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Implementation of a Semi-Automated Bead Extraction Procedure During Analyte Purification for High Throughput Immunogenicity Assays

Divya Pathak, Sophie Corbeil, Anahita Keyhani, Danielle Salha

Altasciences, 575 Armand-Frappier, Laval, QC H7V 4B3, Canada

CONTACT INFORMATION: mdsalha@altasciences.com

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PURPOSE

Biotherapeutics can elicit immune responses that result in the production of anti-drug antibodies (ADAs), which may impact the pharmacokinetics, safety, and efficacy of the treatment. Here, we present a semi-automated biotin-drug bead extraction and acid dissociation (BEAD) procedure coupled with Protein AGL capture to support a clinical immunogenicity program. Traditional magnetic bead-based immunoaffinity techniques are typically manual, time-consuming, and labor-intensive, resulting in extended assay times, reduced throughput, increased variability, and ergonomic risks from repetitive pipetting. To overcome these challenges, we optimized the BEAD-AGL assay for increased throughput and reliability by integrating semi-automation into the streptavidin-biotin bead-based washing and elution steps using the Thermo Scientific KingFisher Flex platform. This semi-automated approach significantly improves efficiency, reducing assay turnaround times, increasing throughput, and enhancing the robustness of the procedure, ultimately leading to more reliable results and improved workflows.

METHODS

Semi-automation of the BEAD Protein AGL assay was achieved by employing the KingFisher Flex Magnetic Particle processor to perform the streptavidin-biotin magnetic bead washing and acidification steps. The different steps of the method and format are detailed in **Figures 1** and **2**.

Figure 1. BEAD-AGL Assay Procedure Steps

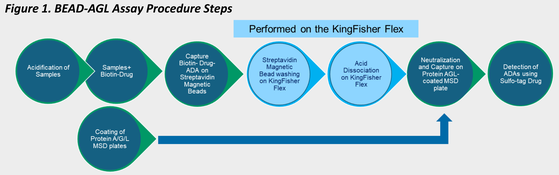
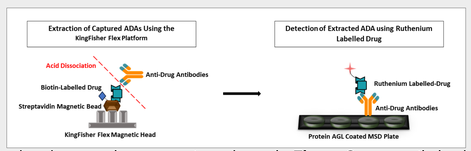


Figure 2. BEAD-AGL Assay Format Using KingFisher Flex Platform



The key bead processing parameters shown in **Figure 3** were optimized during automation to minimize bead loss during washing and elution cycles, thereby improving reproducibility and overall performance.

Figure 3. Bead Processing Parameters to Optimize

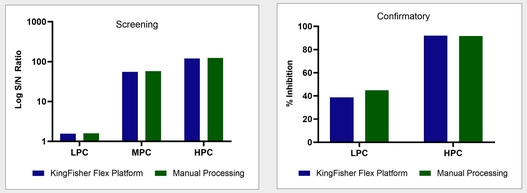
A comparative analysis was performed between manual execution and using the KingFisher Flex platform for the immunoaffinity purification step. This involved assessing cut points and precision metrics to demonstrate performance comparability between the two approaches.

RESULTS

Control and Individual Lot Responses

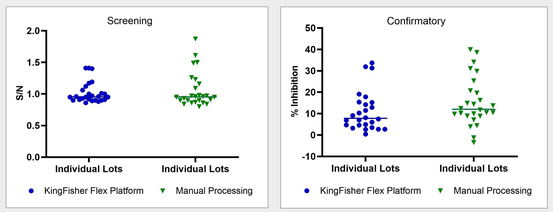
- Comparable assay responses were observed
  - The responses for controls are comparable in the screening and confirmatory assays as demonstrated by a percent difference within 15% between the KingFisher and the manual processing (**Figure 4**).

Figure 4. Control S/N Ratio and % Inhibition Comparison



- The variability between and within individual human serum lots evaluated on the KingFisher Flex platform was reduced compared to manual extraction (**Figure 5**).
  - Standard deviation of signals on the KingFisher Flex platform was 9%, as opposed to 18% with manual extraction.
  - Standard deviation of % inhibition on the KingFisher Flex platform was 9%, as opposed to 11% with manual extraction.

