Technical Overview

Using the Agilent BioTek EL406 and Agilent BioTek BioStack to Automate the Liquid Handling Steps of Assays

Semi-automation of ELISA and colorimetric assays

Author

Paul Held, PhD
Agilent Technologies, Inc.

Abstract

Many biological assays, such as ELISA or colorimetric assays, have been standardized by using microplates. Standardization allows the use of instrumentation to automate specific fluid movement tasks, such as plate washing or the addition of reagents. Typically, each task requires a dedicated instrument to accomplish the fluid handling task with manual intervention to move the plate to each instrument. The Agilent BioTek EL406 washer dispenser is multifunctional and capable of carrying out the different fluid handling tasks for a single assay. This technical overview describes how to use Agilent BioTek liquid handling control (LHC) PC software to control an EL406, in conjunction with an Agilent BioTek BioStack microplate stacker, to automate the liquid handling portion of many assays.

Introduction

Programming the LHC to automate multistep processes is straight forward. LHC handles tasks in a linear fashion from the beginning of the protocol steps towards the end with no multitasking. Once a step has been completed, the next step is initiated. With this in mind, one can automate a series of liquid handling and incubation steps that are performed in succession for a series of plates.

Correct timing between steps is the paramount issue regarding programming automated assays using LHC with the EL406 and BioStack. If repeatable and valid results are to be obtained, differences in timing for process steps and process times with various plate counts need to be accommodated. For example, the time it takes the EL406 to dispense 200 µL of a reagent would be greater than the amount of time required to only add 50 µL of another reagent. Likewise, the time it takes to process one step of an assay for 10 plates would be longer than the time required for five plates. These differences in process times between steps, which need to be accounted for to achieve optimal and repeatable results, can be compensated for using the "Delay" command in LHC. This command is typically used under two circumstances. Primarily, it is used to provide ambient incubations when all plates have been returned to the BioStack and then restacked. The delay time used depends on the number of plates processed. Because the delay needs to start after all plates have been processed then restacked, one needs to calculate a different delay interval for different plate counts.

The equation for delay time is as follows:

Equation 1. $D_n = Inc - (P_n + R_n)$

Where D_n is the delay time for n number of plates, Inc is the intended total incubation time, P_n is the process time required for n number of plates, and R_n is the time necessary to restack n number of plates. For example, if the intended incubation time were 30 minutes, and the preceding process takes 30 seconds per plate, the delay time would be 26 minutes and 31 seconds for a five-plate batch. D_n = 1,800 seconds – ((5 × 30 seconds) + 59 seconds). Therefore, D_n = 1,591 seconds, or 26 minutes and 31 seconds.

Figure 1. Agilent BioTek EL406 washer dispenser and Agilent BioStack microplate stacker.

In addition to delaying the initiation of the next process step to provide the necessary incubation intervals, the delay command can be used to control the specific timing between EL406 procedural steps. With most ELISA process steps, the actual procedure is a repeated task. For example, a dispense step on a microplate can be broken down into 12 separate dispense procedures: one for each 8-well strip of the plate. As dispense-volumes increase, the time to accomplish the procedure increases as well. This difference has to be accommodated for the correct assay timing to be maintained.

The differences between onset of incubation periods and the onset of delay steps is the most complicated feature of LHC automation programming. Incubation steps typically will begin at the completion of a process task on the first plate, while delay steps begin with the completion of the previous step on the last plate. In other words, the incubation for plate-one would naturally begin after the addition of a reagent. For example, the Avian Flu assay requires 30 minutes incubation after the addition of sample (Figure 2), which is a manual process. The initial delay within LHC is 29 minutes and 4 seconds, rather than the 30 minutes suggested by the assay protocol (Figure 3, line 1). This difference is the result of the time required to prime the washer and three dispensers.

