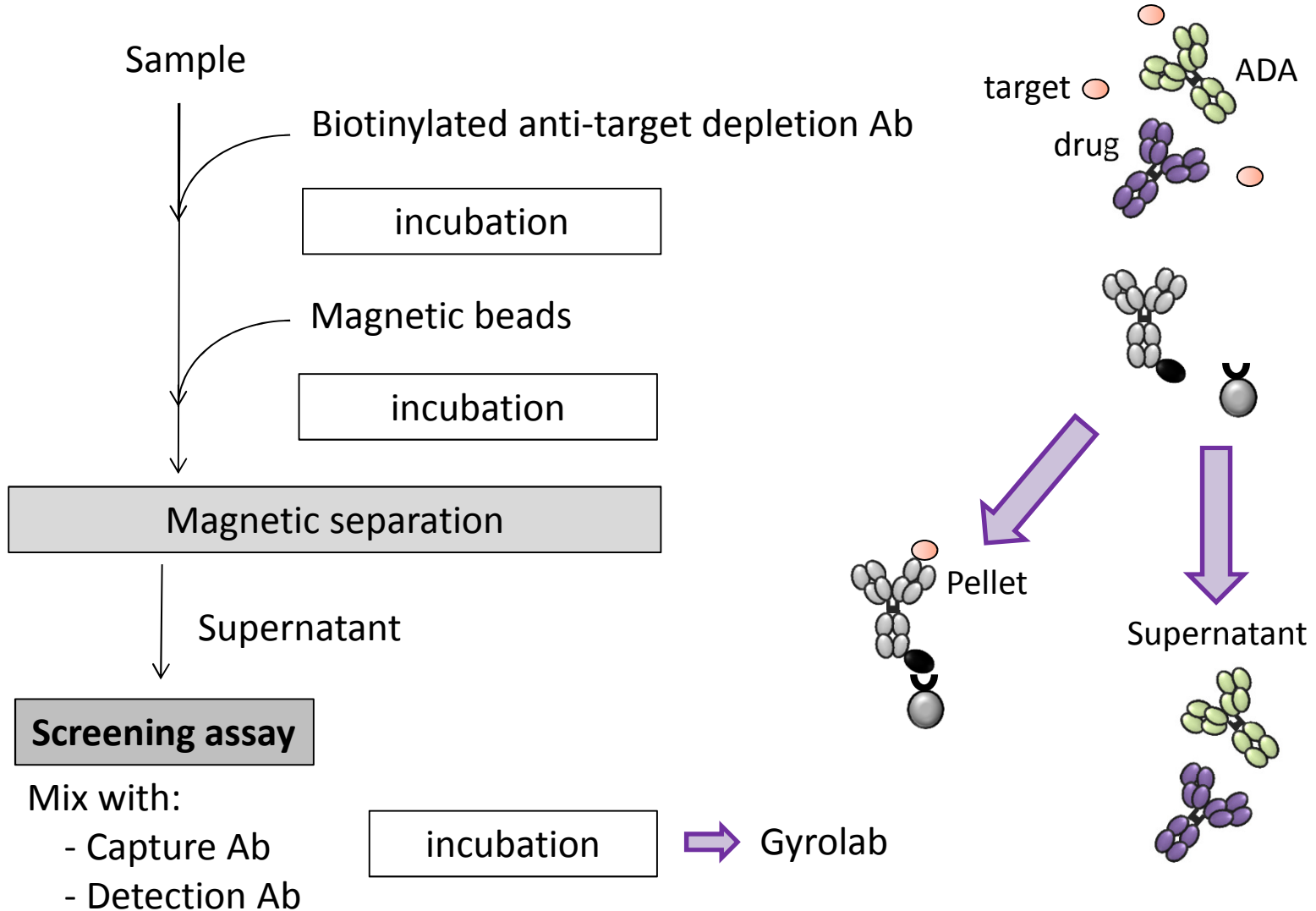
Homogeneous ADA Assay



Target interference

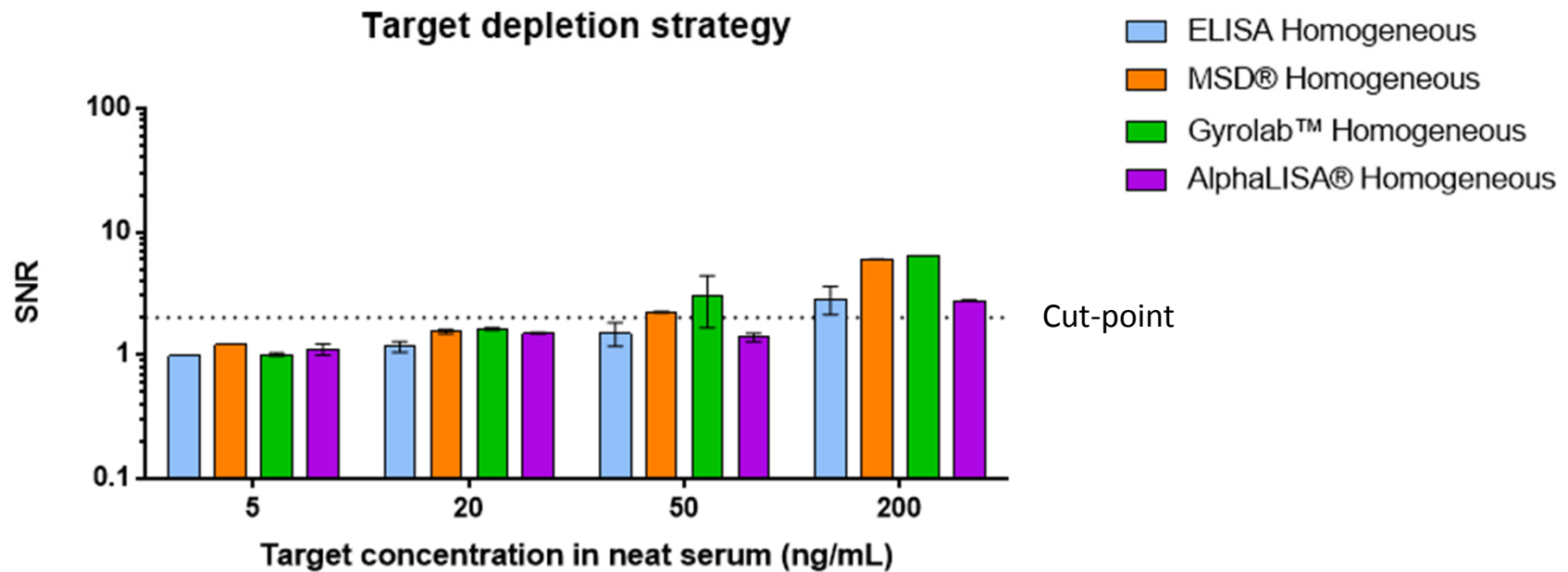


Target Interference: Depletion Strategy



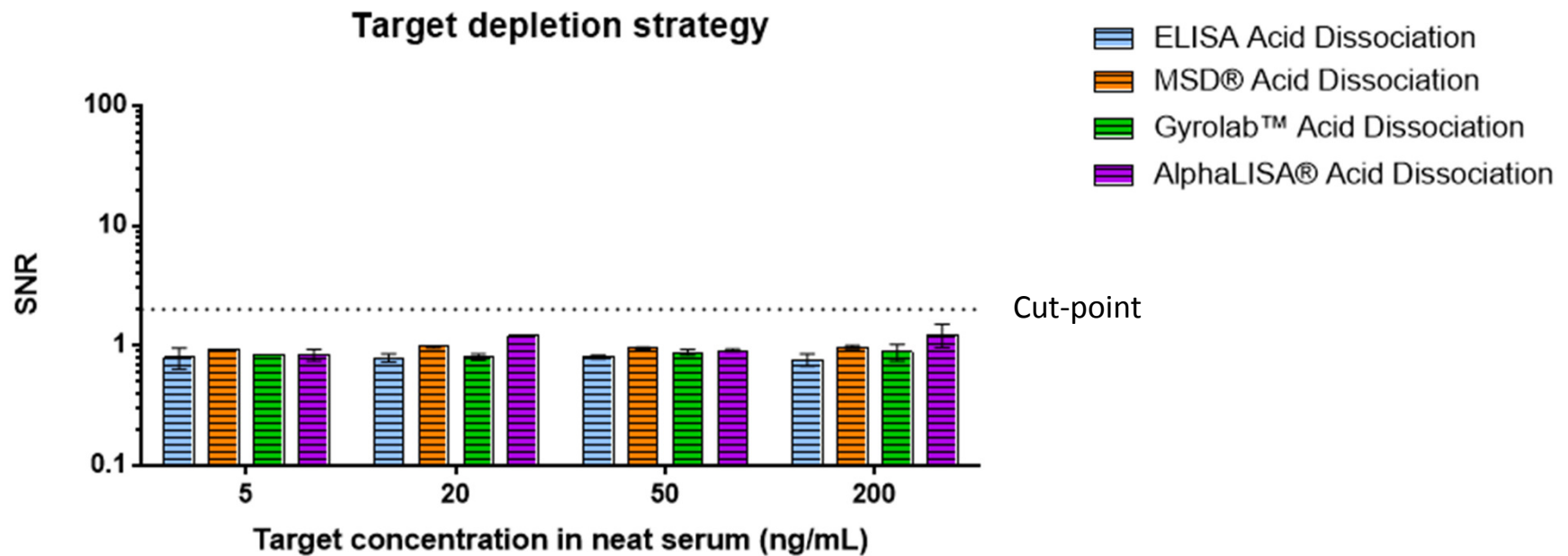
ADA Platform Comparison

Target Interference



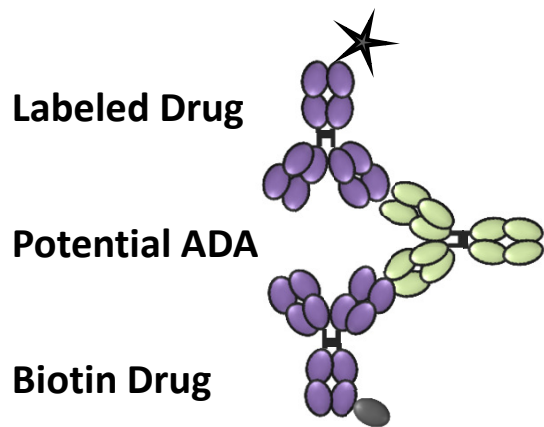
ADA Platform Comparison

