APPLICATION NOTE

Application Workstations

AlphaLISA Automation

Use of the JANUS Automated Workstation to automate AlphaLISA assays

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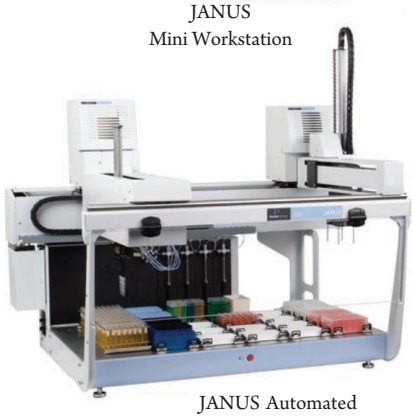
JANUS
Mini Workstation



JANUS Automated
Workstation

Introduction

Immunoassays are a mainstay for the quantification of a variety of bio-molecular analytes in drug discovery, drug development, and life sciences research laboratories. While ELISAs have traditionally been the most popular form of immunoassay, they are limited by the need to perform multiple wash steps. Where high throughput and/or capacity are required, automated systems have been developed, but the many wash steps create challenges and can require re-optimization and revalidation of assay performance.

To overcome these ELISA limitations, PerkinElmer has introduced AlphaLISA®, a novel, homogeneous immunoassay technology that eliminates wash steps. Compared to ELISA assays, AlphaLISA assays generally have a wider dynamic range and at least comparable sensitivity. AlphaLISA assays also can be scaled up from 96-well to 384-well format with no need for re-optimization. AlphaLISA panels are available in the following areas: biologics, angiogenesis, cancer, cardiovascular, inflammation, metabolism, and neurodegeneration.

Using PerkinElmer's family of automated workstations and microplate readers, AlphaLISA assays can be easily and reliably prepared, incubated, and analyzed without the need for complex and error-prone wash steps, time-consuming manual processing, or costly custom automation systems. Preparation of AlphaLISA assays using the JANUS® Automated Workstation can be tailored to the present and future needs of the laboratory with choices of pipetting technologies, level of automation, capacity, and process control. Moreover, the JANUS workstation can be easily set up to process different assay types in a multi-user, multi-assay environment, thus providing a flexible automation solution for many laboratories.

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To demonstrate the performance of the JANUS workstation in automating AlphaLISA assays, four AlphaLISA cytokine assays (IL1β, TNFα, IL17, and IFNγ) were prepared using three automated pipetting technologies. Lower detection limits (LDL) were determined for TNFα tests run in three different microplate types. Additionally, analyte recovery and precision for TNFα, IL17, and IFNγ automated assays were compared with those from cytokine assays prepared manually. Lastly, Z' values were calculated for the automated procedures for each analyte to assess assay reproducibility and robustness.

Materials and Methods

Reagents

Cytokine AlphaLISA kits (TNFα, IL1β, IL17, and IFNγ), available from PerkinElmer, were run as described in the kit inserts [1-4], except where appropriate to optimize for automation. The assay workflow is outlined in Figure 1.

Instrumentation

Sample preparation. Three automated pipetting strategies were used in this study: JANUS Varispan™ with VersaTip® and two JANUS Modular Dispense Technology™ (MDT) approaches. The MDT pipetting arm allows for a suite of pipetting heads to be interchanged without user intervention, and an MDT head and a Serial Dilution Tool (SDT) head were each used to prepare AlphaLISA tests. A summary of the JANUS configurations used in this study is shown in Table 1. Control plates, run concurrently with the respective automated tests, were prepared manually.

JANUS WinPREP® software coordinated all liquid handling and gripper movements used in the automated studies. Assay plates and reagents were covered and uncovered at appropriate times during the assay by use of an integrated gripper arm (Varispan unit) or the MDT gripper tool (MDT unit). WinPREP software accommodated multiple timers to precisely track overlapping incubation durations for simultaneous assays.

Detection. All assay microplates were read on an EnVision® Multilabel Plate Reader with AlphaScreen® option.

Method

All assays were carried out in PerkinElmer white 384-well OptiPlates®. TNFα tests were also performed in PerkinElmer

Figure 1. The AlphaLISA assay workflow is depicted above. All the steps outlined were performed by the JANUS AlphaLISA Workstation and an EnVision reader, as well as manually.

