AlphaLISA Automation for PK Assays: Boosting Throughput While Ensuring Robustness and Precision

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INTRODUCTION

The AlphaLISA immunoassay platform offers a homogeneous, no-wash format that streamlines sample processing and handling compared to conventional ELISAs. By integrating AlphaLISA into an automated platform, laboratories can significantly increase throughput by enabling the processing of large number of samples across multiple plates with minimal hands-on time.

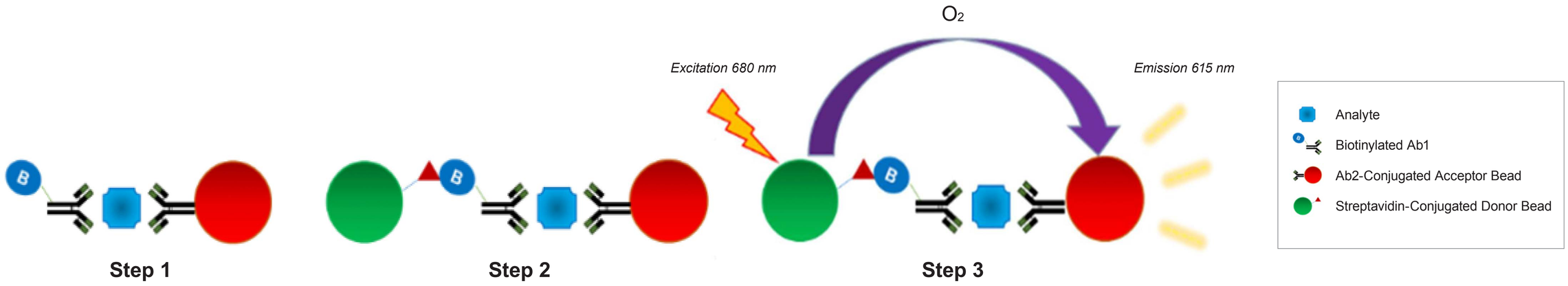

Figure 1. Method principles

Step 1: The analyte form an immunocomplex together with a Biotinylated Antibody (Biotinylated Ab1) and the acceptor beads conjugated with a second antibody (Ab2-Conjugated Acceptor Bead).
Step 2: Streptavidin-conjugated Donor Beads bind to the biotinylated antibody. Via this interaction, acceptor and donor beads are brought in proximity.
Step 3: Upon excitation at 680 nm, the donor beads generate singlet oxygen molecules that diffuse to nearby the acceptor beads leading to an activation and emitted light can be measured at 615 nm. This luminescent signal is proportional to the amount of analyte in the sample.
Note: Step 1, processed by liquid handling system Hamilton Microlab Star; Step 2 and Step3, processed by liquid handling system TecanEVO.

OBJECTIVES

This case study describes the optimization of an automated AlphaLISA PK assay optimized to deliver results equivalent to those obtained with the manual method.

The main objectives were:

- **Match automation—manual performances** by optimizing the automated AlphaLISA PK assay;
- **Identify and address automation-specific challenges** to establish a reliable high-throughput workflow;
- **Enhance scalability and consistency** minimizing operator-dependent variability and guarantee assay reproducibility.

OPTIMIZATION STEPS

Adapting the AlphaLISA Method for Automation:

- **Adapt sample volume** to improve assay precision
- **Verify critical reagents stability** to align with automated pipetting timing and maximize assay throughput

Key Implementation Steps for the Automation Workflow:

- **Adjust pipetting and sample dispensing** for critical reagents and sample replicates;
- **Fine—tune Liquid Class (LC)** parameters to ensure accurate liquid handling;
- **Optimized MRD mixing** to enhance precision and consistency

RESULTS & CONCLUSIONS

Preliminary comparison between manual and automated methods: Accuracy and precision

The initial automated test exhibited poorer precision (%CV) compared to the manual method, highlighting the need for further optimization of robotic handling and pipetting.

