	<h1>ELISA Automation</h1>	Hudson Control Group 10 Stern Avenue Springfield, NJ 07081 TEL 973-376-7400 www.hudsoncontrol.com
AB104A NOV 2002		

INTRODUCTION

The ELISA, or Enzyme-Linked ImmunoSorbent Assay, is a commonly used tool for the detection of small quantities of a target compound in solutions such as serum, urine, cell supernatants, etc. Since this is an immunoassay, the success of an ELISA experiment is dependent on the quality of the antibody in relation to its ability to specifically bind with the target compound, or antigen.

ELISA experiments can be performed in a variety of ways. While the details of ELISA are beyond the scope of this document, we can summarize the concepts of a generic ELISA as follows:

- A microplate is coated with antibody (or antigen).
- Samples, controls, and standards are added to the wells of the microplate, and incubated between ambient and 37° C for some time period. During the incubation, either some amount of the antigen in the sample will bind with the precoated antibody or the antibody in the sample will bind with the precoated antigen. Some may not bind; this is referred to as unbound antigen or antibody.
- After the incubation, the unbound entities are washed away. This wash is typically a repeated cycle of adding wash buffer, soaking, and removing the liquid in each well.
- A secondary antibody called the conjugate is added. (This typically has an enzyme attached that will react with a substrate to produce a color change.) Another incubation period is performed, during which the conjugate will bind to the antibody-antigen complex in the plate.
- After the incubation, another wash is performed to remove any unbound conjugate.
- Substrate is added. The enzyme will react with the substrate and cause a color change in solution, providing a means to measure the amount of conjugate, which in turn will tell how much antigen is present. Another incubation period allows time for this reaction to take place.
- A “stop” reagent may be added at this point to stop the substrate-enzyme reaction and prevent further color development. This stop reagent is typically a dilute acid.
- The wells in the microplate are now “read” and their contents measured with a detector. The values of the readings are used to determine the amounts of antigen in the sample.
- Typically, some of the wells within the microplate are dedicated to standards and controls. The standards are used to generate what is called a standard curve that is used to provide the means to quantify the binding in the sample wells. The controls are known quantities and are used to gauge the success of the assay by evaluating the received data versus the stated concentrations for each control. All of the above steps are also applied to these wells, and these results are used in the quantitation of the final answer.
- There are many variations to the above steps, but the basic procedure is the same.

This document focuses on considerations for automating ELISA experiments using laboratory automation robotics. This automation offers the capability to use ELISA experiments in a high-throughput mode. The ability to automatically perform ELISA on 20-100 microplates per day is necessary to meet the modern demands of high-throughput screening for the development of new medicinal drugs.

AUTOMATION OVERVIEW

ELISA experiments can be broken down into basic steps:



The number of steps and the order will vary depending on the specific assay. But there are 4 basic steps that are always present: Washing, Dispensing, Incubating, and Reading. Dispensing includes pipetting operations for sample preparation. (Transfer of samples, controls, and standards).

Various laboratory microplate-based devices developed for the lab automation market can be used to accomplish these steps:

- **Washing:** Automatic plate washers.
- **Dispensing:** Liquid handlers or dedicated microplate dispensers.
- **Incubating:** Plate stacks (ambient) or automated incubators.
- **Reading:** Microplate readers.

These devices can be connected together in order to automatically process all of the ELISA steps for a batch of microplates without user intervention. This is referred to as “walkaway automation”. In order to automate the process in this manner, two more components are required:

- **Automation robotics:** These are devices that are made specifically to automate the movement among multiple microplate-based lab automation devices.
- **Scheduling software:** The software will allow the user to program a method, which is based on the flow of the ELISA. When the method is run (executed) the scheduling software will direct the robotics to move the plates among the various assays steps in the most time-efficient manner possible.