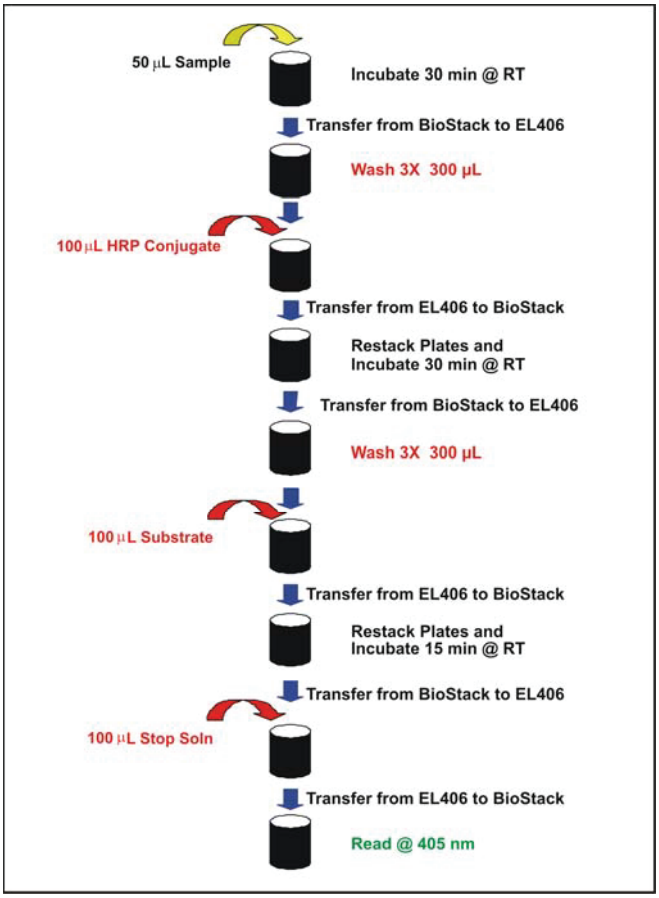

Figure 2. Avian influenza virus antibody assay test procedure steps. Processes carried out by the Agilent BioTek EL406 washer dispenser are indicated in red.

The second incubation, also 30 minutes, is accommodated with a 22-minute and 35-second delay (Figure 3, line 13). The 7-minute and 25-second difference is the time from the addition of conjugate to the first plate to the completion of the process of the wash cycles, reagent addition, and restacking of the five 96-well plates back into the input stack (Table 1).

During that time, the first plate was incubating at ambient temperature, while the other plates were being processed. Once all of the plates are finished, they are restacked. With the completion of the delay, the first plate is again transferred to the EL406 and the "Add Substrate" assay step is initiated.

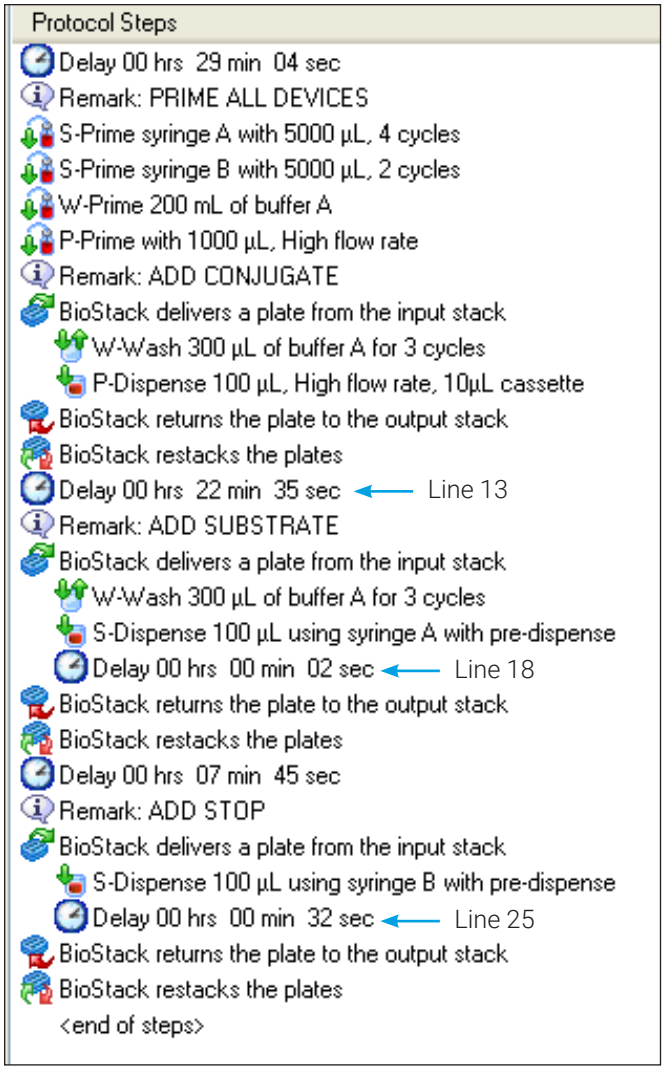

Figure 3. Agilent BioTek LHC process steps required to automate avian influenza antibody assay for a five-plate run.

