

Note: Any problems and/or non-conformance found during the QC test procedure must be repaired and/or replaced by the QC chemist. Notify the QC Lab Supervisor when any problems are noted. If the QC Lab Supervisor is not available, notify the RED group.

Part 1: Initial Set-up and Alignment

Plumbing:

Note: We do not use factory plumbing on the autosampler. Modifications must be made to the autosampler to our specifications as outlined below:

1. Syringe Size = 100 μ L.
2. Loop Size = 20 μ L (Michrom Made)
3. Dispense Tube = 500 μ L (Michrom Made) (Teflon Tubing)
4. Needle = Fused Silica 5 μ L (Michrom Made)
5. Transfer Lines = 5 μ L (Michrom Made) to column
10 μ L (Michrom Made) to pumps
6. Add Titanium filter to the solvent Input line and place in the Wash Bottle. *Note: the Wash Bottle should be filled with 40/40/20 Acetonitrile/n-Propanol/H₂O.*

Wiring:

1. Connect the power cord to the back of the autosampler.
2. Connect the "Markers" Cable to the back of the autosampler (P4).
3. Connect the yellow/green wires to the back of the HPLC under appropriate Input slots.
Note: the "Markers" Cable sends a signal from A/S to HPLC when autosampler has made an injection to tell the HPLC to begin its run.
4. Connect the "Input" Cable to the back of the autosampler (P6). *Note: the "Input" Cable allows an input signal from the HPLC to the A/S to tell the A/S to perform an injection. The black wire is ground. The white wire is for loop injection remote start. The red wire is for starting a User Program.*
5. Connect the red/white/black wires to the back of the HPLC under appropriate Output slots.
6. Connect the RS-232 cable to the back of the autosampler (S2) for software control.
Note: the dip switches above "S2" should be 1=off, 2=on, 3=on, 4=off.
7. Connect the other end of the RS-232 cable to an open COM Port on the PC.

Programming System Settings:

1. Turn the power to the "ON" position on the autosampler. *Note: The autosampler will go through a quick self-test.*
2. Program the General Parameters for the autosampler through the keypad menu. Press the key that says "System" and then choose "General".
3. Program the correct settings according to Michrom's plumbing:
 - Loop Volume = 20 μ L
 - Needle Tubing = 5 μ L
 - Syringe Volume = 100 μ L
 - Syringe Speed = Low with 0.1 Scaling Factor (slowest setting)
 - Needle Height = 5mm

3. Continued
 - Skip Missing Wells = Yes
 - Air Segment = No
 - Headspace Pressure = No
 - Time base Display = H:MM:SS
 - Key Click = On
 - Error Buzzer = On
 - Alarm Buzzer = On
4. Once all of the General Parameters have been set, click the “Escape” key. This should take you back to the System Settings Menu.
5. Choose “Usage” on the System Settings Menu and enable the User Program Methods.
6. Press the Escape Key to go back to the “System Settings” Menu and choose “Plates”.
7. Program the correct settings:
 - Tray Type = 48 Vials
 - Process in Rows
 - First Transport Vial = 1
 - Last Transport Vial = 1
8. Once all of the General Parameters have been set, click the “Escape” key. This should take you back to the System Settings Menu.
9. Choose “Clock” on the System Settings Menu and set the correct date and time.
10. Once all of the General Parameters have been set, click the “Escape” key. This should take you back to the System Settings Menu. Press “Escape” again to go back to the Main Menu.

Priming the Syringe

1. Perform a wash cycle by clicking on “Wash” from the Main Menu. *Note: the autosampler will run the wash program which flushes the syringe, valve, needle, etc. with wash solvent.*
2. Once the wash cycle is complete, check the syringe and associated tubing for air bubbles.
3. If air bubbles are present in the syringe, prime the syringe manually by removing the syringe. Press “SRY END” to move the plunger to the end. Unscrew the syringe from the black syringe valve. Prime the syringe with wash solution by moving the plunger up & down rapidly in the solution and filling the entire syringe with liquid. Screw the syringe back into place and then press “SYR HOME” to set the plunger back to the home position.
4. Once the syringe has been manually primed, perform another wash cycle by clicking on “Wash” on the Main Menu.
5. Repeat steps 3 & 4 if necessary until no air bubbles are present in the syringe and/or visible in the teflon dispenser tube.

