Generic ADA Assay:
a universal approach to enhance early phase and preclinical immunogenicity testing

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1 INTRODUCTION

The anti-drug antibodies (ADAs) detection is critical in the development of biopharmaceuticals because of the potential adverse effects they can cause, including immunogenicity-related safety concerns and reduced drug efficacy. The strategy for immunogenicity assessment is based on risk analysis and the likelihood of eliciting an immune response. If a drug candidate is found to be highly immunogenic, more time and resources are invested in these evaluations, bearing in mind that preclinical ADA are not predictive. For this reason, their determination is not strictly mandatory in the preclinical phase.
By looking at these numbers, generated by our laboratory in a non-clinical environment in 2022, we had an idea:

Total number of ADA samples tested	Total number of ADA method qualified	Average number of sample tested per method used
126	**4**	**31.5**
• ~1% of total samples analyzed • ~8% of studies required ADA testing	• 1 never used • 3 qualified and used	• Development + qualification average exp: 13 • Sample testing average exp: 1

To address this challenge a generic assay was developed to identify ADA against human IgG-like molecules in preliminary murine studies

3 METHOD DEVELOPMENT

During the method development, careful selection of specific capture and detection reagents and a minimal required dilution were essential to ensure accurate and sensitive ADA detection. During this phase, a mouse anti-human H&L chain was defined as positive control for the assay. However, the most important factors defined at this stage were the ADA saturation procedure and the drug tolerance.

A. ADA SATURATION PROCESS

Defined drug concentrations (1, 10, 50, 100 µg/mL) were added to samples containing mock ADA only and mock ADA with drug to mimic a real sample. It was observed that the drug concentration with the best performance was **50 µg/mL**, which was selected for further experiments.

B. DRUG TOLERANCE

Since the drug is already present in the sample after the treatment with the biotherapeutic, the drug tolerance was assessed to define if more drug could interfere with ADA detection.

ADA
HPC 90 µg/mL
LPC 250 ng/mL

→

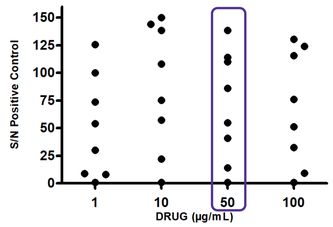
DRUG
0 to 1500 µg/mL

→

ASSAY DAY
Addition of further 50 µg/mL of drug to all samples and analysis

No interference due to the drug, the method can tolerate up to **1.5 mg/mL** of drug.

A



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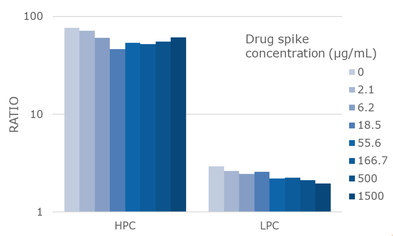
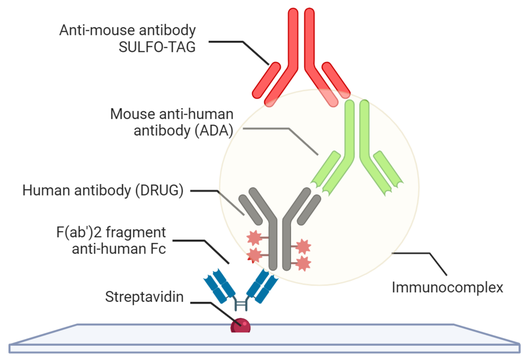


2 INTO THE GENERIC ADA FORMAT

A generic three-step format assay, that uses a standard set of reagents to detect ADA-DRUG immunocomplexes, was developed on MesoScale Discovery (MSD) platform.

- **CAPTURE**: biotinylated F(ab')2 anti-human Fcγ
- **IMMUNOCOMPLEX**: an excess of drug is added to the sample to allow immunocomplex formation at 37°C.
  A mouse anti-human antibody is used as positive control (PC).
- **DETECTION**: anti-mouse antibody labelled with the SULFO tag



ADVANTAGES

- **Unique set of reagents for all drug modalities**: this format provides a standardized set of reagents that can be used for different drug modalities, simplifying the assay development process.
- **No labeling required**: the reagents involved are commercially labelled, saving time and reducing potential batch-to-batch variability.
- **Reduced time and cost**: developing and validating specific assays can be time and cost consuming. Using the generic ADA assay, eliminates the need to develop and validate a specific assay for each drug candidate.

DISADVANTAGES

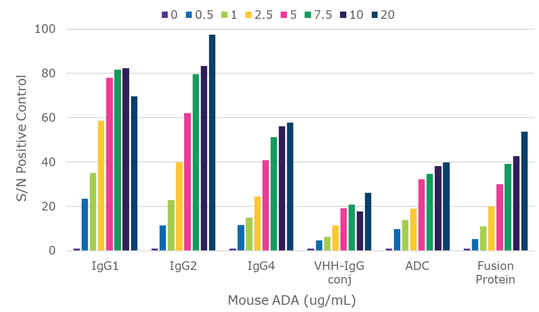
- **IgG-specific method**: this format focuses on the detection of IgG isotype ADA. Other isotypes, such as IgM or IgA, are not captured by the assay.
- **Species-specific method**: the developed assay is specific for mouse samples. Further adaptation of the method will be required to test other species.

4 METHOD APPLICATION

Six drug modalities within Merck's pipeline were tested spiking the PC at different concentrations.

The results are shown as a ratio on the negative control for each drug modality. A concentration dependent S/N increase was observed between different modalities: this effect could be explained by the differences in the available binding space.

The overall method performance is good, indicating that it is suitable for sample analysis.



5 STRATEGY FOR ASSAY APPLICATION

	SHORT WAY – EARLY DISCOVERY STUDIES	LONG WAY – MORE ADVANCED STUDIES
DAY 1	**New drug performance test:** • Checking the best drug concentration to apply • Choice of the LPC and HPC concentration for qualification • Definition of parameters to troubleshoot	
DAY 2	**Sample testing** 15 individual naïve samples + PCs + study samples • In run Dynamic CP • S/N comparison between pre-dose and treated samples responses	**Screening CP and PC** 4 analytical runs, 2 operators, 25 individual samples + NC, LPC and HPC • Definition of Screening CP • Choice of LPC for sample testing
DAY 3		
DAY 4		**Drug tolerance** • Definition of drug tolerance level for LPC and HPC, according to study drug expected levels
DAY 5		**Sample testing**

6 CONCLUSION

- A **generic method for the detection of ADA in murine** discovery studies has been successfully developed:
  - **Unique assay format** applicable to **IgG-like molecules**
  - Definition of **ADA saturation** procedure
  - **Drug tolerance** assessed
- Method performance tested with **6 drug modalities**
- **Assay applicability strategy** defined – short and long way

NEXT STEPS

- Application of the method to **all preclinical matrices** (rat, minipig, rabbit)
- Evaluation of **generic approach for cynomolgus monkey matrix –** generation of a ***ad hoc*** **positive control**

Images adapted from Biorender. Graph A generated with Prism 4.