Automation of Cell-Based Assays

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Introduction

Recently there has been a trend toward more frequent use of cell-based assays for drug discovery. If cell-based assays are to be used as part of the high throughput screening process, where it is desirable to analyze tens of thousands of samples per day, then some type of laboratory automation is required. However, cell-based assays differ from more traditional screening enzyme- or antibody-based assays in that the use of live cells requires special considerations. This bulletin will outline these issues as they relate to the application of robotics-based laboratory automation for cell-based assays.

What are Cell-Based Assays?

The term is used to refer to any of a number of different experiments based on the use of live cells. This is a general definition and can include a variety of assays that measure cell proliferation, toxicity, motility, production of a measurable product, and morphology. Cell-based assays offer a more accurate representation of the real-life model since live cells are used, and also offer the possibility of a dynamic experiment through monitoring the numbers or behavior of the live cells.

Considerations for Cell-Based Assay Automation

Cell-based assays can be conducted in the ubiquitous microplate format that is used for other types of high throughput screening. This means laboratory robotics that are used to move microplates can be used. However, there are a number of issues that need to be considered when developing automation for cell-based assays.

- **Environmental control.** The cells typically require control of temperature, pH, and humidity in order to maintain viability.
- **Contamination protection.** The cells must be protected from contamination of external agents such as bacteria. In some cases, it is necessary to protect laboratory personnel from contamination by the cells (i.e. viral cell lines). If high-sensitivity fluorescence detection is being used, then dust particle contamination must be kept to a minimum, as dust causes false positive readings for these kinds of detection systems.
- **Long incubations.** Some experiments require long incubation times, up to several days, which has an impact on how automation is designed and used.

Optimizing Laboratory Robotics for Cell-Based Assay Automation

It is possible to apply robotic automation for cell-based assays by using some modifications to the system to accommodate these special requirements.

Microplate lid handling

Lidded microplates are frequently used as a measure to protect against contamination. Laboratory robotics are required that can:

- Pick up and move lidded plates.
- Remove, track, and replace lids during the assay steps.

Automated incubators

Automated incubators provide a means for storing microplates under controlled conditions of temperature, pH and humidity combined with the ability to interface with plate-movement robotics. This provides the ability to move plates from the incubation environment to other devices that must act on the sample as part of the assay, then return to the incubator. This is particularly valuable for assays that include multiple incubation steps.

Environmental control

While plates are outside the incubator environment, it is necessary to prevent contamination. This can be accomplished by enclosing the laboratory automation robotics and other devices within a controlled environmental chamber. There are various options available depending on the specific requirements for protection of the samples and the laboratory personnel.

- Laminar flow hoods. These provide a protective air curtain along with positive pressure to protect the inside contents from external contamination, such as from airborne bacteria. These do not protect personnel in the lab.

- Biosafety cabinets. These incorporate a combination of airflow control and HEPA filtration to protect both the contents of the cabinet and the people outside. There are several types of Biosafety cabinets as specified by the CDC (Centers for Disease Control). A common example is the Class II Type A where 70% of the intake air is recirculated through HEPA filtration, with the remaining 30% returned to the room, also with HEPA filtration. This system does not require a dedicated external duct.

Laboratory hoods and Biosafety cabinets are commonly available, however the typical sizes that are used for manual work are too small to contain laboratory automation equipment.

In order to effectively automate a cell-based assay, it may be required to move microplates from devices within the hood to other devices that are outside the hood. In order to accomplish this, the laboratory robotics must be effectively designed, and the hood must be modified in such a way to allow pass-through access while maintaining the specified environmental control.

Components for Cell-Based Assay Automation

Many of the same microplate-based lab automation devices that are used in traditional high-throughput screening are also used in automated CBA.

