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PandA-monium: are we too tolerant in ADA method development?

EBF Open Symposium - Drug Tolerance Session

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Introduction

ADA methods

A variety of ADA methods are available, each with their own performance characteristics

Currently, the bridging method is widely used as the gold standard

- Although superior to antigen-capture, the drug tolerance can be improved by applying pretreatment steps
  - acid dissociation, bead- and surface-based extractions, and other immunoglobulin enrichment techniques.

The precipitation and acid dissociation (PandA) method is a more recent addition to the ADA method toolbox.

- Makes use of the precipitation properties of polyethylene glycol (PEG), followed by an acid dissociation step to coat free ADA
- Specifically developed for its high drug (and target) tolerance
  - often required for toxicokinetic studies.

Introduction

PandA method

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A breakthrough novel method to resolve the drug and target interference problem in immunogenicity assays

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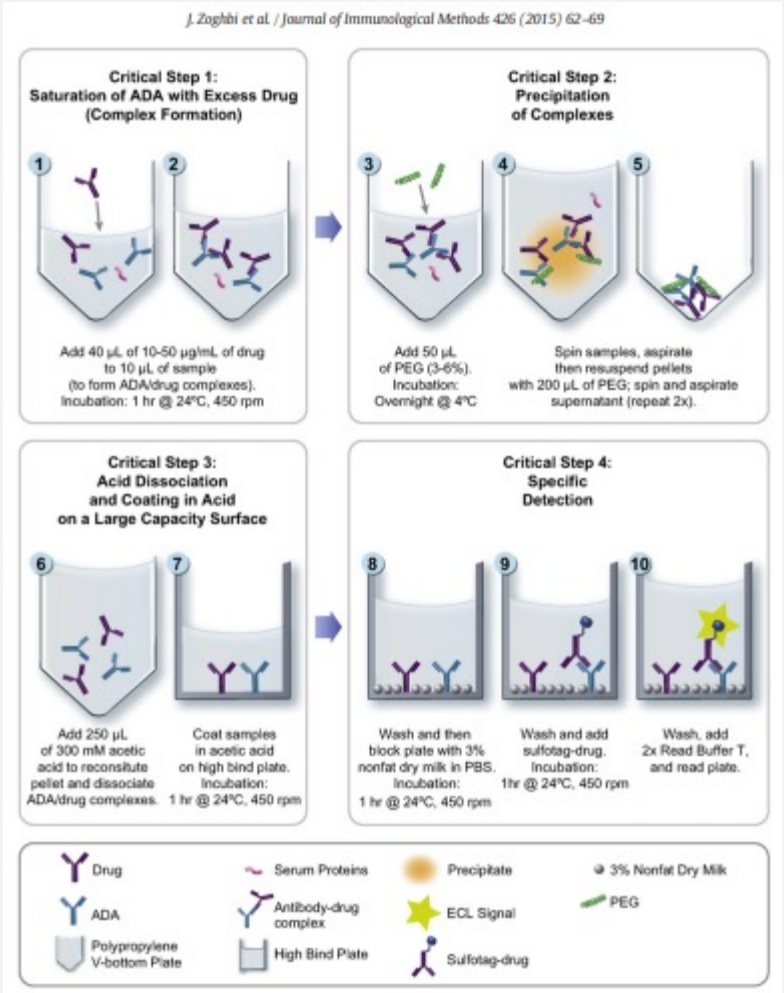


