Magnetic Bead–Based Isolation Enables Kinetic Characterization of HLA-Specific Antibodies by SPR and BLI

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Abstract

Purpose
To evaluate an integrated workflow combining LABScreen™ SAB, One Lambda™ MagSort™, BLI, and SPR for functional characterization of HLA-specific antibodies beyond conventional MFI measurements.

Methods
HLA-specific antibodies were isolated from serum samples using MagSort™ HLA-coated magnetic beads. Purified antibodies were analyzed by BLI to compare dissociation behavior among samples with similar SAB MFI and by SPR (Carterra LSA) to characterize interactions across multiple HLA alleles.

Results
MagSort™ isolation generated HLA-specific antibody preparations suitable for BLI and SPR analysis. BLI revealed differences in dissociation behavior among samples with comparable SAB MFI, while SPR demonstrated distinct kinetic profiles across multiple HLA alleles. These findings support complementary roles for BLI in kinetic screening and SPR in detailed allele-specific characterization.

Introduction

HLA-specific antibodies are routinely assessed using LABScreen™ Single Antigen Bead (SAB) assays, which provide sensitive detection of HLA-specific antibodies and assessment of antibody reactivity based on mean fluorescence intensity (MFI). However, MFI does not directly characterize the stability or kinetics of antibody–HLA interactions.

Real-time biosensor technologies, including biolayer interferometry (BLI) and surface plasmon resonance (SPR), can provide complementary information on antibody association and dissociation behavior. Application of these technologies to HLA-specific antibodies in serum is challenging because abundant non-specific serum components can interfere with biosensor measurements.

One Lambda™ MagSort™ is a research product containing HLA-coated magnetic beads that may help isolate HLA-specific antibodies from serum prior to kinetic analysis. Here, we evaluated an integrated research workflow combining LABScreen™ SAB, MagSort™, BLI, and SPR to extend conventional antibody detection with functional characterization of antibody binding behavior.

Materials and methods

Serum Sample Selection

Serum samples containing HLA-specific antibodies were initially characterized using **LABScreen™ Single Antigen Bead (SAB)** assays. Samples demonstrating comparable MFI toward selected HLA alleles were selected for downstream kinetic analysis.

One Lambda™ MagSort™ Antibody Isolation

HLA-specific antibodies were selectively isolated from serum samples using the **One Lambda™ MagSort™** research product HLA-coated magnetic beads. Following adsorption, non-specific serum proteins were removed by washing, and purified antibodies were eluted for biosensor analysis.

Biolayer Interferometry (BLI)

Purified antibodies were analyzed using BLI to compare antibody dissociation behavior among serum samples targeting the same HLA allele. BLI was evaluated as a platform for comparative kinetic screening of HLA-specific antibodies.

Surface Plasmon Resonance (SPR)

SPR (Carterra LSA) was used to characterize antibody interactions across selected HLA alleles. SPR was evaluated as a platform for detailed kinetic characterization across multiple HLA alleles.

