Generation of anti-drug antibody (ADA) positive control and development of a bridging immunogenicity assay for RNA therapeutics in human serum

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16th EIP conference in Lisbon, February 24-26, 2025

AGENDA

Introduction

ADA positive control generation for ONTs

ADA assay development and validation for ONTs

Summary

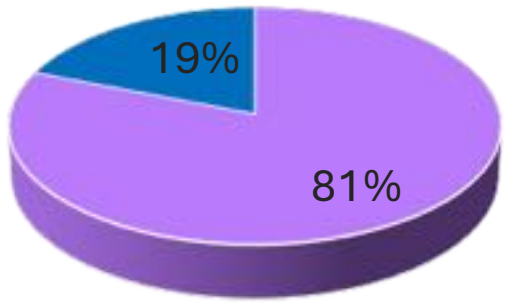
Introduction

RNA oligonucleotides

- Modified nucleic acids, typically designed to bind to a complimentary RNA sequence
- Increase expression of needed proteins
- Silence expression of pathogenic proteins
- Able to be quickly synthesized
- Various backbone chemistries:
    - Phosphorothioate,
    - Phosphorodiamidate morpholino,
    - 2’ Fluro, 2’ MoE

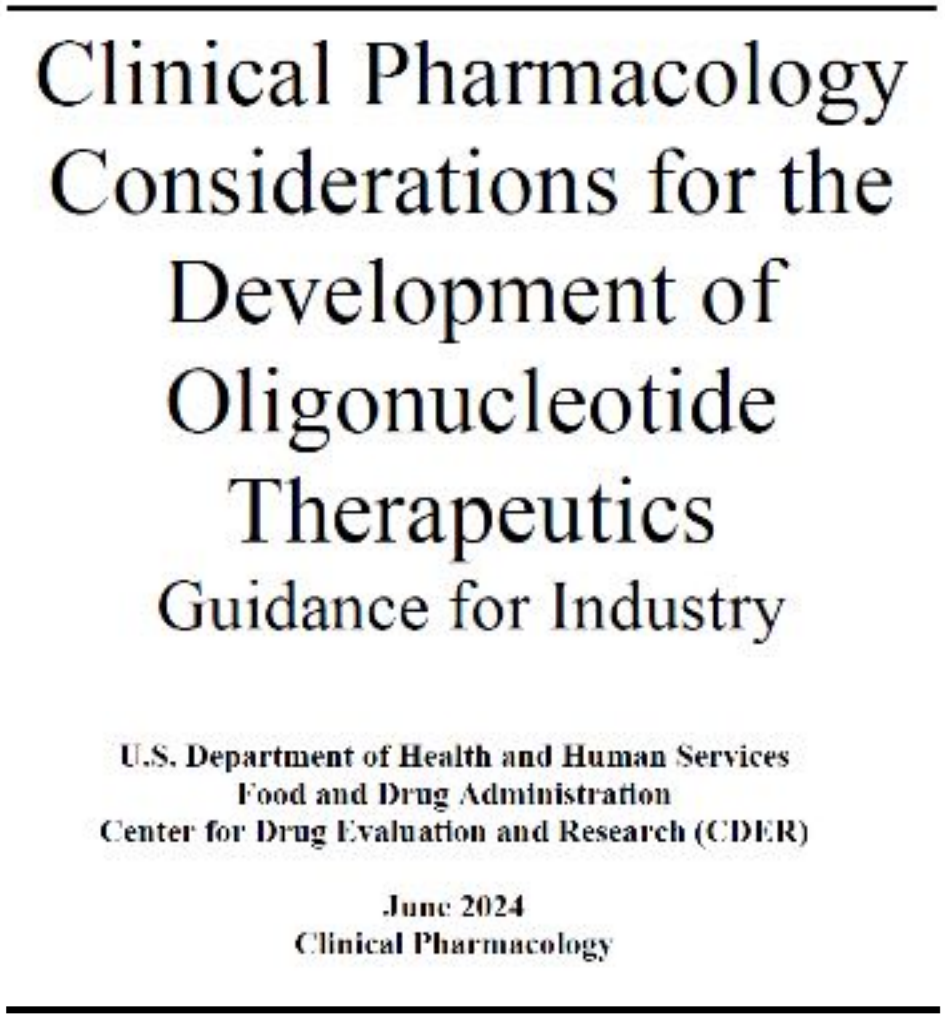
FDA Approved Oligonucleotide Therapeutics (ONTs)

Drug	Mol.Target, Tissue	Class	Sponsor (Year Approved in US)	Indications
VITRAVENE® (fomiversen)*	CMV IE-2, Eye	ASO	Ionis Pharmaceuticals, Inc. (1998)	Cytomegalovirus Retinitis
MACUGEN® (pegaptanib)*	VEGF 165, Eye	Aptamer	Gilead Sciences, Inc. (2004)	Age-Related Macular Degeneration
KYNAMRO® (mipomersen)	ApoB-100, Liver	ASO	Ionis Pharmaceuticals, Inc. (2013)	Homozygous Familial Hypercholesterolemia
SPINRAZA® (nusinersen)	SMN2, spinal cord	SSO	Biogen, Inc. (2016)	Spinal Muscular Atrophy
EXONDYS 51® (eteplirsen)	Dystrophin 51, Muscle	SSO	Sarepta Therapeutics, Inc. (2016)	Duchenne Muscular Dystrophy
DEFITELIO® (defibrotide)	Liver	ss + ds DNA	Jazz Pharmaceuticals, Inc. (2016)	Hepatic Veno-Occlusive Disease
HEPLISAV-B (HepB-CpG)	Hepatitis B	DNA	Dynavax Technologies Corporation (2017)	Hepatitis B
TEGSEDI® (inotersen)	TTR, Liver	ASO	Ionis Pharmaceuticals, Inc. (2018)	Hereditary Transthyretin Amyloidosis, Polyneuropathy
ONPATTRO® (patisiran)	TTR, Liver	siRNA	Alnylam Pharmaceuticals, Inc. (2018)	Hereditary Transthyretin Amyloidosis
WAYLIVRA (Volanesorsen)	Apolipoprot CIII, Liver	ASO	Ionis Pharmaceuticals, Inc (EU, 2019)	Familial chylomicronemia syndrome
VYONDYS 53 (golodirsen)	Dystrophin 53, Muscle	SSO	Sarepta Therapeutics (2019)	Duchenne Muscular Dystrophy
GIVLAARI® (givosiran)	ALAS1, Liver	siRNA	Alnylam Pharmaceuticals, Inc. (2019)	Acute Hepatic Porphyrias
VILTEPSO® (vitolarsen)	Dystrophin 53, Muscle	SSO PMO	Nippon Shinyaku Co., Ltd. (2020)	Duchenne Muscular Dystrophy
OXLUMO® (lumasiran)	HAO1, Liver	siRNA	Alnylam Pharmaceuticals, Inc. (2020)	Primary Hyperoxaluria Type 1 (PH1)
AMONDYS 45® (casimersen)	Dystrophin 45, Muscle	SSO PMO	Sarepta Therapeutics, Inc. (2021)	Duchenne Muscular Dystrophy
LEQVIO® (inclisiran)	PCSK9, Liver	siRNA	Novartis Pharmaceuticals Corporation (2021)	Hypercholesterolemia
AMVUTTRA® (vutrisiran)	TTR, Liver	siRNA	Alnylam Pharmaceuticals, Inc. (2022)	TTR, liver
IZERVEY™ (avacincaptad pegol)	Complement protein C5, Eye	RNA Aptamer	Iveric bio Inc. (2023)	Geographic atrophy (GA) secondary to age-related macular degeneration (AMD)
RIVFLOZA™ (nedosiran)	degradation of LDHA mRNA, Liver	siRNA	Novo Nordisk (2023)	Primary Hyperoxaluria (PH)
QALSODY® (tofersen)	SOD1 mRNA, Muscle	ASO	Biogen, Inc. (2023)	Amyotrophic lateral sclerosis (ALS)
WAINUA™ (eplontersen)	TTR , Neuron	ASO	Ionis Pharmaceuticals, Inc. (2023)	Hereditary Transthyretin Amyloidosis
RYTELO™ (Imetelstat)	Telomerase inhibitor	ASO	Geron Corporation (2024)	Myelodysplastic syndromes (MDS)