PandA method at B&I Ghent

Recently, most ADA methods developed at Sanofi are PandA methods

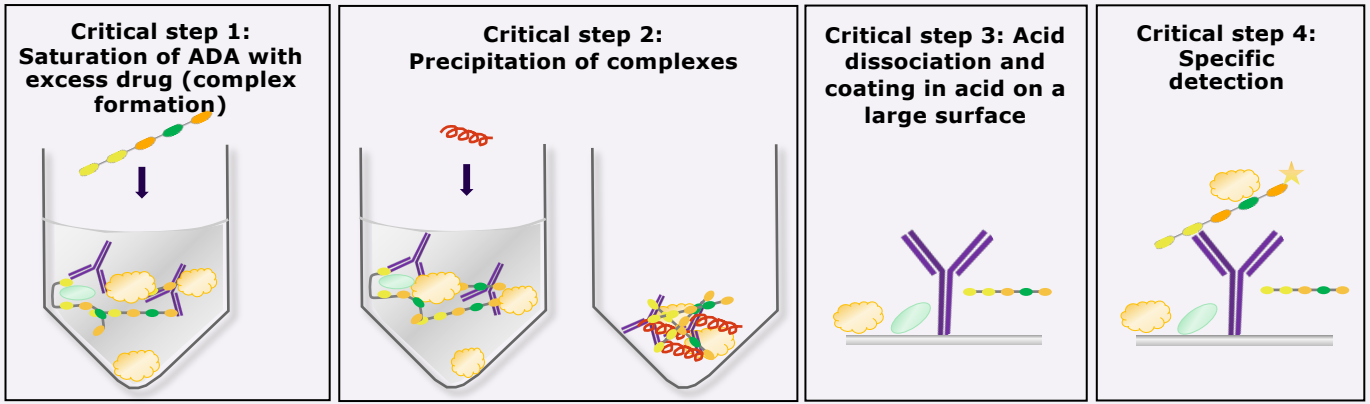
- Significant efforts went into the characterization and optimization of the PandA method for use with NANOBODY® molecules at B&I Ghent
  - Critical parameters were identified and optimized, assay steps simplified and streamlined
  - Availability of a default method that can be used in a plug-and-play fashion for different drug modalities
    - Optimization of a method with limited number of development runs (~3)
    - Drug and target tolerant with robust performance
    - Also, more experience was gained at CROs for development/transfer

- PandA is easily selected as the default method, especially for non-clinical assays, when high drug tolerance is required.
  - Extended to all biologics
- However, concern remains for the incubation with acid (≥ 60 minutes) which might denature some ADAs and contribute to a lower dynamic range.

B&I Ghent: bioanalysis and immunogenicity Ghent, DMPK

PandA method at B&I Ghent

Optimized for NANOBODY® drug compounds

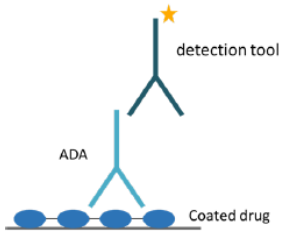
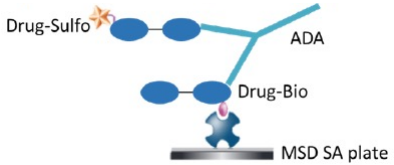


- Optimal PEG concentration needs to be evaluated
- Acids typically used
  - 300 – 500 mM Acetic Acid, 300 mM most common, pH ranges from 2.5 – 3.5
  - Glycine-HCL: 10 mM, pH 3.0; 50 mM Glycine, pH 2.5; 300 mM; 400 mM pH 2.3

Overview ADA methods to be used in support of non-clinical/clinical studies

POC studies

Combined PK-Safety/Toxicology/clinical studies
→ **selection preferably based on required drug/target characteristics**

	Direct binding (ELISA/MSD)	Bridging ADA (MSD)	PandA (MSD)
Assay format			ADA/drug complex with PEG acid 
PRO	• Detection of intramolecular ADA binding • Species-specific detector → No labeling of drug → fast start of development	• Sensitive • All isotypes are detected • Pre-treatments to improve DT	• Sensitive • All isotypes are detected • High drug tolerance
CON	• Detection of specific isotypes → For clinical trials all isotypes are required • Target/drug interfere • Aspecific binding needs to be assessed	• Labeled drug (2) as detector • Target/drug interference possible • No detection of intramolecular ADA binding (reduced sensitivity)	• Labeled drug (1) as detector • Target interference possible • Use of acid, relatively harsh conditions

