Immunogenicity Assessment of Antibody Drug Conjugates in Cancer Studies

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Introduction

Antibody-drug conjugates (ADCs) are heterogeneous molecules composed of:

- An antibody (whole mAb or fragment) assuring the specificity
- A cytotoxic drug conferring the toxicity
- A stable chemical linker

ADCs are becoming increasingly important in oncology due to their specificity for tumor-associated antigens. With their heterogeneous composition, they pose novel challenges in their development. For example, the neo-epitopes formed in the mAb as a result of the conjugation may lead to a significant immune response in the patient population.

Since immunogenicity assessment is a requirement from regulatory agencies in the safety profile evaluation of biopharmaceuticals, it is critical to develop and validate adequate assays.

Here we show the challenges/solutions and special considerations when analyzing the immunogenicity of ADCs in Cancer Studies.

Analytical Methods

For immunogenicity assessment of therapeutic protein products, guidance documents (US FDA 2016) and publications (Shankar, 2008) describe strategies and development as well as validation of assays. A tiered approach often applied to monoclonal antibodies (mAB) could be adapted for ADC immunogenicity appreciation, with the following steps:

- Screening
- Confirmatory
- Titer
- Characterization assay
- Neutralizing assay

Additional assays could complete the immunogenicity evaluation such as Isotyping and Relative affinity assessment.

The stepwise immunogenicity assessment procedure is illustrated in Figure 1. Screening, confirmatory and characterization assays are presented in Figures 2A, 2B and 2C-2E respectively.

- The screening assay presented is a bridging assay, with pre-incubation in presence of conjugates for capture and detection.
- In the confirmatory assay, exogenous ADC added to samples competes for binding to conjugates used for capture and detection. Variation of percent inhibitions in a therapeutic-naïve population is taken into consideration for confirming a positive response.
- With the characterization assay, domain specificity is assessed by using ADC components (whole mAB; cytotoxic drug or linker) as inhibitors. Neo-epitopes formed in the mAb as a result of the conjugation are in this case not depicted. Molar ratio as well as drug-to-antibody ratio (DAR) should be considered to set up the concentrations of inhibitors.