* No longer marketed

Regulatory guidance on Immunogenicity for ONTs

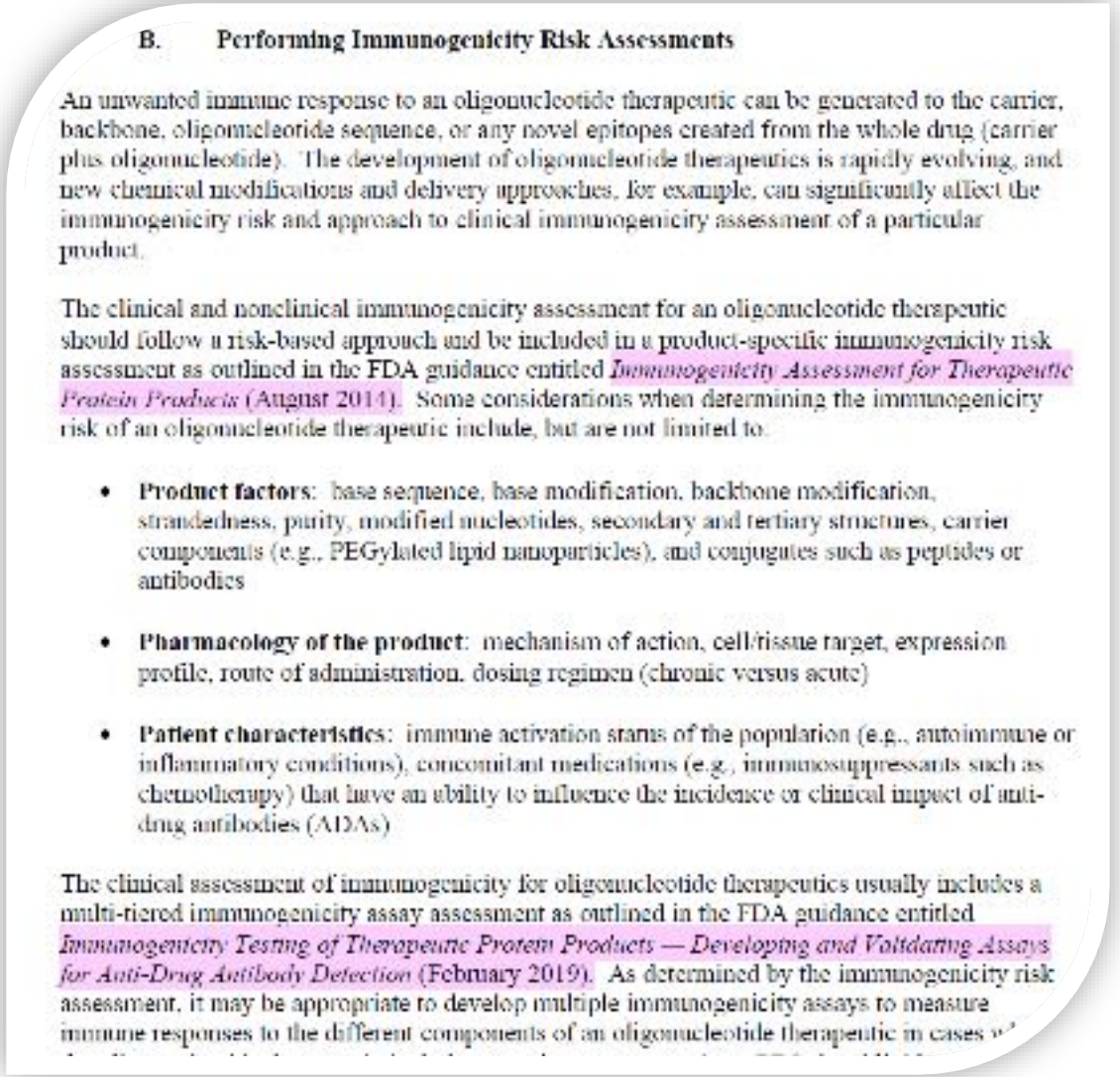


Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

June 2024
Clinical Pharmacology



B. Performing Immunogenicity Risk Assessments

An unwanted immune response to an oligonucleotide therapeutic can be generated to the carrier, backbone, oligonucleotide sequence, or any novel epitopes created from the whole drug (carrier plus oligonucleotide). The development of oligonucleotide therapeutics is rapidly evolving, and new chemical modifications and delivery approaches, for example, can significantly affect the immunogenicity risk and approach to clinical immunogenicity assessment of a particular product.

The clinical and nonclinical immunogenicity assessment for an oligonucleotide therapeutic should follow a risk-based approach and be included in a product-specific immunogenicity risk assessment as outlined in the FDA guidance entitled *Immunogenicity Assessment for Therapeutic Protein Products* (August 2014). Some considerations when determining the immunogenicity risk of an oligonucleotide therapeutic include, but are not limited to:

- **Product factors**: base sequence, base modification, backbone modification, strandedness, purity, modified nucleotides, secondary and tertiary structures, carrier components (e.g., PEGylated lipid nanoparticles), and conjugates such as peptides or antibodies
- **Pharmacology of the product**: mechanism of action, cell/tissue target, expression profile, route of administration, dosing regimen (chronic versus acute)
- **Patient characteristics**: immune activation status of the population (e.g., autoimmune or inflammatory conditions), concomitant medications (e.g., immunosuppressants such as chemotherapy) that have an ability to influence the incidence or clinical impact of anti-drug antibodies (ADAs)

The clinical assessment of immunogenicity for oligonucleotide therapeutics usually includes a multi-tiered immunogenicity assay assessment as outlined in the FDA guidance entitled *Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection* (February 2019). As determined by the immunogenicity risk assessment, it may be appropriate to develop multiple immunogenicity assays to measure immune responses to the different components of an oligonucleotide therapeutic in cases w

White papers on Immunogenicity assessment for ONTs

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Assessment of the Immunogenicity Potential for Oligonucleotide-Based Drugs

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Therapeutic oligonucleotides (ONs) have characteristics of both small molecules and biologics. Although safety assessment of ONs largely follows guidelines established for small molecules, the unique characteristics of ONs often require incorporation of concepts from the safety assessment of biologics. The assessment of immunogenicity for ON therapeutics is one area where the approach is distinct from either established small molecule or biologic platforms. Information regarding immunogenicity of ONs is limited, but indicates that administration of ONs can result in antidrug antibody formation. In this study, we summarize the collective experience of the Oligonucleotide Safety Working Group in designing the immunogenicity assessment appropriate for this class of therapeutic, including advice on assay development, clinical monitoring, and evaluation of the impact of immunogenicity on exposure, efficacy, and safety of therapeutic ONs.