- **Liquid handlers.** Can be used for
  - Cell plating
  - Compound addition
  - Stimulus/inducer/inhibitor addition
  - Substrate and other reagent addition

- **Dispensers.** Can be used for:
  - Media addition
  - Stimulus/inducer addition

- Substrate addition

- **Washers.** Can be used for:
  - Plate washing
  - Media removal and addition

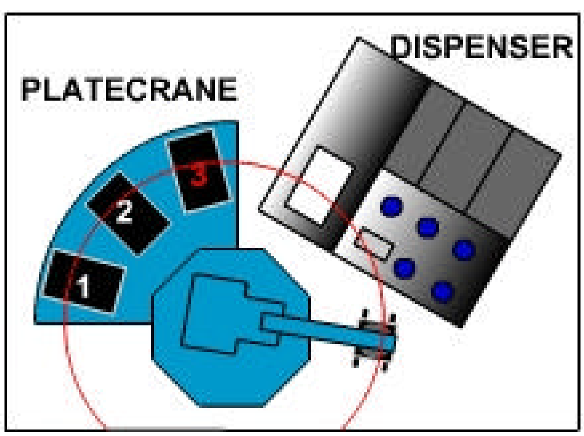
- **Automated incubators**.

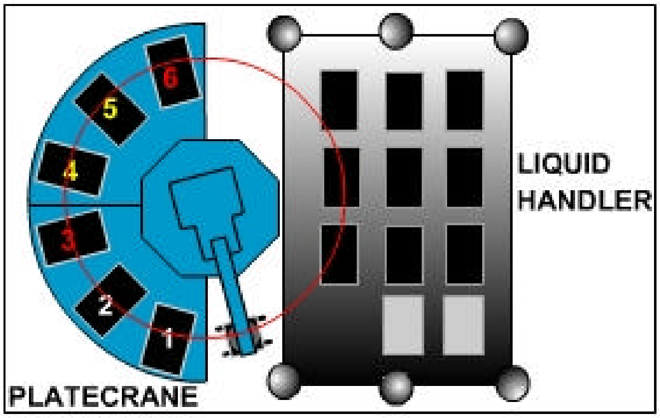
- **Readers and Imaging systems**. Some cell-based assays use basic absorbance, chemiluminescence, or fluorescence readers for the final measurement of the results. There are also a number of high-performance imaging systems that are used. These are paired with special detection reagents and can also perform live, real-time measurements of live cells.

- **Automated Centrifuges**. Used to pellet cells on the bottom of the wells of a plate in order to selectively remove a volume of media. The removed media may be evacuated to waste and replaced using a washer or the volume can be transferred to a separate plate for in order to determine the contents via a binding assay (i.e. ELISA). This is especially useful for non-adherent cell lines that "float" in the media and would be aspirated without centrifugation. Adherent cell lines do not need this equipment since they are only removed by physical or chemical means.

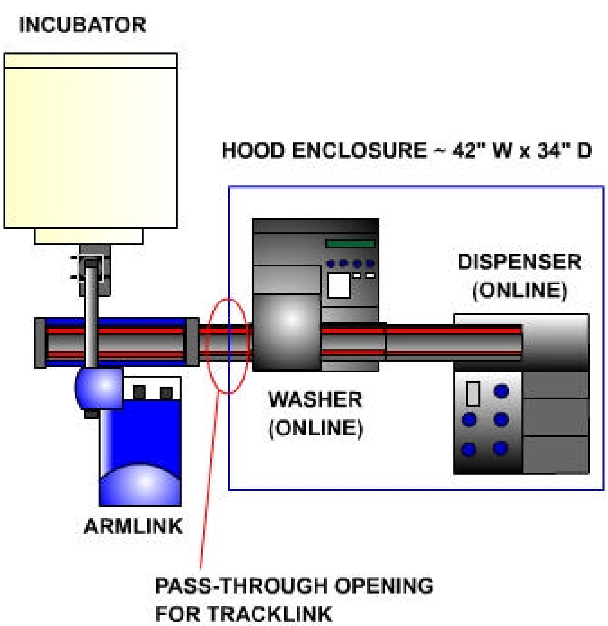
Examples of Cell-Based Assay Automations

Just as there is no one way to accomplish a cell-based assay, there is no one way to automate a cell-based assay. Depending on the structure of the experiment, as well as the available time, space and budget, it might be best to automate a single small portion, automate several portions and link them together manually, or automate an entire assay.