Manual

STD	%CV	%Bias
STD1	0.3	0.5
STD2	3.3	-0.4
STD3	2.5	-1.2
STD4	2.1	1.6
STD5	0.2	0.6
STD6	1.7	-1.4
STD7	2.6	0.1
STD8	0.1	1.6
STD9	4.2	-1.0
blank	BLQ	BLQ

QC	%CV	%Bias
ULOQ _I	2.2	0.3
ULOQ _II	6.0	12.4
ULOQ _III	4.9	-2.2
HQC _I	0.8	1.5
HQC _II	1.6	8.6
HQC _III	1.9	-1.1
MQC _I	1.3	1.9
MQC _II	0.1	7.1
MQC _III	2.8	-2.7
LQC _I	1.3	19.4
LQC _II	0.2	23.1
LQC _III	2.6	11.3
LLOQ _I	1.9	8.7
LLOQ _II	1.6	5.3
LLOQ _III	20.2	-5.9

Manual

Duplicate variability and overall CV%

	1	2	3	4	5	6	7	8	9	10	11	12
A	4.2		0.3		0.7		7.1		0.6		12.4	
B	0.1		9.4		1.1		12.2		1.9		7.4	
C	2.6		1.3		0.2		8.0		25.5		6.2	
D	1.7		1.6		0.2		5.9		5.9		12.7	
E	0.2		1.7		1.6		4.2		0.7		3.1	
F	2.1		0.8		5.0		26.6		14.4		3.7	
G	2.5		115.0		0.0		6.9		8.9		2.8	
H	3.3		0.6		0.5		7.8		5.2		4.5	

overall %CV 7.2

Robotic

STD	%CV	%Bias
STD1	5.4	0.6
STD2	7.9	-0.8
STD3	6.7	-1.1
STD4	4.4	2.1
STD5	9.9	0.2
STD6	5.8	-2.2
STD7	5.1	0.4
STD8	7.6	7.9
STD9	37.7	-1.4
blank	BLQ	BLQ

QC	%CV	%Bias
ULOQ _I	22.3	4.9
ULOQ _II	29.4	6.4
ULOQ _III	13.5	9.0
HQC _I	6.1	1.7
HQC _II	15.2	15.7
HQC _III	11.3	0.1
MQC _I	15.2	2.8
MQC _II	7.1	6.6
MQC _III	10.9	2.1
LQC _I	0.5	21.0
LQC _II	22.2	12.3
LQC _III	23.7	17.6
LLOQ _I	3.5	15.2
LLOQ _II	8.5	11.9
LLOQ III	23.2	11.2

Robotic

Duplicate variability and overall CV%

	1	2	3	4	5	6	7	8	9	10	11	12
A	37.7		5.4		11.9		10.3		1.6		11.2	
B	7.6		2.4		5.5		7.9		2.3		12.7	
C	5.1		2.4		22.1		13.8		1.9		14.8	
D	5.8		0.7		10.6		28.4		14.9		10.2	
E	9.9		22.6		12.6		26.5		30.7		74.8	
F	4.4		5.6		21.0		8.3		16.3		21.8	
G	6.7		15.9		32.4		7.1		10.4		26.6	
H	7.9		1.3		12.7		8.8		9.2		5.8	

overall %CV 13.5

Improving robustness and precision of the automated process by adjusting reagent and samples dispensing parameters, optimizing the Liquid Class (LC) and introducing an extra mixing step:

Optimization of donor-beads dispense parameters (height, vectors)

STD	%CV	%Bias
STD1	13.8	-2.1
STD2	4.3	-0.4
STD3	1.5	4.0
STD4	41.2	-5.2
STD5	37.0	-4.2
STD6	4.6	4.5
STD7	35.8	-3.8
STD8	8.0	2.4
STD9	1.0	-0.9
blank	BLQ	BLQ

QC	%CV	%Bias
ULOQ _I	24.4	-14.0
ULOQ _II	21.3	-7.1
ULOQ _III	7.0	-1.1
HQC _I	0.8	1.7
HQC _II	137.7	-50.0
HQC _III	12.2	-0.4
MQC _I	29.4	6.2
MQC _II	0.3	12.4
MQC _III	17.0	-2.2
LQC _I	13.0	35.4
LQC _II	9.4	38.6
LQC _III	0.0	18.0
LLOQ _I	0.5	4.6
LLOQ _II	24.0	7.6
LLOQ _III	26.5	-19.4