Keywords: antidrug antibodies, immunogenicity testing, oligonucleotides

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REVIEW ARTICLE

Considerations in the Immunogenicity Assessment Strategy for Oligonucleotide Therapeutics (ONTs)

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Abstract
Oligonucleotide therapeutics (ONTs) are a diverse group of short synthetic nucleic acid–based molecules that exploit innovative intracellular molecular strategies to create novel treatments for a variety of medical conditions. ONT molecules (~7–15 kDa) reside between traditional large and small molecules, and there has been debate regarding their immunogenicity risk. To date, 13 ON drugs have been approved, and as the field is relatively new, there are currently no specific regulatory guidelines to indicate how to develop, validate, and interpret the immunogenicity assays of ONTs. Some investigators do not test for immune responses to ONs while others test for antibodies (Abs) to components within the formulation, which may or may not include aspects of characterization such as domain mapping of ONT conjugates. Similar to other biopharmaceuticals, the immunogenic properties of ONTs could be influenced by sequence, route, dosage, target population, co-medications, etc. The current anti-drug antibody (ADA) data for different approved ONTs suggest that their administration poses a low immunogenicity risk without any significant impact on pharmacokinetics (PK), pharmacodynamics (PD), and safety; nevertheless, until the field matures with data from many more ON drugs, it remains prudent to assess immunogenicity. The emphasis of this article is to highlight how current ADA methodologies might be applied to the development of ONTs, discuss factors that may pose immunogenicity risks, and provide the authors' current position on immunogenicity assessment strategies for ONTs. We also discuss assay parameters that may be appropriate for the detection and characterization of ADAs, including the evaluation of neutralizing ADAs, ADA isotyping, Abs to dsDNA, and pre-existing ADA. Immunogenicity risk assessments (IRAs) and early interactions with regulators will inform how to proceed in late stage/pivotal studies.

Keywords Anti-drug antibody · Food and Drug Administration · Immunogenicity · Therapeutic oligonucleotides

Immunogenicity – Current Experience with Approved RNA Oligonucleotides

Drug	RoA	ADAs	Percentage*	ADA labelling language
KYNAMRO® (mipomersen) 2013	SC	Yes	**38%**	Flulike symptoms higher in antibody-positive patients. ADAwere associated with higher trough levels for the drug.
SPINRAZA® (nusinersen) 2016	IT	Yes, **PMR****	**6%**	There are insufficient data to evaluate an effect of ADAs on clinical response, adverse events, or the pharmacokinetic profile of nusinersen.
EXONDYS 51® (eteplirsen) 2016	IV	Yes , **PMR****	**<1%**	Pharmacokinetics, pharmacodynamics, safety, and/or efficacy of EXONDYS 51 is unknown.
TEGSEDI® (inotersen) 2018	SC	Yes	**30%**	the assay measured only IgG isotypes and the existence of other isotypes may be possible.
ONPATTRO® (patisiran) 2018	IV	yes	**3.6%**	No clinically significant differences in the safety, PK/PD
VYONDYS 53 (golodirsen) 2019	IV	Not measured, **PMR****	**---**	
GIVLAARI® (givosiran) 2019	SC	Yes	**1%**	No clinically significant differences in the safety, PK/PD
VILTEPSO® (vitolarsen) 2020	IV	Yes	**0%**	
OXLUMO® (lumasiran) 2020	SC	Yes	**6%**	No clinically significant differences in the safety, PK/PD
AMONDYS 45® (casimersen) 2021	IV	Not measured, **PMR****	**---**	
LEQVIO® (inclisiran) 2021	SC	Yes	**5%**	No effect of ADAs on clinical efficacy, safety, or PK
AMVUTTRA® (vutrisiran) 2022	SC	Yes	**2.5%**	No clinically significant differences in the safety, PK/PD
RIVFLOZA™ (nedosiran) 2023	SC	No		
QALSODY® (tofersen) 2023	IT	Yes	**58.4%**	ADA appeared to decrease plasma tofersen clearance by 32%. Effects of ADA on CSF tofersen clearance is unknown. No discernible effects of ADAs on safety have been observed.
WAINUA™ (eplontersen) 2024	SC	Yes	**37%**	ADA did not affect the PK/PD, safety, or efficacy
RYTELO™ (imetelstat) 2024	IV	Yes	**17%**	There was no clinically significant effect of ADA on the PK, safety, or efficacy

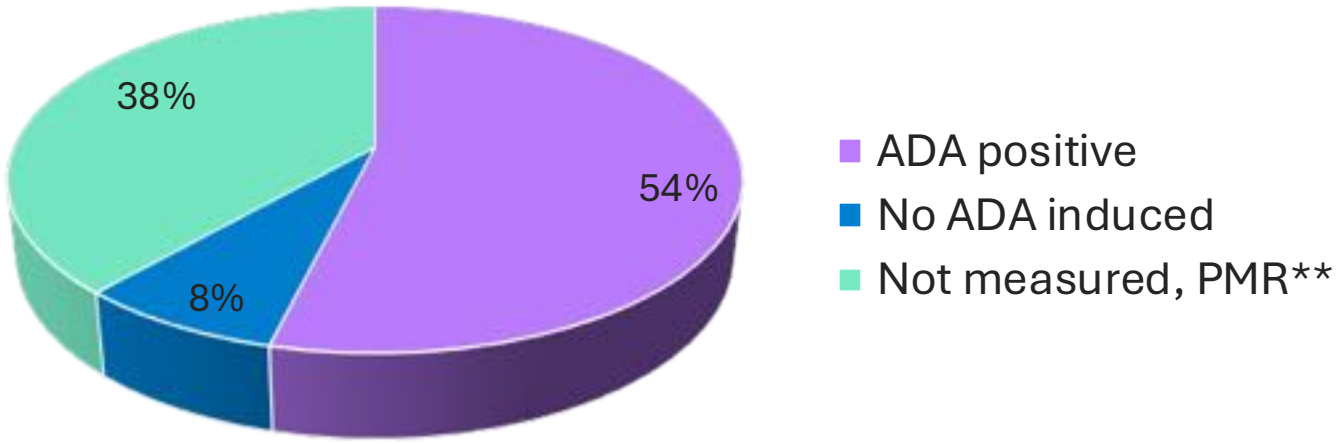
***The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay.**

****Immunogenicity assessments should be required as a post-marketing requirements (PMR) under 505 (o)**

Reference: Drugs@FDA: FDA-Approved Drugs: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm

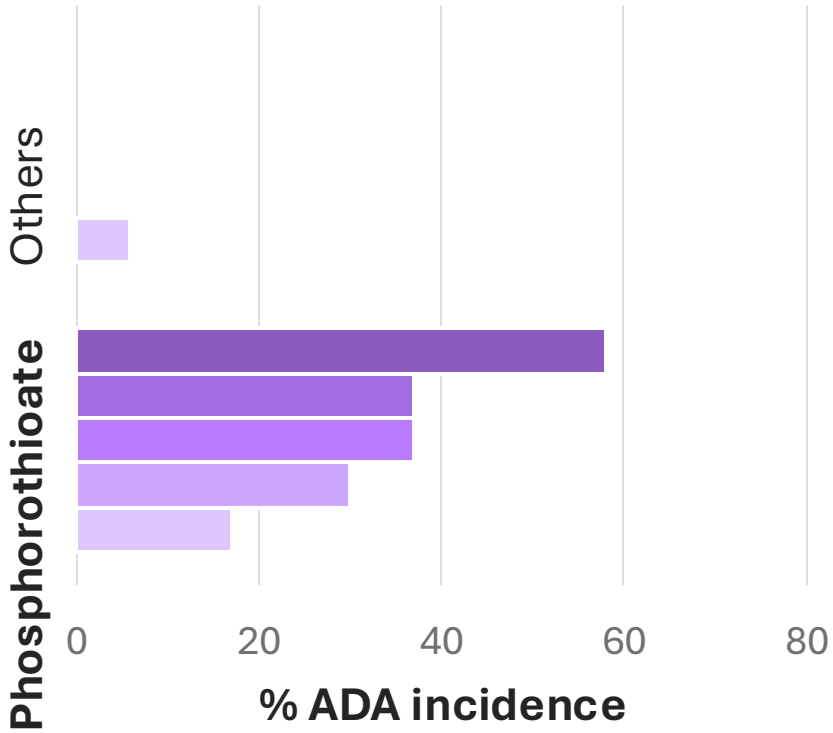
Immunogenicity – Current Experience with Approved RNA Oligonucleotides