Legend: ADA: anti-drug antibodies; PandA: Precipitation and acid dissociation; POC: proof of concept; DT : drug tolerance

Case 1

Project details – non-clinical + clinical assay

Tetravalent NANOBODY® molecule with half-life extention (HLE)

HSA binding domain→ HLE in serum

- target accumulation possible

IO indication: very low drug levels are expected

- Limited drug tolerance needed: 0.5 µg/mL

Legend: HLE: half-life extention; HSA: human serum albumin; IO: immuno-oncology

Case 1

Development & qualification

Bridging format selected

- Based on sensitivity, limited drug tolerance requirements and extensive development experience with this format

Development
Typical parameters:

- Assay diluent, MM, MRD
- Sensitivity, drug- and target tolerance, matrix interference

- Extensive drug labeling optimisation (detectors)
  - Background signal (% inhibition linked to sulfo ME); free drug fraction/overlabelling bio
  - Drug-bio: 5 – **7** – 10 ME label
  - Drug-sulfo: **2** – 3 – 5 – 10 ME label

- Development took ~3 weeks

Legend: MM: master mix concentration ; MRD: minimal required dilution ; ME: molar excess

Case 1

Communication from project team: change in dosing regimen → need for higher drug tolerant assay

- Bridging assay was not sufficiently drug tolerant
- Inceased drug tolerance required for future NHP studies: >2 mg/mL

→ Development of a **PandA ADA method** to support ongoing and planned NHP study
- Starting from the default PandA method conditions
- Fit-for-purpose assay ready for qualification in less than a week

Run	Development run 1	Development run 2	Development run 3
Parameters	Screen individuals in default PandA method	PEG and acid optimization	Test of different blocking buffers

Assay strategy for the first clinical trial:
- Development of a drug tolerant bridging method failed
  - Acid dissociation, ionic strength and heat pretreatment were explored

Case 1

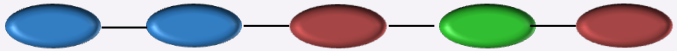
Method characteristics

Parameter	NHP - Bridging	NHP - PandA	Human - PandA
Floating SCP → NF	1.613	1.261	1.081
CCP	NA	NA	21.76 %
Sensitivity (affinity)	500 ng/mL (LPC; mAb)	500 ng/mL (LPC; mAb)	100.0 ng/mL (LPC; pAb) LOD According to 99% consistency: 91.2 ng/mL
Intra-assay precision (screening assay)	HPC: maximum 8.7 % LPC: maximum 4.8 % NC: maximum 8.6 %	HPC: maximum 8.0 % LPC: maximum 9.6 % NC: maximum 8.6 %	HPC: maximum 17.1% LPC: maximum 10.2% NC: maximum 9.4%
Intra-assay precision (confirmatory assay)			HPC: maximum 5.0% LPC: maximum 7.8%
Inter-assay precision (screening assay)	Normalized HPC: 21.1 % Normalized LPC: 11.6 % NC: 5.3 %	Normalized HPC: 16.1 % Normalized LPC: 13.9 % NC: 8.6 %	Normalized HPC: 10.9% Normalized LPC: 5.8% NC: 8.6%
Inter-assay precision (confirmatory assay)	NA	NA	HPC: 0.3% LPC: 9.5%
Drug tolerance (LPC)	10.0 µg/mL	10 mg/mL (max tested)	1.0 mg/mL (max tested)
Target tolerance	500 ng/mL (FN) 250 ng/mL (FP)	500 ng/mL (FN) 50 ng/mL (FP)	400 ng/mL

SCP: screening cut-point; NF: normalisation factor; CCP: confirmatory cut-point; LPC: low positive control; NC: negative control; NA: not applicable; FN: false negative; FP: false positive

Case 2

Project details – clinical assay



Pentavalent NANOBODY® molecule with HLE

HSA binding domain→ HLE in serum

- target accumulation possible
  - Soluble and monomeric targets: no interference expected