Figure 1: Tiered approach for Immunogenicity Assessment

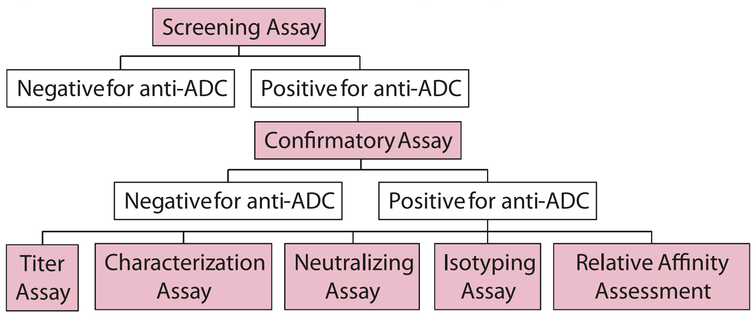
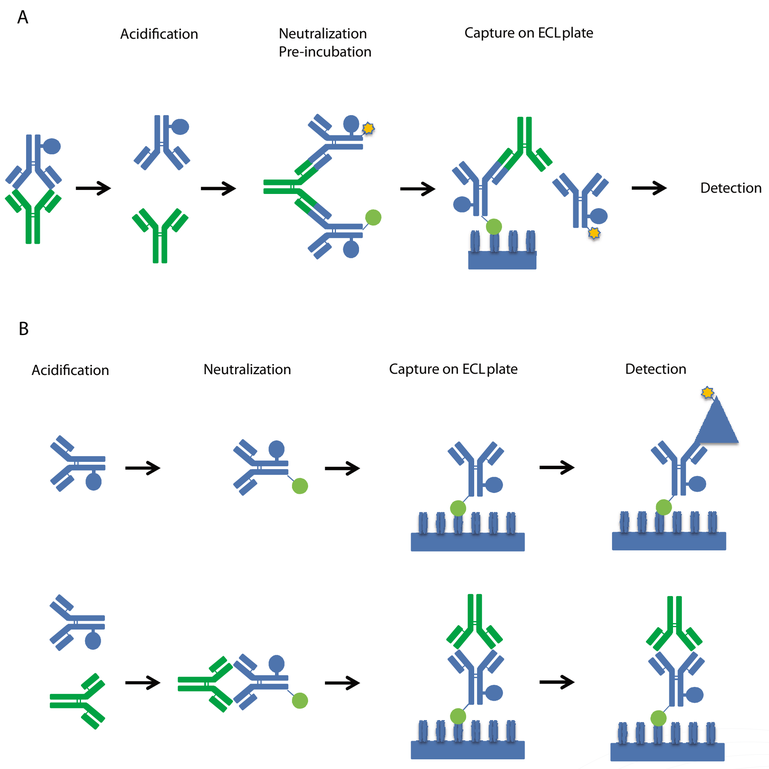
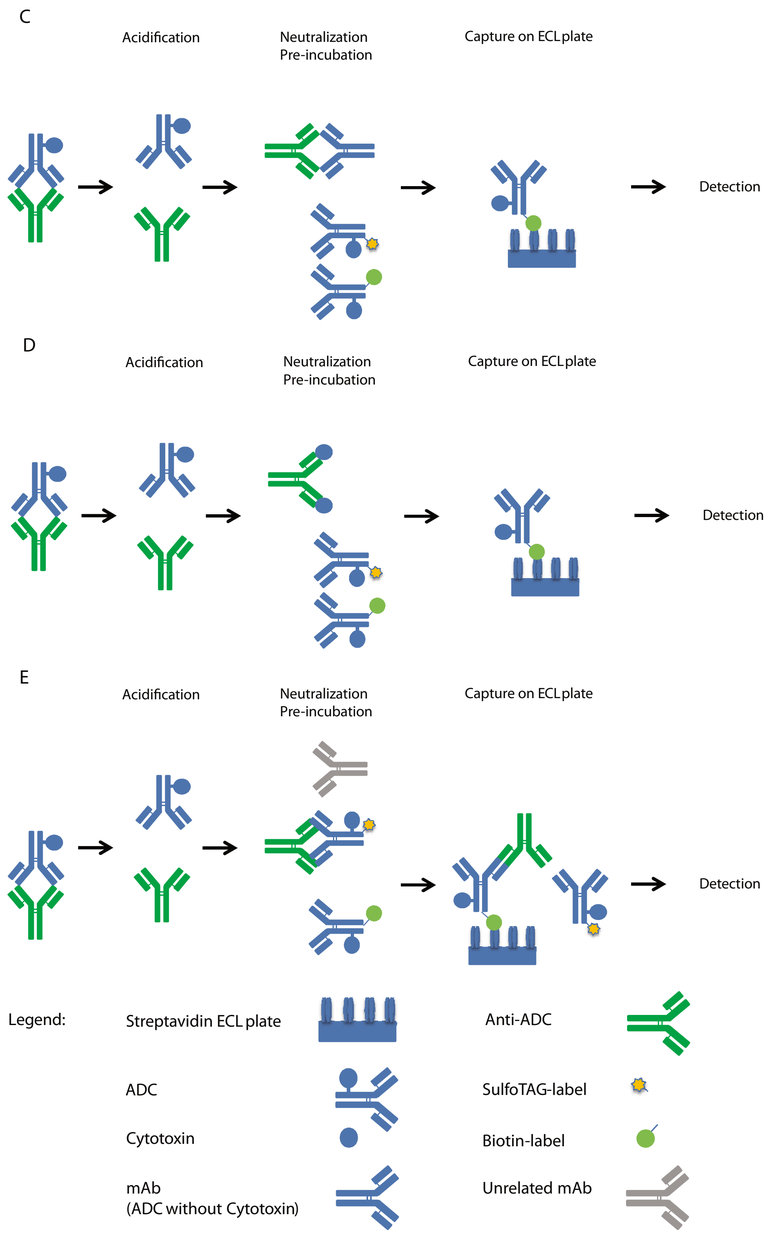