Sample Needle Height Alignment

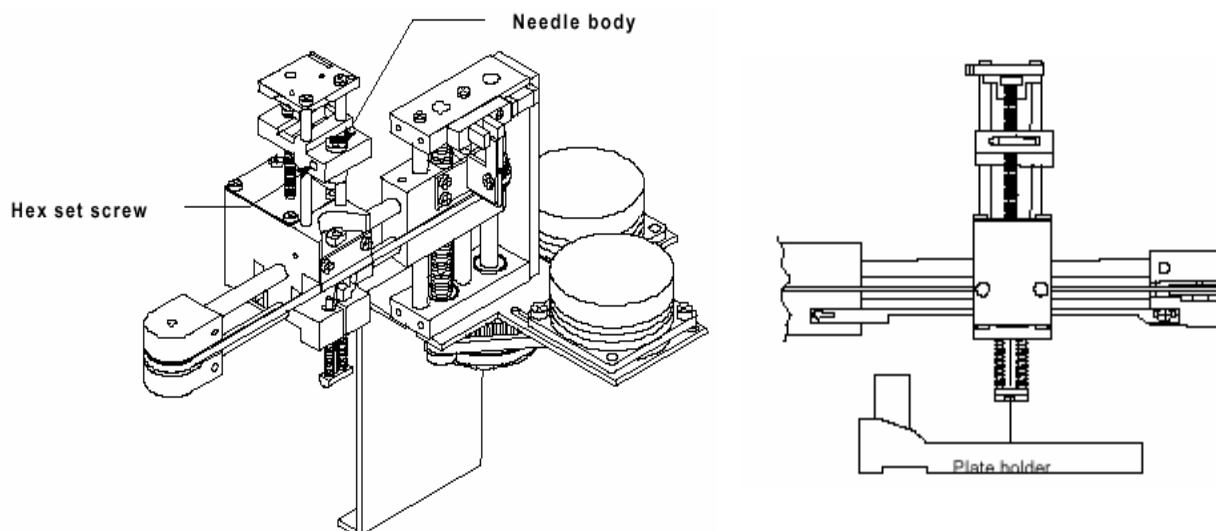
Note: Whenever a new needle is installed, the needle height must be properly aligned.

1. Click on the “Settings” button on the keypad and choose “General”.
2. Change the Needle Height to Zero and press enter. Click Escape twice to go the Main Menu.
3. **Remove the 48-vial tray from the tray holder during the alignment procedure .**
4. Alignment of the needle takes place in the Service Mode. From the Main Menu, click the “Menu” key to see “Service” as an option. Choose “Service”, input 250896 and click enter. *Refer to the Endurance Service Manual (Section 3.11 & 4.4.3) for detailed instructions.*

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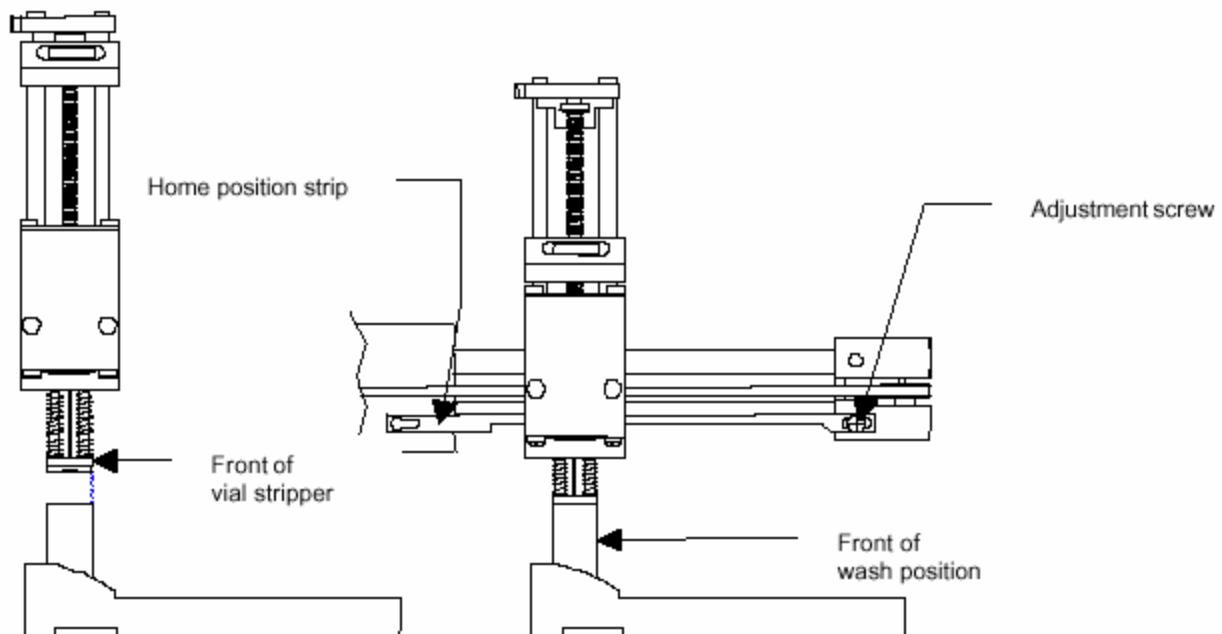
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5. Press the “Menu” key until the heading reads “Needle and Trap Adjustment Page 1 of 6”. Then press the “up arrow” key to get to page 5 of 6. Press the “Row” key until “C” is selected and then press “Start”. This action will bring the needle arm out to Row C.
6. Press the “down arrow” to get to page 4 of 6. Press “down” to move the vertical needle down.
7. Set the needle body of the needle unit as high as possible to avoid bending or crushing the needle during the alignment procedure. Loosen the allen screw and slide the sample needle body up.
8. Press the “up arrow” twice to get to page 6 of 6. Press “down” to move the sample needle down.
9. Adjust the sample needle height up or down so that it is within a papers width from the bottom of the tray holder. Adjustments are made by loosening the allen screw and sliding the sample needle body up or down. Once the adjustments have been made, re-tighten the allen screw and then click “home”.
10. Recheck alignment by clicking on “down” to lower needle. Repeat step 8 if necessary.
11. Once the needle alignment is correct, press “escape” to get back to main menu.
12. Reset the needle height setting in the general parameters back to 5mm so that the needle does not get bent or crushed during typical use with vials and the vial tray in place. To reset the needle height, click on the “System” key from the main menu and then choose “General”. Change the needle height to 5mm and press enter.



