

APPLICATION NOTE

RockTown Tech

HSPA-A universal graphical user interface for the Hamilton Microlab STAR liquid handler

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Hamilton Microlab Star liquid handlers have unique and flexible functionalities such as independent liquid aspiration/dispense in each channel, and untethered access to and from any given location on the machine deck layout. However, the STAR control program (Venus) can be difficult to learn and operate. To simplify STAR programming for analysts, a user friendly interface software called HSPA (Hamilton STAR Program Assistant) was generated. HSPA communicates with Venus during the liquid handling process through a text output file. It leaves the instrument action commands to Venus, and allows users to focus on detailed method information and sample location information.

Introduction

Automated liquid handlers are widely used in sample extraction procedures in bioanalysis. Compared to manual liquid transfer, automated liquid transfer, using a 96-well format, significantly improves efficiency, accuracy, and precision. In addition, automated liquid handlers are evolving constantly through technological advancements in functionality and reliability to address the analytical challenges relevant to Bioanalytical work. The new technology of the Hamilton Microlab STAR liquid handler has unique advantages as a liquid handler designed to provide the analyst with additional flexibility, such as independent liquid aspiration/dispense in each channel, and untethered access to and from any given location on the machine deck layout.

While this technology offers great functionality and flexibility, learning how to operate the Hamilton Star liquid handler through its control program can be quite

complex, requiring extensive programming for a given extraction method. Additionally, the control program operates in such a way that limits the ability to modify or change aspects of any method to accommodate situations that might come up during the course of sample analysis. For instance, if a run was interrupted, the ability to continue from a permutation of a step without having to restart the entire run requires logic specifically built in to the method program file providing run recovery options. To alleviate these difficulties, a solution was developed combining the Hamilton machine control program (Hamilton Venus) and a Microsoft Visual Basic based software user interface.

The new approach divides a typical Hamilton program into two separate parts. All instrument related parameters and permutations are defined in a method file. That includes deck layout, liquid level detection, appropriate tip size for a given volume, specific volume of liquid transfer for each step, target labwares for aspiration and dispense, etc. The individual sample positions for a liquid transfer step are defined at the runtime using the graphical inter-

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face. Using the Hamilton Venus software, a generic Venus program was created containing all possible permutations of sample type, volume, container, and transfer type, to accommodate the demands of any method. By separating a Hamilton program into these two sections, the complex logic for Venus programming is predetermined in the generic Hamilton Venus program along with the HSPA method, leaving the users with the simple task of catering only the sample positions of each step of their analytical method using the graphical interface at run-time. The strain of programming for a given extraction type is drastically reduced, simplifying the overall process.

HSPA returns a Hamilton STAR liquid handler back to its basic functionality which is to move liquid from one position to another. Using the graphical interface of a method editor, the basic transfer steps are entered in the correct order and saved as a method file, the run-independent method commands. Then, using the saved method file at the analytical run level, the specific details of the transfers outlined are entered according to the defined sequence for that run, the run-dependent parameters for the method. The combined run-independent method commands and run-dependent parameters (sample positions) are automatically saved as a text file in a designated location that is accessed at the time the analytical run is performed by the generic Hamilton Venus program.

Experimental

The following example protein precipitation method is depicted using HSPA Method Editor in **Figure 1**:

1. Add 50 μL of internal standard in 50/50 (v/v) acetonitrile/water to each well of a 1.2mL 96-well extraction plate except the wells designated as blank without internal standard.
2. Add 50 μL of 50/50 (v/v) acetonitrile/water as internal standard diluent to the wells of the extraction plate.
3. Add 50 μL of sample to each well of the extraction plate.
4. Vortex the extraction plate at 1200 RPM for 1 minute.
5. Add 400 μL of acetonitrile to each well of the extraction plate.
6. Vortex and centrifuge the extraction plate.
7. Transfer 300 μL of supernatant from the extraction plate to each well of a clean 1.2 mL 96-well injection plate.
8. Dry-down.
9. Add 100 μL of 50/50 (v/v) acetonitrile/water as reconstitution solution to each well of the injection plate.

