

## STarSystem Control Verification (Sheet 1)

Control Microspheres are used on IS version STarSystems to verify calibration and optical integrity. System Verification in STarStation 2.3 is performed via the Script interface. All Control microsphere lot information must be entered via the STarStation Reagent Manager. The steps below outline the procedure required to perform a system control verification of the STarSystem Cytometer.


**NOTE:** System verification should only be attempted when the system has undergone the startup procedure, has been successfully calibrated and the dCal temperature is within range.

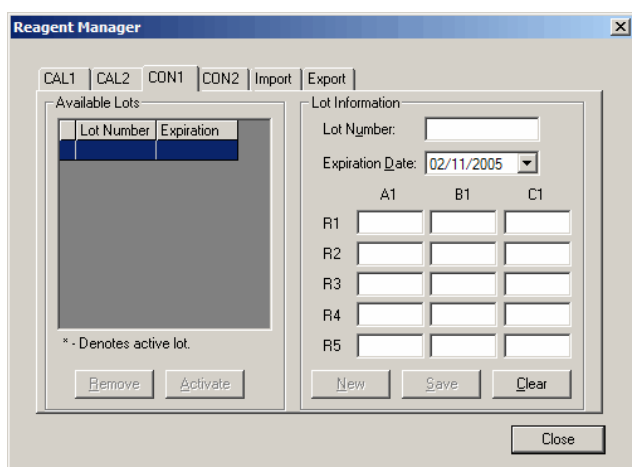
**Step 1**

- a) Remove the CON1 and CON2 microsphere bottles from the refrigerator.
- b) Allow the reagents to reach room temperature before they are used.
- c) Ensure that the Cytometer has been correctly warmed up before attempting calibration.
- d) Ensure that the target values for the specific control microsphere set have been entered correctly into the STarStation Reagent Manager.

If this step has already been performed proceed to step 2.

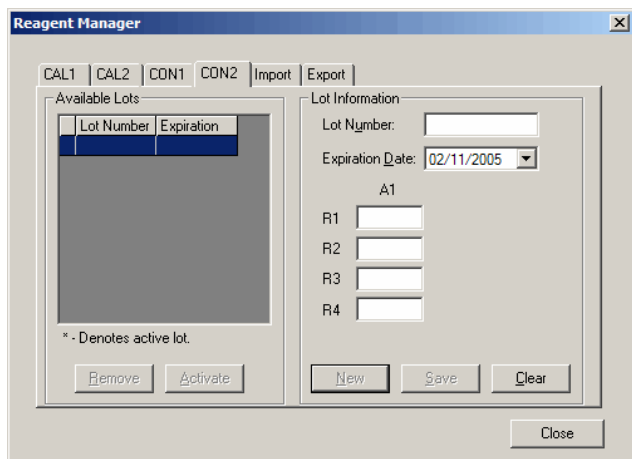
**Entering Control Microsphere Details into the STarStation Reagent Manager**

- e) Display the STarStation Reagent Manager by selecting CTRL + R, clicking the Reagent Manager Icon  from the Manager Toolbar or selecting the **Reagent Manager** option from the **Edit** Menu. Click the **Verification** tab.
- f) Select the **CON1** tab and click the **New** button.




Enter the Lot Number, Expiration Date and Target Values for the CON1 Classification Control microspheres. These values can be found on the Certificate of Analysis data sheets shipped with the control beads. When all information has been entered press the Save button to commit the data to the database.

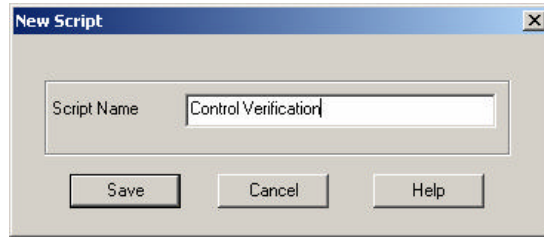
- g) Select the **CON2** tab and then click the **New** button.