The 2-second delay denoted in the "Add Substrate" step addresses the timing differential of adding reagent with the peristaltic pump rather than the syringe pump (Figure 3, line 18). Likewise, the 32-second delay in the "Add Stop" step accommodates the difference in timing from the previous step due to the lack of a wash procedure (Figure 3, line 25).

Table 1. Restack time requirements.

Plate Number	Time Requirement
1	12 s
5	59 s
10	1 min 55 s
15	2 min 54 s
20	3 min 53 s
25	4 min 48 s
30	5 min 45 s
40	7 min 47 s
50	9 min 34 s

The total time required for the EL406 to complete an assay process increases with the plate count, while the assay's incubation time remains constant. For example, it takes more time to wash and add reagent to 10 plates than it does five plates, but the incubation time following that specific procedure in the assay would remain the same. The delay function of LHC is used to account for the difference between the process time and that of the incubation (Equation 1). Because the process time changes with plate count, the delay period that is linked to the incubation steps needs to be changed accordingly for each differing plate count so that the total time used is equal to the incubation time.

This requires that different LHC files are created for each run that uses different numbers of microplates. In other words, a run that has five plates would require an LHC file and a run that had 10 plates would require a separate LHC program file. Each file would have the same process steps, but the delay time would be different to accommodate the different plate counts.

At some point, the process time of the different assay steps needs to be determined experimentally. While it is easiest to time the process steps of a single plate, then multiply that value for the number of intended plates, the most accurate method is to time a dummy run with the exact number of plates required. Using a stop watch to time each of the process steps, one can calculate the delay time required.

Other issues

Loops

Loops should only be used for repetitive tasks by the EL406, such as a wash step and a dispense step that are repeated on the same plate before returning to the BioStack. Using a loop command followed by a BioStack command to define the exact number of plates to be processed is not recommended. The inadvertent use of more plates than outlined in the LHC program would result in plates not being processed. Put only the number of plates to be processed in the input stack and use a "Use BioStack" command, which acts as a loop function, to deliver and retrieve plates. This command will process all of the plates in the stack.

Long runs

Long runs require priming of the peristaltic pump. The tubing of the peristaltic pump dispenser is permeable to gases. Over time, air will diffuse through the tubing and into the fluid lines, resulting in bubble formation in the fluid path. To eliminate this phenomenon, the dispenser needs to be primed periodically. Priming should take place once every hour that fluid is in the tubing, or immediately before use.

Comment lines

The comment line command is a useful tool within the LHC programming to denote specific process steps. Not only does it assist during the programming of the assay, it can also be a useful tool to monitor the assay process during the run.

Conclusion

The combination of the Agilent BioTek EL406 washer dispenser and the Agilent BioStack microplate stacker in conjunction with Agilent BioTek liquid handling control (LHC) software allows the automation of the liquid handling steps for many assays. Assays that only require ambient temperatures are performed with the system located on the lab bench. Temperature control outside of the ambient range would require that the system be placed in some sort of environmental chamber large enough to accommodate the entire configuration. In both instances, only a single temperature setting can be used for all of the assay processing.

The Agilent BioTek EL406/BioStack system does not multitask the plate processing, as would larger, more sophisticated automated systems, but rather processes a single plate at a time. This methodology is best used for low- to medium-throughput assays. The BioStack storage processor only has one input and output stack. The entire batch of plates must be processed by the EL406, returned to the output stack, and then restacked into the input stack for incubation before the next EL406 processing can take place.

Using this system, the throughput is limited by the length of time for the shortest incubation step. While the system can physically hold as many as 50 microplates in the BioStack input stack, the automated system is limited by the interaction of the process steps and the incubations. When the first plate's process has been completed, the ambient incubation for that plate has started. For the automated process to be successful, all tasks on all plates from the batch have to be completed in the time allowed for incubation. If all the process steps are the same, then the shortest incubation step limits the batch size. As indicated in Equation 1, the total process time, along with the time to restack the plates, cannot exceed the subsequent incubation time. In other words, the calculated delay has to be a positive integer.

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