ADA testing status for approved RNA Oligonucleotides



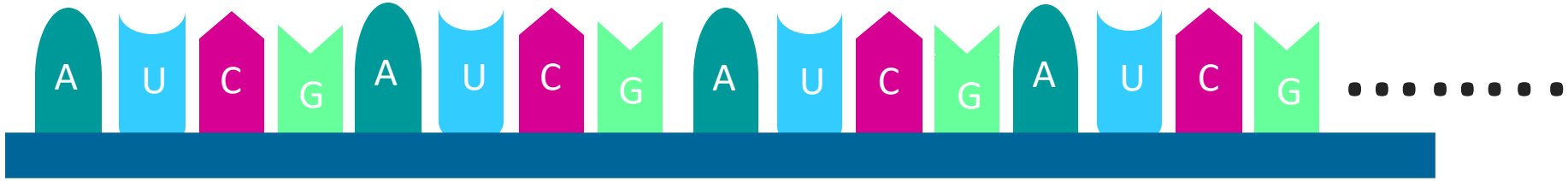
**PMR=post-marketing requirements under 505 (o)

ADA vs Backbone chemistry of Oligonucleotide



AIC Drug A

RNA antisense oligonucleotide; 20-mer



ADA positive control generation

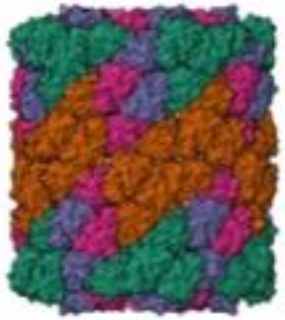
- Drug-linker synthesis
- Immunization program
- Purification of antibody

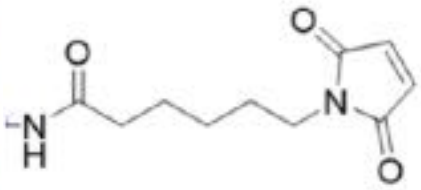
Drug-linker synthesis

Maleimide activated Keyhole limpet hemocyanin (KLH) 390,000 Daltons

This ratio corresponds to an approx. **3-fold excess of drug** material **over reported maleimide sites** on the KLH-monomer was used .

A





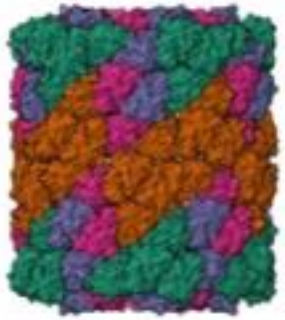
— 5'-(SHC6)—

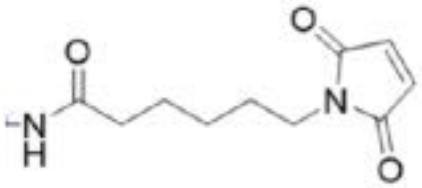
Thiol-modified drug 20mer

160–320 maleimide groups
for each KLH molecule.

Intermediate products were at least >85.0% purity

B





— 5'-(SHC6)—

Thiol-modified drug 20 mer + drug 20mer

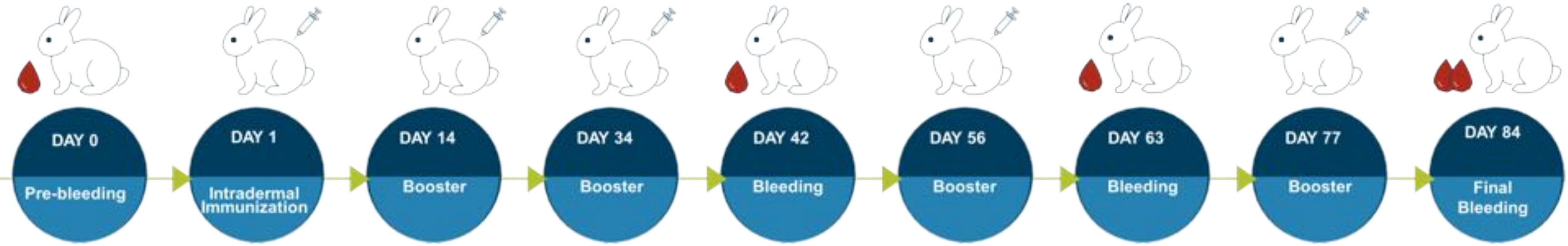
This conjugation process was conducted under experimentally proven condition:

- No protein loss during the conjugation/dialysis process.
- All non-conjugated oligonucleotide material was removed during dialysis.
- KLH-conjugated oligonucleotide = total input oligonucleotide – removed oligonucleotide during dialysis

Immunization program

A KLH-conjugated drug 20mer

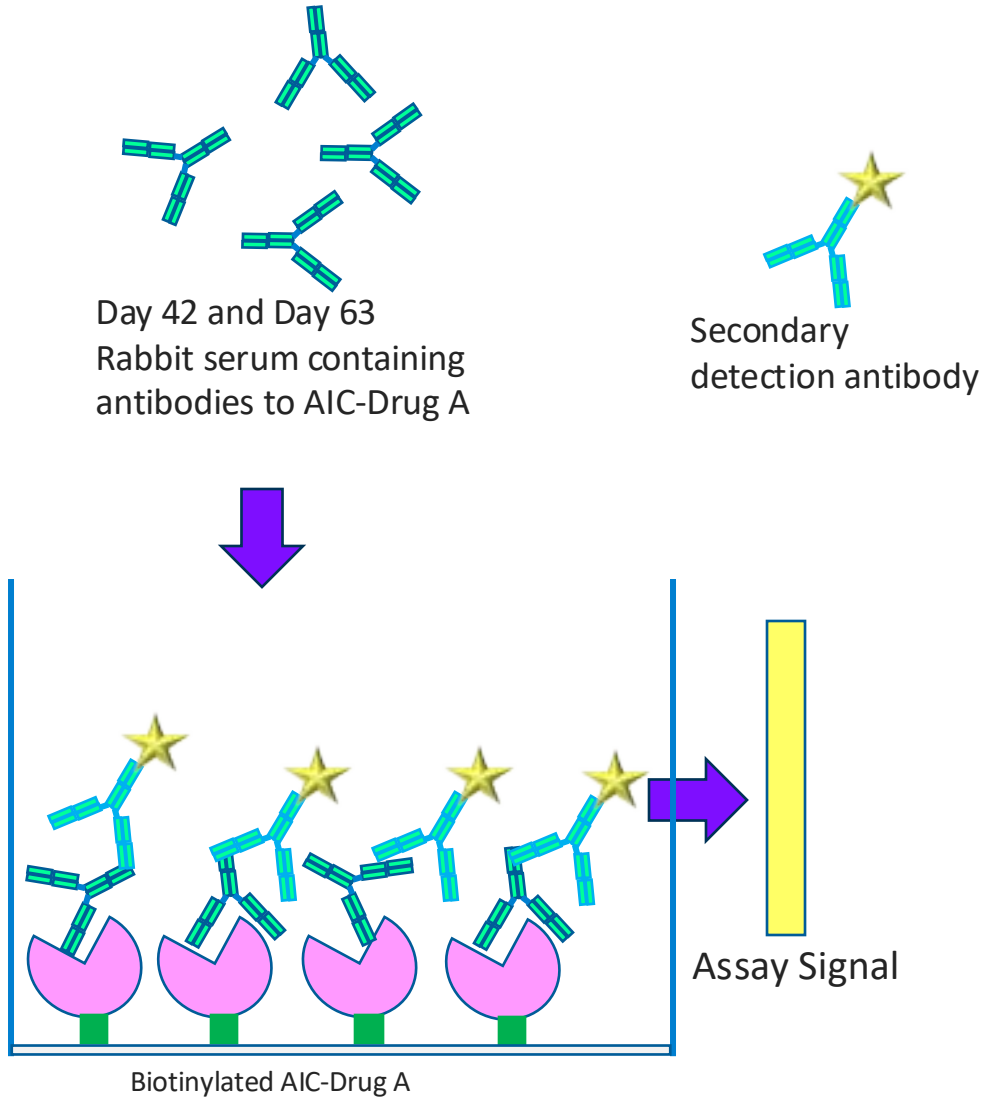
B KLH-conjugated drug 20mer+ drug 20mer(repetitive drug)