Horizontal Needle Adjustment

Horizontal needle alignment may be necessary. It is important to check the horizontal needle alignment to ensure that the needle punctures the vials in the center (from front to back).

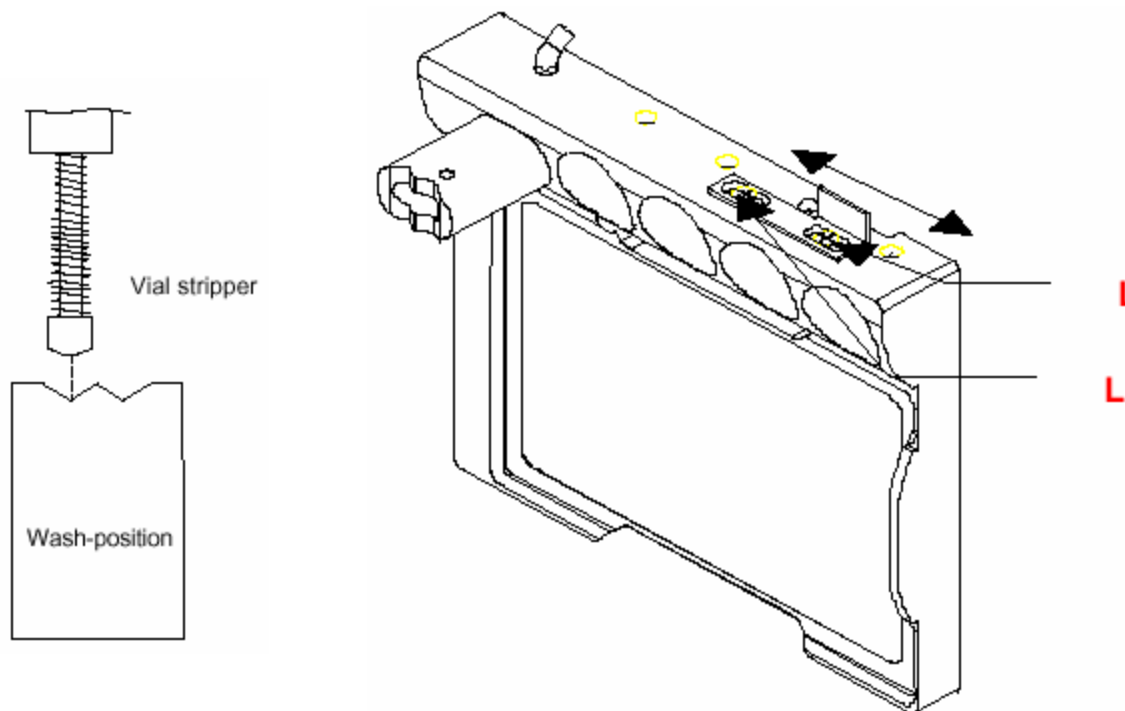
1. The horizontal needle unit movement can be adjusted by sliding the home position strip forwards or backwards. Alignment of the horizontal needle takes place in the Service Mode. From the Main Menu, click the “Menu” key to see “Service” as an option. Choose “Service”, input 250896 and click enter. Refer to the *Endurance Service Manual (Section 3.11 & 4.4.2)* for detailed instructions.
2. Press the “Menu” key until the heading reads “Needle and Trap Adjustment Page 1 of 6”. Move the tray to the Wash position by clicking on “Wash”.
3. Press the “up arrow” key to get to page 5 of 6. Move the needle arm above the wash/waste position by clicking on “Wash”.
4. Press the “down arrow” key to get to page 4 of 6. Move the vertical needle down by clicking “Down”.
5. Check the alignment of the horizontal needle. Proper alignment is when the vial stripper is in-line with the front of the wash position.
6. If necessary, adjust the alignment by loosening the adjustment screw and sliding the Home position strip frontwards or backwards. Once the adjustment is complete, re-tighten the adjustment screw.
7. Move the vertical needle up by clicking “home”.
8. Press the “up arrow” key to get to page 5 of 6. Move the needle arm to the home position by clicking “home”.
9. Press the “down arrow” key to get to page 4 of 6. Move the vertical needle down by clicking “Down”.
10. Re-check the alignment of the horizontal needle and repeat the adjustment if necessary.



