Agilent RapidFire High-throughput MS System

Troubleshooting Guide

How to use this guide

Use the actions given in this guide to troubleshoot problems that may arise while using the Agilent RapidFire High-throughput MS System. If the problem persists after you perform the appropriate troubleshooting routine, please contact Agilent for technical support.

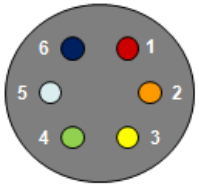
Agilent Technologies

Before you begin: Understand and interpret the flowpath diagrams

To debug most problems that you may encounter on your RapidFire high-throughput mass spectrometry system, you need to understand the following flow diagrams for the instrument, which are shown in this section.

The following tips apply to all flow diagrams:

- The three nanovalves V1, V2, and V3 have six color-coded ports:



- Ports are connected by inner grooves, which are shown as white segments on the flow diagrams. These links are different in the **Inject** and **Load** positions.

  For example:

  - When V1 is in the **Inject** state (blue), V1P6 and V1P1 are connected.
  - When V1 is in the **Load** state (green), V1P1 and V1P2 are connected.

Figure 1 State #1: Aspirate

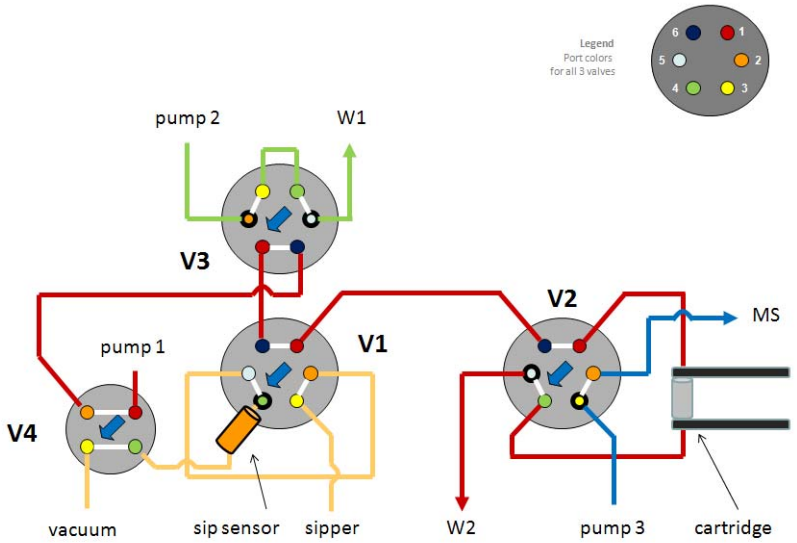


Figure 2 State #2: Wash/Load

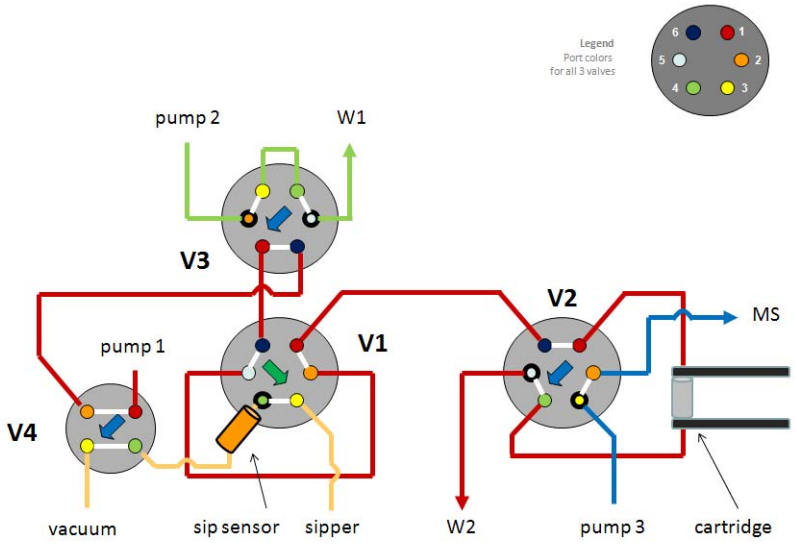


Figure 3 State #3: Elute

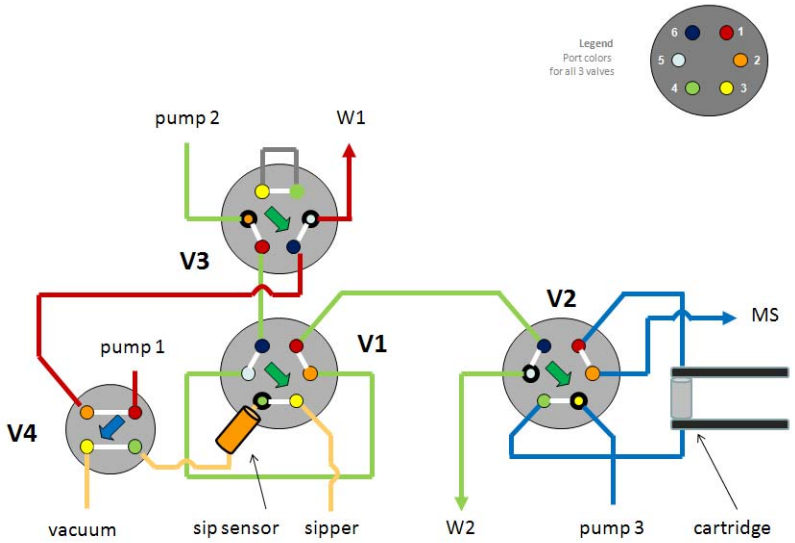


Figure 4 State #4: Re-equilibrate

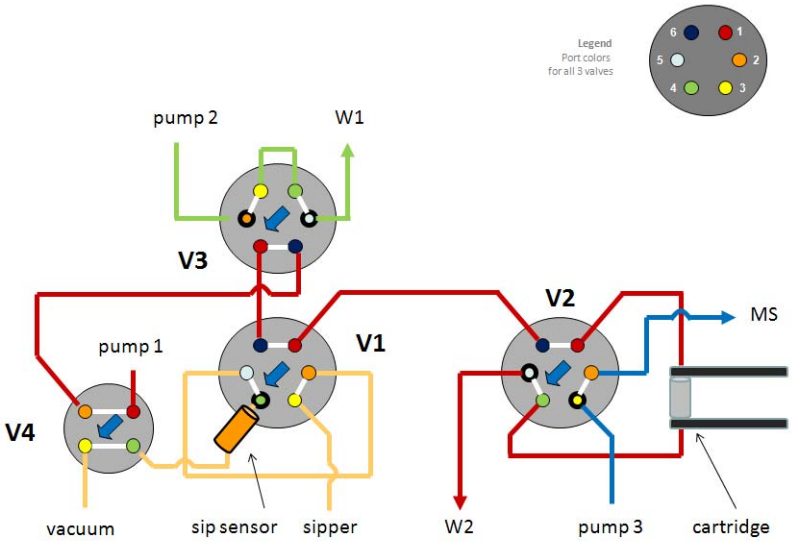


Figure 5 Flush the sipper tube

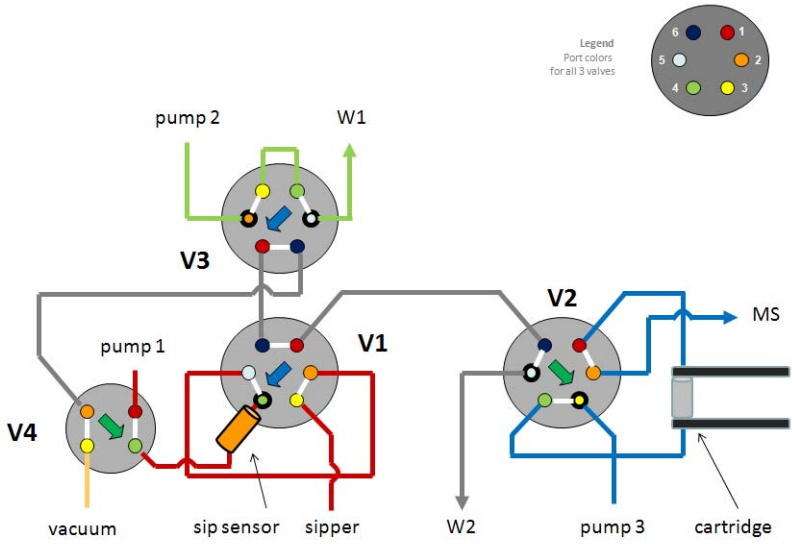
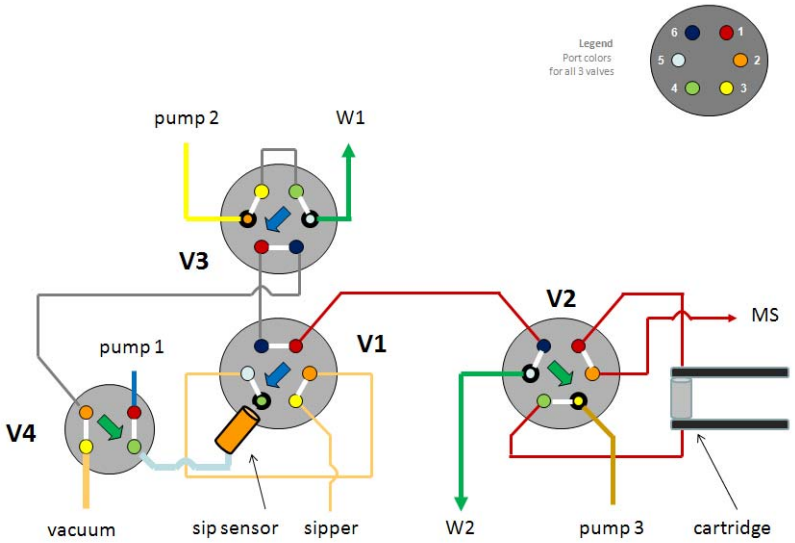
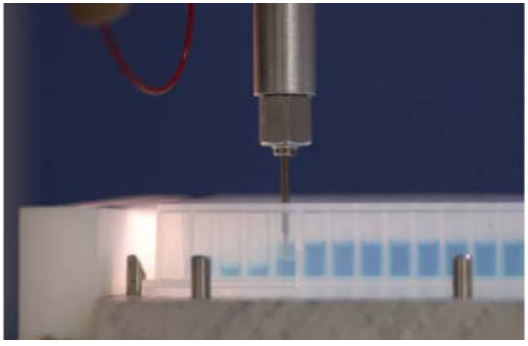
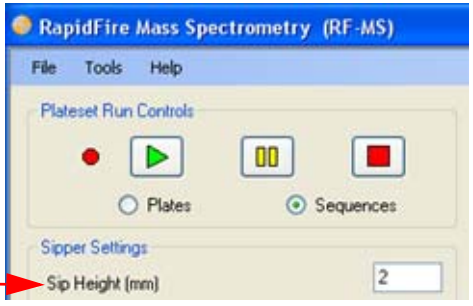
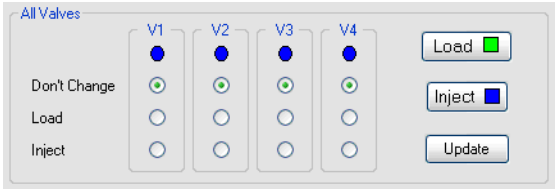
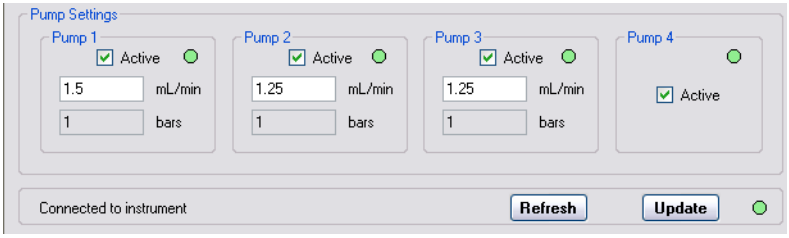