10. Vortex, cover with a sealing mat and inject.

Creating Your Method File

To create a method, a user opens the HSPA Method Editor program and adds each step of the procedure incrementally. Beginning on the top left cell titled "Step Name", a brief description of the step is entered to indicate the purpose of the step. Then enter the appropriate aspiration volume, in this case 50 μL for step 1, in the input box "Volume (μL)". Once a volume is entered, the drop-down boxes "Tip Size" and "Liquid Class" will pre-populate with available values for that volume. A larger tip size can be selected which will automatically repopulate available "Liquid Class" in the selection box. Select an appropriate Liquid Class from the drop down box, and then check the appropriate liquid level detection (LLD) settings: cLLD (capacitive) or pLLD (pressure) for both aspiration and dispense. Enter the desired submerge depth in millimeters (when using liquid level detection), or select a fixed height from the bottom of the well in millimeters (when liquid level detection is not used). Select appropriate containers from the "Aspiration From" and "Dispense To" selection boxes to define the source and destination containers for the step. "The Change Tips" box should be checked if new tips are needed for each sample. This is typically applied for steps of sample addition or transferring samples from one plate to another to prevent contamination. If a step involves transferring samples in volatile solvents such as hexane and ethyl acetate in a liquid/liquid extraction method, liquid often drips out of the pipette tips due to build up vapor pressure inside the tips. By selecting the "Pre-wet Tips" check box and an appropriate solvent trough containing the same volatile solvent, Hamilton STAR liquid handler will perform aspiration and dispense of the solvent in the trough two times prior to transfer samples to prevent samples dripping out of the tips. For the same purpose, the pre-wet tip step automatically applies to any liquid transfer from a solvent trough. Comments of the step can be added by simply typing in the "Comment" box for additional information. After all satisfactory parameters are entered, click the "Add" button to enter the step into the method and then proceed to enter the values for the next step. Pauses can be added as steps in a method with the option of adding a comment as well (recommended since it will appear in the Hamilton runtime message box to indicate the purpose of the "pause" step). When all steps have been entered, scan the method text at the top of the window to verify each step according to the extraction method. If changes are required, click on the step requiring change, and make the appropriate correc-

