# Automation of Scintillation Proximity Assays

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## Introduction

Scintillation Proximity Assay (SPA) technology and LEADseeker™ homogeneous imaging system are used extensively in high throughput screening. When designing an automated SPA or LEADseeker assay several factors must be taken into consideration, for example: the isotope used, the bead type and liquid handling of beads. Perhaps the major consideration is the method of addition of the beads to the assay. Various different liquid handling devices are available which can carry out this step. Here we review a selection of those evaluated to date for compatibility with SPA.

## Method

Polyvinyltoluene (PVT), yttrium silicate (YSi), polystyrene (PS) and yttrium oxide (YOx) streptavidin beads labelled with [$^3$H] Biotin at 0.2mCi/mg beads were prepared. 50mg/ml suspensions of beads in water were dispensed into 384 well plates and plates were counted for 1 minute per well on a microplate counter for PVT and YSi beads, imaged on the LEADseeker instrument for 5 minutes with coincidence averaging for PS beads and 2 minutes with coincidence averaging for YOx beads. The volumes chosen for dispensing: 15, 10 and 5µl are based on the range typically used for bead addition to a 384 well plate in a miniaturised assay.

The dispensing program used on each instrument was individually optimised for best results with beads. In all cases each aliquot was dispensed from a separate aspirate step. For 5µl dispensing the beads were added to a well which already contained 20µl of water, to assist the removal of the beads from the tips and ensure complete coverage of the base of the well. Where the instrument provided the choice of fixed or disposable tips the disposable tip option was chosen for ease of radioactive waste disposal. Tips were changed between bead types by which time a significant build up of bead could be detected on the outside of each tip. All of the instruments with the exception of the Labsystems Multidrop™ (which employs a peristaltic pump) are operated by air displacement. In this study the units were evaluated as stand alone items but all of the instruments are capable of being integrated into a robotic system.

## Results

CCS Packard 384 head

The 384 head is a module option for the CCS Packard PlateTrak™. It is capable of dispensing down to 0.5µl with a quoted precision of 5% coefficient of variation (CV) when dispensing aqueous solutions. As the head has 384 tips, only one trip to the bead reservoir was required per plate, which is a significant benefit if speed and therefore throughput is of great importance. An entire plate can be filled in under 2 minutes. It is possible to fill a plate faster by using higher aspirate and dispense speeds, but in this instance they were slowed to the lowest possible settings for most accurate dispensing of beads. A 5µl air gap was aspirated prior to aspirating an excess of beads. Reverse pipetting was chosen as the most precise way of dispensing beads. Precision data at optimal conditions can be seen in figure 1.

To maintain a suspension the beads were mixed with the tips prior to aspiration, this was however not sufficient for yttrium beads and additional mixing with a hand held pipette was required.

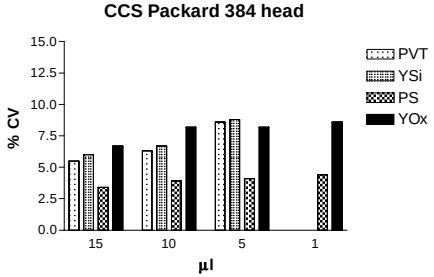


*Figure 1. Precision achieved when dispensing [$^3$H] labelled beads into a 384 well plate using the CCS Packard 384 head.*

Packard Multiprobe™ II

The Multiprobe II is a very flexible instrument with the ability to perform any assay in 96 and 384 well plates. With only 4 probes it was relatively slow to fill a 384 well plate with beads, taking approximately 30 minutes when each dispense required a separate trip to the reagent reservoir. The probes have liquid level sensing which enables beads to be mixed with a small magnetic stirrer, as the tips can compensate for the vortex formed by the stirrer. In this case the tips were programmed to sense the liquid surface and then aspirate from 3mm beneath, ensuring that the liquid at the surface, which tends to contain fewer beads, was avoided. The Multiprobe II was operated using the default speeds for an aqueous solution. The precision achieved with the above settings can be seen in figure 2.