Figure 6 Physical colors of tubing (flush the sipper tube)



Symptom 1. The RapidFire system does not seem to be sipping

Actions	Supporting actions	Comments or Results
1 Check that the vacuum is on.	• Verify the integrity of the connections to the sidearm waste flasks. • Check that the vacuum pressure displayed on the RapidFire UI is in the range of -60 to -70 kPa. • Present liquid such as water from an eppendorf tube to the sipper tube and verify that the liquid is aspirated slowly but noticeably.	
2 Check that the sipper tube is going deep enough in the wells to aspirate the sample. (See the figure on the left below.)	• If necessary, adjust the **Sip Height** on the main RapidFire tab. (See the figure on the right below.) • Make the value smaller to lower the position of the sipper tube in the wells.	• The value sets the distance in millimeters that the tip of the sipper tube is above the plate bottom.
		
3 Flush the sipper tube.	• Click **Flush Now** on the main tab of the RF-MS data acquisition software.	

Actions	Supporting actions	Comments or Results
4 Assess aspiration proficiency by measuring aspiration times.	**a** With the vacuum on, and with all three valves in **Inject** (blue) position, hold an eppendorf tube of water under the sipper tube. • Measure how long it takes to sip 1 mL of water. • The normal range is 25-45 seconds. **b** Disconnect the beige loop at V1P2 and stick the free end into the same little tube of water. • Is aspiration taking place? • Is 1 mL of water sucked up in 35 seconds or less?	If the first action (Step 4a) results in poor, sluggish aspiration, while the second action (Step 4b) reveals efficient, swift aspiration, then: • The obstruction is likely to be in the sipper tube itself, at the V1P3 port, or in the V1P2-V1P3 groove. • Change and flush the sipper tube as described in "Symptom 5. A valve port or groove is clogged" on page 15.
5 If sipping still appears to be inefficient after you've taken actions 1 – 4 above, test for a flow constriction within V1.	**a** Manually actuate V4 to its **Load** (green) position in the Valve Tuner window. **b** Ascertain that all three nanovalves (V1, V2 and V3) are in the **Inject** (blue) position, as show below.	
		
	c Turn on pump 1 (P1) using the commands of the main window. • Set the flow rate to 1.5 mL/min. • Watch the backpressure of P1. • If the backpressure remains low, then ramp up the flow rate incrementally (1.5, then 3, then 5 mL/min).	
		

Actions	Supporting actions	Comments or Results
	d Switch V1 to **Load** (green). **e** Observe the backpressure of P1.	• If P1 overpressures, a clog is likely to exist at V1P3, V1P4 or in the groove connecting these two ports. • See "Symptom 5. A valve port or groove is clogged" on page 15 for information on how to troubleshoot a valve port or groove that is clogged.

Symptom 2. The sip sensor does not detect liquid sample

Actions	Supporting actions	Comments or Results
1 Verify that aspiration is occurring properly.	Follow Steps 1- 4 of "Symptom 1. The RapidFire system does not seem to be sipping" on page 9.	
2 Check the aspiration time set in your RF method (**.rfcfg**).	State # 1 or aspiration time should be set to 600 ms or greater.	
3 Check the digital output display of the sip sensor module located along the inside left wall of your RapidFire High-throughput MS System as shown below. 	**a** With the vacuum on, record the value displayed when air is being sipped. **b** Hold a tube of liquid under the sipper tube and record the value displayed on the sip sensor module. **c** Compare the values recorded above.	The second value (sipping liquid) should be approximately 2x the first value (sipping air).