For purposes of automation, the ELISA is typically broken down into three parts:

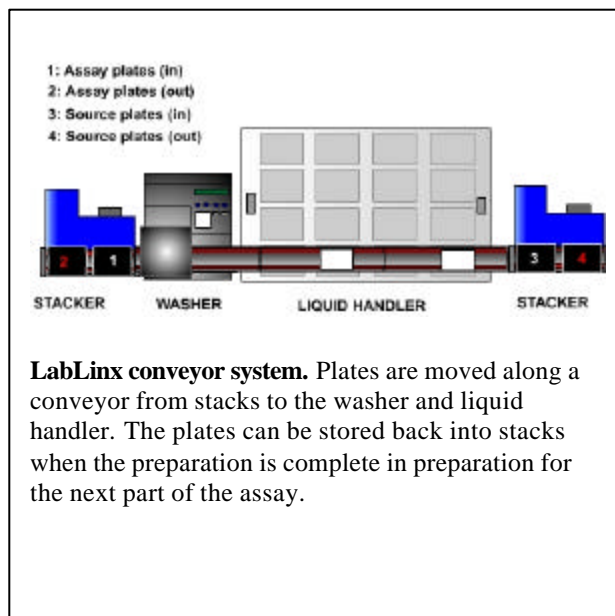
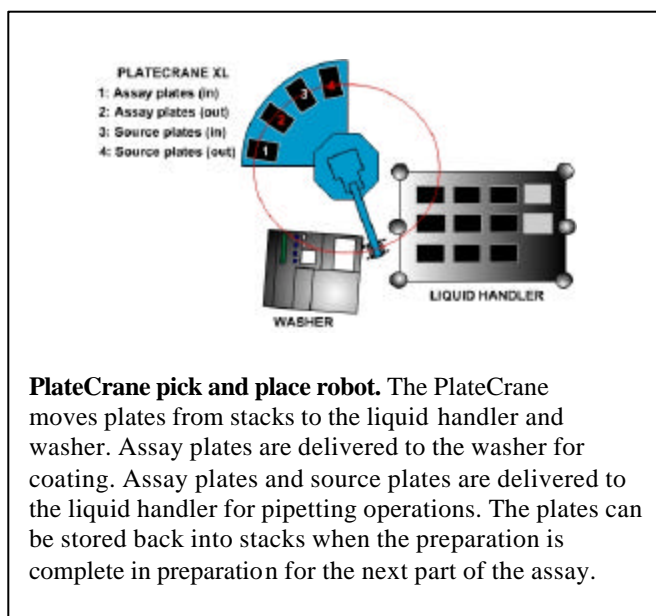
- **Plate Preparation:** Coating of the plate with the required antigen or antibody.
- **Sample, Standards, and Controls Preparation:** Generating the appropriate dilutions of the samples, generating the standard dilution curves, and preparation of the controls to monitor the effectiveness of the assay.
- **Sample Assay:** The plate is subjected to the remaining steps of the assay.

The plate preparation and transfer of the samples is usually done with a liquid handling workstation that can access a variety of source labware, access the required diluents, and transfer the samples to the prepared assay plates. Depending on the assay, the controls are typically prepared manually or are provided in the appropriate dilution as a pre-made reagent. The Sample Assay steps typically can be performed without a liquid handler, using exclusively simpler devices such as washers, dispensers, and readers.

It is possible to develop a complete automation solution that will perform all of the steps, but some laboratories instead choose to separate these parts into 2 different workcells for convenience or space-savings.

PLATE PREPARATION AUTOMATION

A liquid handler and washer are needed to perform the plate preparation operations. Microplates are provided to these devices by robotics in order to achieve unattended automation. Hudson offers two products for this automation.

