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STRATEGY TO INCREASE THROUGHPUT AND METHOD SENSITIVITY FOR CLINICAL IMMUNOGENICITY STUDIES

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INTRODUCTION

As drug development moves past Phase 1 clinical trials into Phase 2 and 3, demonstrating safety and promising therapeutic impact, analysis of a large number of biological samples over several years requires a higher throughput and a thorough life cycle management of critical reagents. Strategies to introduce automation-assisted workflows to increase turnaround time and reduce sources of variability caused by batch-to-batch preparations of critical reagents are critical.

Here, we discuss an approach to increase throughput by coating several plates simultaneously using a coating stabilizer. This stabilizer enhances throughput and efficiency by stabilizing the coated protein, enabling bulk coating of plates and consistency within the coated plate lot. Consequently, assay time is reduced by eliminating the need for additional blocking incubation and washing steps, allowing samples to be directly added to the dried, coated plates. This optimization streamlines the process, improving the overall efficiency and reliability of the method.

Moreover, the use of a coating stabilizer is also beneficial in reducing the non-specific binding observed in the assay due to its additional blocking capacity and significantly improving the assay's drug tolerance and sensitivity by preserving the conformation of coated proteins without requiring too harsh and fastidious acid dissociation steps.

Figure 1. Steps for Coating using a Stabilizer

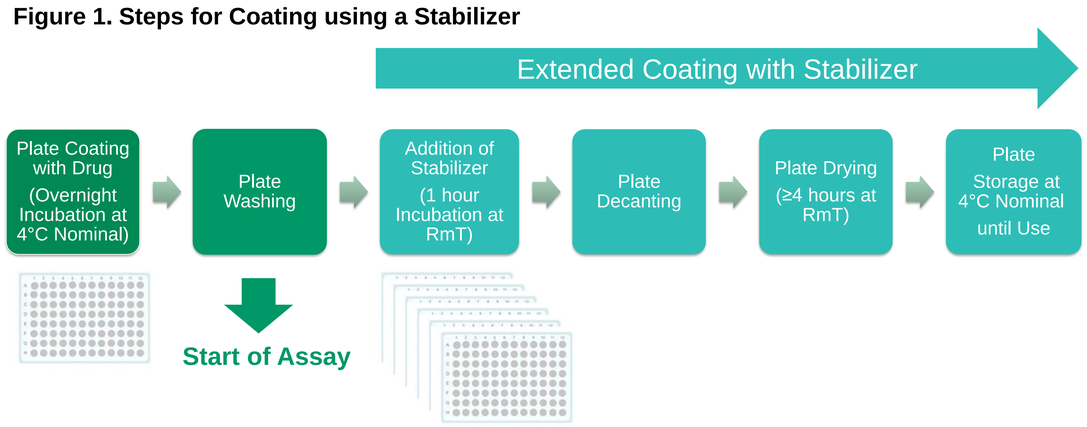


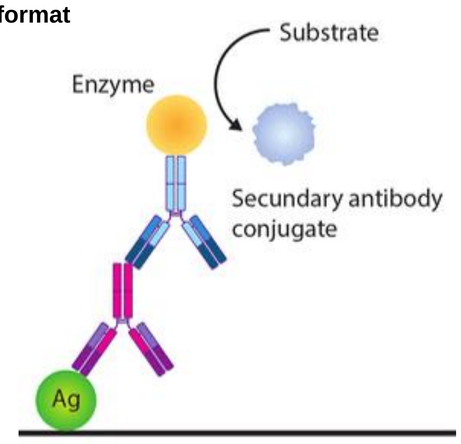
Table 1. Comparison of Assay Specifics and Workflows

	Fresh Coating	Coating Stabilizer
Plate Coating	Prior to assay (2 plates / coating)	Bulk coating in advance (40 plates / coating)
Quantity of Capture Drug for 40 plates	3 mg	2 mg (33% material saved)
No of Coated Plate Lots	40 lots	1 lot
Assay Time	5 hrs	3 hrs (40% time saved)
Reliability	Increased variability and chance of technical oversight	Reduced variability and minimal technical oversight
Throughput Capabilities	Standard	Prone to automation by using LHS

METHOD

In ELISAs, key components such as plate coating and blocking/assay buffers are essential for capturing anti-drug antibodies and reducing nonspecific binding, thereby enhancing sensitivity and drug tolerance. In this indirect ELISA format without acid dissociation, the anti-drug antibodies are captured by an immobilized peptide and detected using an HRP-labeled cocktail of species-specific antibodies. (presented in Figure 2). During peptide immobilization, an immunoassay stabilizer was employed to preserve the peptide's conformation and simultaneously block the plate surface.

Figure 2. Indirect ELISA Assay format



The study evaluated the effects of various blockers and assay diluents, such as Buffer A, Casein Blocker in PBS, and 5% BSA in PBS with and without the stabilizer. Additionally, the long-term stability of the stabilized plates was assessed on Day 1, Day 29, and Day 83 and compared to freshly coated plates to determine their impact on assay sensitivity, individual lot variability, and drug tolerance.