Duplicate variability and overall CV%

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.0		13.8		5.5		0.8		11.3		9.5	
B	8.0		10.3		14.9		10.0		7.8		16.4	
C	39.8		0.3		12.8		0.8		9.4		16.1	
D	4.6		17.6		0.5		5.7		5.7		12.0	
E	37.0		43.5		140.1		12.6		45.8		106.4	
F	41.2		0.9		21.1		10.7		4.0		25.3	
G	1.5		25.4		58.8		8.5		8.8		13.5	
H	4.5		10.9		9.7		7.4		11.9		6.6	

overall %CV 18.5

LC parameters adjustment for dispense samples in duplicate

STD	%CV	%Bias
STD1	8.8	1.3
STD2	22.3	-2.2
STD3	19.0	-1.6
STD4	1.9	0.3
STD5	6.1	2.9
STD6	0.8	-0.7
STD7	8.6	-1.9
STD8	8.2	0.5
STD9	0.9	0.8
blank	BLQ	BLQ

QC	%CV	%Bias
ULOQ _I	4.4	-7.0
ULOQ _II	10.1	-4.8
ULOQ _III	18.6	-5.6
HQC _I	0.8	-1.6
HQC _II	11.9	-7.2
HQC _III	13.0	-2.5
MQC _I	6.7	-4.0
MQC _II	8.1	2.2
MQC _III	10.8	-8.3
LQC _I	4.7	11.7
LQC _II	6.0	14.7
LQC _III	33.7	-2.4
LLOQ _I	6.6	5.4
LLOQ _II	9.0	9.9
LLOQ _III	23.4	-2.9

Duplicate variability and overall CV%

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.0		8.8		2.2		23.7		3.4		2.0	
B	8.4		1.5		6.8		22.5		5.1		14.8	
C	8.4		4.8		7.8		1.0		3.0		31.4	
D	0.9		6.1		11.6		2.6		8.7		16.0	
E	5.8		9.7		13.0		8.5		3.2		41.2	
F	1.9		1.0		3.5		8.9		8.1		15.4	
G	19.0		4.3		7.1		9.7		5.5		13.9	
H	72.3		45.4		10.6		7.3		0.5		17.6	

overall %CV 10.1

Optimization of MRD mixing before transfer on assay plate

STD	%CV	%Bias
STD1	0.2	0.9
STD2	6.0	-1.9
STD3	7.2	2.3
STD4	2.7	-1.8
STD5	2.5	1.5
STD6	1.0	-0.5
STD7	1.8	-0.8
STD8	2.0	1.2
STD9	0.6	-0.6
blank	BLQ	BLQ

QC	%CV	%Bias
ULOQ _I	1.2	-4.3
ULOQ _II	1.5	0.1
ULOQ _III	0.3	-4.9
HQC _I	1.7	2.8
HQC _II	2.4	0.4
HQC _III	1.7	-1.1
MQC _I	3.1	3.7
MQC _II	0.7	7.5
MQC _III	1.4	4.4
LQC _I	1.3	17.8
LQC _II	1.2	17.6
LQC _III	2.8	12.8
LLOQ _I	0.5	9.2
LLOQ _II	4.9	-6.5
LLOQ _III	4.9	-8.6

Duplicate variability and overall CV%

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.6		0.2		3.0		1.0		2.9		2.6	
B	2.2		1.7		1.5		1.4		3.4		1.3	
C	1.9		0.1		1.1		0.1		2.0		2.9	
D	1.1		0.4		2.4		5.4		1.8		6.7	
E	2.6		1.7		1.2		1.5		0.4		7.3	
F	2.7		4.3		2.4		1.0		2.8		4.7	
G	7.2		2.0		0.5		0.2		0.6		1.7	
H	6.0		1.2		1.2		1.1		3.2		1.1	

overall %CV 2.2

Note: The %CV values shown in the tables are based on back-calculated concentrations of STD and QC samples from the analytical run. The %CVs displayed in the plate layout are derived from duplicate raw signal data. While both refer to the same samples, the values may differ slightly due to the calculation method.

The successful implementation of automated AlphaLISA assay resulted in:

- **High throughput:** 8 plates/day (1024 samples/week)
- **Process standardization**
- **Maximum walk away time**

The assay was successfully validated and subsequent sample analysis confirmed robustness, precision and accuracy of the robotic method, highlighting the value of automation in regulated bioanalytical environment, where **data quality, consistency,** and **reproducibility** are essential.