

Automating AlphaLISA[®] Reagent Handling with the Tempest[™] Microfluidic Dispensing System

Johannes Josef Fels, Hanne Skov Pederson, Per Nielsen, Novo Nordisk
Ashton Grandy, Formulatrix

INTRODUCTION

Pharmacokinetic and Pharmacodynamic (PK/PD) studies play a critical role in the drug discovery and development process. The studies typically require characterization of drug and biomarker concentrations from large numbers of samples with high sensitivity and broad analytical range.

The AlphaLISA[®] technology (PerkinElmer, Inc., Waltham, MA) is a homogeneous no-wash alternative to conventional solid-phase ELISA assays that can be applied to biomarker detection in plasma and serum samples.

In the AlphaLISA assay, a biotinylated antibody and an antibody-conjugated Acceptor bead are used to capture the target analyte. The biotinylated antibody associates with a streptavidin-coated Donor bead. When the analyte is present in the sample, the Donor and Acceptor beads are brought together. Upon excitation, a photosensitizer inside the Donor bead converts ambient oxygen to an excited singlet state. Singlet oxygen diffuses up to 200 nm to produce a chemiluminescent reaction in the Acceptor bead, leading to light emission. The amount of light is proportional to the amount of analyte present in your sample, and the assay can be quantified by running against a standard curve.¹

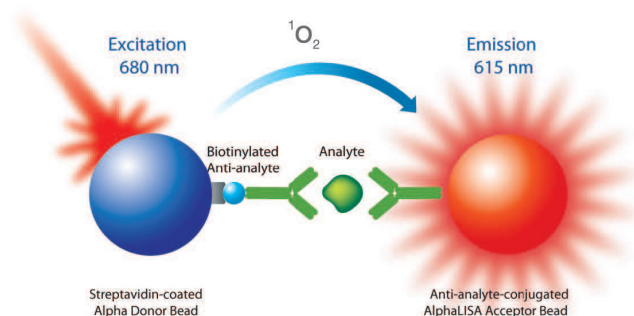


Figure 1. AlphaLISA technology principle.

The AlphaLISA workflow has been demonstrated to provide lower limits of detection, improved assay precision and broader dynamic range compared with conventional ELISA while allowing for a 5-fold reduction in sample volume for measurement of insulin in plasma samples.² The technology has been applied in an automated process supporting high-throughput bioanalysis with a capacity in excess of 10,000 data points in 6 hours.

Automation of the AlphaLISA reagent addition steps utilizing the Tempest[™] microfluidic dispensing instrument allows for a 6-fold reduction in reagent dead volume from 30-65 ml down to 5-10 ml compared with a solenoid valve-based dispensing instrument while maintaining assay performance in the automated workflow.

MATERIALS AND METHODS

Biotinylated antibodies and antibody-conjugated Acceptor beads and assay buffer were prepared as described previously.²

The assay was performed in 384-well plates (Optiplates, PerkinElmer). The assay was run with calibrators and samples in duplicate. The test samples (2 μ L) and standards were added to the wells by transferring from 96-well sample plates to the 384-well assay plate.

15 μ L of premixed antibody-conjugated Acceptor beads and biotinylated antibody in assay buffer were added to the wells using either the Tempest or FlexDrop[™] (PerkinElmer) automated dispensers.

The plates were covered with a lid and incubated at room temperature (RT) for 60 min. At the end of the incubation, Streptavidin Donor beads in assay buffer (30 μ L) were added using either the Tempest or FlexDrop dispensers.

The plates were again covered with a lid and incubated for 30 min at RT. Immediately thereafter, the plates were measured using the EnVision[®] plate reader (PerkinElmer).