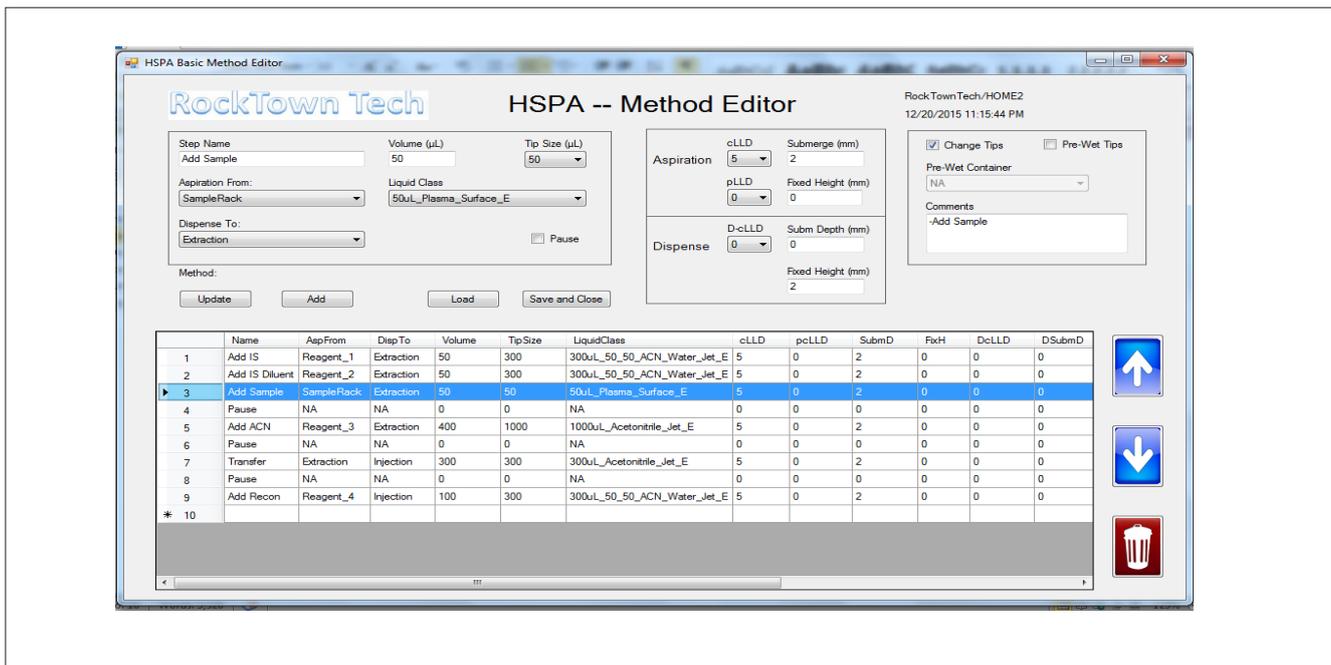


Figure 1. HSPA Method Editor

tion. Then click on the “Update” button to update the modification of the step. After selecting a step, use the “Up Arrow”, “Down Arrow”, or “Delete” buttons on the right side in order to move the step in the appropriate position or delete it. Once all corrections have been made, click the “Save and Close” button and name the file appropriately. The method file is saved in the Microsoft Excel file format. HSPA Method Editor Module has a function to load a previously saved method to modify and save again as a new method (Fig.2).

Understanding the Deck Layout

A standard Hamilton STAR has deck position col-

umns numbered from 1 to 30 for STARlet, 1 to 54 for STAR, and 1 to 71 for STAR Plus, going from left to right on the deck. STARlet is the smallest model while STAR Plus is the largest model. There are a limitless number of permutations for the types of carriers that can be placed in the Hamilton deck, from tip carriers, to plate carriers, to tube holders and reagent troughs. In HSPA the deck layout of the Hamilton STAR is standardized and defined in the HSPA Configuration file. The correspondent labwares and sequences are set in the STAR liquid handler deck layout file as well. This specific deck layout file is used in the generic Venus program together with HSPA.

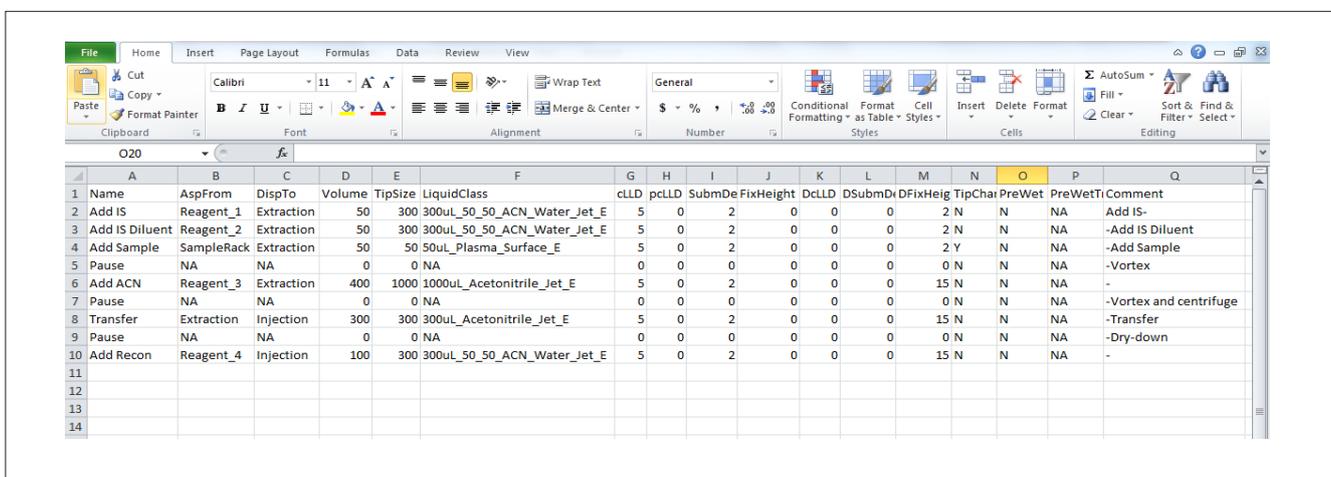


Figure 2. A saved method file

A specific container, e.g. extraction plate, can be a combination of multiple labware (96-well plates). HSPA uses position number to identify each sample, so consecutive numbers are assigned to the container from the Hamilton STAR Venus software's deck layout sequence section. Pipette tips are tracked in HSPA program by entering the initial tip position at the beginning of a run. The total number of available tips is defined in the configuration file.