RESULTS

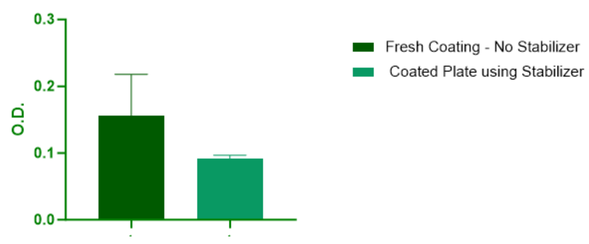
Impact of Coating Stabilizer on Non-Specific-Binding

- Comparison of assay buffers to the matrix (negative control) using no stabilizer (fresh condition) and using a coating stabilizer for up to 83 days. Refer to Table 2 and Figure 3.
  - The stabilizer's impact on reducing the non-specific binding is buffer-dependent: there was an enhanced blocking effect while using the blocker casein and 5% BSA in PBS.
  - Testing various assay buffers in conjunction with the coating stabilizer during method development is beneficial.
  - Reduced variability was observed between individual matrix lots, thus confirming the advantage of using a coating stabilizer.

Table 2. Impact of Stabilizer and various Buffer on the Non-Specific Binding

Assay / Blocking Buffer	Coating Condition	Assay / Blocking Buffer	Negative Control (serum)
		Signal (OD)	Signal (OD)
Buffer A	Fresh – No Stabilizer	0.068	0.069
	83 days– With Stabilizer	0.081	0.111
Blocker Casein in PBS	Fresh – No Stabilizer	0.091	0.138
	83 days– With Stabilizer	0.078	0.104
5% BSA in PBS	Fresh – No Stabilizer	0.130	0.352
	83 days– With Stabilizer	0.099	0.354

Figure 3. Individual Lots Responses with and without a Coating Stabilizer



Impact of Coating Stabilizer on Sensitivity and Drug Tolerance

- A 40% increase in S/N was observed at LPC and HPC levels with the coating stabilizer. Refer to Figure 4.
  - Significant improvement of sensitivity which indicates a better access of the positive control to the coated peptide.
- Drug tolerance was evaluated with and without the coating stabilizer. Refer to Table 3.
  - The required drug tolerance was met without the need to implement an additional sample pre-treatment such as acidification.

Figure 4. Positive control Responses with and without a Coating Stabilizer

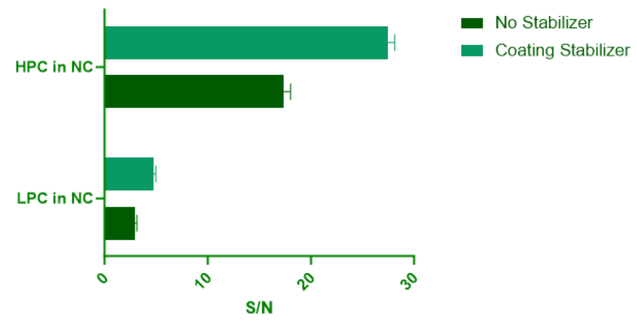


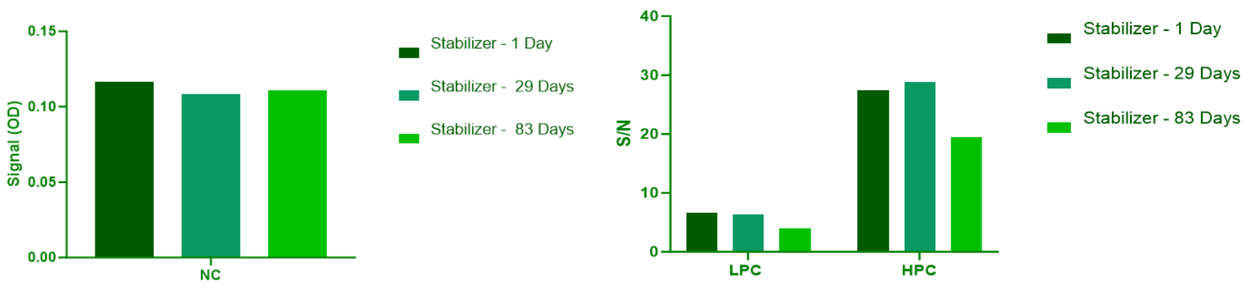
Table 3. Impact of Stabilizer on the Drug Tolerance

Assay Buffer/ Blocking Buffer	Coating condition	LPC in the presence of 25 µg/mL of drug
		S/N
Blocker Casein in PBS	Fresh – No Stabilizer	1.1
	With Coating Stabilizer	2.0

Long-Term Stability Assessment

- Responses of the controls were monitored over time when using a coating stabilizer (refer to Fig 5.).
  - NC signals remain stable up to 83 days.
  - PC S/N remains stable up to 29 days. Significant drop at 83 days for this method.
  - Time stressing is crucial to establish the stability of the coated plates and can be influenced by the type of assay buffers, drug, and positive controls used.

Figure 5. Control Responses over Time when using Stabilizer



CONCLUSION AND CLOSING STATEMENT

Throughput is an important consideration for immunogenicity studies considering the 3-tier analysis, which requires multiple rounds of analysis. Any strategy to reduce timelines is worth considering. The coating stabilizer was proven to be effective up to 29 days for this specific method which resulted in a 25% increased throughput as it allows coating large batches of plates ready to be used. Consideration should be made during method development and confirmed during method validation to properly establish the stability of the coated plates. Furthermore, using a coating stabilizer facilitates the implementation of automation as it removes the need for overnight coating under refrigerated conditions, which could be challenging on such a platform.

Moreover, the coating stabilizer has contributed to achieving the required drug tolerance without necessitating the use of harsher mitigation strategies such as acidification, which can often denature acid labile ADA during sample testing, leading to underestimating the overall immunogenicity; moreover, this results in a much simpler strategy compared to acidification.