Antibody response by indirect ELISA titer determination

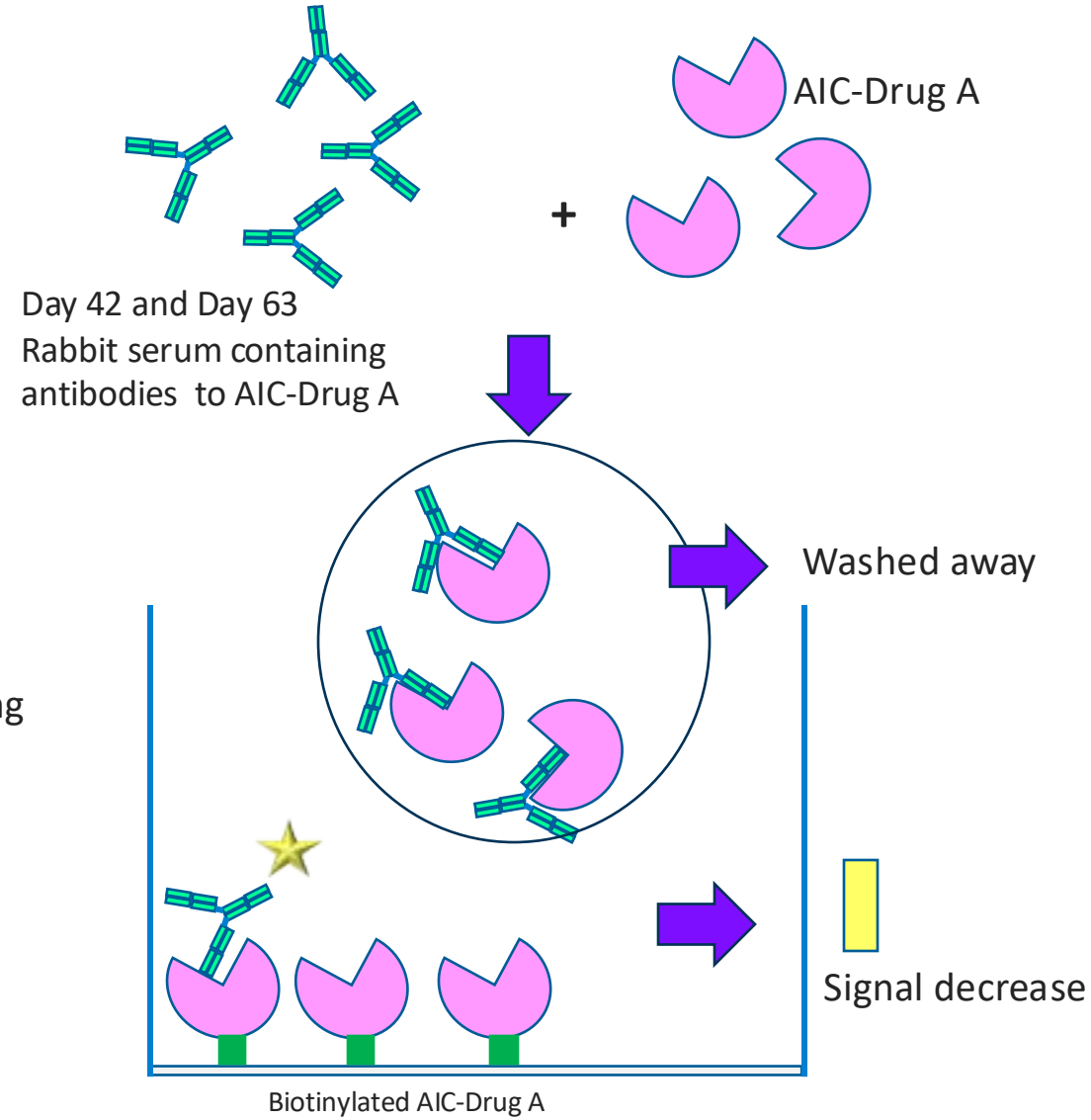
- The rabbit serum from day 42 and 63 from group A and B samples were added to the well plate coated with 5'Biotin-labelled- AIC-Drug A individually and incubated.
- After incubation, unbound antigens are washed away.
- The antibody on the plate detected by color changing reagent using a spectrophotometer at 450 nm.
- The intensity of the color correlates with the concentration of the target antibody in the sample.

- **Both groups showed significant antibody titer, with Group B achieving slightly higher antibody titers.**



Antibody response by competitive ELISA for inhibition determination

- The rabbit serum from day 42 and 63 from group A and B samples were mixed with a known quantity of free-20-mer AIC-Drug A individually and incubated.
- This mixture is then added to the well plate coated with 5'Biotin-labelled-AIC-Drug A.
- The antibody in the sample competes with the AIC-Drug A for binding to the 5'Biotin-labelled- AIC-Drug A coated plate.
- After incubation, unbound antigens are washed away.
- The antibody on the plate detected by color changing reagent using a spectrophotometer at 450 nm.
- The intensity of the color inversely correlates with the concentration of the target antibody in the sample.