Using Your Method-Running Your Extraction

At the time a run is to be performed, a user starts the generic Hamilton Venus program which will initiate HSPA runtime module automatically (Fig. 3).

The user will click on the "Select Method" button first to choose the appropriate method file for the run. The steps for the method will appear in a list format at the top of the screen. The step names (descriptions) and parameters should be reviewed prior to proceed the run. The starting tip columns for the available tips should be entered at this time. Click the "Next" button to continue setting up sample positions for each step. Figure 4 shows the graphic interface of defining sample positions. There are two options to enter sample positions. The first option is to enter a block of consecutive samples with number of samples, aspiration and dispense starting positions. Click the "Add Sample" button to commit the entry. A list the samples will appear on the right side of the screen. Sample names, positions can be mod-

ified in the list. For samples aspirated from a reagent trough, the aspiration position is assigned to 1 for every sample. After all samples are added to the list, click the "OK" button to complete the step. The other option to assign sample positions is to import it from a sequence file. HSPA accepts excel, csv, and tab delimited text files. Columns for sample names, aspiration, and dispense positions are selectable from the sequence file at the runtime. The sequence file can be generated by users or exported from LIMS (Laboratory Information Management System) or ELN (electronic Laboratory Notebook). Figure 5 shows the result from importing a sample sequence file.

Importing sample positions from a sequence file provides the most flexibility in using Hamilton STAR liquid handler. Notice how the samples are reused in Figure 5 as the same aspiration position for different repeats of QC samples. Matching aspiration and dispense positions is no longer necessary. Samples can be at any position within the labware container. It is extremely useful to method development and validation work since some odd arrangements happen often.

If a step in the method is not needed, simply click the "OK" button. Not adding a sample to the list will make HSPA to skip the step. This function may be used to resume a run after an unexpected interruption. Pre-

