
Immunogenicity Testing of Therapeutic Antibodies in Ocular Fluids After Intravitreal Injection

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Diabetic Macular Edema (DME) and Age Related Macular Degeneration (AMD) Are Leading Causes of Vision Loss



Normal Vision

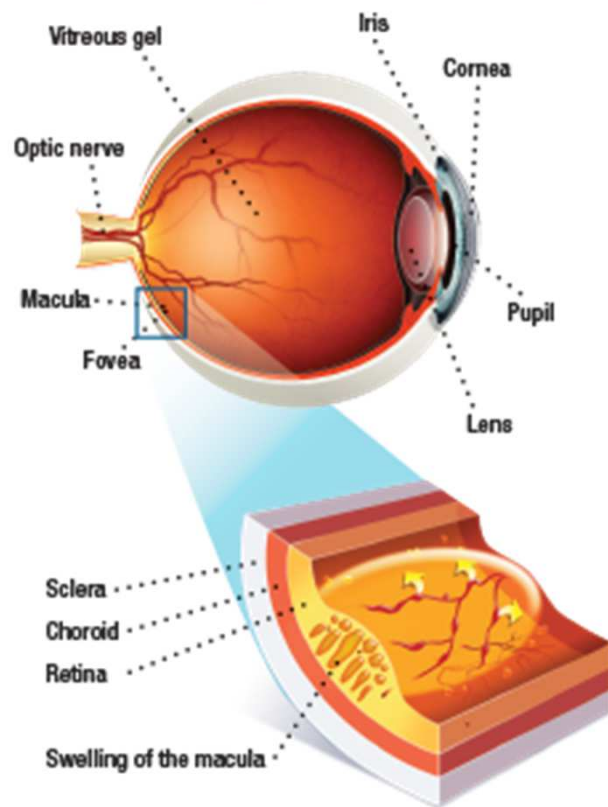


Diabetic Retinopathy

Vision is obstructed by macula edema

- Around 200 millions patients in 2020
- Increasing numbers are expected due to aging of the population and the diabetes epidemic

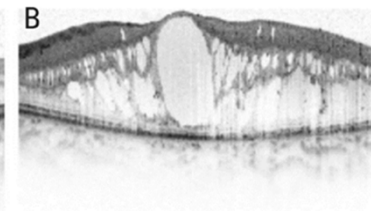
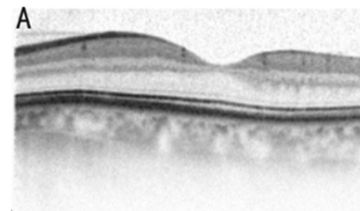
Growth of Abnormal Blood Vessels in Macula Region



