

**BURECO AG is a GMP and GLP certified bioanalytical service organization for the Life Science Industry worldwide. We offer biochemical, radiometric, immunological and cell based analytical technologies since 1992.**

## IMMUNOGENICITY TESTING OF BIOPHARMACEUTICALS

Biopharmaceuticals may elicit an immune response in pre-clinical animal and in clinical studies. An immune response may affect the efficacy of a biopharmaceutical by altering its pharmacokinetics and/or neutralizing its activity. In the worst case, an endogenous counterpart of the drug may be neutralized with disastrous consequences. It is therefore mandatory to monitor immunogenicity adequately during all phases of product development <sup>(1-3)</sup>.

## TESTING STRATEGY

Immunogenicity testing should be done on the basis of a risk assessment and is usually done in several stages:

- SCREENING ASSAY that detects all antibodies binding to the biopharmaceutical (ADA; anti-drug antibodies) in serum samples of animals or patients,
- CONFIRMATORY ASSAY to eliminate false positive samples,
- ASSAY FOR NEUTRALIZING ANTIBODIES that detects those serum samples that contain neutralizing antibodies, and
- CHARACTERIZATION of anti-drug antibodies detected in serum samples.

BURECO AG has extensive experience in developing suitable, project specific immunogenicity testing strategies for animal as well as for clinical studies.

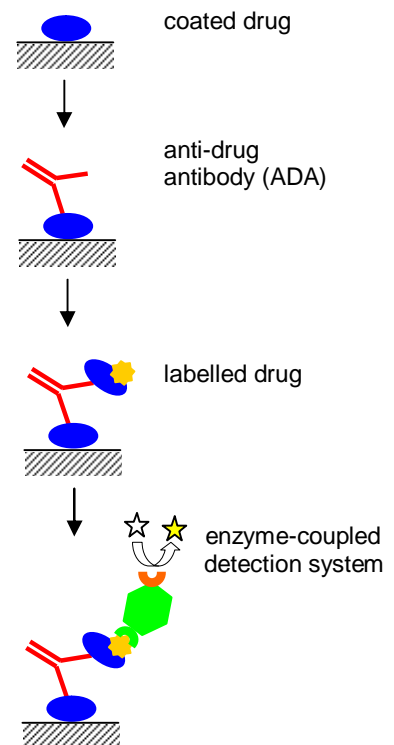
## SCREENING AND CONFIRMATORY ASSAYS

Screening for ADA is usually done using suitable ELISA formats, radio-immune precipitation (RIPA), e.g. with <sup>125</sup>I labeled antibodies, or surface plasmon resonance (SPR). The same assays are used for the confirmation step, e.g. by demonstrating inhibition of binding by excess of the drug.

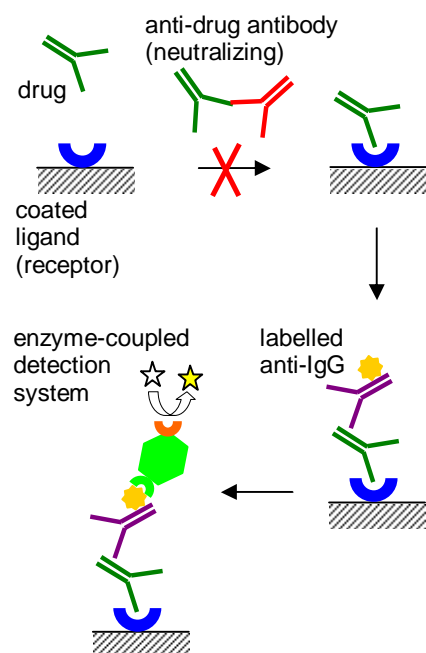
BURECO AG offers all suitable ELISA formats, as well as RIPA, for screening for ADA.

Bridging ELISA formats are particularly useful in early project stages and for different animal species when project specific immunological reagents are not available. To run a bridging ELISA, labeled drug is needed, however. The label may be detected directly (e.g. fluoresceine) or indirectly via an amplification system (see schematic depiction at the right).

### Bridging ELISA format:



### Competitive Ligand Binding (CLB) assay format



## ASSAYS FOR NEUTRALIZING ANTIBODIES

Neutralizing antibodies are usually detected using functional cell culture based assays. Cell based potency assays used for release and stability testing can usually be adapted for detecting neutralizing antibodies. High serum concentrations in the assay may present special challenges. In the case of some monoclonal antibody drugs, a competitive ligand binding (CLB) assay may be adequate to detect neutralizing antibodies. This is true for antibodies where the mechanism of action consists only in antagonizing a binding event.

Development and validation of NAb assays may be challenging. Matrix effects and the presence of residual drug can interfere with the assay. Needed specific reagents, e.g. control antibodies, may not be available. If possible an existing potency assay is adapted in a first step and a NAb assay is developed in a second step using a suitable surrogate antibody. Appropriate controls and predetermined criteria for the evaluation of results must be established.

BURECO AG offers all common assay formats for cell based assays, such as:

- Proliferation or proliferation inhibition assays
- Reporter gene assays
- Kinase receptor activation (KIRA) assays
- Phospho-specific antibody cell ELISA (PACE)
- Cell binding assays (cell ELISA).

Readout systems include UV/visible spectrophotometry, chemiluminescence and fluorescence techniques.

BURECO AG has extensive experience in developing suitable, project specific NAb assays for animal and clinical studies.

## Outline of development strategy for a cell-based NAb assay

Develop potency assay for the drug or optimize existing potency assay. Key criteria: Adequate signal-to-noise ratio, reproducibility, precision.

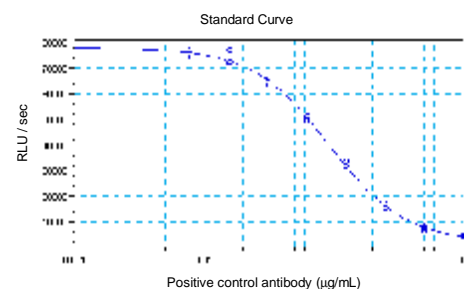
Determine matrix effects (pretreatment of samples / maximum tolerated sample concentration in assay).

Develop NAb assay using surrogate neutralizing antibody. Define final assay format (appropriate controls, plate design, assay conditions, acceptance criteria, etc.). Determine influence of residual drug.

Validate NAb assay; define cut-point using 20 – 50 samples from naive subjects (e.g. pretest samples).

Test samples

Run confirmatory and characterization experiments as needed.



## References

- Ref. 1 EMEA CHMP/BMWP/14327/2006; Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins (April 2008)
- Ref. 2 Gupta, Shalini, et al. (2007); Recommendations for the design, optimization, and qualification of cell-based assays used for the detection of neutralizing antibody responses elicited to biological therapeutics; J. Immunol. Meth. 321: 1-18
- Ref. 3 Mire-Sluis, Anthony R., et al. (2004); Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products; J. Immunol. Meth. 289: 1 - 16