Tray Holder Alignment

Tray alignment was done at the Spark Holland factory, however, minor adjustments may still be necessary. **Since the adjustment of the tray holder is done on the tray holder itself, the tray holder “belongs” to the autosampler. Do not interchange tray holders on different autosamplers!**

1. The Tray Holder alignment can be adjusted by sliding the sensor vane to the right or left. Alignment of the tray holder takes place in the Service Mode. From the Main Menu, click the “Menu” key to see “Service” as an option. Choose “Service”, input 250896 and click enter. Refer to the *Endurance Service Manual (Section 3.11 & 4.4.4)* for detailed instructions.
2. Press the “Menu” key until the heading reads “Needle and Trap Adjustment Page 1 of 6”. Move the tray to the Wash position by clicking on “Wash”.
3. Press the “up arrow” key to get to page 5 of 6. Move the needle arm above the wash/waste position by clicking on “Wash”.
4. Check the alignment of the horizontal needle with the wash position on the tray holder. Proper alignment is when the vial stripper is in-line with the “V” wash position.
5. If necessary, adjust the alignment by removing the tray holder and loosening the adjustment screws and sliding the sensor vane to the right or left. Once the adjustment is complete, re-tighten the adjustment screws and reinstall the tray.
6. Move the needle arm to the home position by clicking “home”.
7. Press the “down arrow” to get to page 1 of 6. Move the tray to the home position by clicking “home”.
8. Move the tray to the Wash position by clicking on “Wash”. Press the “up arrow” key to get to page 5 of 6. Move the needle arm above the wash/waste position by clicking on “Wash”. Re-check the alignment of the horizontal needle and repeat the adjustment if necessary. Press “Escape” to get back to the Main Menu.



Tray Type and Tray Alignment Tests

Once all alignments are complete, it is very important to confirm that the auto sampler can run all of the tray types, and that the tray alignment on 96-low, 384-low and 48-vial trays is set properly.

96-Low Tray

1. Place tape over the 4 corner wells (A1, A12, H1, H12) of the 96-low tray, and then place the tray on the tray holder.
2. Press the “System” button on the keypad, and then choose “Plates”.
3. Program the correct tray settings:
 - 96-low
 - Process in Rows
 - First Transport Vial = 1
 - Last Transport Vial = 1
4. Once all of the General Parameters have been set for the correct tray type, click the “Escape” key. This should take you back to the System Settings Menu. Press “Escape” again to go back to the Main Menu.
5. The tray alignment test will take place using the Service Mode. From the Main Menu, click the “Menu” key to see “Service” as an option. Choose “Service”, input 250896 and click enter. *Refer to the Endurance Service Manual (Section 3.11 & 4.4.2 & 4.4.4) for detailed instructions.*
6. Press the “Menu” key until the heading reads “Needle and Trap Adjustment Page 1 of 6”.
7. To move the needle assembly to Column 1, input “1” under “Searching Column” and click “Start”.
8. Press the “up arrow” key to page 5 of 6. To move the needle assembly to Column A, input “A” under “Searching Row” and click “Start”.
9. Press the “down arrow” key to page 4 of 6. To move the vertical needle down and puncture the tape, click “Down”.
10. Press “Home” to move the needle back up. Visually inspect the puncture hole for proper alignment and make any necessary adjustments.
11. Repeat the process until the hole is perfectly in the center of the well.
12. Start back at #7 and repeat procedure for the other 3 corners of the plate (A12, H1, and H12).