Data Analysis

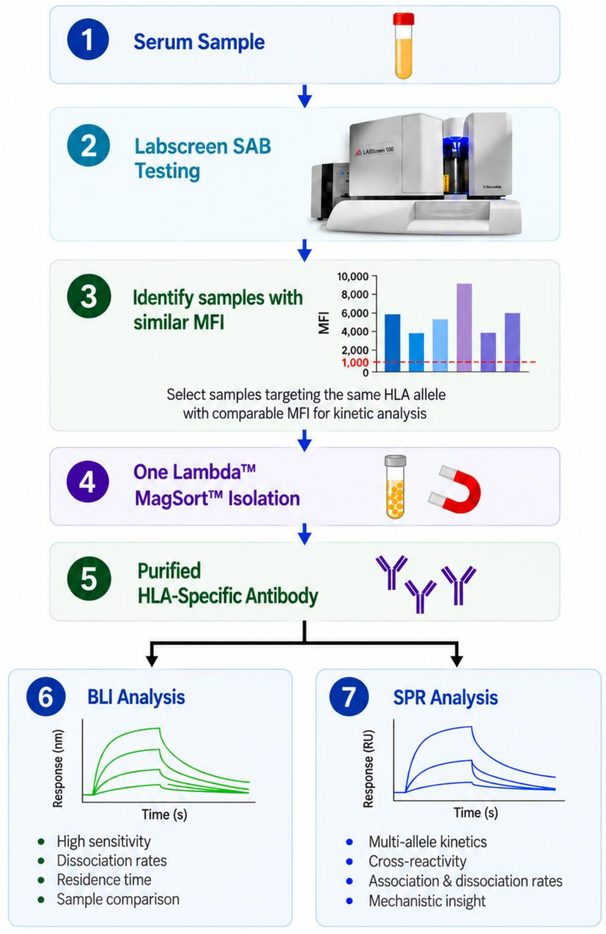
LABScreen™ SAB results were used as the reference method for identifying HLA-specific antibodies and selecting samples with comparable antibody reactivity.

BLI sensorgrams were analyzed to compare dissociation behavior among serum samples targeting the same HLA allele. Antibody residence time and relative dissociation behavior were compared across samples exhibiting similar LABScreen™ SAB MFI.

SPR sensorgrams were analyzed to compare antibody interactions across multiple HLA alleles. Because BLI and SPR employ different biosensor configurations and surface chemistries, kinetic parameters were interpreted **within each platform** rather than directly compared numerically between platforms. Instead, the complementary analytical roles of each platform were evaluated.

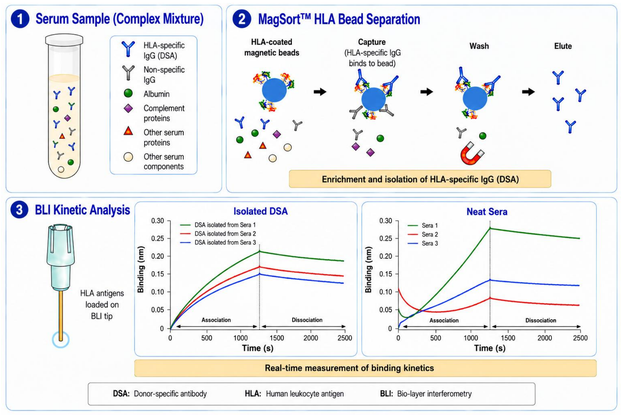
Results

Figure 1. Integrated Research Workflow for Kinetic Characterization of HLA-Specific Antibodies



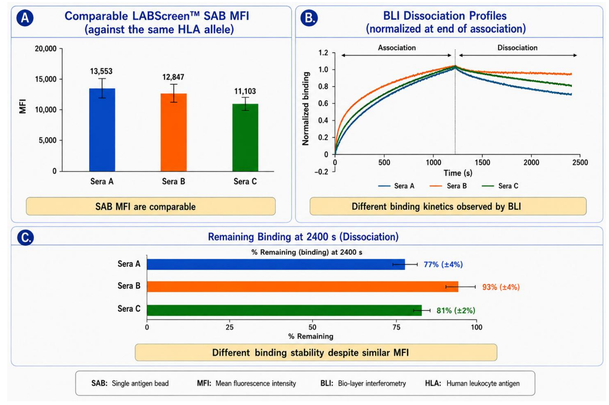
In this study, serum samples were first characterized by LABScreen™ Single Antigen Bead (SAB) testing, and samples targeting the same HLA allele with comparable MFI were selected for further investigation. HLA-specific antibodies were isolated from selected sera using One Lambda™ MagSort™ HLA-coated magnetic beads to reduce interference from non-specific serum components. The isolated antibodies were subsequently evaluated using label-free BLI and SPR approaches to investigate antibody–HLA binding behavior. BLI was used to compare binding and dissociation profiles among selected samples, while SPR enabled evaluation of allele-specific interactions across multiple HLA antigens. Together, these research approaches provide complementary kinetic information for characterizing HLA-specific antibodies beyond MFI-based serologic measurements.

