

## Purpose

Therapeutic proteins can induce immune response, leading to neutralization of the therapeutic effect or even autoimmune reactions and serious health problems.

For application of immunoassays in immunogenicity testing, especially the following parameters are recommended [1]:

- Good sensitivity to assess low affinity anti-drug antibodies (ADA)
- Excellent tolerance for the presence of the free drug
- High tolerance towards interfering matrix components

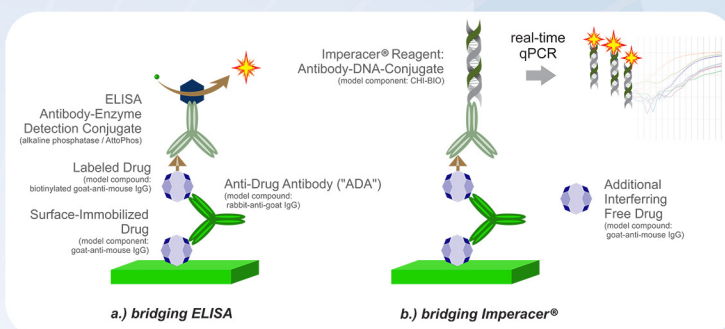
To address these recommendations, high-performance technologies are needed to circumvent limitations in conventional immunoassays.

Therefore, we here report on the application of the Immuno-PCR (IPCR) methodology to combine the advantages of ultra-sensitive detection with state-of-the-art sample dilution technology.

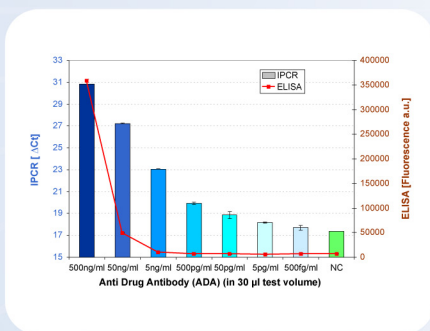
## Methods

Immuno-PCR (IPCR) combines the excellent specificity of ligand binding assays with quantitative real-time PCR (qPCR) as today's most sensitive detection technology in molecular biology. IPCR typically enables a 20 – 10,000-fold increase in sensitivity compared to classical ELISA or ECL immunoassays in combination with a broad detection window and high tolerance against individual matrix effects [2].

Here we describe a model assay to demonstrate drug tolerance levels of the IPCR based Imperacer® immunoassay system in a bridging assay format. This typical immunogenicity testing assay set-up was conducted in buffer and standardized human serum. A model IgG system was studied to investigate compatibility and potential of ultra-sensitive analytics in combination with immunogenicity applications, e.g. for the detection of ADA raised against therapeutic antibodies.

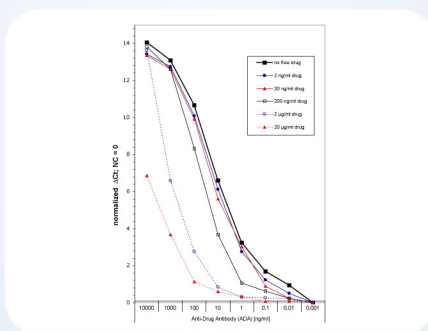


## Results



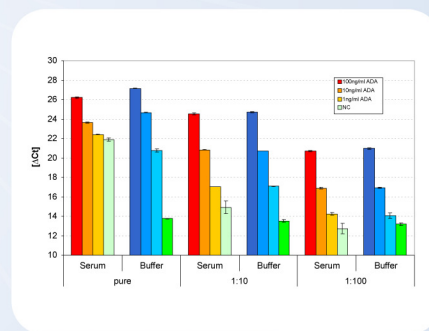
A) Sensitivity and Detection Range

Similar to classical assay set-ups, the novel bridging Imperacer® again revealed a more than 1000-fold increased sensitivity in comparison to an ELISA carried out under identical conditions. Correlation of measured Imperacer® signals ( $\Delta C_t$ ) and spiked concentrations revealed a dynamic range for quantification of 6 orders of magnitude spiked ADA with an intra-assay standard deviation below 5%.



B) Drug Tolerance

In this Imperacer® bridging assay format, an initial drug tolerance ratio was found at 2000-fold excess of the interfering drug compound (detection of 10 ng/ml ADA in the presence of 20 µg/ml free drug). In an additional set of experiments, a cell-free neutralizing Imperacer® model assay revealed a basic tolerance ratio towards the presence of a neutralizing ADA (Nab) of nearly 1000-fold.



C) Matrix Tolerance

Additional experiments were carried out to investigate sample dilution strategies to minimize disturbing effects of the biological matrix and to match assay performance in different matrices. For this purpose, an ADA concentration series spiked in either pure buffer or human serum (BISEKO, Biotest, Germany) was analyzed after 1:1, 1:10 and 1:100 dilution in AnySource® sample dilution buffer. At an adequate sample dilution ratio, the Imperacer® assay revealed identical performance in serum and buffer.

## Conclusion

- Imperacer® revealed a drug tolerance ratio of up to 1:2000 in this model study.
- Imperacer® demonstrated a 1000-fold increased sensitivity compared to ELISA and excellent matrix tolerance.
- Sample purification, to extract free drug in order to increase drug tolerance levels can be omitted simply by appropriate sample dilution in combination with ultra-sensitive analyte detection.
- Imperacer® therefore has great potential as a novel ligand binding assay format for challenging immunogenicity applications without any sample pre-treatment or purification procedures.