Required drug tolerance for FIH

- Anticipated Cmax of ~75 µg/mL

Legend: FIH: first-in-human

Case 2

Development & qualification

Development of a **bridging format selected** based on

- Sensitivity
- Reach required drug tolerance by pretreatment, if needed

Development

Typical parameters:

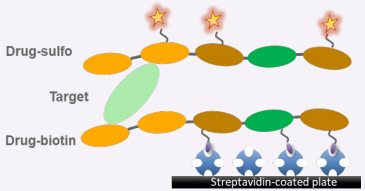
- Assay diluent, MM, MRD
- Focus on assay sensitivity, drug tolerance, matrix interference
- 10x molar excess of biotin and sulfo-tag selected
- MRD: 25 - **50** - 100
- MM: 1 - **2** - 4 µg/mL (Nb-bio and Nb-sulfo)
- With and without 50 µg/mL drug (drug tolerance)

Development took ~2 weeks

- No issues encountered

Legend: MM: master mix concentration ; MRD: minimal required dilution

Case 2

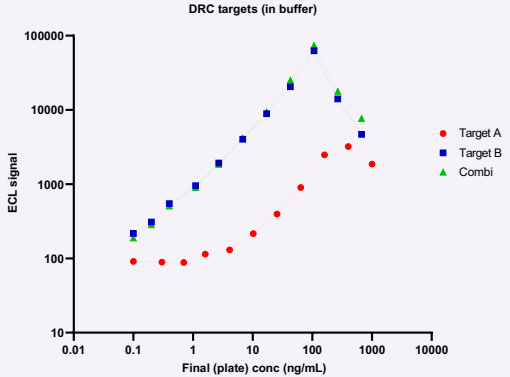


Target interference

Target interference was assessed during development

- Expected to be a formality due to the monovalency of the targets

- Maximum simulated target levels during FIH were:
  - target A: 250 ng/mL
  - target B: 300 ng/mL



- Test DRC of each target (in buffer, see figure)
- At max conc (individual and combined) in PC samples
  - PC = pAb and mAb for each target

- False positive signal generated!

- Investigation:
  - No aggregation of the targets
  - The target-binding building blocks are not identical, bind different epitopes on target

  → possibility that **1 target** is bound by **2 drug molecules**

Legend: DRC: dose response curve ; PC: positive control ; FIH: first-in-human

Case 2

Evaluation of pretreatments

The team preferred to test pre-treatments in the bridging assay first, before considering a PandA method

Pre-treatment options to solve target interference:

- Acid and heat pretreatments explored

Selection based on destruction of both targets

- Using a panel of pAb and mAbs

~2 weeks of intensive additional assay development

Ultimately, the **bridging method with pretreatment failed and PandA method was developed**

Case 2

PandA method

A drug- and target tolerant PandA method was developed and qualified (ready for transfer) in 2-3 weeks

- Starting from the default PandA method conditions
- 3 optimisation runs: PEG, drug and confirmatory drug

A lot of time and resources could have been saved if PandA method was developed immediately!

Parameter	Human PandA
Floating SCP → NF	SCP Healthy => Additive SCPF (5% false positives) = 5.0187 SCP diseased => multiplicative SCPF (5% false positives) = 1.081
CCP	CCP Healthy (1% false positives) = 13.73% CCP Diseased (1% false positives) = 13.28%
Sensitivity	100 ng/mL (LPC; affinity purified pAb) Healthy: individual interpolated LOD values are ≤ 15.7 ng/mL. Diseased: LOD According to 99% consistency: 23.0 ng/mL
Intra-assay precision (screening assay)	HPC: maximum 12.7% LPC: maximum 7.8% NC: maximum 4.0%
Intra-assay precision (confirmatory assay)	HPC: maximum 4.6% LPC: maximum 3.4%
Inter-assay precision (screening assay)	Normalized HPC: 11.1% Normalized LPC: 7.8% NC: 4.7%
Inter-assay precision (confirmatory assay)	Normalized HPC: 0.1% Normalized LPC: 7.0%
Drug tolerance (LPC)	300.0 µg/mL (highest tested conc)
Target tolerance	Target A: 250 ng/mL Target B: 300 ng/mL