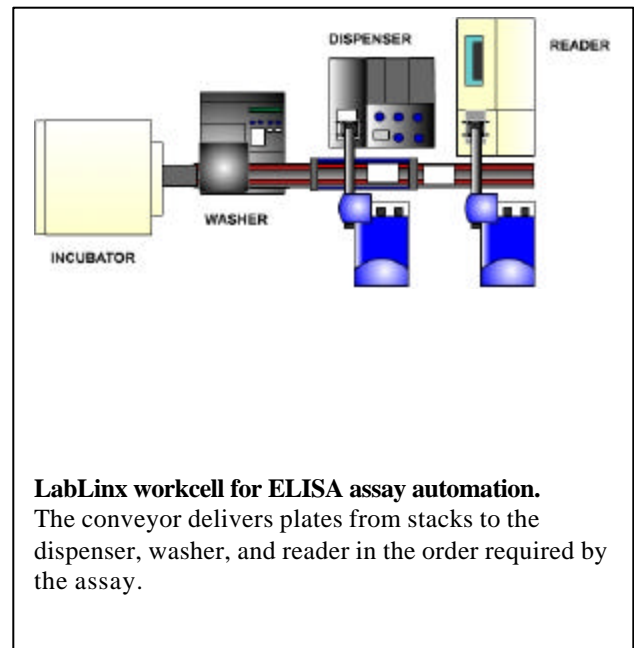
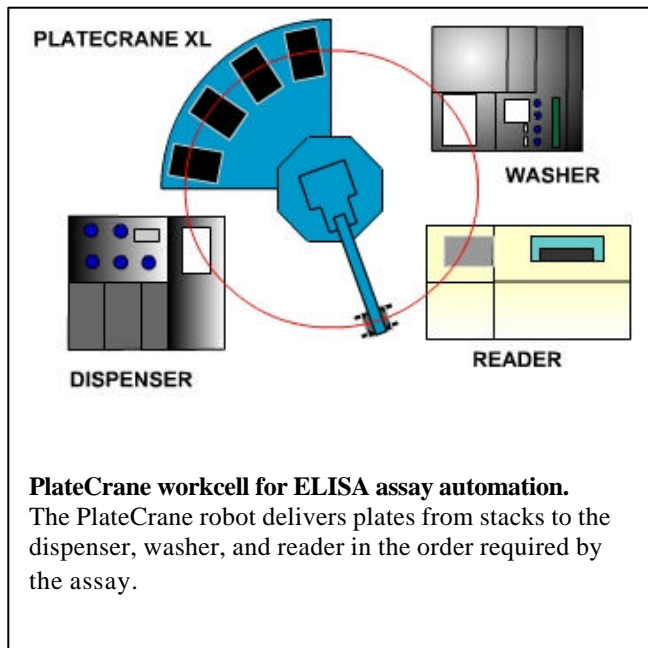


The PlateCrane workcell provides a simple and economical solution for automation plate preparation. The LabLinX workcell is capable of higher throughput operation since no arms are required to move the plates. Plates are

rapidly dispensed from the stacks to the washer or liquid handler, where they are processed while remaining on the track. LabLinX also offers more expandability, should more devices such as sealers need to be added. The LabLinX workcell can also be expanded to automate the complete ELISA process. (see below).

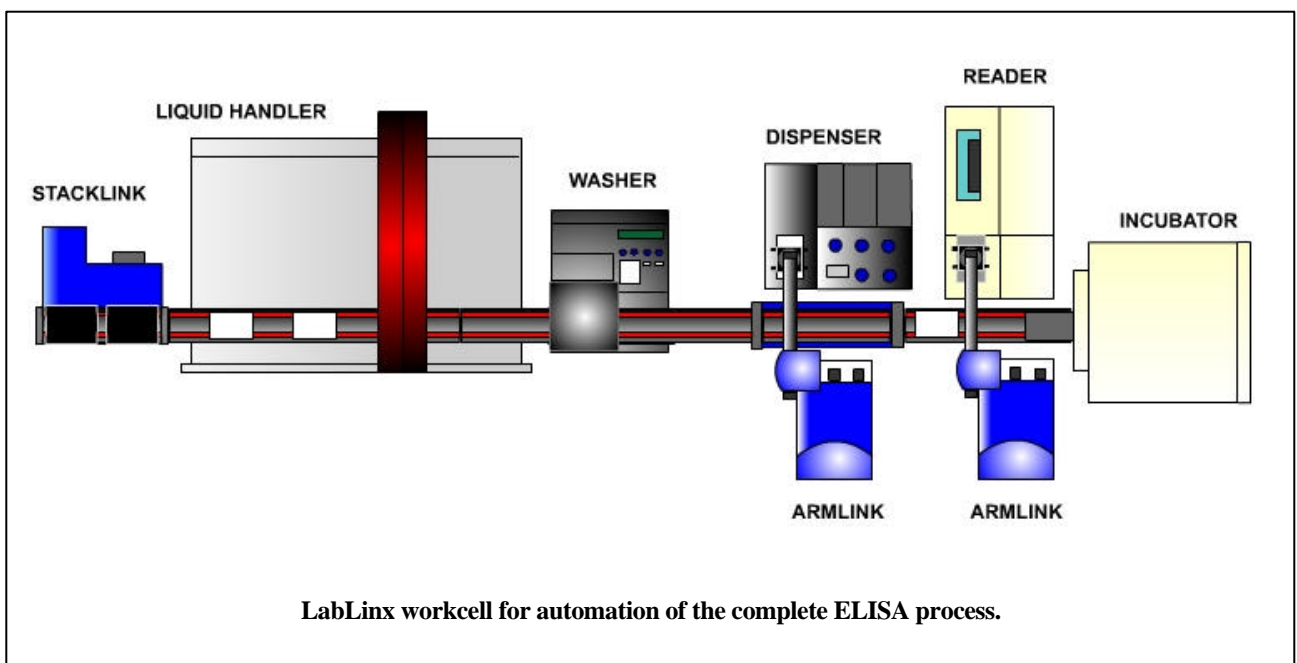
SAMPLE ASSAY AUTOMATION

Once the plate is prepared, the subsequent liquid handling steps of the assay can be performed with a plate dispenser, since all wells of each plate will be subjected to the same liquid dispensing. A washer is added for plate washing and a reader is added for the final detection step.