Target Interference

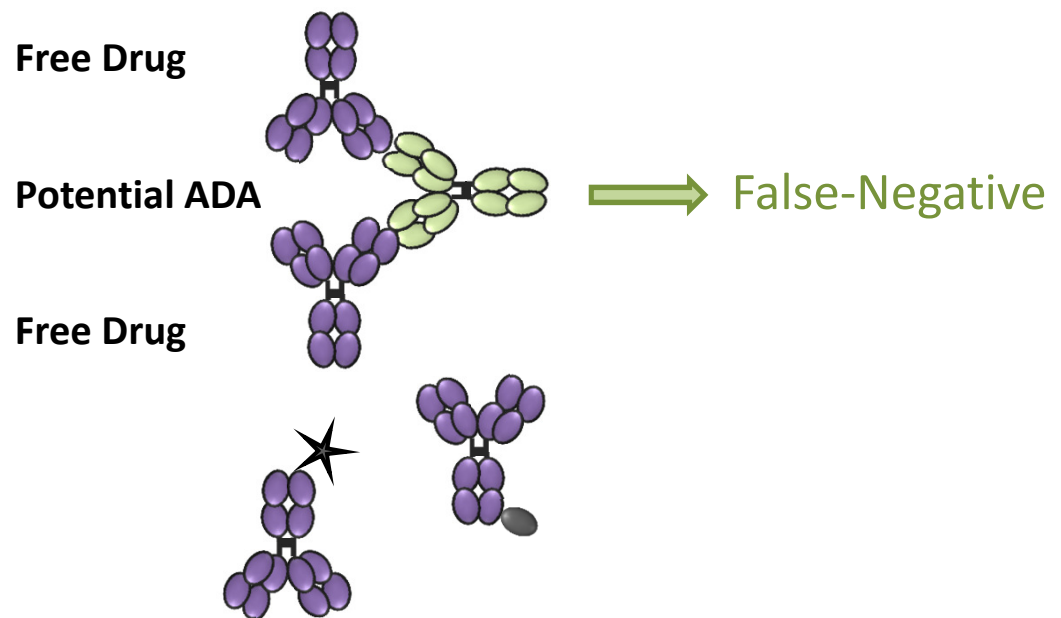


Concept of Drug Interference

Homogeneous ADA Assay

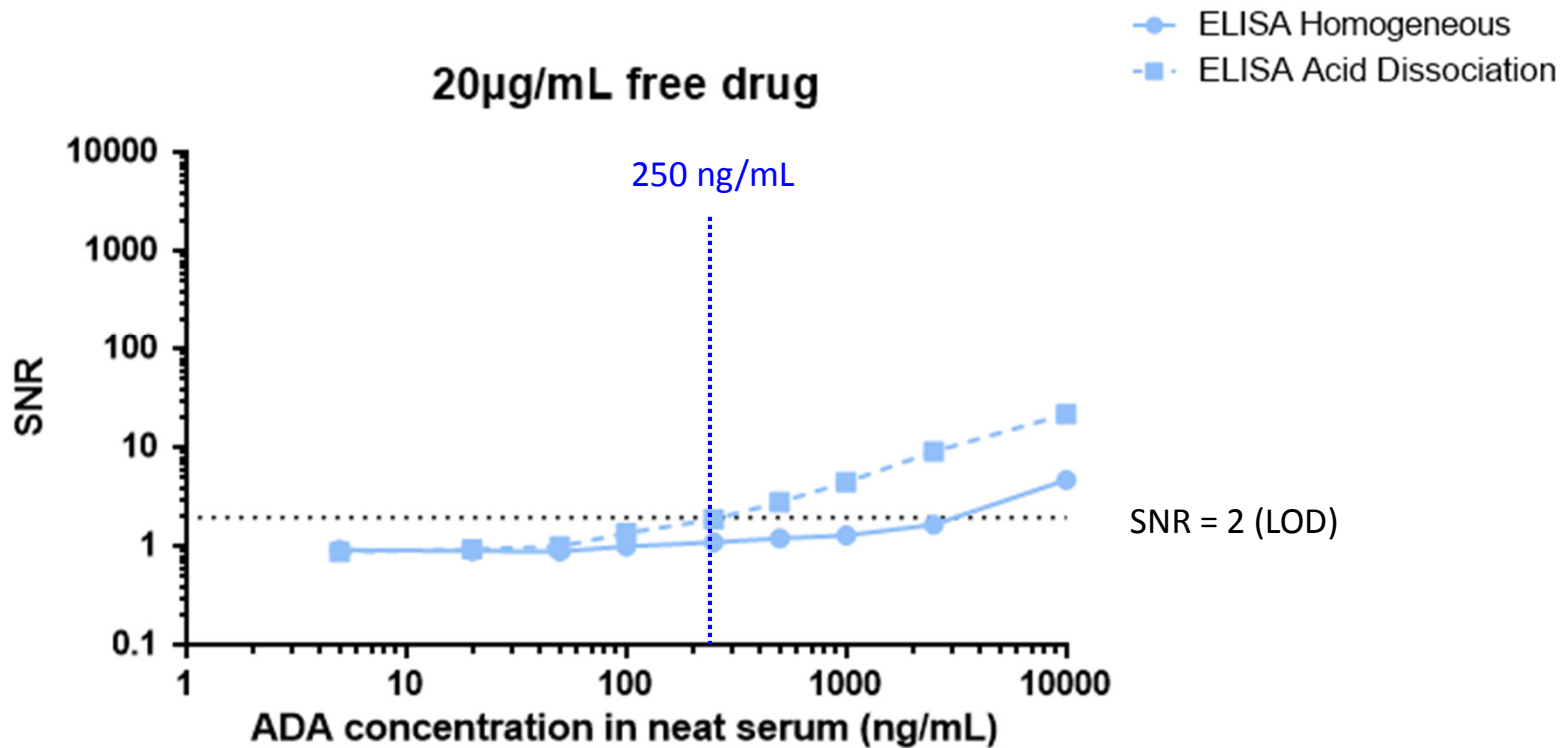


Drug interference



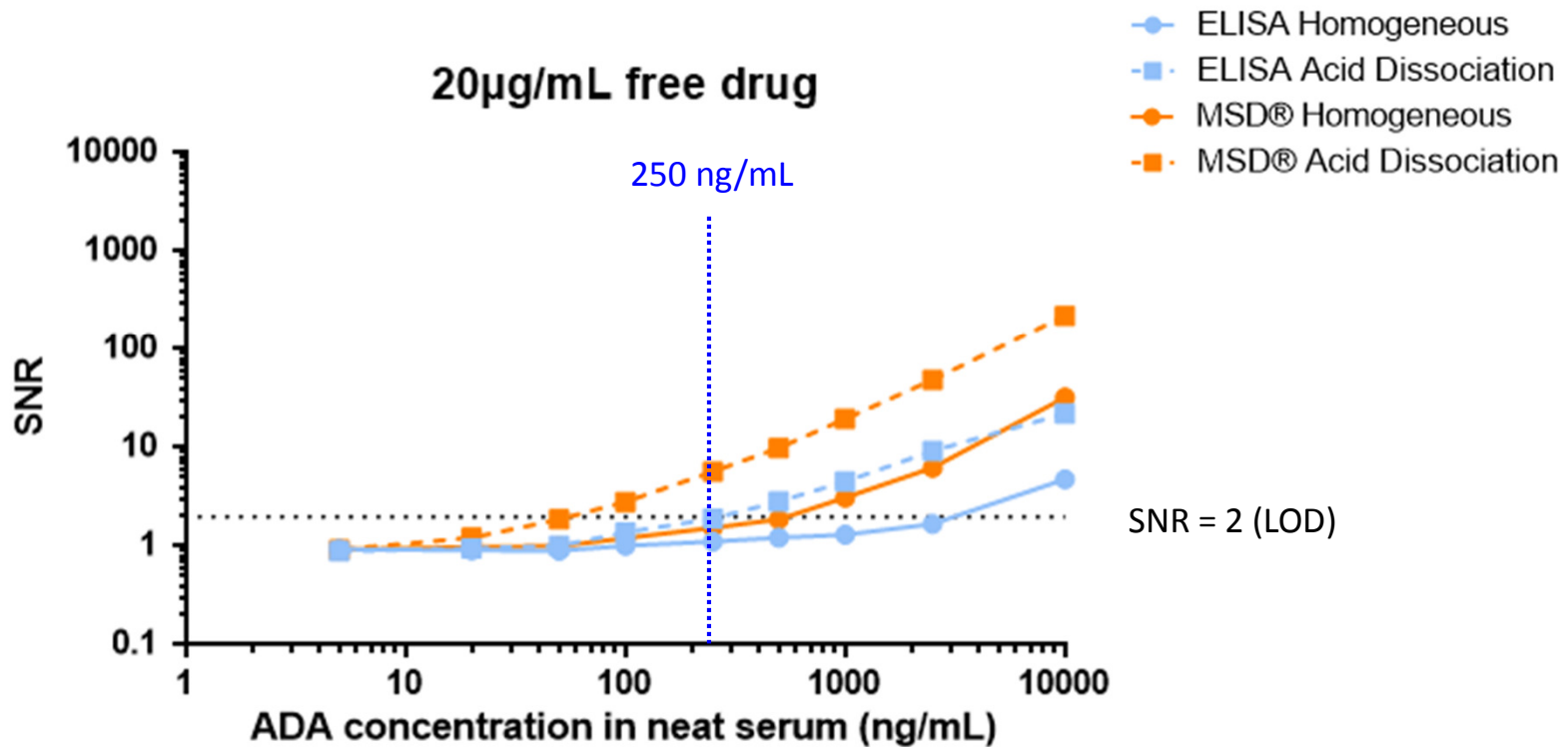
ADA Platform Comparison

Drug interference



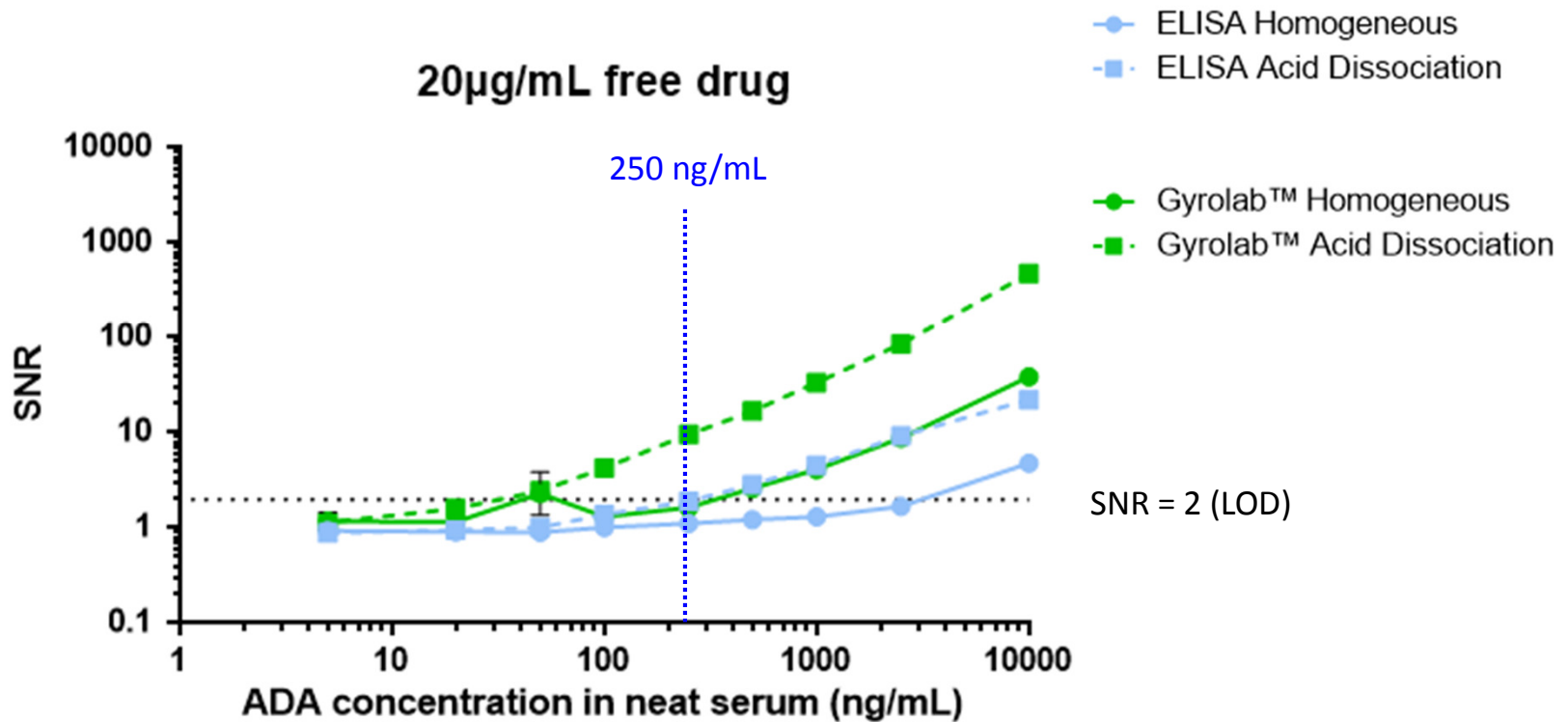
ADA Platform Comparison

Drug interference



