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Review

# Recommendations for the design, optimization, and qualification of cell-based assays used for the detection of neutralizing antibody responses elicited to biological therapeutics

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**Abstract**

The administration of biological therapeutics can evoke some level of immune response to the drug product in the receiving subjects. An immune response comprised of neutralizing antibodies can lead to loss of efficacy or potentially more serious clinical sequelae. Therefore, it is important to monitor the immunogenicity of biological therapeutics throughout the drug product development cycle. Immunoassays are typically used to screen for the presence and development of anti-drug product antibodies. However, in-vitro cell-based assays prove extremely useful for the characterization of immunoassay-positive samples to determine if the detected antibodies have neutralizing properties. This document provides scientific recommendations based on the experience of the authors for the development of cell-based assays for the detection of neutralizing antibodies in non-clinical and clinical studies.


*Keywords:* Neutralizing antibody bioassay; Cell-based assay; Serum-based bioassay; Immunogenicity assay; NAb assay

*Abbreviations:* BrdU, bromodeoxyuridine; CAT, chloramphenicol acetyl transferase; CV, coefficient of variance; ECL, electrochemiluminescence; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; EPO, erythropoietin; FACS, flow activated cell sorting; GFP, Green Fluorescent Protein; HSA, human serum albumin; Ig, immunoglobulin; IL-1, interleukin-1; KIRA, kinase induced receptor activation; MAb, monoclonal antibody; MGDF, megakaryocyte growth and development factor; mRNA, messenger ribonucleic acid; MTT, [3-(4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide]; NAb, neutralizing antibody; RIA, radioimmunoassay; PRCA, pure red cell aplasia; rHuEPO, recombinant human EPO; SPA, scintillation proximity assay; TNF, tumor necrosis factor.

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[1] The views expressed herein reflect the opinions of the author and not necessarily those of the FDA.

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## 1. Introduction

The development of antibodies to biological therapeutics upon their administration in study subjects is not an uncommon occurrence (Porter, 2000). On a case-by-case basis, the antibody response may be inconsequential or may have a dramatic effect on the efficacy and/or safety of the drug product if the antibodies alter drug pharmacokinetics or are of a neutralizing nature. Neutralizing antibodies (NAbs) block the biological activity of the therapeutic molecule by either binding directly to epitope(s) that lie within the active site of the therapeutic molecule or by blocking its active site by steric hindrance due to binding to epitope(s) that may lie in close proximity to the active site. While, in certain cases NAb presence may not result in a clinical effect, at sufficient NAb levels in other cases, a decrease in efficacy may be observed which may require administration of higher doses of the