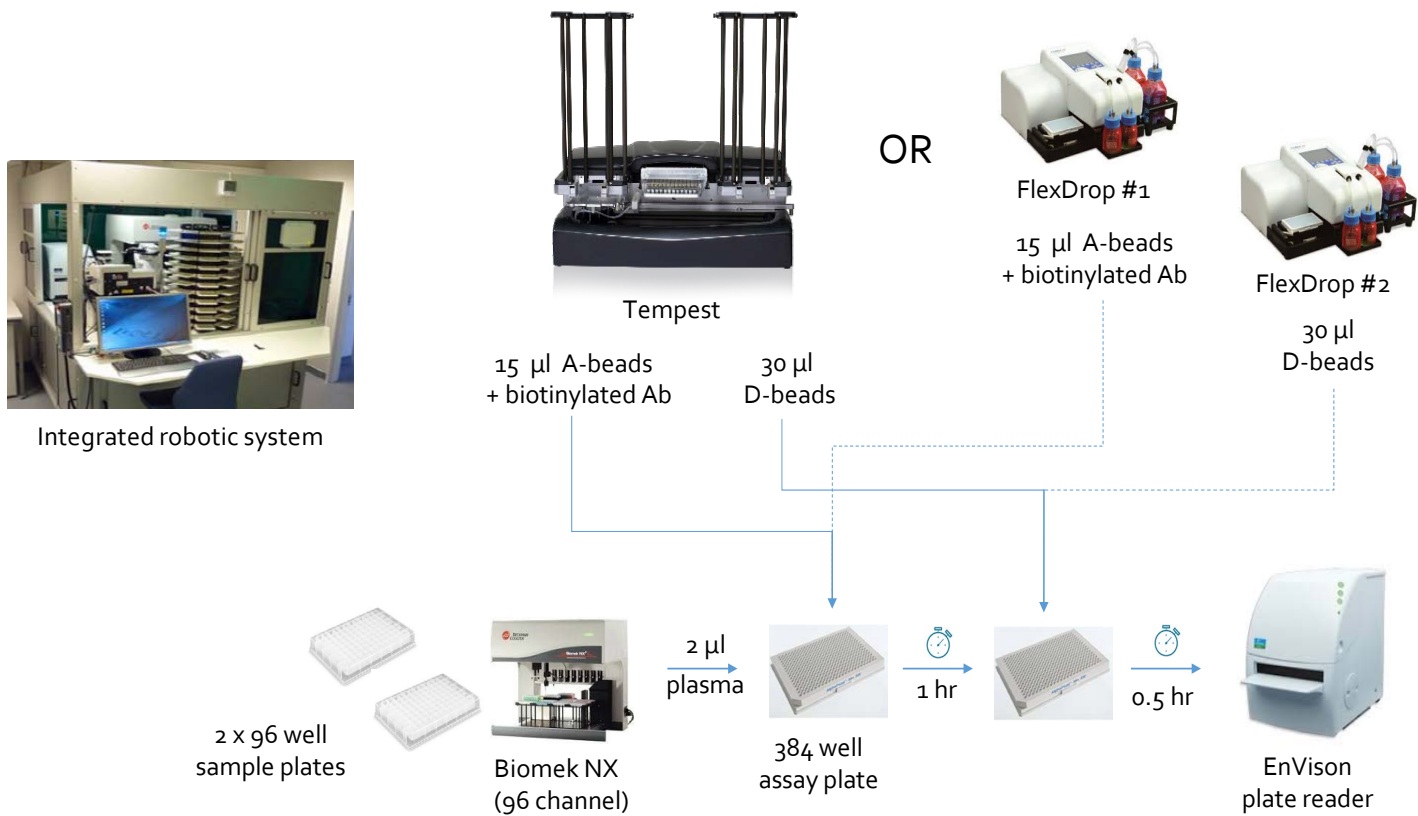


Figure 2. Automated AlphaLISA workflow. Plasma samples are transferred in duplicate from 96-well source plates to 384-well assay plate along with calibrators and controls for a total of 144 samples per plate. Assay reagents (Acceptor beads + biotinylated antibody and Donor beads) are added to assay plate using either one Tempest or two FlexDrop dispensers. Photo of the integrated system with robotic arm and incubation/storage racks capable of running 20 assay plates in 6 hours.

RESULTS

736 samples were processed through the automated AlphaLISA assay using two separate runs on consecutive days. Day 1 samples were processed using the two FlexDrop units to dispense the antibodies and beads to the assay plate. Day 2 samples were processed using the Tempest to dispense the reagents and the resulting concentration values were compared (Figure 3). R^2 value of 0.9741 was obtained with an average recovery of 101%.

A second experiment was performed to measure the robustness of the assay using the Tempest for reagent dispensing. 20 plates were processed using samples of 50 pM. The measured plates CVs were less than 5% with average CVs for double determination of less than 3% across the 20 plates (Figure 4).

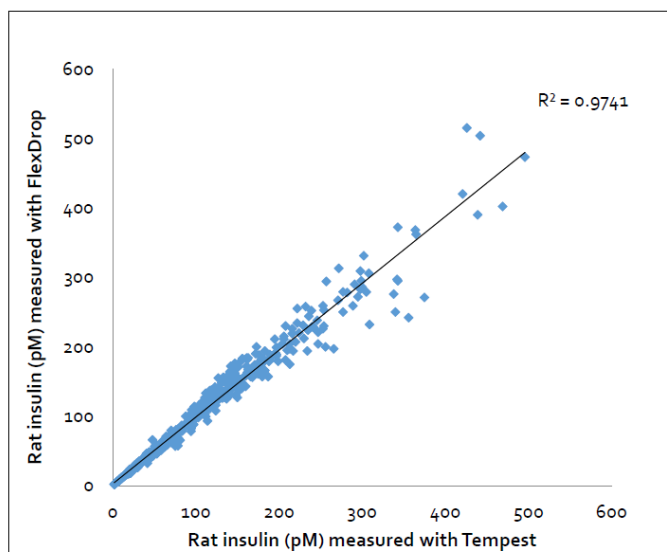


Figure 3. Comparison of automated AlphaLISA results measuring insulin concentration in plasma using either Tempest or FlexDrop instruments for the addition of reagents to assay plates.