	IL1β, TNFα	IL17, IFNγ	IL17, IFNγ
Deck Capacity	Expanded platform	Standard platform	Standard platform
Pipetting Arm	Varispan - 8	Modular Dispense Technology	
Pipetting Tool	VersaTip	MDT P30/384 P30/RC	SDTool
Disposable Tip	P20, P200	P30	P30
Number of Tips	8	384	16
Tip Capacity	11 ul, 200 ul	30 ul	30 ul

Table 1. JANUS Automated Workstation configurations used to prepare AlphaLISA standards and samples. The JANUS Varispan system was equipped with an integrated Gripper arm, whereas the JANUS MDT instrument was fitted with an MDT Gripper Tool.

384-well AlphaPlates® and 96-well ½ Area Plates. Standards for the JANUS Varispan study were automatically diluted with assay buffer in 1.5 ml microfuge tubes on the instrument deck before reformatting to assay plates, while standard material for the MDT runs was placed on the JANUS deck in a polypropylene 384-well plate. Low profile pyramid-bottomed multi-trough reagent reservoirs, which limited dead volume, contained the Acceptor bead-biotinylated antibody mixture and Donor beads used for the assays. The small footprint and multiple reservoir troughs facilitated parallel preparation of multiple assays by the JANUS workstation. Reagents and assay plates were covered with black lids during incubation periods.

Recovery, Precision, Reproducibility. Controls for the TNFα and IL17assays were produced by dilution of analyte with AlphaLISA assay buffer, while AlphaLISA HiBlock buffer was used for the IFNγ assay. A set of controls was included, in triplicate, with each TNFα, IL17, and IFNγ standard curve, and control concentrations interpolated from the standard curves were compared with the known control concentrations to evaluate assay recovery. Standard deviation was calculated for replicate recovery results to assess precision.

Sensitivity. Lower detection limits were calculated for each plate type used, whether prepared by the JANUS workstation or manually, for the TNFα tests. The value resulting from mean background counts ("zero", or buffer, standard) plus three standard deviations was interpolated from the standard curve to determine LDL.

Z' tests were performed to determine assay reproducibility. Pooled high assay standards and assay buffer were each used as AlphaLISA samples in 48 wells of the appropriate assay plate to collect the signal and background data required to calculate Z'.

Results and Discussion

Comparison of results based on sample preparation method

tandard curves for IL1β (JANUS Varispan), IL17 (MDT and SDT), and IFNγ (MDT and SDT) are displayed in Figure 2, accompanied by standard curves resulting from the corresponding manually-prepared assays. All standard curves exhibit similar profiles with the wide dynamic range characteristic of AlphaLISA. In cases in which multiple automated approaches were used, curves are virtually identical.

		96-well	384-well	
		½ Area Plate	OptiPlate	AlphaPlate
Manual	LDL	2.1 pg/ml	3.0 pg/ml	2.3 pg/ml
	Z'	.89	.84	.83
JANUS Varispan	LDL	2.7 pg/ml	2.1 pg/ml	2.5 pg/ml
	Z'	.88	.88	.86

Table 2. AlphaLISA TNFα assays gave comparable sensitivity and Z' values when performed in 96-well or higher density 384-well format plates. No assay re-optimization was required. All assays were done using 50 ul total volumes.

