Biomarker Screening: Automated Sample Preparation for AlphaLISA® Cytokine Assays using JANUS® AlphaLISA Workstation

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1 Introduction

Biomarkers function in normal physiological processes and they have been implicated in disease etiology. They are therefore studied extensively in drug discovery, drug development, and life science research laboratories. Enzyme-linked immunosorbent assays (ELISAs) are widely used for biomarker concentration determinations, but their limitations include: high cost, low throughput, low dynamic range, mediocre sensitivity, and ELISAs are labor-intensive due to the need to wash microplate wells between assay steps.

PerkinElmer's AlphaLISA is a novel, homogeneous, bead-based immunoassay technology that is capable of biomarker concentration measurements with high sensitivity and specificity, and it can be scaled for use with 96-, 384-, and 1536 well microplate formats. AlphaLISA uses smaller sample volume and features increased dynamic range relative to traditional ELISA techniques.

AlphaLISA Assay Technology
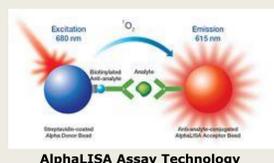
Because AlphaLISA is homogeneous (requiring no wash steps), it is easily automated. Assay automation provides sample preparation consistency and reliability, ensuring high quality data.

JANUS® AlphaLISA Workstation has been designed to automate AlphaLISA. It is available in a wide variety of configurations in order to suit user needs. The ideal JANUS system required for a given situation depends on the degree of throughput, integration needs, and deck capacity required. AlphaLISA performance with JANUS Modular Dispense Technology® (MDT) automated sample preparation is described in this poster. Data from interferon gamma (IFNγ) and interleukin 17 (IL 17) cytokine AlphaLISAs prepared with the high throughput JANUS MDT head and the flexible SDTool are compared with those from manually prepared assays.

2 JANUS AlphaLISA Automation

JANUS MDT System

Automated AlphaLISA assays were prepared using a JANUS with a Modular Dispense Technology (MDT) pipetting arm. Modular Dispense Technology features dispense heads with 96- and 384-tip capacity, and a suite of SDTools, all of which can be interchanged on the fly.

4 IFNγ Immunoassay Results
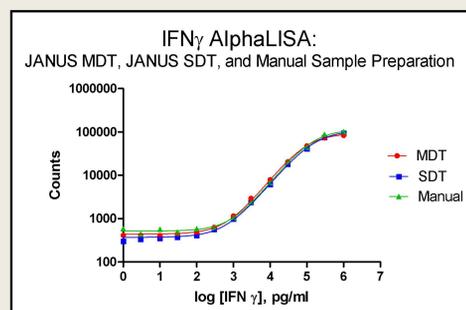
Results for IFNγ AlphaLISA liquid handling by JANUS MDT, JANUS SDT, and manually, depicted at left, demonstrate that automated preparation produces identical standard curves.

Controls of known IFNγ concentration were formulated by dilution in assay buffer and run in parallel with the standard curves. Values interpolated from the standard curves were evaluated for recovery and precision.

Data displayed in the chart at right indicate similar precision among all sample preparation methods; recovery was more consistent when automated methods were used.
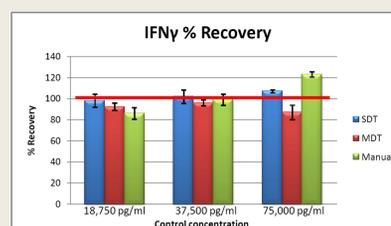
## 3 JANUS Modular Dispense Technology
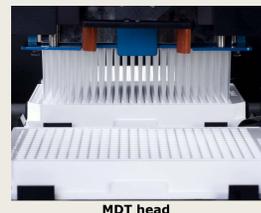
MDT head

The JANUS MDT P30/384 Head was used for all reagent additions for one series of AlphaLISA tests. Equipped with up to 384 P30 30ul tips, the MDT head is capable of ultra high throughput AlphaLISA sample preparation. It is optimal for use with samples that are already formatted in microplates.

JANUS MDT Serial Dilution Tool. JANUS Serial Dilution Tools (SDT) are a suite of new interchangeable dispense heads that allow the JANUS MDT arm to acquire a single row or column of disposable tips from a standard box of tips, discard the single row or column of tips, and continue to acquire single rows or columns of tips from the box until the box is exhausted.


SDTool
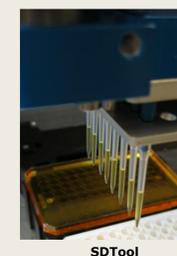
5 IL 17 Immunoassay Results

Good agreement is also shown among IL 17 standard curves generated with JANUS MDT, JANUS SDT, and manual sample preparation.
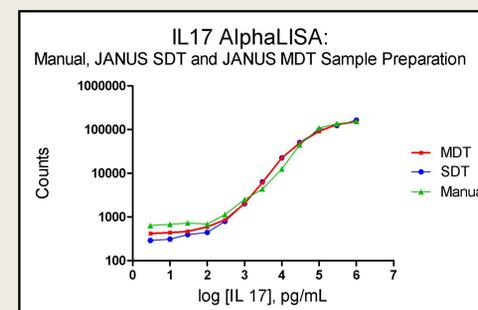

Controls of known IL 17 concentration were formulated by dilution in assay buffer and run in parallel with standard curves. Values interpolated from the standard curves were evaluated for recovery and precision.
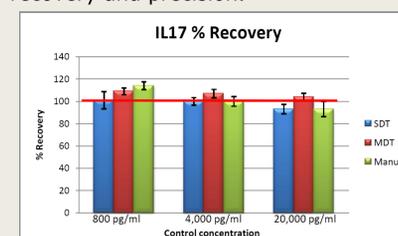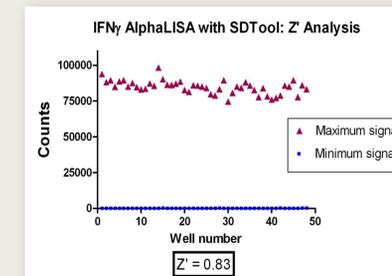

As with the IFNγ assay, precision values were similar among IL 17 AlphaLISA assays, but analyte recovery was more consistent when controls were run using JANUS automated sample preparation.

6 Z prime (Z′) Analysis
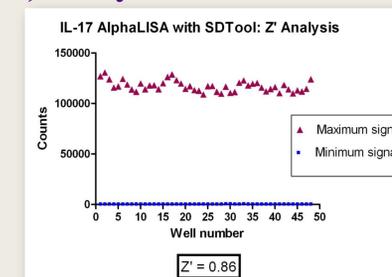
AlphaLISAs performed with JANUS SDT give Z′ values better than 0.8, indicating robust assays.

7 Summary

- In this study, IFNγ and IL 17 AlphaLISA cytokine assays have been automated on JANUS utilizing the MDT pipetting arm with an MDT head, an SDTool, and the MDT Gripper tool. Assay performance characteristics were compared with those from assays prepared manually.

- Analyte recovery was more consistent for the automated runs, and the assays are highly robust with Z′ values >0.80. Precision obtained from the automated assays was comparable to manually prepared runs.

- Both AlphaLISA chemistry and JANUS liquid handling workstations are flexible and scalable. AlphaLISA assays can be scaled to 96-, 384-, and 1536-well microplates without loss of assay sensitivity, and JANUS accommodates all of those formats.