Automation of AlphaLISA Insulin and VEGF Immunoassays on JANUS Liquid Handling Workstation

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1 Introduction

Insulin and vascular endothelial growth factor (VEGF) have emerged as molecules of interest in clinical, pharmaceutical, biotech, and basic research settings.

Insulin has long been studied with regard to its role in diabetes, and the care and management of that disease, but it is now also measured to elucidate its effect in the development of metabolic syndrome, and it has been suggested as a biomarker for other conditions, such as prostate cancer risk and the aging process.

VEGF's action as a potent vascular growth promoter is implicated in developmental events, such as embryogenesis, wound and bone healing, and the establishment of lactation, but also in the angiogenesis of tumors. Since it has been implicated in a number of cancers, VEGF is measured in studies aimed at oncological diagnostics, prognostics, and therapy development.

ELISA has traditionally been used for the measurement of insulin and VEGF. AlphaLISA™ technology is a novel, homogeneous, bead-based immunoassay technology that can be used for a variety of biochemical determinations, including insulin and VEGF screening. AlphaLISA assay format has the ability to run large numbers of samples with a small sample volume, excellent sensitivity, and an expanded dynamic range, relative to ELISA. As an added value, AlphaLISA technology has been demonstrated to successfully work in high density formats (384- and 1536-well plates). Assay miniaturization – use of small sample and reagent volumes in high density microplates – is an excellent way to realize cost savings and high throughput. Manual processing of miniaturized assays is cumbersome and error-prone, however. Because AlphaLISA assays are homogeneous, they can be easily automated.

Here we present data demonstrating the effectiveness of AlphaLISA insulin and VEGF immunoassays automation. The studies have been performed on two configurations of the PerkinElmer JANUS® family of liquid handling workstations.

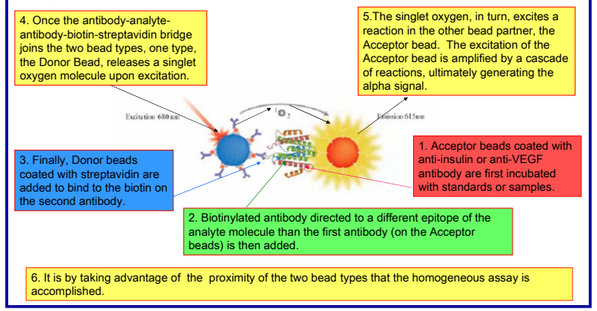
2 Comparison: ELISA & AlphaLISA

AlphaLISA is designed with many advantages over the traditional ELISA approach

	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 Chemistry

AlphaLISA immunoassays can be designed using either the competitive or sandwich approach. The insulin and VEGF AlphaLISA's described here are designed with the sandwich method.



4 Insulin Immunoassay

Sample or standard and Acceptor beads (5µL and 10µL, respectively) were added to wells of white 384-well OptiPlates, using a tip touch after each addition. After a 30 min incubation, 10 µL biotinylated antibody was added, and wells were incubated another 60 min. Finally, 25 µL Donor beads were dispensed to wells, and there was a 30 min incubation before alpha signal was read. All incubations were carried out at room temperature (23^{0}C). The entire assay protocol was carried out on the deck of JANUS Mini MDT.

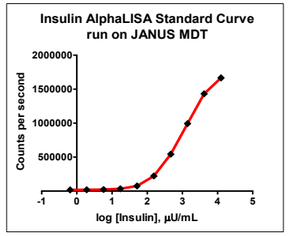
JANUS Mini MDT with PlateStak®

JANUS Modular Dispense Technology® (MDT) features dispense heads with 96- or 384- disposable tip capacity that can be interchanged on the fly. Here, only the I30 head was needed, and it was used with P30 30 µL tips throughout the assay.

The JANUS MDT AlphaLISA workstation is ideal for high throughput situations in which samples are already formatted in microplates.

5 Insulin Immunoassay Results

The standard curve obtained by JANUS automated liquid handling is shown at right. Interpolated recoveries for sham samples are from 88-98%, and precision for standard replicates range from 0.39 to 4.40 %CV. This standard curve covers 4.5 orders of magnitude, and the lowest detectable dose, based on mean buffer sample counts plus three standard deviations is 11.4 µU/mL.



6 Precision and Timing, Carryover Elimination

PerkinElmer Omnibeads were used to model several automation performance studies. Omnibeads generate alpha signal in the absence of AlphaLISA biochemical reaction.

Precision and Timing. To demonstrate precision and throughput, 15 µL Omnibeads was delivered to three 384-well plates using a JANUS MDT unit, and to one control plate using a multi-channel manual pipette. During the automated run, the tips were washed in one two-cycle procedure between each of the three plate additions. Both plate types were read on a PerkinElmer Alpha reader.

The Table shows that the within-plate precision for automated bead delivery is better than the plate that was hand-pipetted. The automated method also proved to be much faster than the manual delivery.

Within-plate Precision				
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*
Total time (automated)				220
Manual	58236	2112	3.62	427

Comparison of precision and timing between automated- and manually-dispensed Omnibeads in 384-well Optiplates.
**Time includes 2-cycle wash procedure between automated plate dispenses*

Carryover. To determine the degree of bead carryover, 25 uL of Omnibeads were added to the Row B (24 wells) of a white OptiPlate, after assay buffer had been added to Row A. Assay buffer was then added to Rows C, D, and E. Between each Row, a single 2-cycle tip rinse with deionized water was performed using the JANUS MDT TipWash. Mean counts per sec for Rows A->E were: 210, 104660, 213, 205, and 210. So a single 2-cycle tip wash is sufficient to eliminate bead carryover, and tips can be washed and re-used for bead additions.