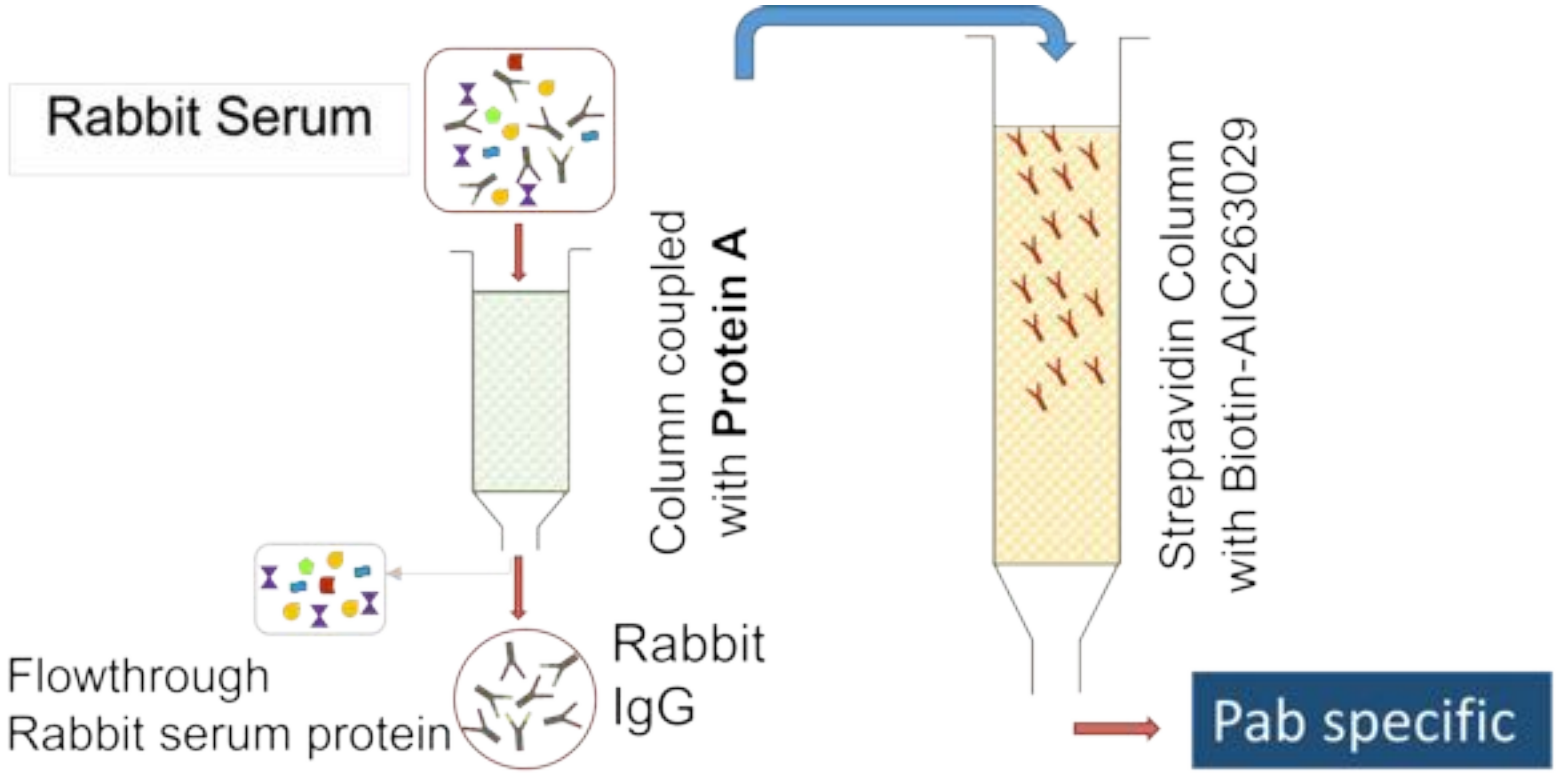


Antibody response by competitive ELISA for inhibition determination

- **Inhibition of rabbit antibody serum on 5'Biotin-AIC-Drug A was assessed using competitive ELISA with free-20-mer AIC-Drug A at two time points.**
- **Group A exhibited greater inhibition (64.50%) than group B (42.10%), particularly at 1300 nM concentrations.**

Purification of antibody

Serum Volume : 200ml: Rabbit 1: 100ml, Rabbit 2: 100ml



Purification of antibody

A KLH-conjugated drug 20mer

- Total rabbit IgG : 1239 mg;
- Total anti-AIC-Drug A-antibody : 82mg
- Yield : 41%

SPR characterization by BIACORE T200 TM

- Rabbit anti-AIC-Drug A antibodies display a good affinities for AIC-Drug A with equilibrium dissociation constants (KD) of 32.4 nM

B KLH-conjugated drug 20mer+ drug 20mer(repetitive drug)

- Total rabbit IgG : 1483 mg;
- Total anti-AIC-Drug A-antibody: 147mg
- Yield : 73%

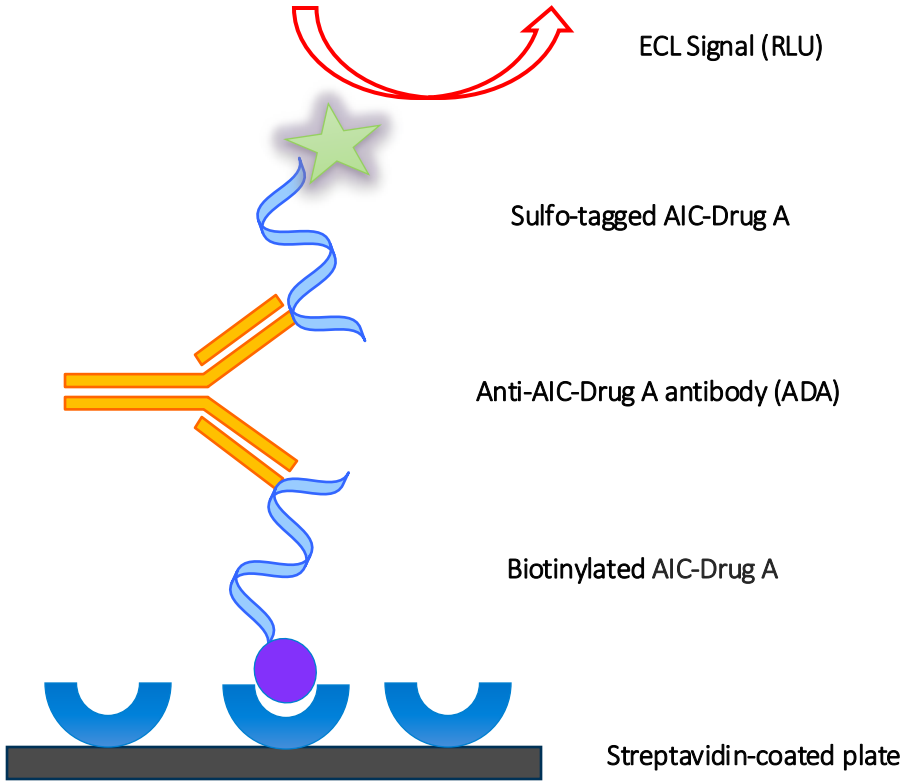
- Rabbit anti-AIC-Drug A antibodies display a good affinities for AIC-Drug A with equilibrium dissociation constants (KD) of 46 nM

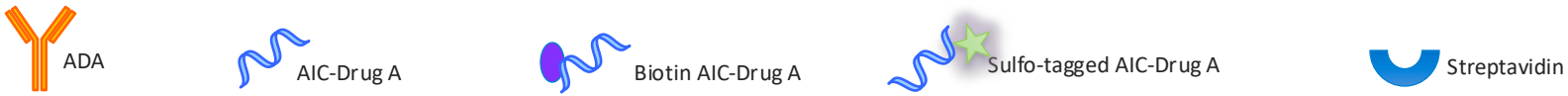
ADA assay development and validation

ADA assay development

Critical reagents

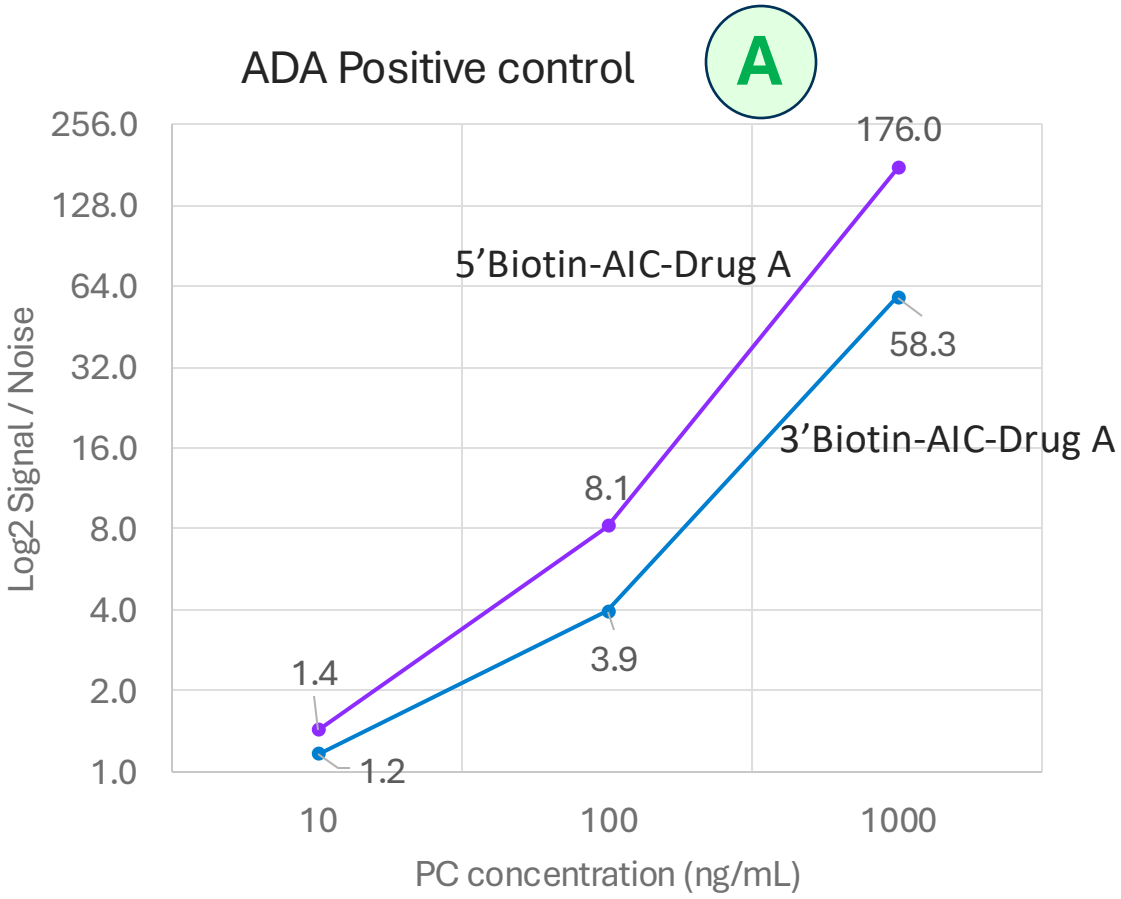
- ADA Positive control **A**
- ADA Positive control **B**
- 3’ Biotin-labelled drug
- 5’ Biotin-labelled drug
- 5’ Sulfo TAG labelled drug