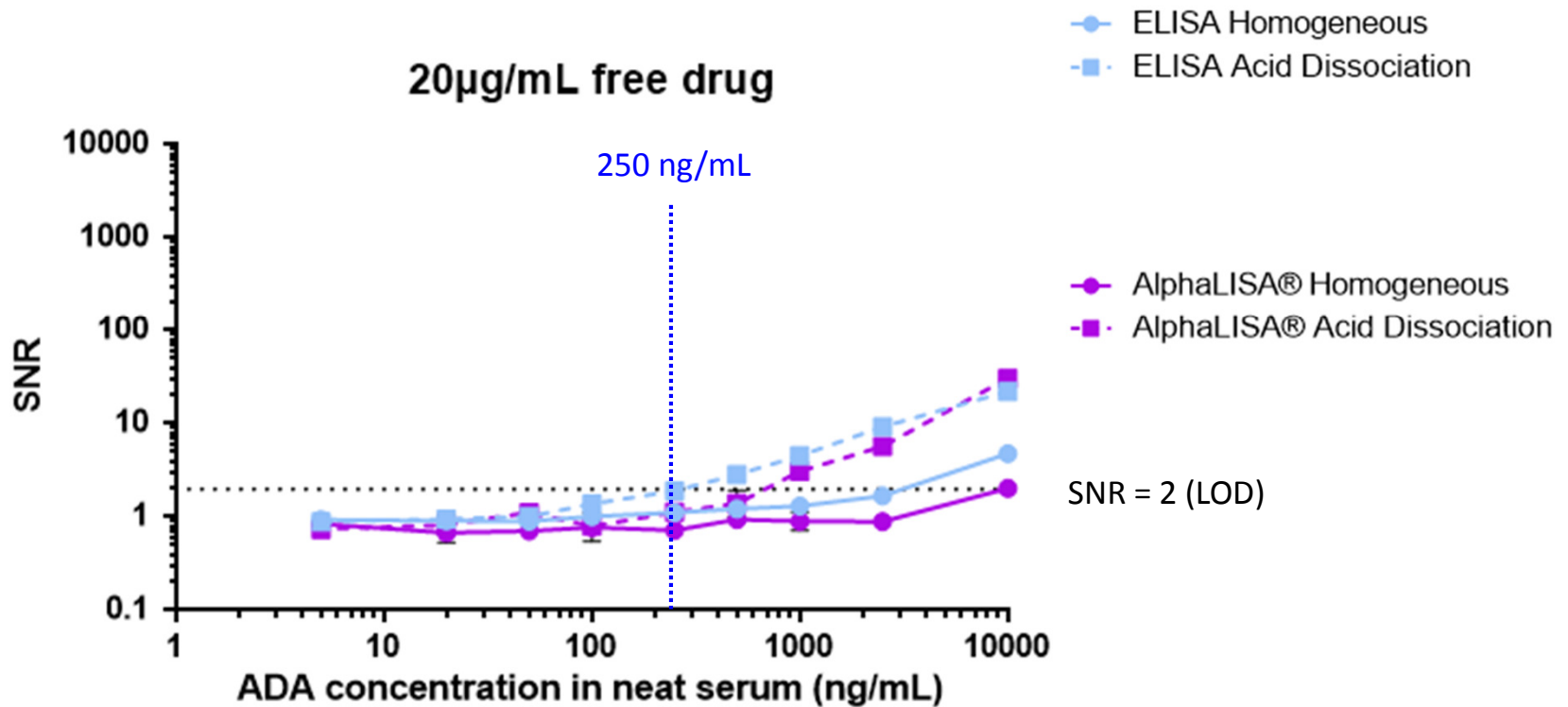
ADA Platform Comparison

Drug interference



ADA Platform Comparison

Drug interference



ADA Platform Comparison: Summary

Format	Sensitivity	Target interference	Drug interference
ELISA Homogeneous	+	+	+
ELISA Acid dissoc.	-	+++	+++
MSD® Homogeneous	+++	+	+
MSD® Acid dissoc.	+	+++	++
Gyrolab™ Homogeneous	+++	+	+
Gyrolab™ Acid dissoc.	++	+++	+++
AlphaLISA® Homogeneous	+	++	-
AlphaLISA® Acid dissoc.	++	+++	+

ADA Platform Comparison: Conclusions

- Choice of **technology platform** and **assay format** can influence:
 - sensitivity, target interference and drug tolerance
- In this case study:
 - the **Gyrolab™** and the **MSD®** platforms with the **acid dissociation** protocol met our ADA assay development goals
- Sensitivity and drug interference influenced by the **positive control** selection
 - may not truly reflect the heterogeneity of the immune system response in patients

Final Conclusions

- With our assays:
 - most often achieve our assay development goals on the **Gyrolab™** and the **MSD®** platforms
 - reagents that give good results on one platform will generally also be good on another technology
 - **one technology** does not always give the best results
- Sample volume, assay throughput and cost are important considerations
- To develop the most suitable large molecule bioanalytical assay, a **range of technology platforms** should be assessed

Acknowledgments

- Bioanalytical Assay Laboratory, present and past :
 - Joris Venet
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 - Sabrina Lory
- Maureen Deehan
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Thank you for your attention!