7 VEGF Immunoassay

The VEGF AlphaLISA was performed using the JANUS Varispan™ pipetting arm equipped with eight VersaTips®. Stock VEGF solution was serially diluted in a deepwell microplate using 200 µL disposable tips, and 5 µL of the resulting standards was transferred, in triplicate, to a white 384-well OptiPlate with 20 µL disposable tips. Thereafter, the assay scheme was the same as that for the Insulin AlphaLISA, described in Panel 4. Additions of the Acceptor beads, biotinylated antibody, and Donor beads were performed with 200 µL disposable tips, in multi-dispense mode.

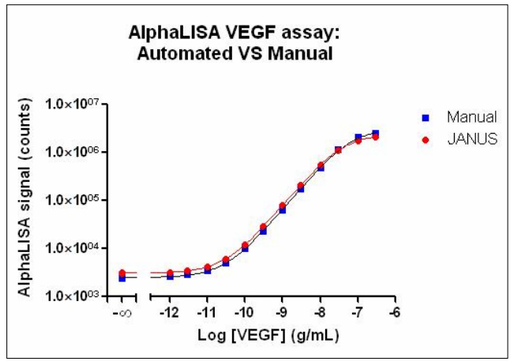
The Varispan arm employs positive displacement pipetting, with variable tip spacing that allows for reformatting of samples, such as transfer from test tubes or microfuge tubes to microplates, and "cherry-picking". Also, with the Versatip option, the user can elect to use fixed tips or disposable tips, and switch modes within a test.

As with the MDT, addition of PlateStaks and other integrations are possible, so the degree of automation is scalable.

JANUS Varispan Mini

8 VEGF Immunoassay Results

Standard curves for VEGF generated manually or by JANUS Varispan pipetting arm are depicted below, where a favorable comparison between the two approaches is evident.

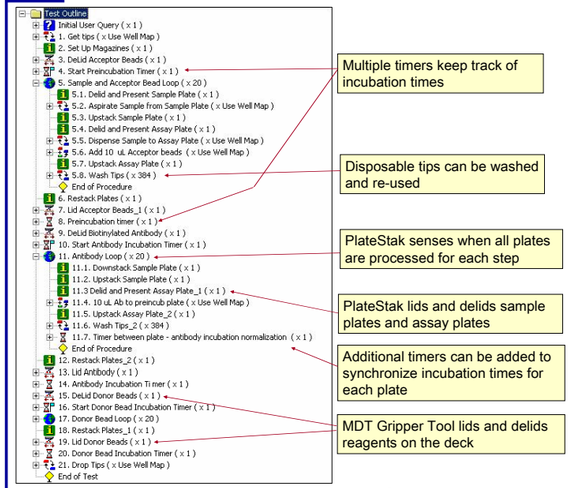


Standard	Manual	Automated
[VEGF], g/mL	%CV	%CV
3.E-07	1.27	2.33
1.E-07	0.43	1.18
3.E-08	2.92	1.08
1.E-08	5.66	0.91
3.E-09	1.80	1.64
1.E-09	6.21	3.37
3.E-10	4.65	2.09
1.E-10	5.63	5.72
3.E-11	7.64	5.44
1.E-11	8.62	5.51
3.E-12	2.49	4.61
1.E-12	8.35	4.61

At left, replicate precision for the standards is listed for both manual and automated runs. Data show similar precision values between the two curves.

Lowest detectable dose was determined by interpolation of mean buffer sample counts plus three standard deviations. The resultant values are 3.49 x 10^{-12} and 3.42 x 10^{-12} g/mL for the manual and automated standard curves, respectively. Therefore excellent sensitivity and dynamic range are demonstrated.

9 WinPREP® for JANUS



This sample of WinPREP software depicts the AlphaLISA programming protocol of a JANUS MDT integrated to a PlateStak, which stacks plates and shuttles them onto the JANUS deck, as directed by WinPREP. Multiple AlphaLISA plates are therefore processed automatically. AlphaLISA can also be run in semi-automated mode, with plates introduced manually.

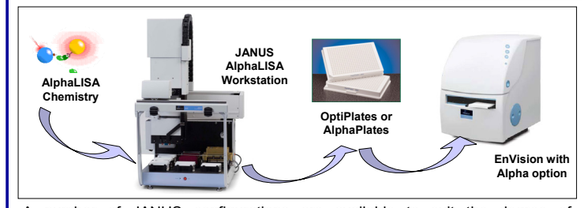
10 Conclusion: Complete Solution

AlphaLISA Chemistry:

- Variety of analytes can be measured
- Improved performance relative to traditional counterparts
- Homogenous – no need for washing, shaking, separations, etc.
- Easily miniaturized to realize cost savings and high throughput.

AlphaLISA on JANUS ALH Workstations:

- Accommodates assay miniaturization, which is cumbersome and error-prone when performed manually
- Choice of kind and number of pipetting arms
- Degree of automation is customizable (Gripper, PlateStak(s), EnVision integration can be added)
- Various deck sizes are available
- Excels at consistent, high throughput performance



A number of JANUS configurations are available to suit the degree of throughput, integration, available space, and deck capacity required by the user. For more information about the JANUS family of AlphaLISA Workstations, see the poster, "**Automation of AlphaLISA Immunodetection Assays: JANUS Family of Automated Liquid Handling Workstations**". With AlphaLISA, the JANUS Automated Workstation, OptiPlates or AlphaPlates, and the EnVision with Alpha plate reader, PerkinElmer offers the complete solution for easy, reliable, and robust immunoassay determinations.