Figure 2: Assay Formats



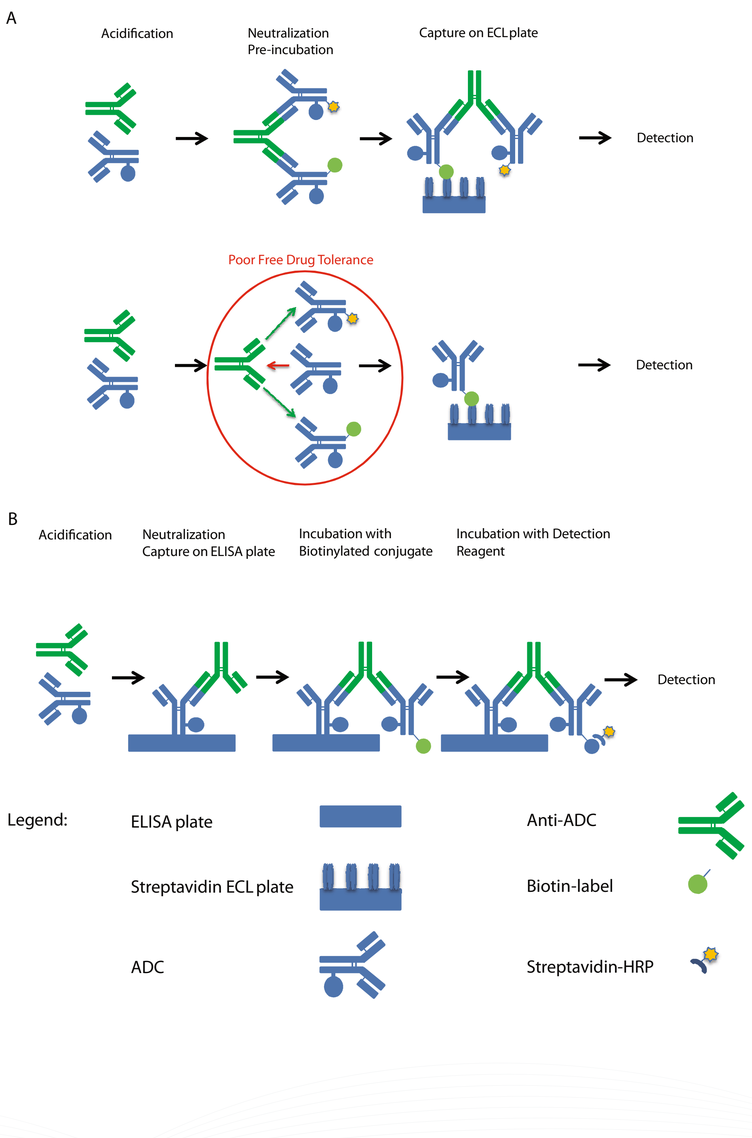


Analytical Challenges and Solutions

Case Study 1: Screening Assay

- Challenge: During the early feasibility phase of a method development to detect anti-ADC antibodies including a pre-incubation with conjugates on a deep-well plate, a poor free drug tolerance was found (Figure 3A).
- Solution: The format was reconsidered and neutralized samples were directly incubated on ADC coated plates. The free drug tolerance was improved (Figure 3B).