Normal Intact Vision



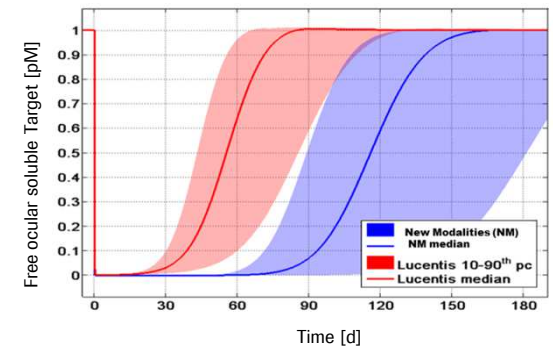
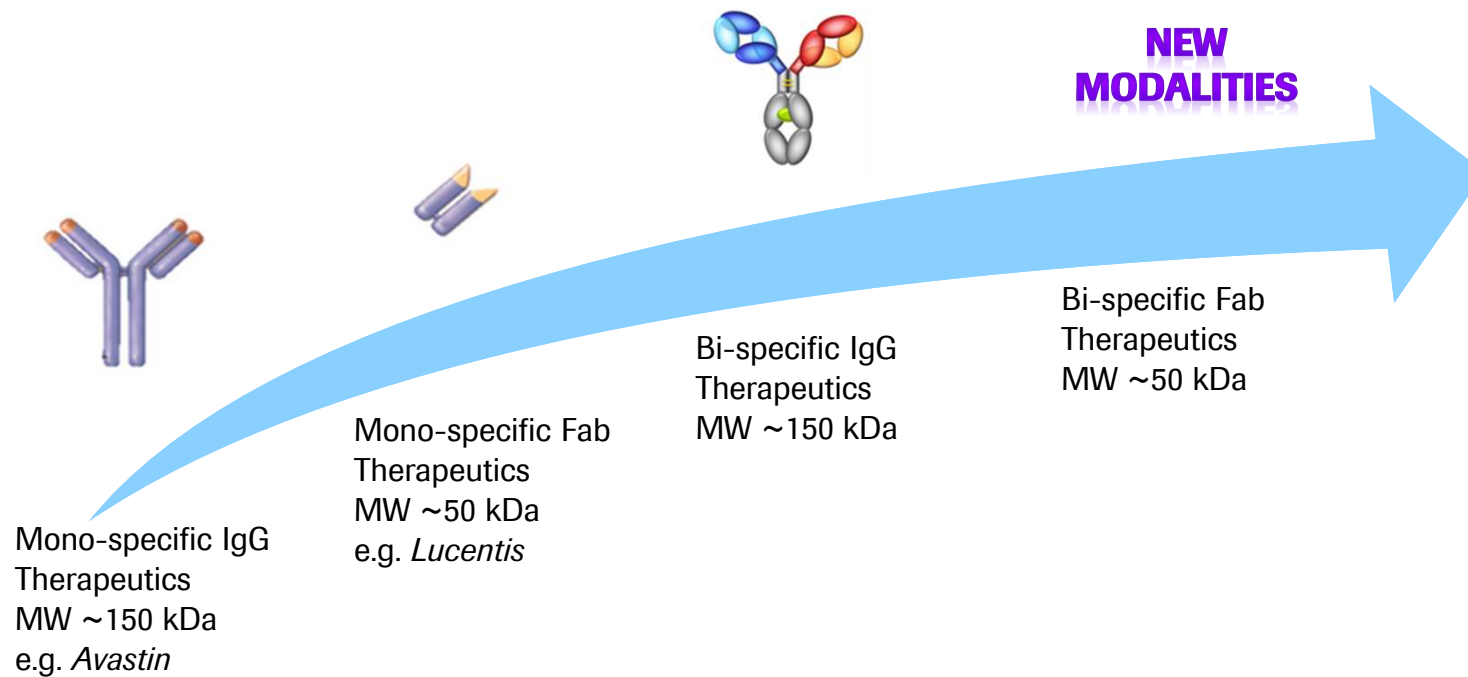
Diabetic Retinopathy



- Current Standard-of-Care (SoC) is intravitreal injection of anti-vascular endothelial growth factor (VEGF) drugs (e.g. Lucentis)

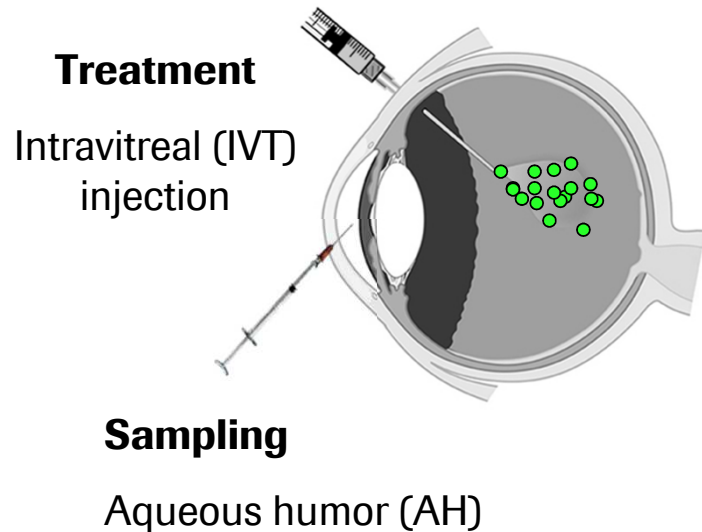
Innovative Molecule Modalities in Ophthalmology

New Modalities allow longer duration in the eye with less frequent IVT injections



- Improved Properties**
- High affinity
 - High concentrations
 - Increased durability
 - Longer duration of action
 - Less frequent dosing

Route of Administration and Challenges in Sampling of Ocular Matrices in Ophthalmology Studies