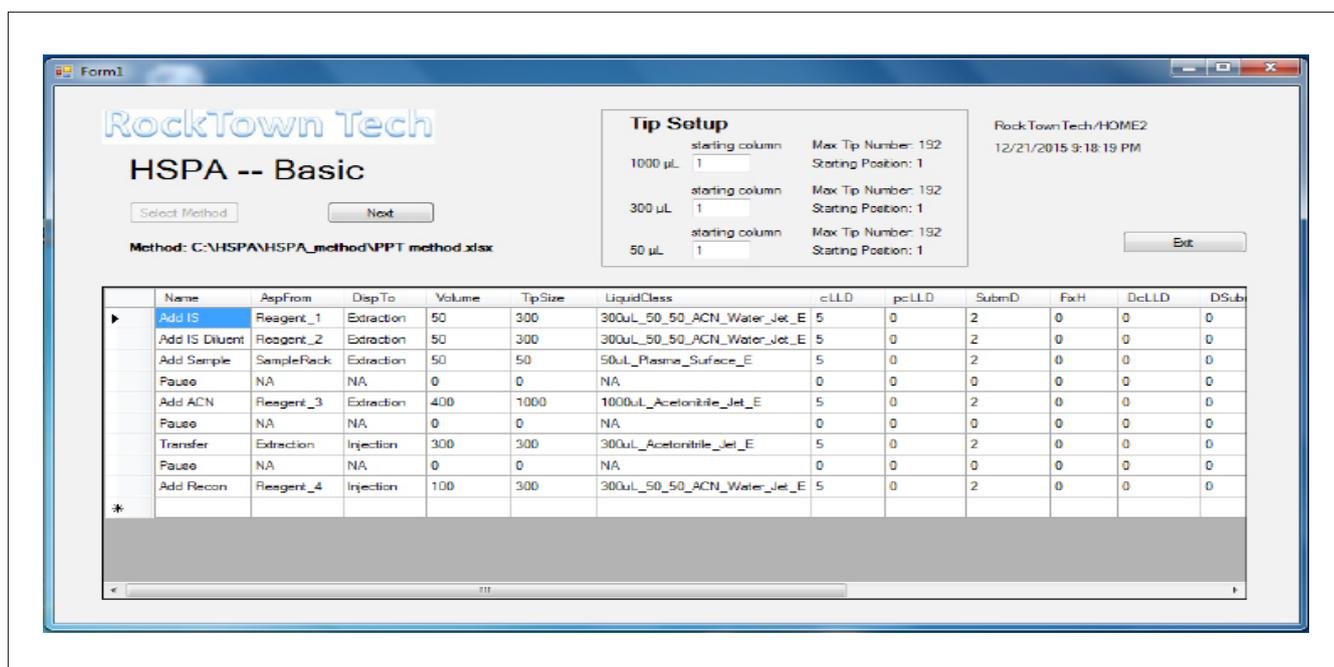


Figure 3. User interface of HSPA runtime module

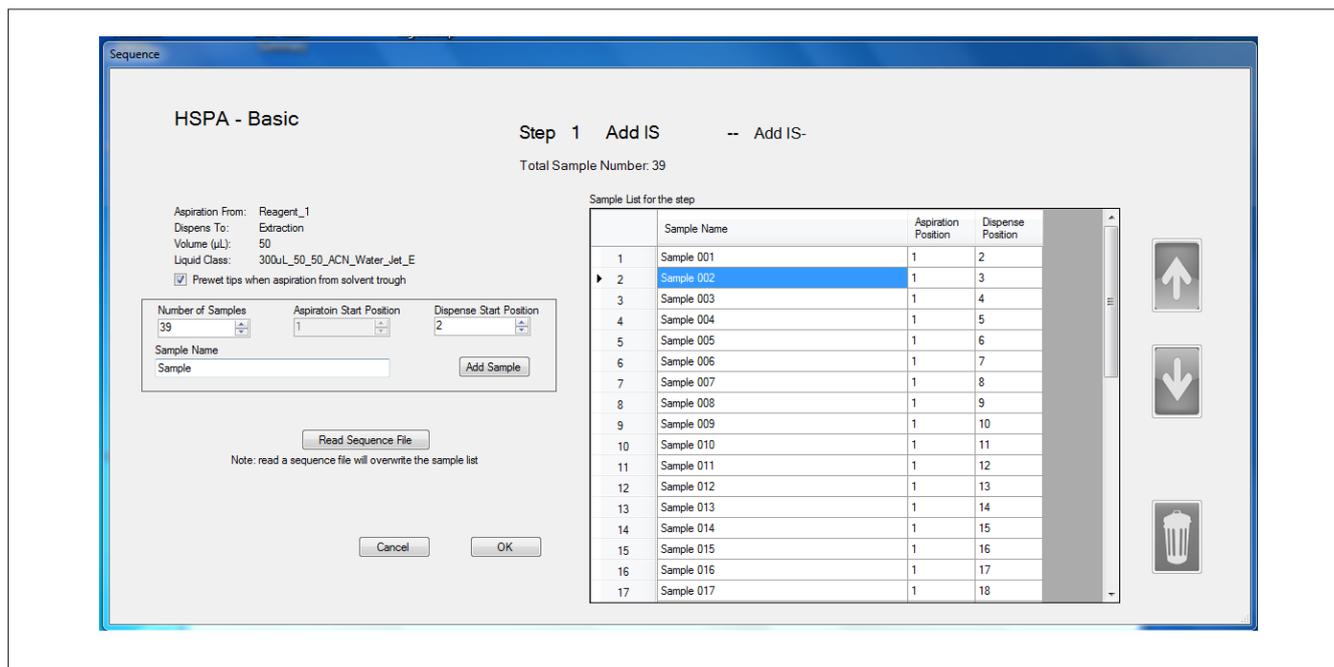


Figure 4. Entering sample positions for a block of samples

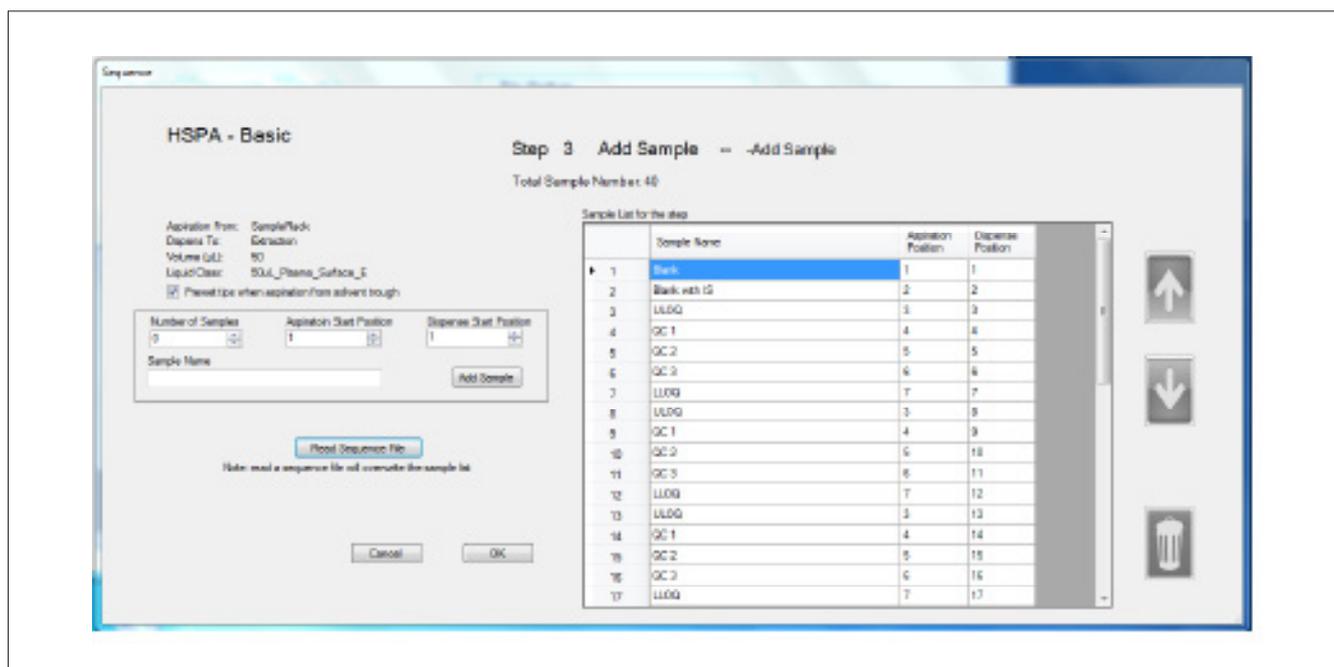


Figure 5. Entering Sample Positions by Importing from a Sequence File

viously executed steps are omitted so the remaining steps can be performed from the interruption point. When all of the method steps have been completed, an output text file is generated by HSPA to record all input commands of the run. The text file is in the comma delimited format with the first cell for the command name and the rest for the input values (Fig. 6). The rows of the text

file are divided into blocks with each step of the HSPA method grouped together in one block. The method parameters such as volume, liquid class, tip type, liquid level detection, etc. are listed at the beginning of each block. The aspiration and dispense positions follow. The generic Hamilton Venus program reads the commands from this output file and performs the liquid transfer actions.

A

```

output - Notepad
File Edit Format View Help
|Trace,welcome to HSPA-Basic
|Trace,User Name: RockTownTech/HOME2
|Trace,Time: 12/21/2015 10:09:01 PM
|Trace,***HSPA-Basic Trial Version***
|Trace,-----
|Trace, Method Name: C:\HSPA\HSPA_method\PPT method.xlsx
|Trace,-----
|Trace, Name: Add IS, AspFrom: Reagent_1, Dispto: Extraction, volume: 50, TipSize:
|Trace, Name: Add IS Diluent, AspFrom: Reagent_2, Dispto: Extraction, volume: 50, Tip
|Trace, Name: Add Sample, AspFrom: SampleRack, Dispto: Extraction, volume: 50, Tip
|Trace, Name: Pause, AspFrom: NA, Dispto: NA, volume: 0, TipSize: 0, LiquidClass:
|Trace, Name: Add ACN, AspFrom: Reagent_3, Dispto: Extraction, volume: 400, TipSize:
|Trace, Name: Pause, AspFrom: NA, Dispto: NA, volume: 0, TipSize: 0, LiquidClass:
|Trace, Name: Transfer, AspFrom: Extraction, Dispto: Injection, volume: 300, TipSize:
|Trace, Name: Pause, AspFrom: NA, Dispto: NA, volume: 0, TipSize: 0, LiquidClass:
|Trace, Name: Add Recon, AspFrom: Reagent_4, Dispto: Injection, volume: 100, TipSize:
|Trace,-----
|Trace, Sample Name: 001, AspFrom: 1, Dispto: 2
|Trace, Sample Name: 002, AspFrom: 1, Dispto: 3
|Trace, Sample Name: 003, AspFrom: 1, Dispto: 4
|Trace, Sample Name: 004, AspFrom: 1, Dispto: 5
|Trace, Sample Name: 005, AspFrom: 1, Dispto: 6
|Trace, Sample Name: 006, AspFrom: 1, Dispto: 7
|Trace, Sample Name: 007, AspFrom: 1, Dispto: 8
|Trace, Sample Name: 008, AspFrom: 1, Dispto: 9
|Trace, Sample Name: 009, AspFrom: 1, Dispto: 10
|Trace, Sample Name: 010, AspFrom: 1, Dispto: 11
|Trace, Sample Name: 011, AspFrom: 1, Dispto: 12
|Trace, Sample Name: 012, AspFrom: 1, Dispto: 13
|Trace, Sample Name: 013, AspFrom: 1, Dispto: 14
|Trace, Sample Name: 014, AspFrom: 1, Dispto: 15
|Trace, Sample Name: 015, AspFrom: 1, Dispto: 16
|Trace, Sample Name: 016, AspFrom: 1, Dispto: 17

