Overcoming drug & target interference in ADA and nAb assays

Robert Nelson Ph.D. MRQA
Head of Bioanalytical Laboratory, Novimmune SA
Presentation Outline

- Case Study 1
  - Drug & target interference in a clinical anti-drug antibody (ADA) assay

- Case Study 2
  - Drug & target interference in a clinical neutralizing antibody (nAb) assay
Case Study 1
Drug & target interference in a clinical anti-drug antibody (ADA) assay

- **Background**
  - Monoclonal antibody (mAb) therapeutic with a target in inflammatory disease
  - Dosing levels in mg/kg range
  - Dimeric soluble target
Case Study 1: ADA Assay Considerations

- Anticipate drug levels based on dosing regimen

- Predict target levels
  - Access to healthy volunteer samples
  - Limited access to patient samples
  - Accumulation of target in presence of drug
    - Literature
    - Exploratory data
    - PK-PD modelling
Case Study 1: ADA Assay Development

- **Bridging format**
  - Gyrolab platform (Bioaffy 200 nL CD)

![Diagram](image)

- Sample
  - Target depletion
  - Acid dissociation
  - ADA assay

- Labelled Drug
- ADA
- Biotin Drug
Case Study 1: ADA Assay Development

- **Bridging format**
  - Gyrolab platform (Bioaffy 200 nL CD)

- **Acid dissociation**
  - 300 mM acetic acid
  - Drug tolerance of 20 µg/mL

![Diagram of ADA:Drug complexes](image)

False negative results
Case Study 1: ADA Assay Development

- **Bridging format**
  - Gyrolab platform (Bioaffy 200 nL CD)

- **Acid dissociation**
  - 300 mM acetic acid
  - Drug tolerance of 20 µg/mL

- **Target Depletion**
  - Target tolerance of 50 ng/mL

Labeled Drug:Target complexes

- Sample
- Target depletion
- Acid dissociation
- ADA assay

False positive results

Robert Nelson, EBF Immunogenicity Focus Workshop
Lisbon, September 2016
For further details see:

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 5069678, 15 pages
http://dx.doi.org/10.1155/2016/5069678

Research Article
Evaluation of Multiple Immunoassay Technology Platforms to Select the Anti-Drug Antibody Assay Exhibiting the Most Appropriate Drug and Target Tolerance


Novimmune SA, 14 Chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland
Case Study 1: ADA Assay Development

- **Target Depletion Protocol**
  - Anti-target antibody **non-competitive** with drug
  - **Solid phase extraction (SPE)** with magnetic beads
  - Anti-target Ab **competitive** with drug added to capture-detection mixture
Case Study 1: ADA Assay Development

- Target Depletion Protocol
  - Target interference addressed effectively
Case Study 2
Drug & target interference in a clinical neutralizing antibody (nAb) assay

- **Background**
  - Monoclonal antibody (mAb) therapeutic with a target in inflammatory disease
  - Dosing levels in mg/kg range
  - Soluble target
Case Study 2: nAb Assay Considerations

- **Cell-based nAb assay**
  - Most appropriate format based on mechanism of action
  - Matrix interference

- **Drug interference**
  - May be complexed with nAb (false negative)
  - May cause shift in cell-based assay parameters

- **Target interference**
  - May cause shift in cell-based assay parameters
Case Study 2: nAb Assay Considerations

- Cell-based nAb assay

Adapted from Shuai & Liu, Nature Reviews Immunology 3, 900-911 (2003)
Case Study 2: nAb Assay Considerations

- Cell-based nAb assay

Adapted from Shuai & Liu, Nature Reviews Immunology 3, 900-911 (2003)

EC₅₀: Approx. 0.1 nM (3.3 ng/mL)
Case Study 2: nAb Assay Considerations

- Cell-based nAb assay

Adapted from Shuai & Liu, Nature Reviews Immunology 3, 900-911 (2003)

IC$_{50}$: Approx. 0.4 nM (60 ng/mL)
Case Study 2: nAb Assay Considerations

- Cell-based nAb assay

Adapted from Shuai & Liu, Nature Reviews Immunology 3, 900-911 (2003)
Case Study 2: nAb Assay Development

- **Drug and Target Depletion Protocol**

  - **Acid dissociation**
    - Dissociate nAb
    - Denature target

  - **Neutralization & depletion**
    - Remove drug (anti-idiotypic)

  - **Separation**
    - Isolate nAb
    - Remove target (MW cut-off)
    - Buffer exchange

  - **nAb assay**
    - Immunogenic response: neutralizing or non-neutralizing
Case Study 2: nAb Assay Development

- **Without** Drug and Target Depletion Protocol
  - Low levels of drug interfere with nAb detection

3.3 nM = 500 ng/mL, 10 nM = 1500 ng/mL, 33.3 nM = 5,000 ng/mL, 133.3 nM = 20,000 ng/mL
Case Study 2: nAb Assay Development

- **With** Drug and Target Depletion Protocol
  - Sensitivity of assay retained

![Graph showing luminescence levels](image)

3.3 nM = 500 ng/mL, 10 nM = 1500 ng/mL, 33.3 nM = 5,000 ng/mL, 133.3 nM = 20,000 ng/mL
Case Study 2: nAb Assay Development

- With Drug and Target Depletion Protocol
  - Drug interference greatly reduced

3.3 nM = 500 ng/mL, 10 nM = 1500 ng/mL, 33.3 nM = 5,000 ng/mL, 133.3 nM = 20,000 ng/mL
Thank you for your attention!

rnelson@novimmune.com