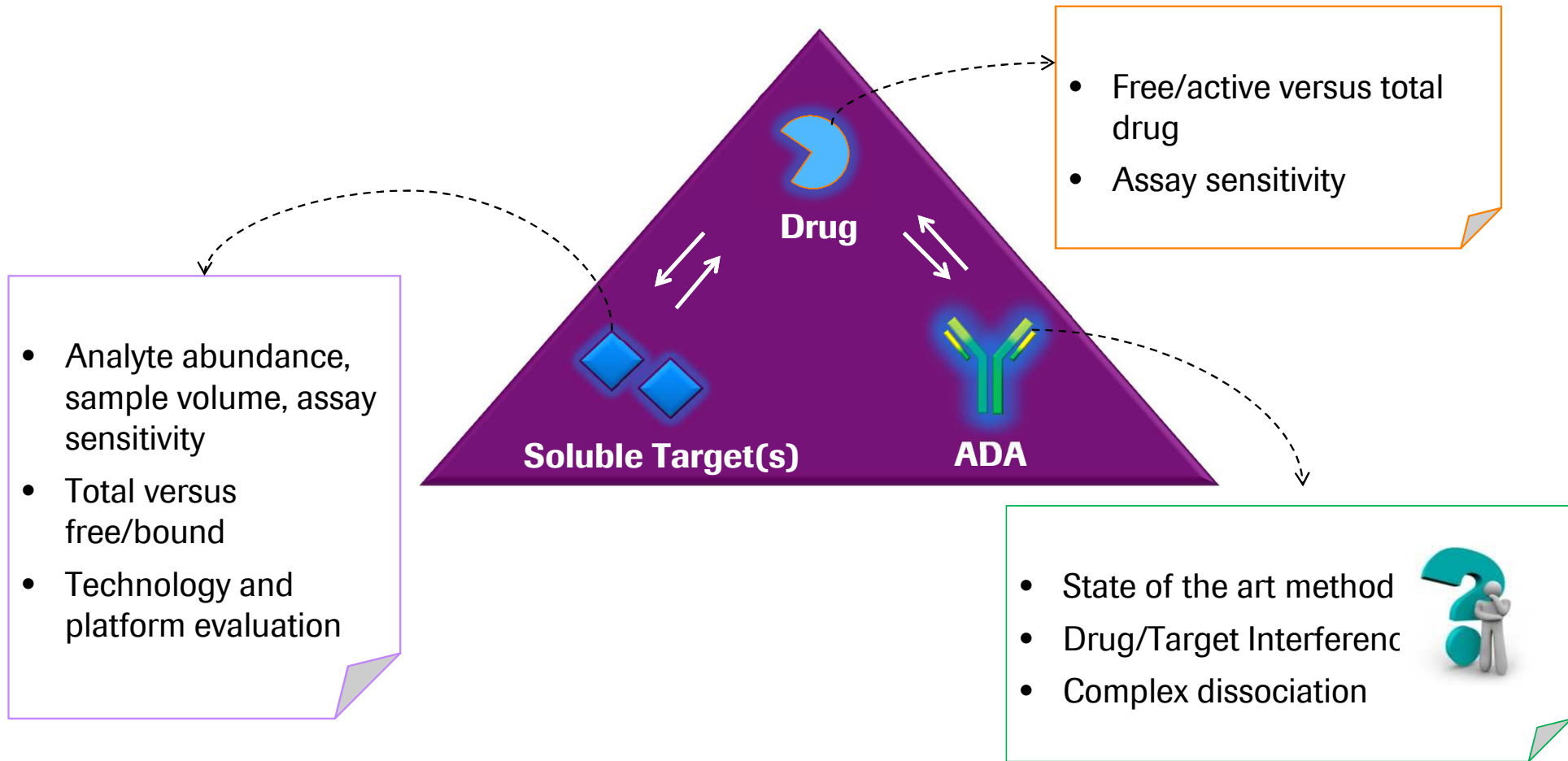


IVT administration is a burden for patients

Challenges in ophthalmology studies

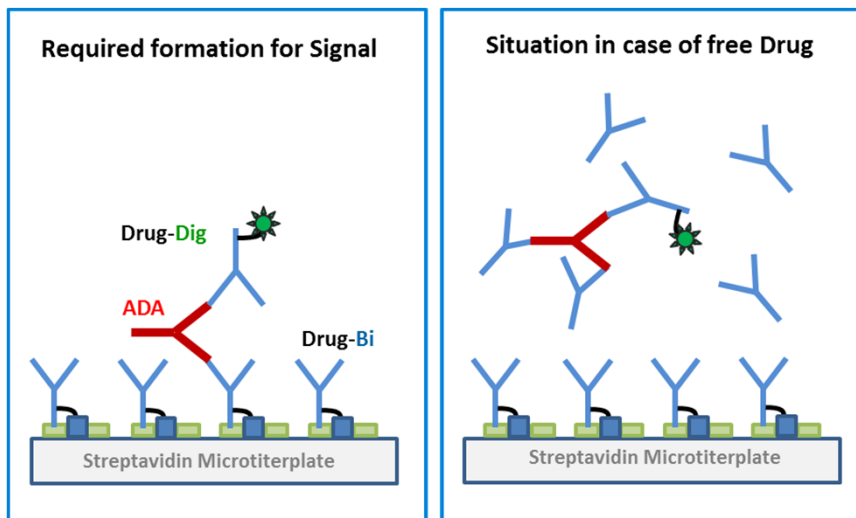
- Multiple ocular sample types with limited volume
 - Aqueous Humor: 20-100 μ l
 - Vitreous Humor: ~500 μ l
 - Retina (tissue): 1 mg
 - Choroid, etc.
- Difficult to access
 - Aqueous Humor: Low sample volume
 - Vitreous Humor: species specific diversity, usually post mortem samples
 - Retina: standardized tissue preparation, post mortem samples
- High sensitivity demands for soluble targets
- Non-Clinical Studies:
 - Different anatomy of the eye in different species
 - Animal AH can only be sampled under narcotic conditions