```

B

```

output - Notepad
File Edit Format View Help
|Trace, Sample Name: ULOQ, AspFrom: 3, Dispto: 31
|Trace, Sample Name: QC 1, AspFrom: 4, Dispto: 32
|Trace, Sample Name: QC 2, AspFrom: 5, Dispto: 33
|Trace, Sample Name: QC 3, AspFrom: 6, Dispto: 34
|Trace, Sample Name: LLOQ, AspFrom: 7, Dispto: 35
|Trace, Sample Name: ULOQ, AspFrom: 3, Dispto: 36
|Trace, Sample Name: QC 1, AspFrom: 4, Dispto: 37
|Trace, Sample Name: QC 2, AspFrom: 5, Dispto: 38
|Trace, Sample Name: QC 3, AspFrom: 6, Dispto: 39
|Trace, Sample Name: LLOQ, AspFrom: 7, Dispto: 40
|Trace,-----
|TipPickup,50,1111111100000000,1
|TransferBegin,TransferBegin
|Transfer,8,SampleRack,Extraction,50,50,50uL_Plasma_Surface_E,5,0,2,0,0,0,2
|TransferPosition,1,1,2,2,3,3,4,4,5,5,6,6,7,7,3,8,
|TransferEnd,TransferEnd
|TipEject,TipEject
|TipPickup,50,1111111100000000,9
|TransferBegin,TransferBegin
|Transfer,8,SampleRack,Extraction,50,50,50uL_Plasma_Surface_E,5,0,2,0,0,0,2
|TransferPosition,4,9,5,10,6,11,7,12,3,13,4,14,5,15,6,16,
|TransferEnd,TransferEnd
|TipEject,TipEject
|TipPickup,50,1111111100000000,17
|TransferBegin,TransferBegin
|Transfer,8,SampleRack,Extraction,50,50,50uL_Plasma_Surface_E,5,0,2,0,0,0,2
|TransferPosition,7,17,8,18,9,19,10,20,11,21,12,22,13,23,14,24,
|TransferEnd,TransferEnd
|TipEject,TipEject
|TipPickup,50,1111111100000000,25
|TransferBegin,TransferBegin
|Transfer,8,SampleRack,Extraction,50,50,50uL_Plasma_Surface_E,5,0,2,0,0,0,2
|TransferPosition,15,25,3,26,4,27,5,28,6,29,7,30,3,31,4,32,
|TransferEnd,TransferEnd
|TipEject,TipEject

```

Figure 6. Sections of an Output File. (A) Trace section to list step name and parameters to be printed in the STAR runtime trace file; (B) liquid transfer section with trace of sample names and individual command containing position information and parameters

Generic Hamilton Venus Program

Venus is the programming package provided by Hamilton for the Microlab Star liquid handler. It is a very powerful, complicated, and flexible computer language. Every necessary parameter of the liquid handler can be programmed into a method. However, due to its design, the liquid transfer procedural information such as volume, liquid class, tip type are mixed with the individual run action information such as the aspiration and dispense positions. In our effort to simplify the use of Hamilton STAR we moved all programming of parameters to the HSPA software. To work together with our HSPA program, a fixed generic Venus program was used to translate commands read from the HSPA output file. The generic Venus program has a loop to read each block of instructions from the HSPA output file sequentially. Inside the loop, the mode of liquid level detection, tip size based on volume, and whether or not tip changes are populated. For the basic edition of HSPA, those advanced parameters from Venus are set to their default values. They are not changed in normal operation mode most of time.

