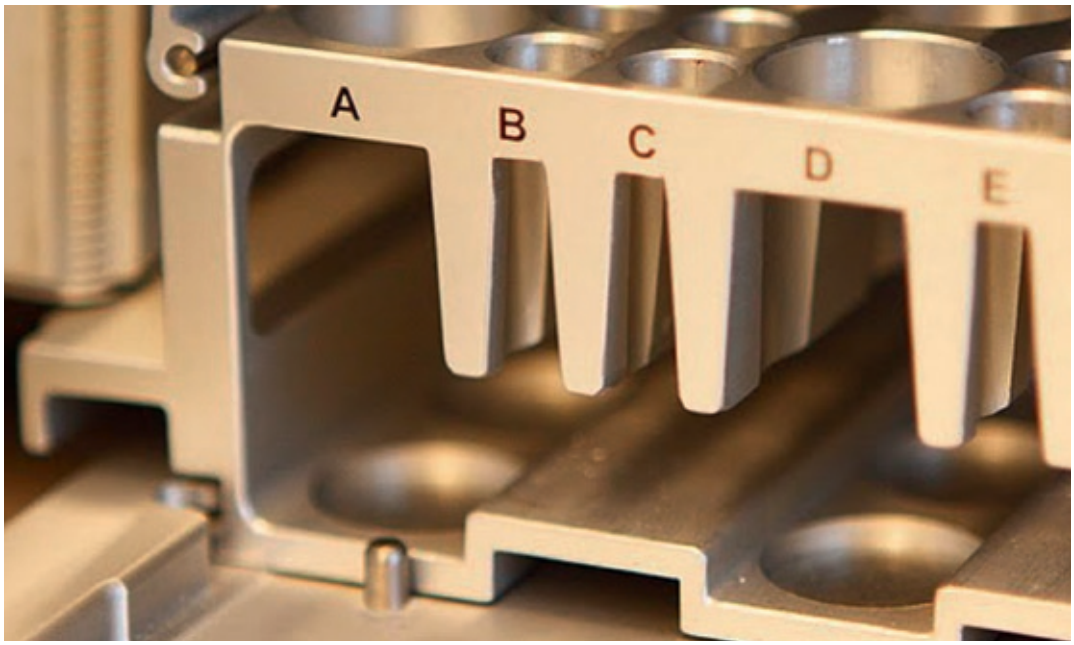
# IMMUNOGENICITY TESTING




TNO triskelion bv

Since biopharmaceuticals and biologics are dominating the pharmaceutical pipeline, the subject of immunogenicity has become of large importance in the (pre)-clinical phases of drug development. When the biopharmaceutical is inducing an immune-reaction this antibody response can lead to a reduction of efficacy of the drug and/or to serious clinical consequences. Therefore, the immunogenicity of every biopharmaceutical has to be characterized in detail. Appropriate assay's for screening and confirmation are mandatory.

TNO has designed a general strategy (using ELISA, RIA, BIACORE and MSD) to assist in the determination and characterization of antibodies that might be induced by your biotherapeutics.

## ANALYTICAL SERVICES

Over the past decade many studies related to immunogenicity screening and or confirmation were performed at the Analytical Research department of TNO Quality of Life. Our immunogenicity group is well experienced and is using state-of the art technologies. All assay's can be performed in accordance to GLP compliancy and following the latest white papers and FDA/EMEA guidance's on immunogenicity.

## THREE STEP APPROACH

TNO offers a three-step approach to determine the antibody titres, antibody characteristics and their neutralizing properties for both the pre-clinical as well as the clinical part of your drug discovery program.

## STEP 1: ANTI-DRUG ANTIBODY ASSAYS

Based on the characteristics of your protein or peptide or other biological drug, one or more binding antibody assays can be developed and validated to determine the presence of anti-drug antibodies (ADA) in samples of the host species. At TNO the following ADA assays are available:

- Direct ELISA.
- Indirect ELISA.
- Bridging ELISA.
- Time resolved fluorescence ELISA (DELFIA).
- Radio Immunoprecipitation (RIP) Assay.
- Surface Plasmon Resonance (SPR; Biacore).
- Electrochemiluminescence detection, Meso Scale Discovery (MSD).

The choice of the ADA assay for your specific question might also be based on the advantages of the different assay types:

- Advantages of direct ELISA: high throughput, ability to detect low affinity antibodies.
- Advantages of indirect ELISA: oriented drug exposure, high through-put.
- Advantages of bridging ELISA: High throughput, high specificity, species independent.
- Advantages if time resolved fluorescence: broad dynamic range.
- Advantages of RIP Assay: high sensitivity, moderate to high through-put, solution phase.
- Advantages of SPR/Biacore: detection of low and high affinity antibodies, direct isotyping, solution phase, realt time dissociation, calibration free concentration analysis.
- Advantages of MSD electroluminescence; most sensitive for some compounds, less sample handeling, multiplexing.

If no antibodies are available as positive control, immunization of one or more animal species can be included in the method development.

### STEP 2: CHARACTERIZATION ASSAYS

In case binding antibodies are found and confirmed as such, they subsequently can be characterized using SPR/Biacore (isotype and affinity profiling) or an immunoassay (isotyping only). IgG, IgM and IgA including further subclasses can specifically be determined.

### STEP 3: NAb ASSAYS

Finally, the neutralizing properties of the binding antibodies can be investigated using a cell-based assay. Based on the biological activity of the drug, the appropriate bioassay will be selected. TNO has extensive experience with different type of bioassays, such as proliferation assays, cytotoxicity assays and reporter gene assays. An example of a bioassay in which the activity of a biotherapeutic and the effect of neutralizing antibodies can be analyzed is presented below. All assays can be validated and performed in accordance with the OECD Principles of Good Laboratory Practice and in line with the latest recommendations, white papers and/or guidelines of the FDA/EMEA on immunogenicity.

### EQUIPMENT

- Biacore T100 (GE Healthcare).
- Meso Scale Discovery (MSD) Sector Imager 2400.
- SPECTRAmax® M5 (Molecular Devices Ltd.).
- Victor2 Multilabel Counter (Wallac, PerkinElmer B.V.).
- Luminex, LINCOplex200.
- 1414 Scintillation Counter (Wallac, PerkinElmer B.V.).
- 1450 Microbeta Plus Scintillation Counter (Wallac, Perkin Elmer B.V.).

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