The Interplay Between Drug, Target and ADAs in Ophthalmology Studies



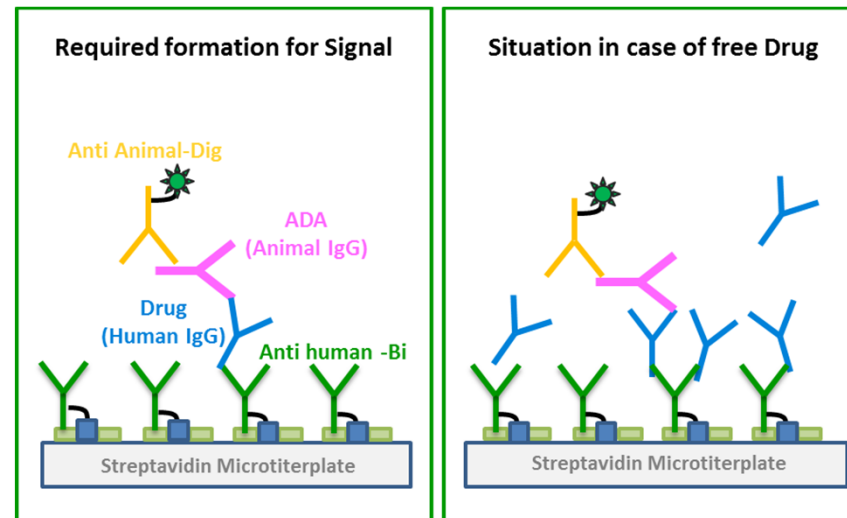
Comparison of Bridging and Immune Complex ADA Assay Principles

Bridging ADA Assay



The Bridging ADA assay shows signal interference in samples with high drug level.

