HELP: A novel immunogenicity method to improve drug tolerance of Monoclonal Antibody Therapeutic

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Overview

- Background
- Assay formats and challenges
- Developed assay
- ADA and Nabs lessons
- HELP Method
Background

- OKT3 was the first monoclonal antibody therapeutic to be approved in 1985
- Murine antibody which was used as an immunosuppressant drug given to reduce acute rejection in patients with organ transplants
- Highly immunogenic
- Led to improvement and creation of fully humanised antibodies
- Fully humanised antibodies can still elicit ADA in some patients
MSD Semi Homogenous Bridging Format

- **Advantages**
  - Improved sensitivity and larger dynamic range
  - Improved drug tolerance

- Method transferred to us lacked the required drug tolerance
Bridging Format in the presence of drug with no acid dissociation
Bridging Format in the presence of drug with acid dissociation
Master Mix assessment

- The transfer method used 2 µg/mL of biotinylated drug and 2 µg/mL of ruthenium labelled drug.
- Increase to 4 µg/mL of biotinylated drug and 4 µg/mL of ruthenium labelled drug did not compromise the assay sensitivity.
Acid dissociation assessment

- Glycine improved the drug tolerance to the required level of \(~100 \mu g/mL\).
Acid dissociation

- Glycine HCl improved the drug tolerance but lacked assay reproducibility
Nabs assay labelled drug optimisation

Nabs for ligand binding assay Sulfo-Tag drug optimisation

![Diagram of Nabs assay and labelled drug optimisation](image.png)
Nabs assay in the presence of PC

![Graph showing Nabs for ligand binding assay](chart.png)

- **Sulfo-tag-Drug**
- **Bio-Target**
- PC

**Graph Details:**
- **Y-axis:** RLU Response
- **X-axis:** Sulfo-Tag Drug/Nabs Concentration ng/mL
- **Legend:**
  - Blue line: Sulfo-Tag Drug
  - Red line: Nabs
Lessons from the Nabs assay

• Nabs assay are more susceptible to the drug as the assay format only uses lower concentration of drug if it is cell based assay or lower concentration of labelled drug if it a ligand binding assay
• Drug depletion or competition approaches needs to be considered
• Drug depletion using the target
• Excess biotinylated drug competition
• However, each of these two method have there own complication.
Drug Depletion using the target complication

Neutralising PC can bind to the second binding site of the drug as much as the target

Possible outcome 01: No loss in assay sensitivity in Nabs assay.

Possible outcome 02: Loss in assay sensitivity in Nabs assay.
Drug Depletion using excess Bio drug complication

Pre existing drug also will compete to binding to the PC. Therefore, excess of biotinylated drug needs to be added to over compete the pre-existing drug.
Lessons from the Nabs assay

- While trying to solve the matrix interference in the Nabs assay where samples were heated at 56°C for 30 minutes.
- An improvement in drug tolerance was also observed.
- Sample heat treatment followed by an acid dissociation step was further carried out in the ADA assay.
- The assay sensitivity was not compromised, and drug tolerance was improved.
- We named it ‘HELP’ method, which stands for ‘Heat Enhanced Low pH’.
**HELP Method assay procedure**

1. Prepare samples and incubate at 37°C for 30 min.
2. Heat inactivate samples in transfer plate by incubating at 56°C for 30 min, shaking at 250 rpm.
3. Perform a 10-fold acid dissociation with 300 mM acetic acid in new transfer plates.
4. Incubate plate at RT for 1hr at 100 rpm.
5. Add 35 µL of acid dissociated samples to a new transfer Plate.
6. Add 35 µL of MMX to the samples and then Incubate.
7. Incubate at RT overnight shaking at 100 rpm.
Drug tolerance using HELP Method

- Drug tolerance improved up to 25 fold compared to both methods where acetic acid and Glycine HCl were used alone.
Summary of the method developed

<table>
<thead>
<tr>
<th>Sample Matrix</th>
<th>Human Serum</th>
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<tbody>
<tr>
<td>Minimum required dilution</td>
<td>10 fold in 300 mM acetic acid</td>
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<tr>
<td>Positive control Antibody</td>
<td>Polyclonal anti-Drug antibody</td>
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<table>
<thead>
<tr>
<th>PC samples</th>
<th>HPC= 10000 ng/mL</th>
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<tbody>
<tr>
<td></td>
<td>MPC= 250 ng/mL</td>
</tr>
<tr>
<td></td>
<td>LPC= 100 ng/mL</td>
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<tr>
<td></td>
<td>NC= Pooled human serum</td>
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<table>
<thead>
<tr>
<th>Preliminary assay sensitivity</th>
<th>PCs and selectivity data generated demonstrated that the assay is sensitive to detect 100% of samples spiked with 100 ng/mL positive control.</th>
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</thead>
<tbody>
<tr>
<td>Intra-assay precision for PC samples (%CV)</td>
<td>Precision based upon raw instrument responses were within 6.5, 7.0, 9.9 and 6.6 %CV for HPC, MPC, LPC and NC respectively.</td>
</tr>
<tr>
<td>Inter-assay precision for PC samples (%CV)</td>
<td>Inter assay precision based upon raw instrument across all batches for the HPC, MPC, LPC, and NC responses were 10.5 , 11.7, 15.5 and 22.7 %CV respectively</td>
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<tr>
<th>Preliminary Screening Cut Point Factor (SCPF)</th>
<th>30</th>
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<tbody>
<tr>
<td>Preliminary Confirmatory Cut Point (CCP)</td>
<td>26.2</td>
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<tr>
<td>BPS804 drug Interference</td>
<td>Samples containing 10000 ng/mL, 250 ng/mL and 100 ng/mL positive control antibody showed tolerance to all concentrations of drug assessed (up to 2.5 mg/mL).</td>
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</tbody>
</table>
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