Beckman Multimek™

The Multimek in this evaluation had 50µl tips loaded onto a 200µl head. The head had 96 tips and filled a 384 well plate by quadrant positioning. The beads were dispensed by reverse pipetting with a pre-aspirate air gap. The aspirate and dispense speeds were set at 5% of maximum speed to ensure precise aliquots. The precision achieved when the instrument was operated as described can be seen in figure 3. Running this program it took less than 5 minutes to fill a plate. Unfortunately beads had to be mixed by hand as tip mixing was insufficient to maintain a homogeneous suspension of yttrium beads and no other option was available.

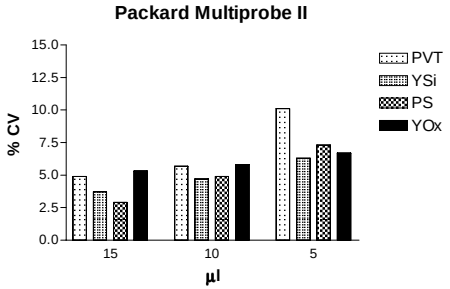


*Figure 2. Precision achieved when dispensing [$^3$H] labelled beads into a 384 well plate using the Packard Multiprobe II.*

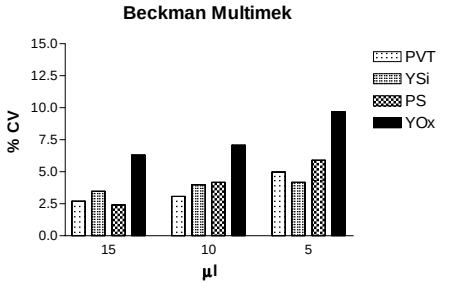


*Figure 3. Precision achieved when dispensing [$^3$H] labelled beads into a 384 well plate using the Beckman Multimek.*

Labsystems Multidrop

The Multidrop is a simple instrument for liquid addition and does not have the diversity of features found on the other instruments. This makes it very small in comparison and relatively inexpensive. It has a head of eight fixed tips fed by eight lines of tubing passing through a peristaltic pump. The beads are held in a stirred reservoir into which the tubing is fed. As the pump operated very quickly a plate can be filled with beads in under 30 seconds. However beads did have a tendency to settle in the tubing in between each plate dispensed. The settled beads were flushed from the tubing by including a prime step between each plate dispensed. The Multidrop is limited to dispensing in increments of 5µl.

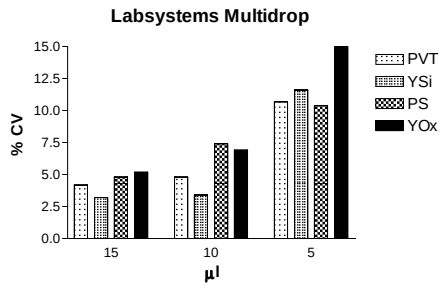


*Figure 4. Precision achieved when dispensing [$^3$H] labelled beads into a 384 well plate using the Labsystems Multidrop.*

## Discussion

The instruments described here cater for different needs within the screening environment. The 384 head is one of several modules available for the PlateTrak and, as such, forms part of a high throughput screening instrument. The Packard Multiprobe is ideally suited to assay development and due to it's flexibility can perform a wide range of assays. The Labsystems Multidrop is a rapid instrument for reagent addition and is often used for the addition of beads to plates which have been prepared on another instrument. The Beckman Multimek is a precise instrument that can be operated as a stand alone item or integrated into a robotic system. All of the instruments achieved similar levels of precision for 10 and 15µl dispensing, 5µl dispensing is however more challenging. With all of the instruments the precision achieved was poorer than that quoted by the manufacturer for aqueous solutions. This is perhaps not surprising as there are additional errors associated with dispensing beads e.g. maintaining a homogeneous suspension and build up of beads on tips. There are various ways of meeting the challenge of maintaining a bead suspension. The Multiprobe due to it's liquid level sensing can aspirate from a stirred vessel. A mixing module can be purchased for the PlateTrak which is designed to handle beads. The Multidrop can be fed from a stirred vessel and if plates are replaced rapidly bead settling is not an issue. The Multimek has been successfully used with custom built bead mixing reservoirs. To remove bead build up tips can be washed in a sonic bath for a few seconds after every 3 – 4 plates.



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