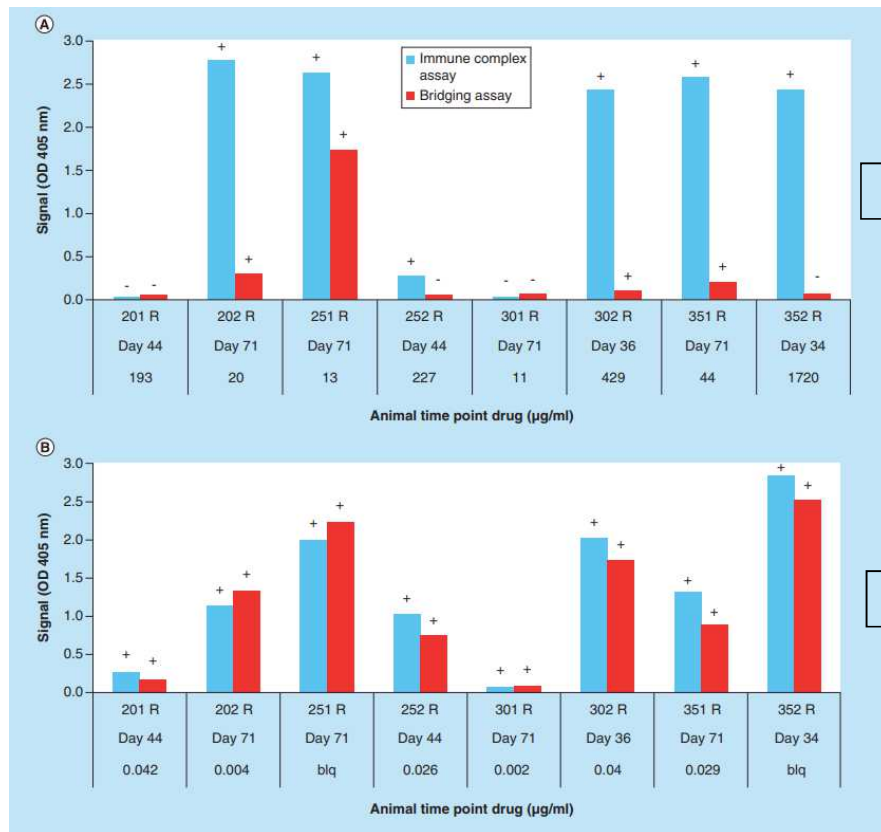
Immune Complex ADA Assay



The Immune Complex ADA assay enables reliable ADA detection in samples with high drug levels as aqueous and vitreous humor after IVT injection.

Stubenrauch K. et al, 2012 Bioanalysis

Comparison of ADA Results Generated by Bridging and Immune Complex ADA Assays



Vitreous humor samples (high drug level)

- IC ADA assay is superior and enables ADA detection in ocular fluids for the first time.
- Bridging assay strongly influenced by high drug concentration, resulting in false negative ADA response.

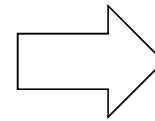
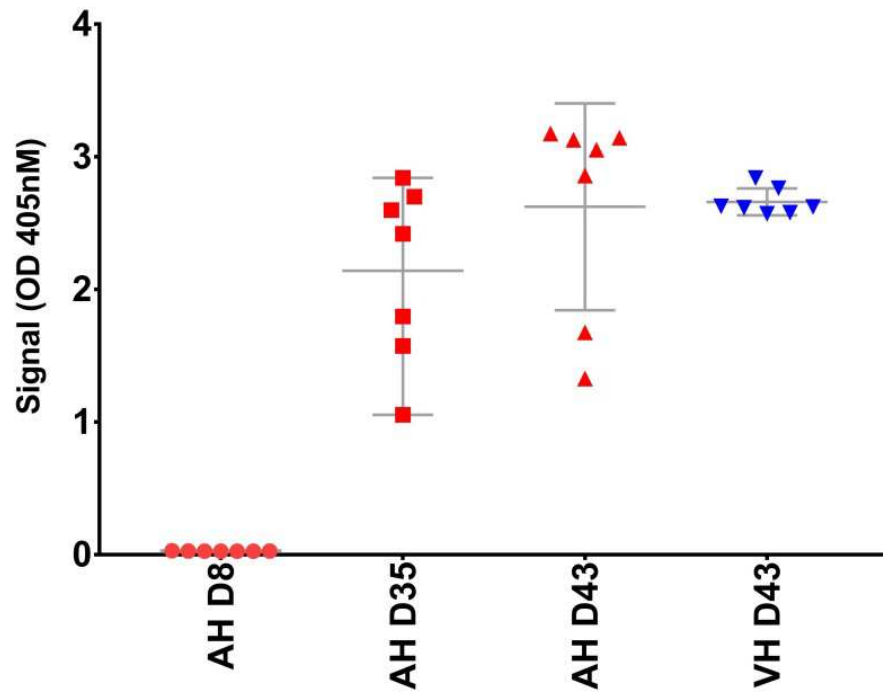
Serum samples (low drug level)

- Excellent correlation of bridging assay with immune complex ADA assay.

„Immunogenicity testing of therapeutic antibodies in ocular fluids after intravitreal injection“; Uwe Wessels et al, 2018 Bioanalysis

