FyoniBio

## Immunogenicity Quick Facts

### What's about?
- testing for drug therapy initiated generation of anti-drug-antibodies in animals or patients

### Which drugs?
- all biotherapeutics from oligonucleotides to antibodies

### Why?
- ADA may impact drug safety and efficacy

### When?
- in preclinical toxicology and clinical studies

### Who?
- GxP certified labs like FyoniBio

# HOW TO PERFORM IMMUNOGENICITY TESTING

## What is immunogenicity?

Biotherapeutics from small oligonucleotides to large proteins are able to elicit immune responses when applied in animals or humans. The assessment of immunogenicity addresses the anti-drug antibody (ADA) response after drug application. Binding of ADA at the drug results in clinical responses that range from no symptoms to severe side effects.

In preclinical animal studies the occurrence of ADA is mainly analyzed to evaluate any effects on the bioavailability of the drug. In humans, the presence of ADA may also alter the pharmacokinetics, may neutralize the drug's activity and affect the mode of action, may support hypersensitivity or autoimmunity reactions, or may cross-react with endogenous counterparts of the drug.

The probability to develop ADAs depends on the application route and ranges from a few patients to high incidences in the mid double-digit range.

## ANALYTICAL APPROACH

Samples are usually analyzed in a three tier approach using a screening, a confirmation and a titration assay. Major challenges which have to be solved during ADA assay development are the availability of a suitable positive control, sensitivity, drug tolerance, matrix effects including pre-existing antibodies, and the determination of the assay cut point.

Clinical samples are analyzed by using all three assays of this approach whereas preclinical samples are typically only analyzed using the screening and confirmation assay.

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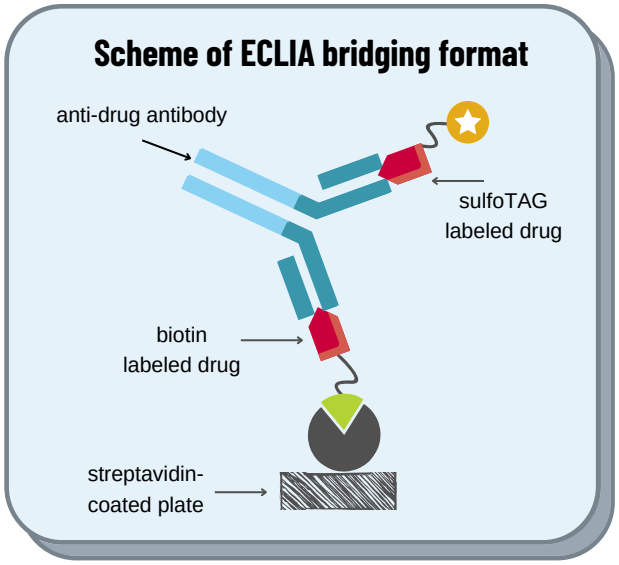
|---|---|
| **Screening assay** | to identify negative and potentially ADA positive samples |
| **Confirmation assay** | to verify potentially positive samples as negative or truly positive |
| **Titration assay** | to determine a serological ADA titer |

# Analytical Services

## ASSAY DESIGN

At FyoniBio, ADAs are detected using an electrochemiluminescence (ECL) assay in a homogenous bridging format.
Samples are diluted at the minimally required dilution to overcome potential matrix effects. Diluted samples are subjected to an acid dissociation step to dissolve complexes of ADA and drug, thereby improving greatly the drug tolerance. Next, samples are neutralized and simultaneously incubated with biotinylated and sulfoTAG-labelled drug to start immune complexes formation. Subsequently, the solution is transferred to a blocked streptavidin coated 96-well plate. Unbound proteins are removed by washing and ECL is measured on a MESO QuickPlex SQ 120 (MSD Inc.).
The measured light signal is proportional to the ADA concentration in the sample.




## RESULTS

Evaluation of the measured signal is done qualitatively, using assay specific cut-points established during validation. As a result of the assay, a sample's signal may be above or below the cut point, classifying the sample as positive or negative. Screening-positive samples are subsequently analyzed in a confirmation assay in absence and presence of the drug. Sufficient ADA signal inhibition by the drug verifies a sample as truly positive. Finally, confirmed positive samples are analyzed in the semi-quantitative titration assay where the Log2 titer of a sample is determined from a 2-fold serial dilution.
For positive samples, further characterization of the isotype and the neutralizing activity of the ADA might be performed.

## FyoniBio SERVICES

**FyoniBio has many years of experience in assay development, validation and bioanalysis in the course of clinical trials. All steps are performed according to the current ICH, FDA and EMA guidelines in our ISO 9001 certified and GCLP compliant laboratories.**

**THE FyoniBio TEAM IS GLAD TO SUPPORT YOU THROUGHOUT YOUR PROJECTS.**

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