Microarray Core UCSF Comprehensive Cancer Center			
Standard Operating Procedure			
Title: Operation of the Hydra Liquid Handling System			
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This SOP will guide a user through the process of turning on, running and shutting off the HYDRA Microdispenser. This liquid handling system allows a user to transfer samples from one plate to another (96well, 384 well, 864 well and 1536 well plates) depending on the user's program specifications. Each program can be written manually or through a guided Protocol wizard. For a more in depth manual please refer the Robbins Operation and Service Manual next to the actual instrument.

Contents:

1.0 HYDRA start

The Hydra should be covered with the dust cover with the needles soaking in a liquid (unless the Hydra has not been in use for more than a week). The tubing that runs water and bleach into the peristaltic pump should have been taken out of the carboys and placed in a beaker next to the carboys. The tubing should also be clear of any liquid.

1. **Remove the dust cover.**
2. **Turn on the laptop (no password is necessary) and start the "Hydra96HTS" software.** The power button is located on the top of the keyboard.
3. **Turn on the Hydra instrument.** The "Emergency Stop" button should be in the off or down position. Twist the button as indicated with arrows, to inactivate the emergency stop to turn the Hydra on. Please note that the Hydra stage will home if needles are submerged into a trough.

4. **Turn on the peristaltic pump.** The power switch is located on the lower right hand corner on the front of the machine.
5. **Check the levels of the carboys (located below the Hydra table).** There are three carboys: water, 2% bleach solution, and a waste container. Make sure that the water and bleach solution are filled and the waste container is empty before commencing. The water can be filled using the Millipore system in the lab. The 2% bleach solution needs to be made by diluting Clorox bleach (initially around 6%; check with the bottle's label) with Millipore water. The Clorox bleach is located under the sink in the Albertson Lab. The waste carboy can be discarded down the sink unless any hazardous chemicals were used while using the Hydra. If so, the waste should be discarded according to Hazardous waste guidelines.
6. **Prime the pumps.** The switches for filling the tubing and emptying the trough are located in front of the peristaltic pump. *Fill 1* is associated with the bleach line and *Fill 2* is associated with the water line. Note that the trough has three lines connected to it: two intake lines for the bleach and water, and one outlet line used to empty the liquid from the trough into the waste carboy.
7. Draw liquid through the intake tubing (for both the 2% bleach solution and the water) until the air has been pushed through the tubing and liquid is flowing freely into the wash trough. The trough should be emptied in intervals while drawing liquid from each solution as well as at the end of the process.
8. **Run the morning wash protocol.** This protocol is located in the "User Protocols" folder and is called "Wash.hsc". The liquid in the syringes (if any) should be expelled and disposed of properly. The first step of the wash run will dispense the liquid from the syringes. The liquid should be caught by a separate trough. The hydra will provide an explanation for this and all of the following steps of the wash cycle. If there is water or Coulter Clenz in the syringes, the liquid can be dumped down the sink. Otherwise please follow Hazardous waste guidelines for chemical disposal.

2.0 Programs

The Hydra can run manually or with the "Hydra96HTS" software. For most procedures the software can be used. Each program is created either with the help of Protocol wizard (recommended for beginners and intermediates) or written in code (for individuals very familiar with the code). Users may find already existing programs that will work for their procedure(s). Each program created becomes an .hsc file.

1. **Create a new program.** Open File/New/Protocol/Editor to create a program by manually writing the code. Open File/New/Protocol/Protocol Wizard for a step-by-step interface that allows a user to create a program without knowing the code.
2. **Creating and defining labware.** Open File/New/Labware Wizard. Labware wizard is a step by step interface that allows a user to define and store parameters for microplates and reservoirs used in protocols. Most types of plates have been previously defined. This step will not be applicable for most users.
3. **Edit a program.** Open File/Editor/Protocol/Editor or Protocol Wizard to edit a program manually or through the interface of the Protocol wizard. File/Editor/Labware Wizard will edit the previously defined program.
4. **Running a program.** Click the triangle "run" button to start a program or open File/Run. Open the .hsc file intended. After starting, the user may pause or stop the run the program during the course of the run

3.0 HYDRA shutdown

The last user of the day should follow the shutdown procedure. If another user is signed up for later use, run the Wash protocol "Wash.hsc" with water and leave the Hydra dry with the dust cover on.

1. **Run the wash protocol with water.** The wash protocol should run its entirety to clean out any residue that might have been left during use.
2. **Run the wash protocol again with the appropriate solution; stop the Hydra when syringes are full.** The solution used in this wash step is dependent on the length of time the Hydra will remain dormant (See directly below). The syringes will pick up the solution from a reservoir. Once filled hit the red "Emergency Stop" button.

Overnight: water

Over the weekend or for a few days: Coulter Clenz (Fisher)

Few weeks to one month: 0.3% Sodium Azide

Indefinite amount of time (at least one month): store dry (skip this step and continue).

3. **Empty the peristaltic pump and tubing.** First remove both the 2% bleach and water tubing out of the carboys and place them into the adjacent beaker. Press the Fill switch for the 2% bleach pump (Fill 1) until the tubing runs dry. Periodically empty the trough, through this process, to keep the trough from overflowing. This is done by pressing the Empty switch. Repeat this process for the Fill 2 line (the water line). Make sure to drain the waste tubing of all liquid as well.
4. **Turn off the peristaltic pump.**
5. **Close "Hydra96HTS" software and turn off the computer.**
6. **Place the dust cover over the Hydra.**
7. **Carboy handling.** Empty out the Waste carboy either down the sink or discard as hazardous waste, depending on the hazardous waste guidelines associated to the solutions used during the run. Refill the water and 2% bleach carboys if necessary.

4.0 Calibration/Troubleshooting

There are two types of calibration: stage calibration, and plate/reservoir calibration. The stage calibration was done at the time of installation. Calibration is required only once at the initial set-up. The stage should only be recalibrated if the system is disassembled and reassembled. As for the plate/reservoir calibrations, when a new labware is defined, Labware Wizard goes through the steps defining each specification. During this process the Wizard is ask to place the plate on the source position and then assign locations of the wells. If a plate has already been defined, but the pins are no longer aligning to the center of the wells, Edit Labware Wizard will allow the user to go back and redefine alignments. For more details, please consult the "HYDRA-96 Automated Plated Positioning System: Installation and Operation Manual" (a thin manual located to the left of the laptop) pages 17-25.

When troubleshooting, the user may refer to the three manuals located on the left of the laptop to retrieve solutions. The "HYPRA Microdispenser: Operation and Service Manual" (thick blue binder) has a section of troubleshooting and tips from 7-1 to 7-12. The following are notes to ensure safe use of the instrument.

1. In case of any emergency, shut down the instrument immediately by using the Emergency Shutoff Switch.
2. Take into account the viscosity of the solutions before using the system. Look at the Table 6-7 on pages 6-28 of the "HYPRA Microdispenser: Operation and Service Manual."
3. Do not allow the pins to contact the bottom of any plate, reservoir, or wash bin. If this does occur, go to Edit Labware Wizard and redefine the depths of the pins for the plate. When the pins hit the bottom of the plate/reservoir, the pins are likely to bend and potentially break.
4. During the initial wash cycle, look at the syringes when they are full. Each syringe should have a small air bubble. This prevents cross contamination during the use. If any of the syringes do not have an air bubble, they will need to be restored. Refer to page 6-30 of the "HYPRA Microdispenser: Operation and Service Manual."
5. For any questions that cannot be answered by the manuals or this protocol, please call the Robbins representative. The information is located on the inside of the front cover