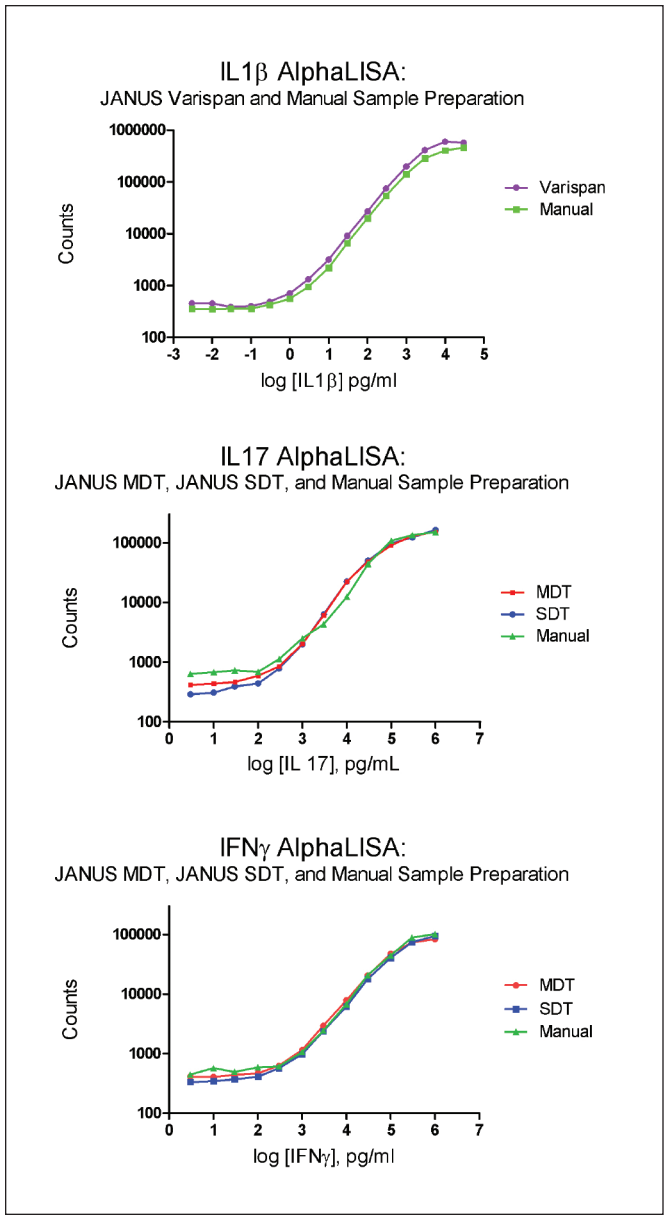

Figure 2. AlphaLISA standard curves prepared with JANUS Varispan (IL1β, A), JANUS MDT, and JANUS SDT (IL17, B, and IFNγ, C) are shown above.

Recovery and precision, Z'

Recovery and precision data calculated from the interpolated IL17, TNFα, and IFNγ control values are shown in Figure 3. Precision is generally similar between automated and manually-prepared assays, except automation enhances precision for sticky molecules such as TNFα. Recovery was more consistent when JANUS automated liquid handling was used.

Calculated Z' values for JANUS SDT-prepared IL17 and IFNγ, and Z' values, along with LDL data for TNFα runs, are listed in Table 2. Results from three different plate types representing two different well densities demonstrate highly sensitive and robust assays, as indicated by the LDL and Z' values for both automated and manual TNFα runs.

