Automation of AlphaLISA Immunodetection Assays: JANUS Family of Automated Liquid Handling Workstations

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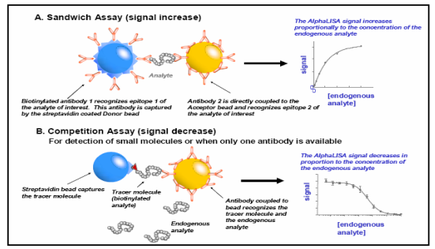
1 Introduction

The need for immunoassays assays at low cost, high throughput and quality, and simplified method exists in a variety of fields, including drug discovery, HTS, and basic research. ELISA is a method that has historically been used for these assays but it has a number of significant limitations. PerkinElmer® developed the AlphaLISA™ chemistry to assay a number of immunoanalytes. AlphaLISA allows for the ability to run large numbers of samples with a small sample volume, excellent sensitivity, and an expanded dynamic range relative to ELISA, all at room temperature and without the need for plate washing or shaking. Plate densities of 96-, 384-, and 1536-wells are accommodated. These characteristics make AlphaLISA chemistry ideal for high throughput screening, and JANUS® automated liquid handling workstations can be used to fully realize the power of AlphaLISA. Here we present several solutions for the automation of the AlphaLISA assay as well as performance data that have been generated using JANUS automated liquid handling workstation.

2 AlphaLISA and Its Advantages

AlphaLISA is a homogenous proximity assay that can be designed as a sandwich immunoassay, or using the competitive technique.



	ELISA	AlphaLISA
Homogenous	No, several wash steps	Yes
Automation	*	***
Throughput	Low	High
Sensitivity	***	***
Dynamic range	2 logs	2,5-5 logs
Microplate format	96-well plate	96- 384- 1536-well
Multiplexing	No	Not yet
Substate sizes	Small molecules to whole cells	Small molecules to large complexes
Use of polyclonal antibodies	Yes	Yes
Assay steps	more than 4	3 to 4
Total assay time	2h to Overnight	2h to Overnight (less hands on time)
Reader	ELISA readers	EnVision

3 AlphaLISA is versatile and scalable

AlphaLISA technology has been used to successfully assay a variety of analyte types: small molecules, polypeptides, proteins, chunky proteins, and even particulates, in matrices such as serum and cell culture media.

AlphaLISA is also scaleable: assays can be converted to different plate densities (96-, 384-, and 1536 well plates) without loss of assay sensitivity. Miniaturization, or the use of smaller reagent volumes in higher density plates – is an excellent strategy for achieving high throughput and costs savings.

Why Automate?

Manual assay performance of small volume, high-density tests, however, can be cumbersome and error-prone. PerkinElmer's JANUS automated liquid handling systems are used to easily realize the power of the AlphaLISA platform.

4 JANUS Mini

- Occupies less than 28" bench space
- Equipped with one pipetting arm: Varispan (4 or 8 channels) or MDT (96- or 384-well format)
- Shares many features with larger JANUS units
- Deck capacity enhanced by addition of PlateStak®(s)
- The JANUS Mini **cannot** be fully integrated to the EnVision® Plate Reader

JANUS Mini MDT

JANUS Mini Varispan

5 JANUS Standard Deck/ Integrator

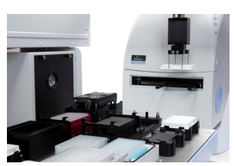
Enables AlphaLISA application automation in a fully integrated "walk-away" mode when the EnVision plate reader, PlateStak and FlexDrop™ non-contact reagent dispenser are added

- One/two pipetting arms (Varispan, MDT, or both) and a Gripper arm
- Allows for plate bar code reading
- Can lid and de-lid plates
- Can shuttle plates and disposable tips to and from the PlateStak(s)