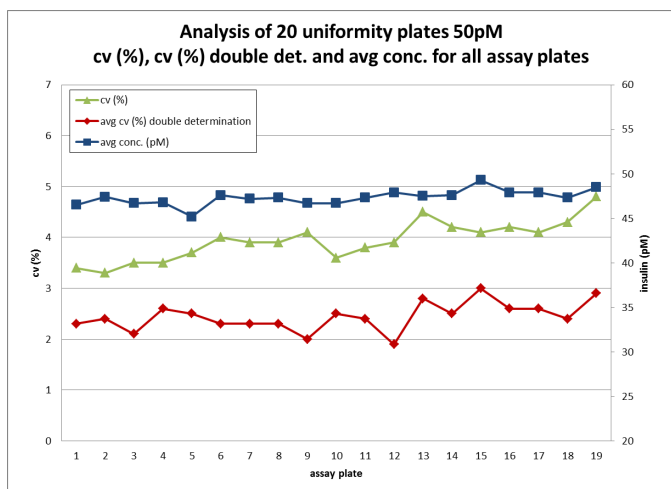
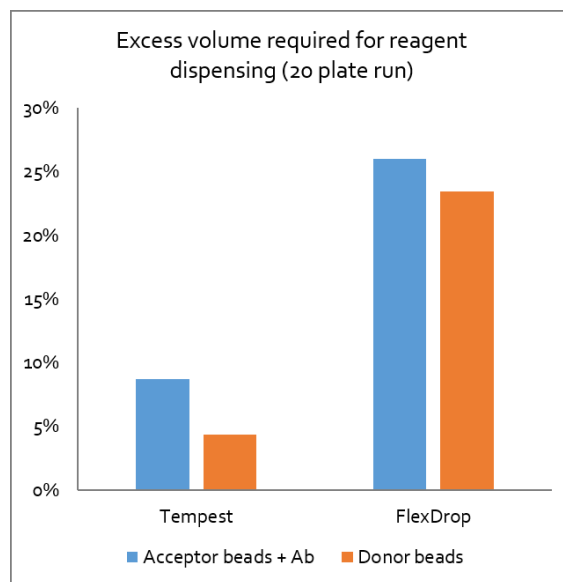


Figure 4. Reproducibility of automated AlphaLISA assay using Tempest for reagent dispensing.



Instrument	Acceptor Bead + Ab Dead Volume	Donor Bead Dead Volume
Tempest	10 ml	10 ml
FlexDrop	30 ml	54 ml

Figure 5. Comparison of reagent utilization between Tempest and FlexDrop instruments for automated AlphaLISA (20 plate run).

DISCUSSION

The homogeneous no-wash format of AlphaLISA immunoassays facilitates implementation of high-throughput automated workflows; however the cost of reagents must be considered when scaling a process to support the demands of biomarker measurements in large-scale PK/PD studies.

An integrated robotic workstation was designed to process up to 25 384-well plates in a 6-hour shift. In order to meet the required assay flexibility, the initial system design utilized two FlexDrop instruments for dispensing the AlphaLISA reagents. While this system design was capable of delivering the required throughput, the FlexDrop dispensers consumed up to 50 mL of reagents during the priming steps resulting in a significant increase in the reagent cost per plate.

For Research use only. Not for use in diagnostic procedures.

Tempest® is a registered trademark of Formulatrix, Inc. AlphaLISA®, EnVision® and FlexDrop® are registered trademarks of PerkinElmer, Inc.

The microfluidic diaphragm pump technology utilized in the Tempest instrument enabled a 3-5 fold reduction (Figure 5) in priming volume of the AlphaLISA reagents while maintaining assay performance and reproducibility required for PK/PD studies.

In addition, the ability of the Tempest to dispense up to 12 reagents from 96 nozzles allows for a single Tempest instrument to replace two FlexDrop instruments, reducing the complexity of the system hardware. Finally, the increased capacity of the Tempest enables the option to run up to 8 different assays compared with only 6 assays with the alternative dispensing instruments.

REFERENCES

1. AlphaLISA Immunoassay Development Quick Guide. www.perkinelmer.com
2. Poulsen F, Borres Jensen K. A Luminescent Oxygen Channeling Immunoassay for the Determination of Insulin in Human Plasma. *Journal of Biomolecular Screening*. 12(2); 2007. doi:10.1177/1087057106297566.