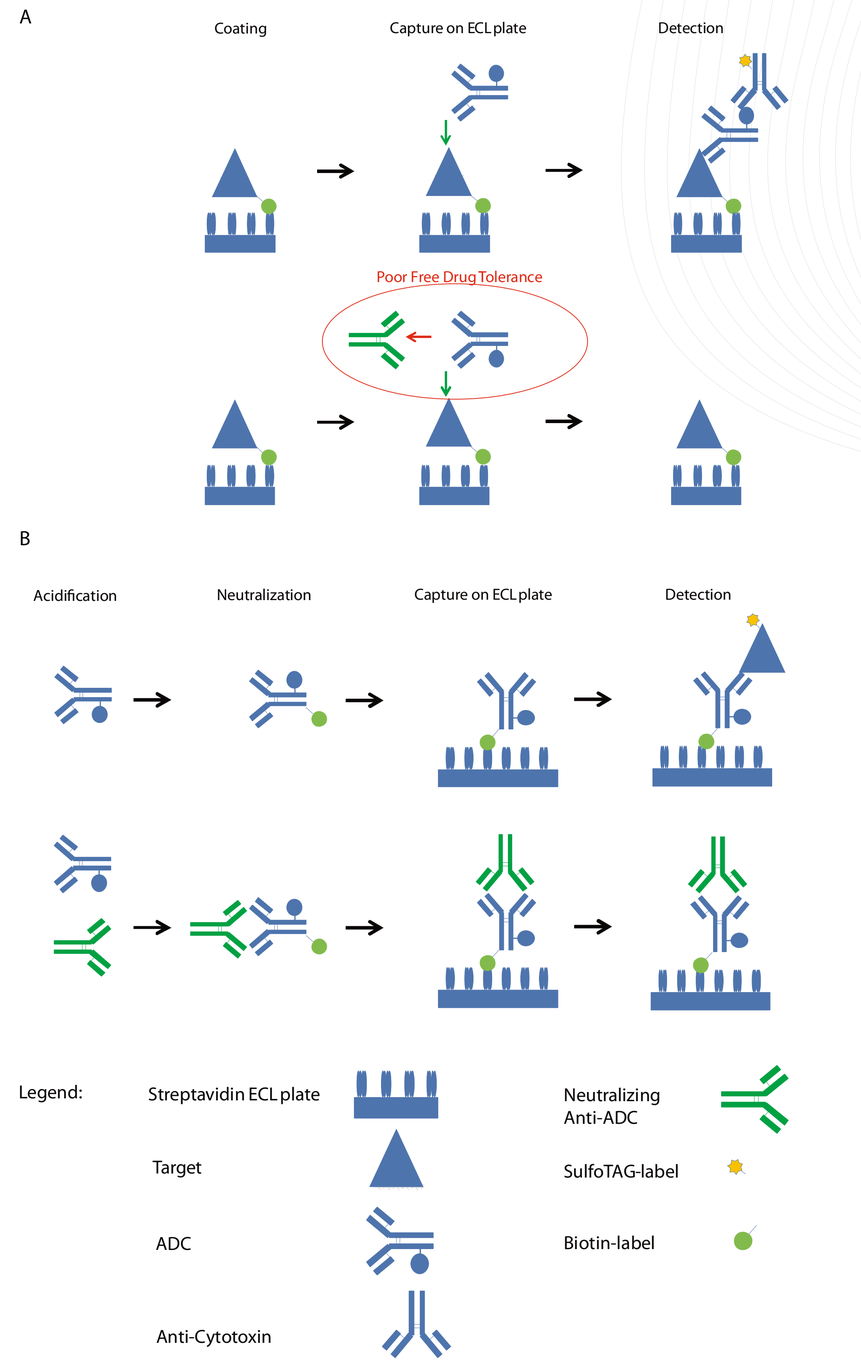
Figure 3: Screening Assay Optimization



Case Study 2: Neutralizing Assay

- Challenge: During the early feasibility phase of a method transfer to detect neutralizing antibodies using a competitive ligand binding assay, a poor free drug tolerance was assessed. The initial format required the spiking of each sample with a defined concentration of ADC (Figure 4A).
- Solution: The format was reconsidered and neutralizing antibodies were captured on streptavidin plates by biotinylated ADC. The free drug tolerance was improved. Moreover, a specific tuning was performed to optimize the labeling conditions of the target used for detection (Figure 4B).

Figure 4: Neutralizing Antibody Assay Optimization



Discussion

Immunogenicity Ligand Binding Assays for ADCs are more complex than for mAB and therefore require specific considerations which comprise:

- Choice of reference items
- Choice of inhibitors
- Labeling conditions
- Immunogenic reactivity of key reagents
- Linker chemistry
- Outlier(s) elimination for cut point determination

Conclusion

Ligand binding assays for ADCs present specific bioanalytical challenges. They are addressed successfully by optimizing the following steps:

- Definition of the approach
- Selection of the Assay format
- Assay development

The Immunogenicity and Pharmacokinetic (PK) assays developed at Celerion Switzerland AG are outstanding tools for early clinical development of ADCs with improved reliability, maximal reproducibility and robustness.

References

- Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products. Guidance for Industry. Draft Guidance. US FDA 2016
- Shankar G et al., Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal. 2008 Dec 15;48(5):1267-81

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