ADA method selection strategy

1. Select format based on expected drug level
- Consider homogeneous MSD bridging, SPEAD or ACE format if DTL < 500 µg/mL
- If DTL >500 µg/mL, develop PandA

2. When developing a PandA method
- evaluate the appropriateness of the PEG concentration by analyzing PC samples with decreasing PEG concentrations
- Apply high drug spiking concentration to enable analysis of high ADA levels
- Test different acid types and concentrations, use mildest condition possible (check pH, pH at 3.0 should be sufficient)

Thoughts on this approach:
Bridging method has a drug tolerance of maximally low µg/mL, not even 100 µg/mL
Proposed alternative methods: bridging, BEAD, SPEAD (1 acid step), ACE (2 acid steps)
- Acids typically used: glycine, citric acid and acetic acid; pH 2.5 or 3.0
- Can be more cumbersome than PandA method
- Just as susceptible to missing low-affinity ADA

Legend: DTL: drug tolerance limit; PC: positive control; PEG: polyethylene glycol

Summary

The PandA method has been extensively characterised and optimised making it possible to develop a sensitive, drug- and target tolerant ADA assay in a plug-and-play fashion in a limited number of development runs.

- The optimized method has streamlined assay steps, with complex steps removed, no exotic reagents and has a robust assay performance
- Valuable addition to the list of ADA methods

Given the limited knowledge on the required drug tolerance at the start of assay development and the often very tight project timelines, people prefer to develop a PandA method over the classical bridging ADA method (even when DTL< 500 µg/mL)

- The most cost and time effective route
- High drug- and target tolerance to accomodate changes in required drug tolerance between start of assay development and start of the study
  - Bridging assays have small tolerance margins
- Less of a risk for non-clinical assays

All these things make the PandA method the go-to format for non-clinical methods at B&I Ghent

Acknowledgements

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Global Bioanalysis & Immunogenicity Team!

Thank you

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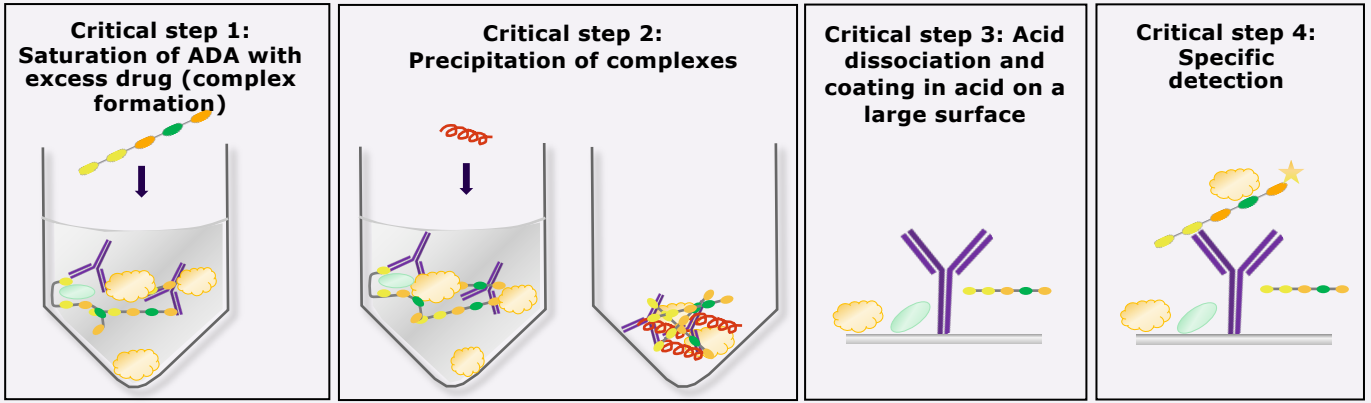
PandA method

PandA method optimized for NANOBODY® drug compounds



Add 20 µL serum sample + 80 µL drug (12.5 µg/mL) in PBS/0.1%Cas

Incubate 1h at RT while shaking (600 rpm)

Add 100 µL of cold 6% PEG to all samples.