Figure 5. Individual Human Serum Lot Results



Cut-Points

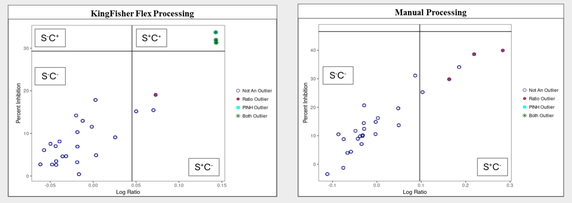
- The screening and titration cut points obtained are within a 25% difference. (**Figure 6**)
- Confirmatory cut-points differ significantly, with a 46% difference.
- Lower cut-points obtained with the KingFisher Flex platform result in an improved assay sensitivity, specifically in the confirmatory tier.

Figure 6. Cut-point Comparison

Specificity Plots

- The kingfisher platform demonstrated reduced non-specific binding compared to the manual processing (**Figure 7**)
  - This is attributed to a more effective and uniform bead washing by the KingFisher Flex platform.

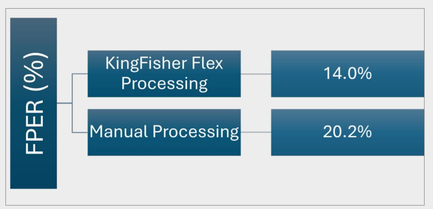
Figure 7. Plot of Percent Inhibition versus Log Ratio Values



False Positive Error Rates

- KingFisher resulted in lower false positive error rates (FPER) in comparison to the manual processing of the beads (**Figure 8**) as a result of more effective washing of the beads
  - The lower FPER results in a reduced number of samples analyzed in the next tier, thus increasing throughput and turnaround time.

Figure 8. False Positive Error Rate Comparison



Precision

- Comparable assay responses were obtained in the screening and confirmatory tiers.
  - Both approaches demonstrated inter and intra-assay precision below 20% (Table 1).

Table 1. Inter and Intra-Assay Precision

	Assay tier	Parameter	Control ID	King Fisher Flex Platform %CV	Manual Processing %CV
Intra-Assay Precision	Screening	Signal	NC	3.3 to 15.7	2.4 to 9.8
		S/N	LPC	2.0 to 7.5	3.4 to 6.7
			MPC	4.1 to 9.0	1.4 to 9.2
			HPC	4.2 to 14.9	1.1 to 9.1
	Confirmatory	%Inhibition	ILPC	7.8 to 11.9	2.2 to 10.5
			IHPC	0.1 to 1.3	0.1 to 1.0
	Assay tier	**Parameter**	**Control ID**	**%CV**	**%CV**
Inter-Assay Precision	Screening	Signal	NC	10.8	8.8
		S/N	LPC	8.7	5.3
			MPC	15.6	8.9
			HPC	16.2	15.2
	Confirmatory	%Inhibition	ILPC	17.8	7.7
			IHPC	1.0	0.9

CONCLUSION

The optimized and validated semi-automated BEAD Protein AGL assay overcomes the key challenges associated with magnetic bead-based immunogenicity assays, such as ensuring robustness, eliminating labor-intensive procedures, and minimizing assay duration. Leveraging the KingFisher platform has proven to be a game-changer, enabling streamlined, high-throughput immunogenicity testing with enhanced efficiency and reliability.

Figure 9. Key Benefits of KingFisher Platform in Assay Efficiency and Ergonomics

Utilizing the Thermo Scientific KingFisher Flex platform, the assay achieves significant improvements:

- Bead washing: Improved efficiency and consistency in bead washing minimizes non-specific binding and variability between individual lots, resulting in improved assay sensitivity due to lower cut points and reduced FPER.
- Efficiency: Reduction in assay time by at least 2 hours per operator when handling multiple plates resulted in a 30% reduction in assay duration.
- Throughput: Reduction in assay time supports higher throughput and allows an increase of 25% in the number of plates performed per analyst.
- Timeline: Increase throughput and lower FPER, which improves turnaround time by 30%.
- Reduced ergonomic risks: By minimizing the need for repetitive pipetting during manual bead processing, we enhance both the robustness of the procedure and the ergonomic safety for users, particularly during extended clinical studies.

ACKNOWLEDGEMENTS

Altasciences Laboratory Team

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