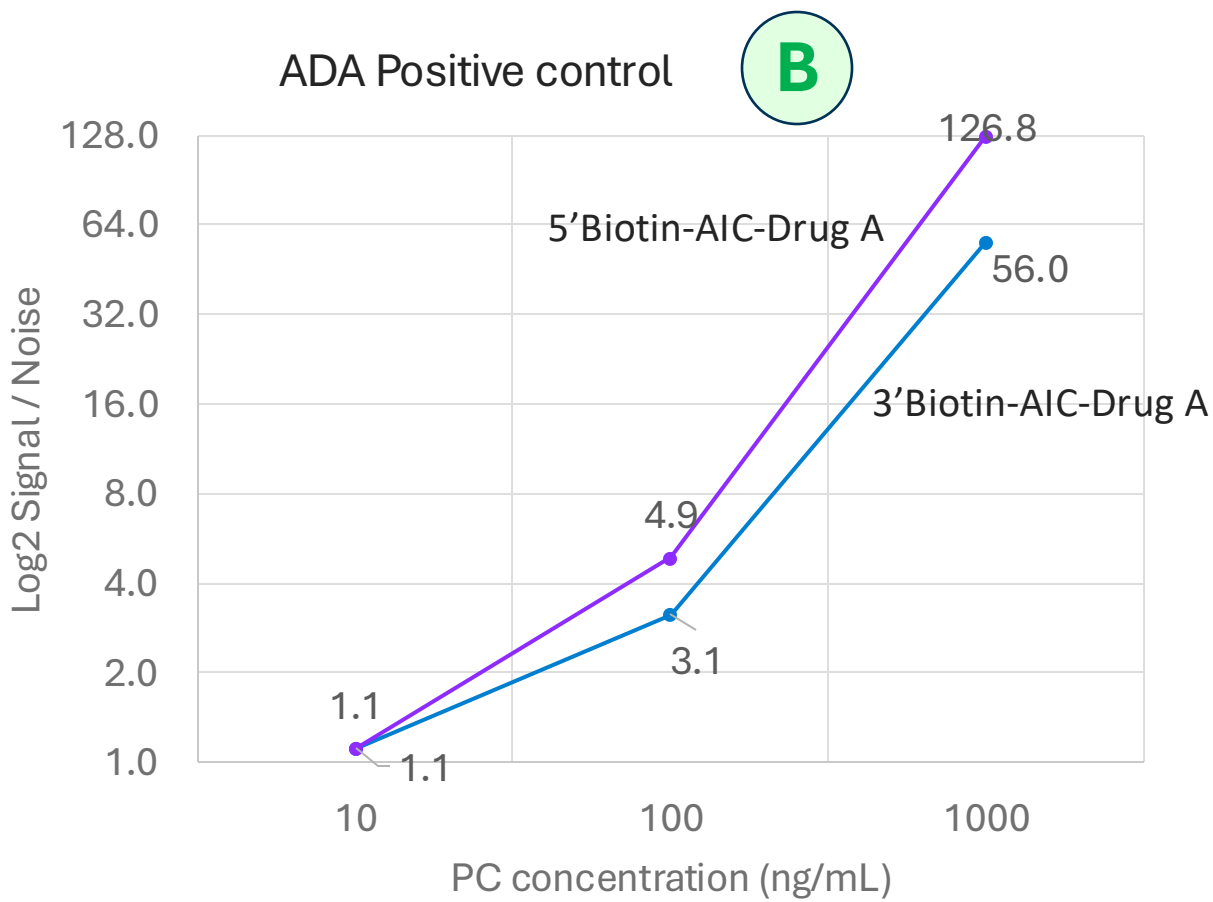




ADA assay development

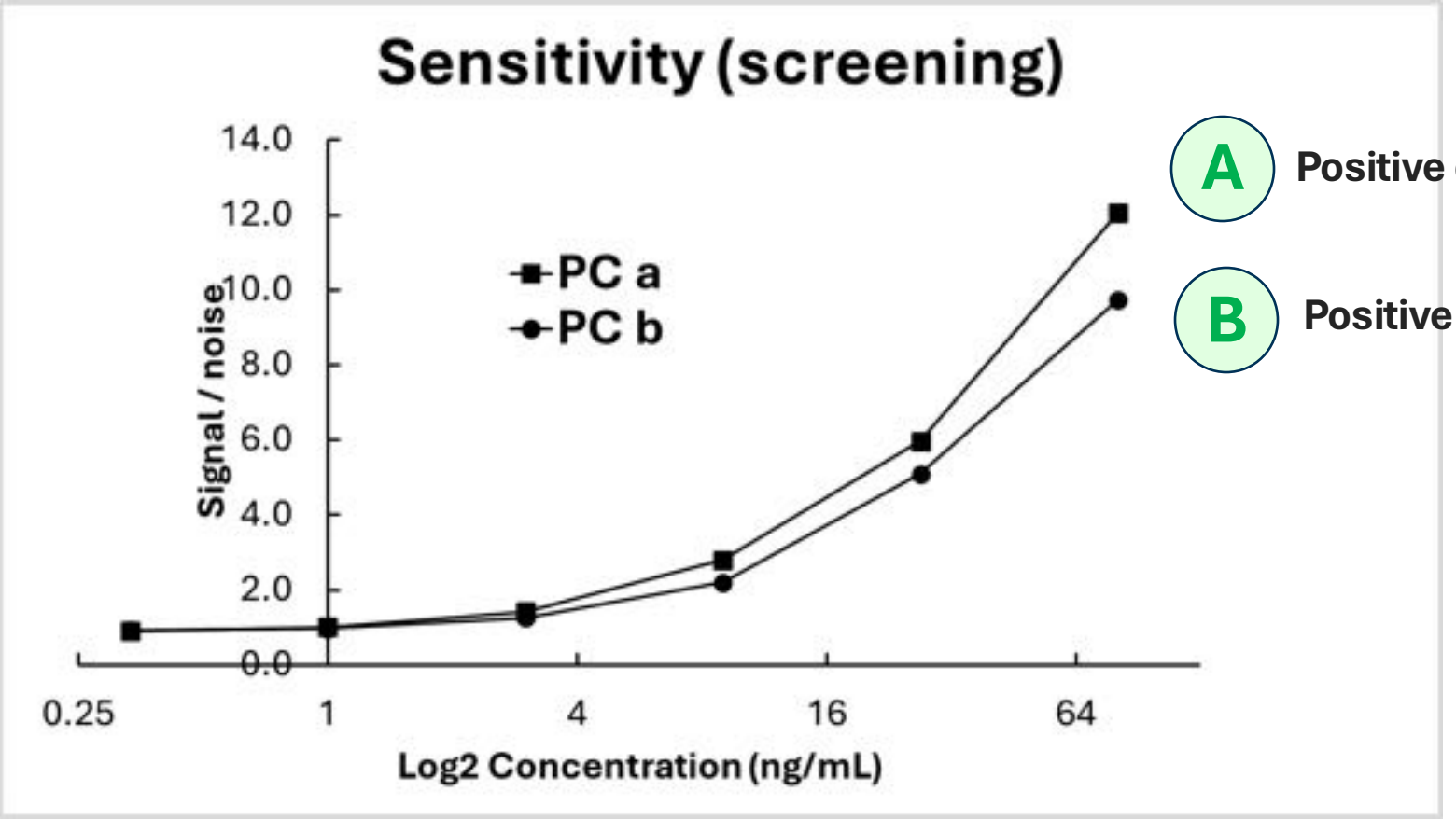
Comparison of **3'** vs **5'** Biotin labelled drug for assay suitability