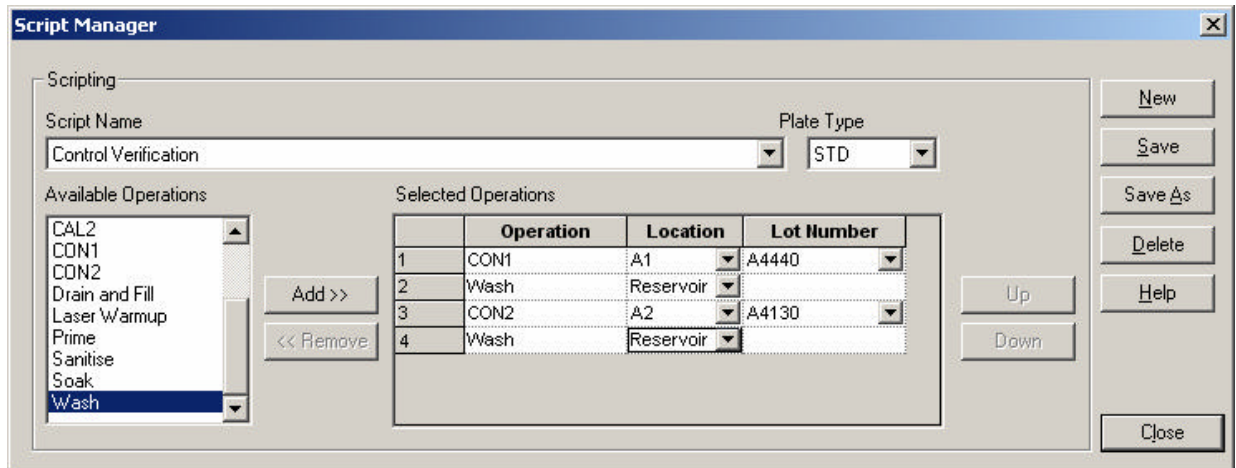
Enter the Lot Number, Expiration Date and Ref Target Values (RP1) for the CON2 Reporter Control Microspheres. When all information has been entered press the Save button to commit the data to the database.

**Step 2** Creating/Modifying a Control Verification Script

- a) If you have already created your control script proceed to step3 (Note: Each control verification script is specific to a particular set of control reagents and target values, if using new or different lots of control reagents a new script will need to be created or an existing script modified).
- b) Launch the STarStation Script Manager by pressing CTRL + B, selecting the Script Manager Icon  from the Manager Toolbar or choosing the **Script Manager** option from the **Edit** menu.
- c) Existing scripts can be modified to use a different lot of control reagent. First select the script from the script drop-down list and modify accordingly. **NOTE:** scripts are specific to and only accessible from the STarStation user account that created them.
- d) To create a new script, click the **New** button and enter a name for the script in the Script Name field and click the **OK** button.




- e) Create a sequence of operations by selecting operations from the *available operations* list on the left of the Script Manager use the  button to add operations to the Selected Operations list. The order of operations in a script can be modified by selecting an operation by clicking the numbered row and using the **Up** and **Down** buttons to alter the commands relative position in the script.
- f) Ensure that all commands requiring a Well Location have been programmed, and that the correct microtiter plate format (Standard 96 well plate, or ACS QC plate) is selected in the **Plate Type** field. NOTE: CON1 and CON2 operations must be performed from different wells.
- g) Select the relevant lot of CON1 or CON2 reagent from the Lot Number drop down list. NOTE: when preparing the plate ensure that five drops of fresh control reagent is used for each Control operation.
- h) Add a single wash operation between the CON1 and CON2 operations. An example script is shown below.



## STarSystem Control Verification (Sheet 2)

### Step 3

#### Performing STarSystem Verification

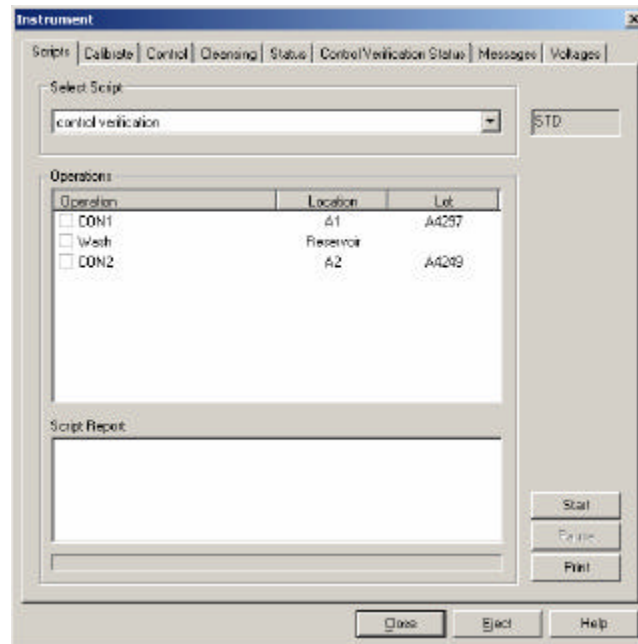
Before attempting system verification ensure that the laser status icon indicates that the system has been warmed . The Sample Probe height and cleanliness should also be verified before verification.

a) Display the STarStation Instrument Controls by selecting the Instrument Controls option from the **View** Menu, using the CTRL + I shortcut key sequence or clicking the Instrument Controls icon.