Cover plate and incubate overnight at refrigerator temperature.

Spin down at cooled conditions (4°C) at 2916 g.

Flick away supernatant.

Add 300 µL cold 3% PEG, wash 5 min at RT on a shaker (750 rpm), perform cold spin (20 min at 2916 g) and flick away supernatant.

Repeat wash + spin once.

After flicking, add 100 µL of 300 mM acetic acid (pH 2.5) to reconstitute the pellet.

Perform 1/10 dilution in acid

Coat 25 µL on a high bind plate.

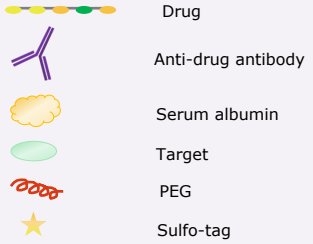
Incubate 1h at RT while shaking

Wash and add 150 µL 3% Amersham ECL blocking agent.

Incubate 1h at RT while shaking.

Wash and add 50 µL detection solution (Drug-Sulfo + HSA (+ 200 µg/mL Drug for confirmatory purposes), in 3% Amersham ECL blocking agent).

Wash and add 150 µL 2x Read Buffer T and read plate.



A breakthrough novel method to resolve the drug and target interference problem in immunogenicity assays; J Immunol Methods. 2015 Nov;426:62-9; Jad Zoghbi *et. al.*

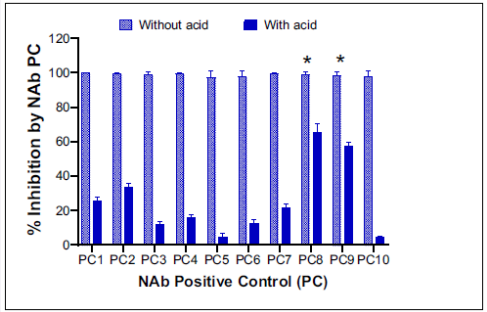
Impact of acid on ADA

Guidance for acid selection

- Common acid concentrations for other methods might be too harsh due to >60 min incubation
  - In other methods acid is added to the matrix, which buffers the acid already

- Try different acids, pH and concentrations e.g.:
  - 100 – 300 mM Acetic Acid
  - 50 – 200 mM Lactic Acid
  - 100 - 200 mM Glycine-HCL, pH 2.3-3.0
- Used mildest condition possible

Acid stability test: PCs were treated at extreme conditions with 600 mM acetic acid and incubated at 37°C for 60 min, then neutralized and tested



Acid-sensitive and acid-stable ADAs were incubated with different acids with varying concentrations and pH for 15 min. Concentrations shows the obtained sensitivity.
30 min incubation time had lower assay sensitivity (data not shown).

	Acid-sensitive PC		Acid-stable PC	
	PC1 (ng/mL)	PC2 (ng/mL)	PC3 (ng/mL)	PC4 (ng/mL)
200 mM glycine pH 2.0	2500	2500	625	156
200 mM glycine pH 2.3	625	156	313	156
200 mM glycine pH 2.5	313	156	313	156
150 mM glycine pH 2.3	2500	156	313	156
150 mM glycine pH 2.5	313	156	625	156
100 mM glycine pH 2.3	313	156	313	156
100 mM glycine pH 2.5	156	156	313	156
300 mM acetic acid	313	156	313	156
50 mM lactic acid	156	156	156	156
100 mM lactic acid	156	156	156	156
150 mM lactic acid	313	156	625	156
200 mM lactic acid	1250	156	313	156

Wickramarachchi D et al. A novel neutralization antibody assay method to overcome drug interference with better compatibility with acid-sensitive neutralizing antibodies. AAPS Journal. 2023; 25:18