<table>
<tr><th>Cell dispensing workcell</th><th>Equipment</th><th>Process Steps</th></tr>
<tr><td>
</td><td>• PlateCrane
• Dispenser
• Stack1 – input up to 30 microplates; 25 with lids
• Stack2 – input up to 30 microplates; 25 with lids
• Stack3 - output</td><td>1. PlateCrane moves plate from input stack to dispenser
2. PlateCrane removes lid from plate if present
3. Dispense cells
4. PlateCrane replaces lid
5. PlateCrane moves plate to output stack</td></tr>
<tr><td colspan="3">This small workcell uses a dispenser to plate cells. A dispenser that can be programmed for gentle delivery may be required for some cell solutions to prevent mechanical damage from the dispense operation. This automated workcell can fit inside a hood enclosure with dimensions of 44"W x 30"D x 30"H. (Inside dimensions).</td></tr>
</table>

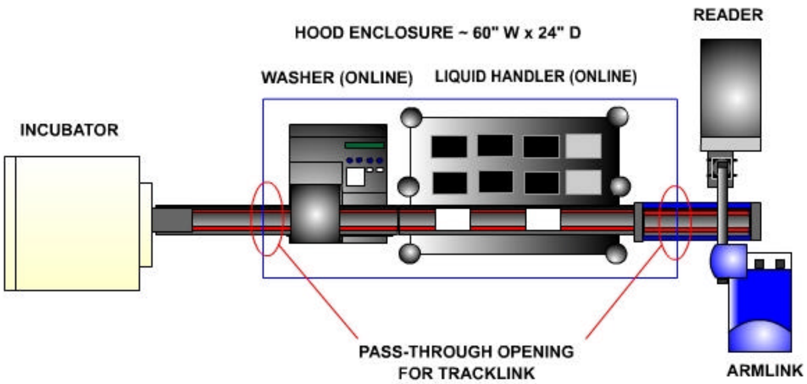
Compound predilution workcell	Equipment	Process Steps
	• PlateCrane • Liquid handler • Stacks1-2 – input up to 30 master microplates; 25 with lids • Stacks4-5 – input up to 30 dilution microplates; 25 with lids • Stacks3&6 - output	1. PlateCrane moves master plate from input stack to liquid handler 2. PlateCrane moves dilution plate from input stack to liquid handler 3. Liquid handler performs dilution of master plates 4. PlateCrane moves master plate from liquid handler to output stack 5. PlateCrane moves dilution plate from liquid handler to output stack

This compound predilution workcell can fit inside of a hood environment, protecting the cells from airborne contamination. Any other portions of a cell-based assay that require a liquid handler can also be performed with this workcell, such as reagent additions. By using a small-footprint liquid handler, this workcell can fit within hood dimensions of 56"W x 36"D x 30"H. (Inside dimensions).

Washing & dispensing workcell	Equipment	Process Steps
	• LabLinx with ArmLink and TrackLink • Incubator • Washer • Dispenser	1. Incubator presents plate to ArmLink 2. Move plate to washer 3. Wash plate 4. Move plate to dispenser 5. Dispense solution 6. Move plate to ArmLink 7. Incubator returns plate to controlled environment

The plates are stored in a controlled environment within the incubator. When liquid handling steps are required, the plate is moved from the incubator directly to the hood enclosure, minimizing exposure to the outside environment. This is accomplished with a special modification to the hood that allows the LabLinx conveyor to pass directly through, connecting the incubator to the components inside.

<table>
<tr><th>Cell-based assay workcell</th><th>Equipment</th><th>Process Steps</th></tr>
<tr><td>



</td><td>

- LabLinx with ArmLink and TrackLink
- Incubator
- Liquid handler
- Washer
- Reader

</td><td>

1. Move plate from incubator to washer
2. Plate wash
3. Move plate to liquid handler
4. Reagent dispense
5. Move plate to reader
6. Read results

</td></tr>
<tr><td colspan="3">This is an example of how a major portion of a cell-based assay can be automated. The plates are stored within the controlled environment of the incubator. Plates are moved from the incubator to within the hood enclosure via the LabLinx conveyor system. Washing and dispensing occur within the hood. If required, plate delidding can occur within the hood. Once the final reagents have been added, the plates are passed back out of the hood enclosure to the reader. After the read operation, the plates can be moved back to the incubator, or if desired, can be discarded.</td></tr>
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