b) Select the **Scripts** tab of the Instrument Controls dialog.

c) Select the Control script you wish to run from the *Select Script* drop-down-list.



d) Vortex the Control Microsphere bottles and place 5 drops of CON1 into the well selected in the script e.g. A1.

e) Place 5 drops of CON2 into the appropriate well e.g. A2.

f) Ensure that the programmed wash location (for the ACS QC plate) or the Reservoir contains sufficient Sheath Buffer to perform the required number of washes. NOTE: a single wash command requires 200µl of fluid.

g) Click the **Eject** button and place the Microtitre plate onto the XY platform.

h) Retract the XY platform tray by clicking the **Retract** button.

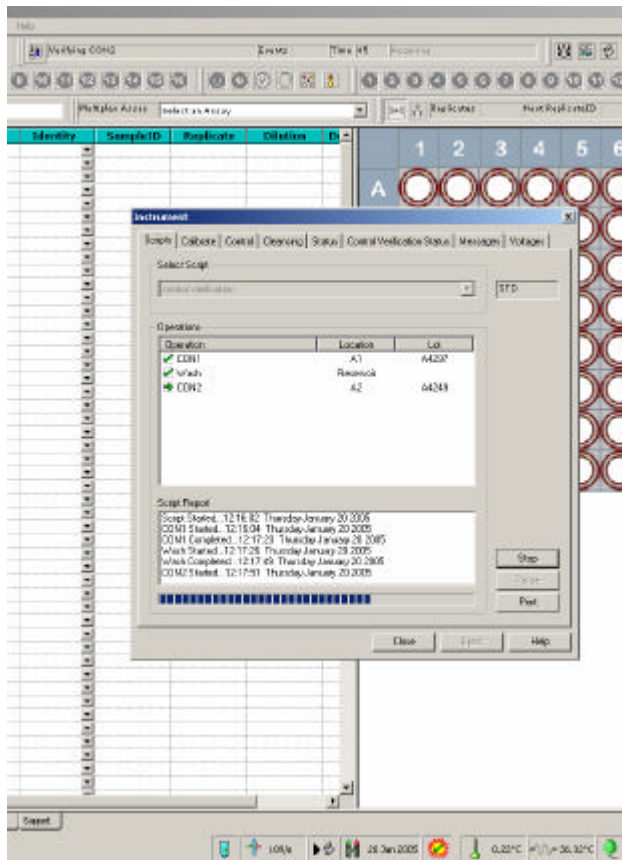
i) Press the **Start** button to commence the control script.

Refer to Step 4 for troubleshooting information.

**Step4**

**Troubleshooting**

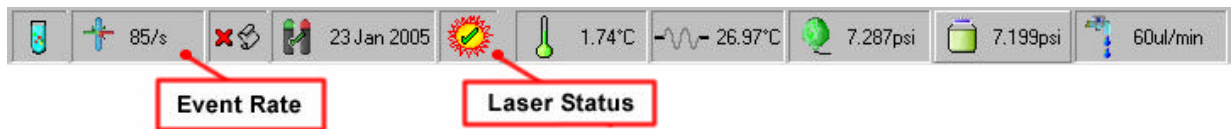
The Instrument Status Toolbar at the top of the Main Window will indicate the progress of control verification and report on the success or failure. View the Control Verification tab of the Instrument Control to review the instrument Control verification status.



The event rate during control verification can be observed via the Instrument Status bar. The highest event rate achieved during verification should be in the range 100 to 300 events/sec.

A low event rate indicates sub optimal probe height adjustment or a blockage in the sample probe/sample tubing.

The Control Verification History for the System can be reviewed from the LXR DataFiles folder which is located at C:\Program Files\Luminex\LXR\DataFiles on the System PC. The Control History can also be viewed via the LX Reports utility.



If the System fails control verification follow the procedure below:

1. Verify that the correct Lot numbers and Target Values are selected.
2. Vortex the control microsphere bottles.
3. Verify that the correct wells have been selected.
4. Remove the sample probe and sonicate the narrow end for 2-3 minutes.
5. Using a syringe, flush the sample probe with distilled water from the narrow end out through the larger end.
6. Readjust the sample probe height upon replacement in the arm.
7. Complete a maintenance wash; 3 Backflush, 3 Drain, 2 Alcohol Flush and 3 Washes with water.
8. Repeat verification. Verify that the pressure readings are between 6 and 9 psi and the Events/Second rate reaches at least 200.

If verification continues to fail, record which parameters have failed and contact Applied Cytometry Systems Customer Support.