5' Biotin labelled drug resulted in higher Ratio (Signal/Noise)

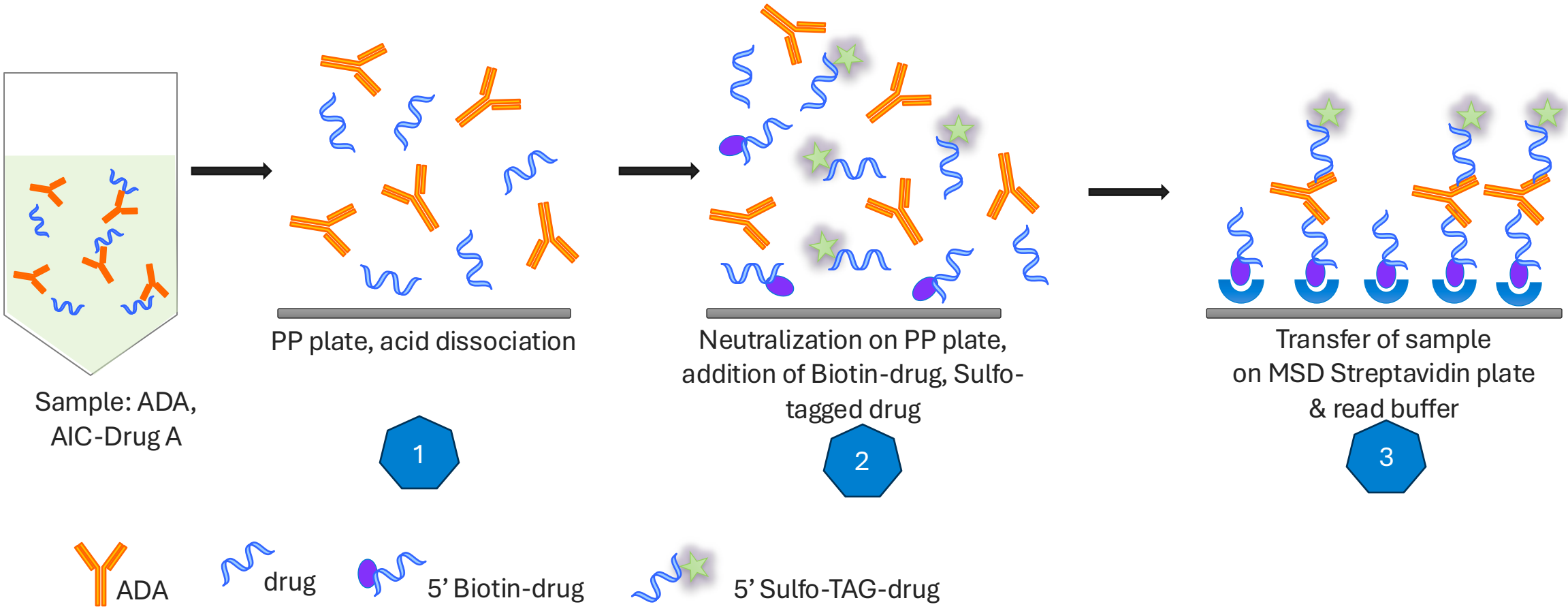
ADA assay development



The PCs produced with both approaches exhibited similar sensitivity during ADA assay development and were therefore combined to create a final PC for the assay.

ADA assay development

an acid dissociation step was included to improve drug tolerance of the assay.



ADA assay validation

Parameters	Result	Pass / fail
Cut Point	Screening Cut Point was established to be 1.110 normalized signal. Confirmatory Cut Point was established to be a value of 16.3% inhibition. Titer Cut Point was established to be a value of 1.236 normalized signal.	**Pass**
Sensitivity	Confidence Interval (CI) at 99%: **16.03** ng/mL (screening LPC) Confidence Interval (CI) at 99%: **20.72** ng/mL (confirmatory LPC).	**Pass**
Selectivity	Selectivity fulfilled the predefined acceptance criteria (with SPC at 50 ng/mL) —100% of samples met acceptance criteria at the 100ng/mL spike level.	**Pass**
Specificity	Drug Tolerance: was shown to be 1250 ng/mL of drug at 100ng/mL of the SPC, and 156.25 ng/mL at the LPC level.	**Pass**
Prozone (Hook) Effect	Prozone effect: Samples spiked with three ultra-high concentrations of the SPC, all screened and confirmed positive. No prozone effect is observed.	**Pass**
Screening and Confirmatory Precision	Inter-assay Precision: HPC, MPC, and LPC met acceptance criteria CV less than 20% Intra-assay Precision: HPC, MPC, and LPC met acceptance criteria CV less than 20%	**Pass**
Titer Precision	all titer precision curves yielded results within ± one dilution-fold of the median dilution (1:16).	**Pass**
Stability:	Short Term Stability: met acceptance criteria for 2-8°C storage up to 48 hours and room temperature for up to 24 hours. Freeze and Thaw Stability: met acceptance criteria for 9 FT cycles.	**Pass**
Robustness	The acid dissociation performed 30 min ± 5 min The incubation of samples with mastermix performed 2 hours ± 15 min The incubation time of samples on the MSD plate performed 1 hour ± 10 mins.	**Pass**

Summary

ADA Positive control generation

- **High quality KLH-Drug conjugates** was synthesized
  - A KLH-conjugated drug 20mer
  - B KLH-conjugated drug 20mer+ drug 20mer(repetitive drug)
- Immunization program was **conducted for 84 days** and successfully monitored for antibody responses
- **Biotinylated-drug immobilized streptavidin column** was used for successful purification of **drug specific polyclonal antibody**
- Group B **yielded 73%** (147 mg from 200 mL)
- Purified polyclonal antibody from **both groups showed good affinity with drug**

Summary

ADA assay development and validation

- Comparison of 3’ vs 5’ Biotin labelled drug for assay response concluded that **5‘ Biotin labelled drug resulted in higher Ratio** (Signal/Noise) during bridging assay development
- The **PCs produced with both approaches** exhibited **high sensitivity** during ADA assay development and were therefore combined to create a final PC for the assay.
- Additionally, **an acid dissociation step** was included **to improve drug tolerance** of the assay.
- Assay **validation results fulfilled pre-defined acceptance criteria**
- Overall, a **highly sensitive** and **specific, solution-based bridging assay** was developed and validated **to detect ADA** against **RNA oligonucleotides AIC-Drug A** in **human serum**

aicuris

Thank you!

Acknowledgments

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Robert Nelson

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