Figure 2. One Lambda™ MagSort™ Enables Kinetic Analysis of HLA-Specific Antibody in Serum Samples



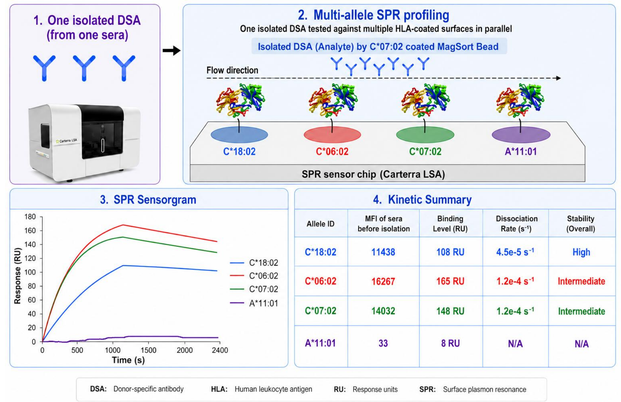
Direct kinetic analysis of serum samples is challenging because abundant non-specific immunoglobulins and other serum proteins can interfere with biosensor measurements. One Lambda™ MagSort™ uses HLA-coated magnetic beads to selectively capture HLA-specific antibodies from serum samples. Following removal of non-specific serum components and elution of the captured antibody, the resulting preparation can be analyzed by both BLI and SPR, providing a practical bridge between conventional HLA antibody testing and downstream kinetic characterization research.

Figure 3. BLI Reveals Distinct Dissociation Behavior Among Samples



HLA-specific antibodies isolated from serum samples exhibiting comparable LABScreen™ SAB MFI toward the same HLA allele (A*66:02) were analyzed by BLI. Despite similar levels of serologic reactivity, the samples demonstrated measurable differences in their dissociation profiles, indicating heterogeneous antibody–HLA complex stability. These differences were not apparent from SAB MFI alone and demonstrate the ability of BLI to distinguish functional binding behavior among antibodies with otherwise similar conventional assay results. **Similar SAB MFI does not necessarily indicate similar antibody binding stability, supporting BLI as a sensitive tool for functional comparison of HLA-specific antibody.**

Figure 4. SPR Characterizes Kinetic Differences Across Multiple HLA Alleles



Allele ID	MFI of sera before isolation	Binding Level (RU)	Dissociation Rate (s^{-1})	Stability (Overall)
C*18:02	11438	108 RU	4.5e-5 s^{-1}	High
C*06:02	16267	165 RU	1.2e-4 s^{-1}	Intermediate
C*07:02	14032	148 RU	1.2e-4 s^{-1}	Intermediate
A*11:01	33	8 RU	N/A	N/A

Isolated HLA-specific antibodies were analyzed by SPR against multiple HLA alleles to evaluate allele-dependent binding behavior. SPR sensorgrams demonstrated distinct kinetic profiles across related HLA antigens, indicating differences in antibody–antigen interaction behavior that were not fully represented by conventional serologic reactivity. The ability to evaluate multiple HLA interactions provides a complementary approach for investigating the kinetic basis of HLA antibody cross-reactivity. **SPR provides detailed allele-specific kinetic characterization that complements conventional assessment of HLA antibody cross-reactivity**

Conclusions

- **One Lambda™ MagSort™ enables selective isolation of HLA-specific antibodies suitable for downstream kinetic characterization research.**
- **MagSort-isolated antibodies can be analyzed by both BLI and SPR following removal of non-specific serum components.**
- **BLI reveals heterogeneous dissociation behavior among antibodies with similar LABScreen SAB MFI**
- **SPR reveals distinct kinetic behavior across multiple HLA alleles.**
- **Kinetic characterization provides complementary information beyond MFI reading**

References

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