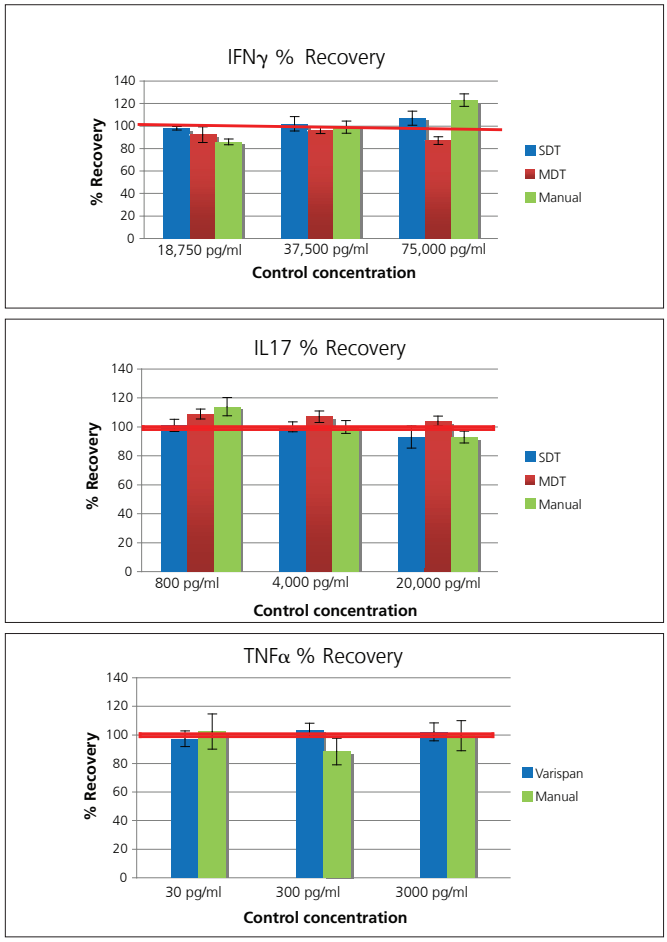

Figure 3. Three control levels were run in triplicate with the TNFα, IL17 and IFNγ AlphaLISAs prepared with JANUS MDT, JANUS SDT, and manually. Automated sample preparation resulted in more accurate results, as evidenced by more consistent recovery values.

Conclusions

We have demonstrated powerful AlphaLISA assay versatility and performance using several JANUS sample preparation configurations. All sample preparation strategies resulted in standard curves with wide dynamic range and good precision. In fact, when the tests were run with two different automated procedures, resulting standard curves were virtually identical. Automated sample preparation also enhanced the accuracy of results, as evidenced by more consistent recovery than when plates were prepared manually. Robust and reproducible automated assay performance for each analyte was found, with Z' values of 0.83 or better.

In addition to high quality liquid handling, the JANUS workstations and WinPREP software accomplished all plate and lid movement, incubation timings, and integration necessary for the scope of this study. WinPREP templates for all of the automated tests described in this study are available to provide quick and easy automated AlphaLISA start-up.

Full automation of AlphaLISA procedures is achieved by integration of the JANUS system to an EnVision or EnSpire AlphaPLUS Multilabel Plate Reader. Additional integration with a plate washer can provide a system capable of full automation of both ELISA and AlphaLISA, since EnVision and EnSpire AlphaPLUS readers are both capable of reading signal for each assay type. With these readers, the versatile and flexible JANUS workstation is certain to enhance immunoassay performance in a wide range of laboratory settings.

References

1 AlphaLISA IL1β Kit: Technical Data Sheet, Certificate of Analysis.

2. AlphaLISA IL17 Kit: Technical Data Sheet, Certificate of Analysis.

3. AlphaLISA IFNγ Kit: Technical Data Sheet, Certificate of Analysis.

4. AlphaLISA TNFα Kit: Technical Data Sheet, Certificate of Analysis.

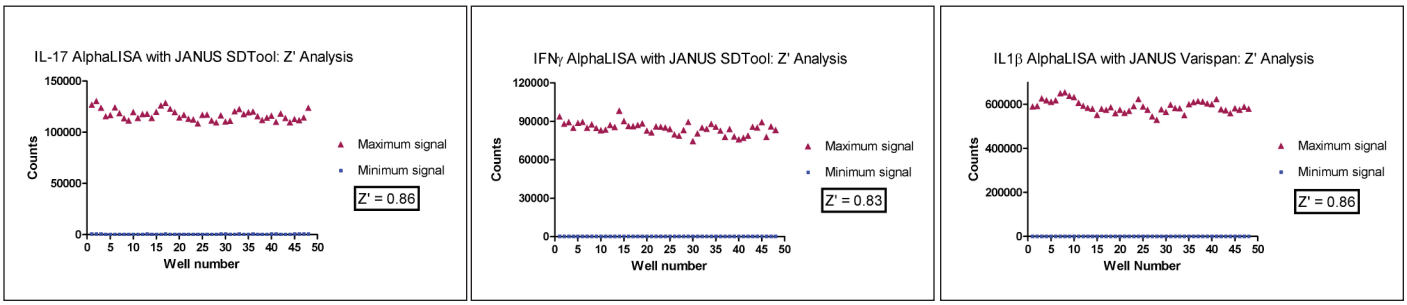

Figure 4. Pooled high-concentration analyte standards and buffer were run as samples in automated AlphaLISA assays (48 wells apiece for each assay) to generate signal and background values for Z′ calculations. The signal and background levels were plotted (above), and highly favorable Z′ values were obtained.

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