ADA Signals in Ocular Fluids after IVT Injection

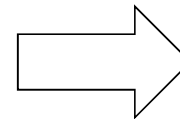
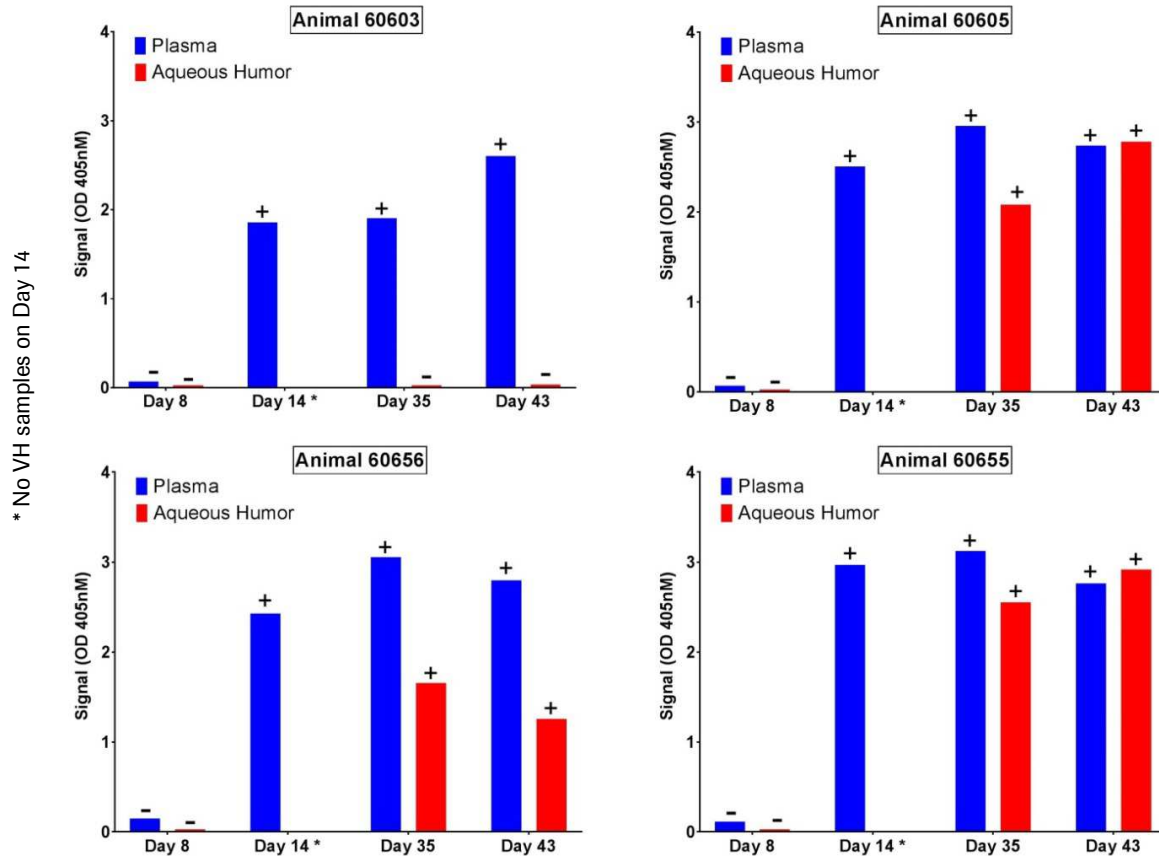
ADA results for Aqueous (AH) and Vitreous Humor (VH)



Good correlation of aqueous humor versus vitreous humor ADA data allows the design of future preclinical ophthalmological studies without the need for vitreous humor sampling.

Uwe Wessels et al, 2018 Bioanalysis

ADA Signals in Plasma Samples and Ocular Fluids after IVT Injection



The good correlation of ADA results from ocular fluids to serum samples indicate that ADA analysis in serum may be predictive for ocular fluids.

Uwe Wessels et al, 2018 Bioanalysis

Evaluation of an Appropriate Matrix for Immunogenicity Testing after IVT Injection in Non-Clinical Studies

	Vitreous Humor	Aqueous Humor	Serum/Plasma
Accessibility	<ul style="list-style-type: none"> • Post mortem • No time course 	<ul style="list-style-type: none"> • Difficult sampling • Limited time points • Low sample volume 	<ul style="list-style-type: none"> • Easy to access • Time course possible • Higher sample volume
Drug level after IVT injection	<ul style="list-style-type: none"> • Very High 	<ul style="list-style-type: none"> • High 	<ul style="list-style-type: none"> • Low
Correlation to Vitreous Humor ADA	---	<ul style="list-style-type: none"> • Given 	<ul style="list-style-type: none"> • Given • Early onset
ADA Assay	<ul style="list-style-type: none"> • Immune Complex Assay 	<ul style="list-style-type: none"> • Immune Complex Assay 	<ul style="list-style-type: none"> • Bridging Assay • Immune Complex Assay

Summary and Conclusion

- Sensitive and drug tolerant immune complex assay enabled detection of ADAs in ocular samples for the first time
- Good correlation of aqueous and vitreous humor ADA data allows immunogenicity monitoring without termination of the animals
- We conclude that systemic ADA analysis might be sufficient for evaluation of immunogenicity in non-clinical and clinical ophthalmology studies

Roche Ophthalmology LMBA Team

Supporting Ophthalmology Projects from early non-clinical studies to Post Marketing



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Uwe Wessels

Doing now what patients need next