Either workcell will perform the assay automation. LabLinX offers higher throughput track-based plate movement as well as more expandability. The modular design of LabLinX can be used to build an expanded system that can automate all aspects of the ELISA process:

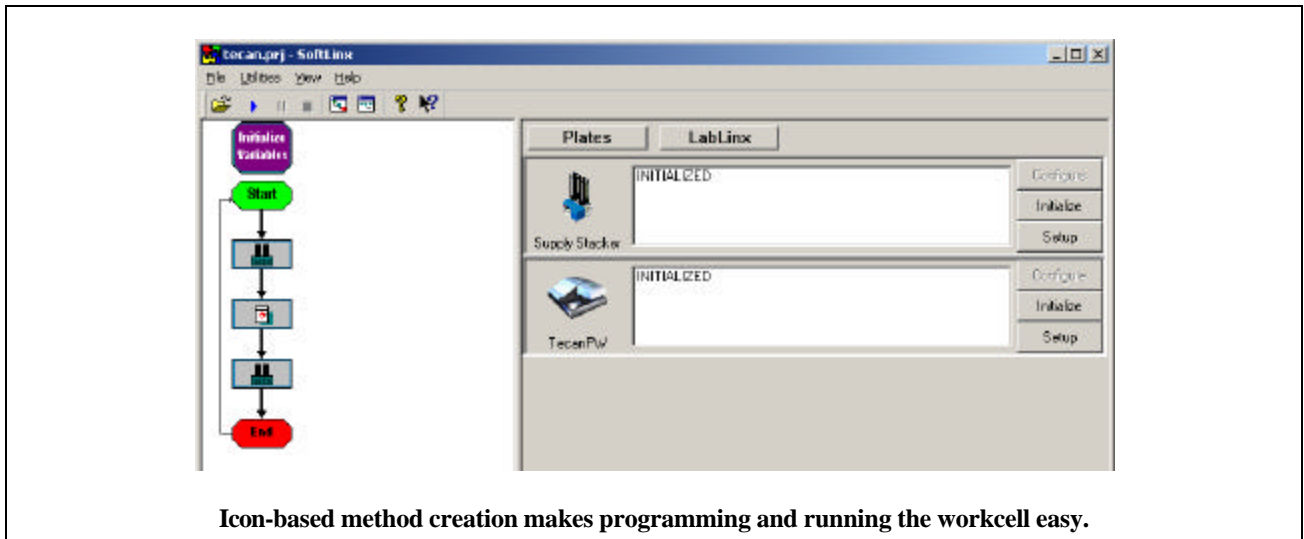
COMPLETE ELISA AUTOMATION



The conveyor-based LabLinx system is integrated with all of the devices needed to perform both the plate preparation and sample assay parts of the ELISA. Plates are moved among the liquid handler, dispenser, washer, and reader. The above system also illustrates the integration of an automated incubator, providing the ability to perform incubation steps in a temperature- and humidity-controlled environment.

SOFTWARE

SoftLinx software from Hudson is used to program the LabLinx modules, and also performs supervisory control of the entire system, including any other devices that are part of the workcell. Based on Hudson's experience with robotic integration of third-party devices since 1983, SoftLinx is designed to make the programming and automation of lab automation workcells easy for routine operation by any lab personnel and flexible for custom modification by programmers. SoftLinx features a drag-and-drop method editor and operates the system via event-driven dynamic scheduling



Icon-based method creation makes programming and running the workcell easy.

CONCLUSION

Hudson offers lab automation robotics that can be configured with other microplate-based devices to build ELISA automation workcells. The PlateCrane can be used to configure simple, economical workcells that provide unattended operation for segments of an ELISA experiment, and LabLinx can be used to build higher speed workcells, or even workcells that can automate the entire process including plate preparation.

For Information about ELISA automation, please contact Hudson at 973-376-7400 or visit www.hudsoncontrol.com