Discussion

Hamilton STAR: Advantages as a Liquid Handler

The Hamilton Microlab STAR liquid handler is exceptionally flexible and powerful due to the ability of each of its channels to independently access any point on the

deck layout (untethered access). This allows the aspiration positions and dispense positions to be in different orders. For example, samples aspirated from A1, B1, C1 of a source plate can be dispensed to A1, C1, B1 of a destination plate. This added flexibility comes at the cost of increased programming, where the programming of a liquid transfer sequence is no longer the simple process of defining the start position and length of the sequence. The untethered access of the Hamilton STAR affords an operator the opportunity to generate a sequence dynamically at the time of the run. In conjunction with the use of 2D barcodes and an information management system (LIMS), untethered access is a rather important function that can be utilized to help eliminate sample position errors. Functions of 2D barcode and LIMS communication will be implemented in the next version of HSPA.

Liquid Classes

To accurately transfer different types of liquids, Hamilton STAR uses distinct liquid classes to store a specific set of parameters for a given liquid. Variations of liquid properties such as density and viscosity are compensated by volume calibration curves and aspiration and dispense speed, among other parameters. Liquid classes are defined according to the tip size that a liquid transfer step is to use. For example, the transfer of a plasma sample would require different liquid classes for transferring

20 μL and 800 μL , as different tip sizes would be used for each. Thus in selecting the correct liquid class for a particular liquid, the volume to be transferred must be considered. HSPA helps ensure that the correct liquid class is selected for the appropriate liquid to be transferred. When the volume for a step in a HSPA method is entered, HSPA software automatically narrows down the liquid classes, displaying only those that are applicable for the entered volume. HSPA also allows for the easy programming of other parameters involved in a liquid transfer step: aspiration and dispense labware containers, liquid level detection (for both aspiration and dispense), tip change requirement, and tip height (for both aspiration and dispense if liquid level detection is not used).

Programming with Venus simplified with HSPA

To take full advantage of the STAR's advanced functions, Hamilton provided a new programming tool – Venus. Venus is a high level computer language that can translate user commands to STAR understandable computer commands. It contains functions that normal computer languages have, for instance: loops, if/then decisions, variables, string manipulations, etc. While this might seem basic to a computer programmer, writing a program using Venus in the associated programming language can be a daunting task for a laboratory analyst. The learning curve is very steep, and programming errors are fairly common. As a result of this, some companies are even training employees to program Venus methods full time. This is just not possible for many organizations. HSPA was developed as a simple and efficient way to resolve this issue. With HSPA, proficiency in the Hamilton Venus programming environment is no longer necessary. HSPA methods are organized stepwise in an intuitive fashion. Sample positions are entered either by block using sample numbers and starting position or by importing a sequence file. Furthermore in the next version of HSPA, with the use of 2D barcodes in worklists and on sample tubes, the aspiration and dispense positions will be mapped automatically. If an instrument error were to occur during a run, HSPA enables the operator to resume the extraction from the point of failure by simply re-entering the positions of the interrupted step and the following steps from the method.

Automatic liquid handlers play an important role in today's Bioanalytical work by drastically improving both efficiency and accuracy. With the added capability of tracking samples with 2D barcodes, liquid handlers can help improve the quality of analytical data as well. HSPA helps to unlock the full potential of the Hamilton STAR

by simplifying the programming in an intuitive and user-friendly fashion.

Summary

- HSPA is a graphical user friendly interface for the Hamilton STAR liquid handler.
- Using HSPA, user does not need to worry about programming complicated Hamilton Venus programs.
- HSPA treats method parameters and run specific information separately.
- HSPA generates a traceable output file with commands that Hamilton Venus program can read.

Website

Hamilton STAR liquid handler

<http://www.hamiltoncompany.com/products/automated-liquid-handling/liquid-handling-workstations>

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