384-Low Tray

1. Place tape over the 4 corner wells (A1, A24, P1, P24) of the 384-low tray, and then place the tray on the tray holder.
2. Press the “System” button on the keypad, and then choose “Plates”.
3. Program the correct tray settings:
 - 384-low
 - Process in Rows
 - First Transport Vial = 1
 - Last Transport Vial = 1
4. Once all of the General Parameters have been set for the correct tray type, click the “Escape” key. This should take you back to the System Settings Menu. Press “Escape” again to go back to the Main Menu.

Tray Type and Tray Alignment Tests Continued

384-Low Tray Continued

5. The tray alignment test will take place using the Service Mode. From the Main Menu, click the “Menu” key to see “Service” as an option. Choose “Service”, input 250896 and click enter. *Refer to the Endurance Service Manual (Section 3.11 & 4.4.2 & 4.4.4) for detailed instructions.*
6. Press the “Menu” key until the heading reads “Needle and Trap Adjustment Page 1 of 6”.
7. To move the needle assembly to Column 1, input “1” under “Searching Column” and click “Start”.
8. Press the “up arrow” key to page 5 of 6. To move the needle assembly to Column A, input “A” under “Searching Row” and click “Start”.
9. Press the “down arrow” key to page 4 of 6. To move the vertical needle down and puncture the tape, click “Down”.
10. Press “Home” to move the needle back up. Visually inspect the puncture hole for proper alignment and make any necessary adjustments.
11. Repeat the process until the hole is perfectly in the center of the well.
12. Start back at #7 and repeat procedure for the other 3 corners of the plate (A24, P1, and P24).

48-Vial Tray

1. Place tape over 4 vials and place them in the 4 corner wells (A1, A8, F1, F8) of the 96-low tray, and then place the tray on the tray holder.
2. Press the “System” button on the keypad, and then choose “Plates”.
3. Program the correct tray settings:
 - 48-Vials
 - Process in Rows
 - First Transport Vial = 1
 - Last Transport Vial = 1
4. Once all of the General Parameters have been set for the correct tray type, click the “Escape” key. This should take you back to the System Settings Menu. Press “Escape” again to go back to the Main Menu.
5. The tray alignment test will take place using the Service Mode. From the Main Menu, click the “Menu” key to see “Service” as an option. Choose “Service”, input 250896 and click enter. *Refer to the Endurance Service Manual (Section 3.11 & 4.4.2 & 4.4.4) for detailed instructions.*
6. Press the “Menu” key until the heading reads “Needle and Trap Adjustment Page 1 of 6”.
7. To move the needle assembly to Column 1, input “1” under “Searching Column” and click “Start”.
8. Press the “up arrow” key to page 5 of 6. To move the needle assembly to Column A, input “A” under “Searching Row” and click “Start”.
9. Press the “down arrow” key to page 4 of 6. To move the vertical needle down and puncture the tape, click “Down”.
10. Press “Home” to move the needle back up. Visually inspect the puncture hole for proper alignment. Only make adjustments if absolutely necessary since this could affect the 96-well and 384-well tray’s alignment.
11. Start back at #7 and repeat procedure for the other 3 corners of the plate (A8, F1, and F8). Press “Escape” to get back to the Main Menu.

Part 2: Analytical QC

Note: Analysis is performed using a QC Bullet column (Magic C18 5u 100A)

Solvent A: 2/98/0.1% Acetonitrile/H₂O/TFA

Solvent B: 90/10/0.1% Acetonitrile/H₂O/TFA

Flow Rate: 250 ul/min

Gradient: 5-95%B in 3 minutes

Detection: UV @ 205nm

Standard: Small Molecule Test Mixture (4-PB4)(10ng/μL)

Partial Loop Injection: 10 μL

Standard Michrom Auto sampler Plumbing & Settings as listed in Part 1.

Plumb transfer lines 5μL & 10μL to HPLC valve (keep HPLC valve in INJECT entire run)

1. Blank Analysis
2. Standard Analysis
3. Reproducibility Study (+/- 0.5%RSD 5 runs in a row)
4. Carry-over Analysis