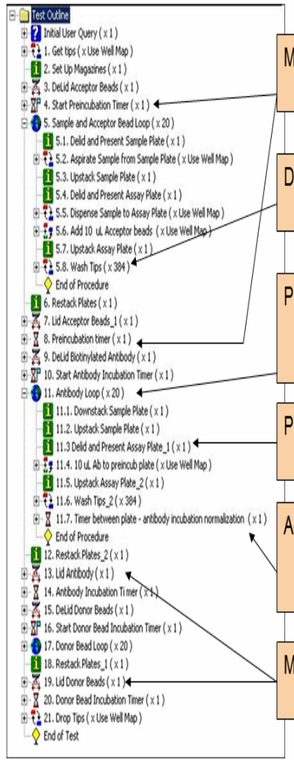
6 JANUS –EnVision Integration

The Gripper Arm's rotational ability is used to deliver the OptiPlate™ or AlphaPlate to the EnVision Alpha reader in a JANUS-EnVision Integrated system.

Gripper arm capability is available as an optional second arm, as shown in Panel 5, or as a Gripper Integrated Platform (right), as the third arm of the system.



7 AlphaLISA Assay WinPREP® Setup Test



Multiple timers keep track of incubation times

Disposable tips can be washed and re-used

PlateStak senses when all plates have been processed for each step

PlateStak lids and delids sample plates and assay plates

Additional timers can be added to synchronize incubation times for each plate

MDT Gripper Tool lids and delids reagents on the deck

8 Automated vs. Manual Sample Preparation Pipetting:

Within-the-plate Precision and throughput Studies

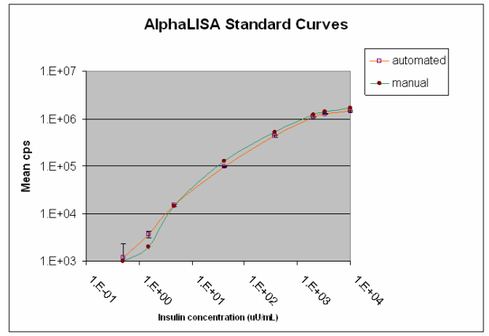
Plate preparation	mean cps	Std. Dev.	%CV	Timing (sec)
Automated	63500	1856	2.92	74*
Automated	63474	1476	2.32	73*
Automated	61466	1662	2.70	73*
Automated Total time (three 384-well plates)				220
Manual (one 384-well plate)	58236	2112	3.62	427

- Beads were pipetted into 384-well Optiplate microplates

Conclusions

- Automatic pipetting is **6 times faster than manual** dispensing
- Precision of automated bead dispensing is better than manual (%CV)

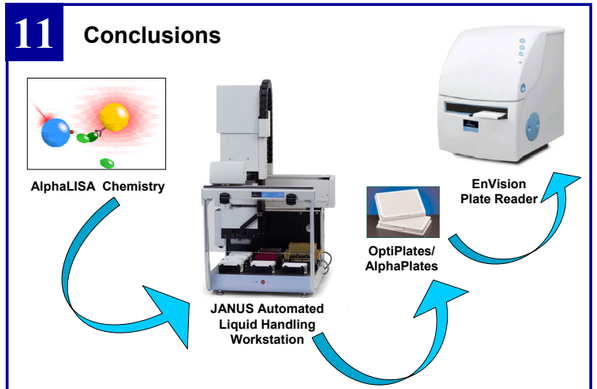
10 Comparison Between the Manual and Automated Runs



Interpolated Values % Recovery		
Control concentration (uU/mL)	Automated	Manual
80	92.6	93.5
123	98.0	98.4
400	88.5	99.2

- Beads were pipetted into 384-well Optiplates plates both manually and using JANUS automated liquid handling robot
- Standard curves were generated for Insulin A Ab-coated AlphaLISA beads. Beads were pipetted manually and using automated dispensing

11 Conclusions



AlphaLISA chemistry is easily adapted to JANUS automated liquid handling workstations, and favorable comparison with data from the manual runs is demonstrated in terms of standard curves, precision, and interpolated analyte recovery. Automation resulted in reduced assay runtime, reduced error, and significantly increased throughput. Automation of AlphaLISA immuno-detection applications on JANUS Automated Workstations offers robust, cost-effective assay performance, improved productivity, and a complete assay setup (reagents, consumables, hardware, and software) supplied by PerkinElmer.