Symptom 3. A pump is experiencing Underpressure

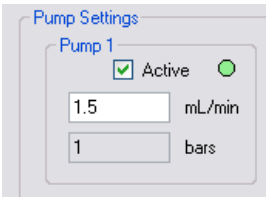
Actions	Supporting actions	Comments or Results
1 Check for leaks along the flow path of this pump.	**a** Check first with all valves in their **Inject** position (blue). **b** Then check with all valves in their **Load** (green) positions.	If you notice a leak: • Make sure all nuts and ferrules are tightly in place and hold the PEEK tubing firmly. • Make sure the end cuts of all tubing are "square" (sharp cuts, perfectly perpendicular to the tubing axis) and bottomed out in their respective ports. • Replace fittings and/or pieces of tubing as needed.
2 Check for air bubbles along the fluidics lines.		If you observe some air bubbles: • Whirl the solvent bottles to let air bubbles escape. • Prime the pumps, or flush them for a couple of minutes at a flow rate of 10 mL/min with the diversionary valves open. • Resume your RapidFire run.

Symptom 4. The mass spectrometer signal is very low

Actions	Supporting actions	Comments or Results
1 Check that the sample is getting sipped out of the plate.	See "Symptom 1. The RapidFire system does not seem to be sipping" on page 9.	
2 Check that all pumps are turned on (P3 in particular), including the peristaltic pump (P4).		
3 Check the back pressure of the pumps.	• If the pressures are *lower than normal*, check that no leak is occurring along the fluidics lines. Look for large air bubbles and get rid of them (see "Symptom 3. A pump is experiencing Underpressure" on page 13). • If the pressures are *higher than normal*, try to pinpoint the location of the clog(s).	
4 Check that you are using MS methods adequate for your assay.	Verify that you are filtering for the correct Q1 and/or Q3 *m/z*.	
5 Check that the MS plate is clean.	If needed, remove the plate from the MS head and wipe it gently with methanol and clean Kimwipes.	
6 Check that you have inserted the cartridge adapted to your specific experiment.		Column affinity to the analytes can greatly influence MS signal quality and intensity.
7 Check that you are using the proper set of aqueous and organic solvents for your assay.		Adding 0.1% TEA or TFA sometimes causes significant changes in MS signal quality and intensity.
8 Consider changing method parameters.	• The wash/load time (State # 2 duration in the RapidFire Cycle Durations section of the RF-MS software page) and the flow rate of P1. • The elution time (State # 3) together with the flow rate of P3. • Add **Blank Injections Between Wells** (on main RF-MS software page) to reduce carryover. • Widen the resolution of quadrupoles Q1 and Q3.	

Symptom 5. A valve port or groove is clogged

This section assumes that previous troubleshooting points to a clog in the V1P2-V1P3 groove (between the sipper tube and the injection loop). The affected valve port or groove may be different on your system.

Actions	Supporting actions	Comments or Results
1 Flush the groove in both directions with aqueous solvent.	**a** Manually actuate V4 to its **Load** (green) position in Valve Tuner. **b** Ascertain that the three nanovalves (V1, V2 and V3) are in the **Inject** (blue) position. **c** Disconnect the sipper tube at V1P3. **d** Turn on P1 only using the command of the Pump Settings section. Keep its flow rate at 1.5 mL/min at first. 	• When you try to flush the V1P2-V1P3 groove, liquid will drip out of V1P3. The presumed clog must have an exit path.
	e If the back pressure of P1 remains low, then ramp up its flow rate incrementally (1.5, then 3, then 5 mL/min). **f** If the clog fails to disappear, now flush in the other direction. **g** Turn on P1 only. If the back pressure of P1 remains low, then ramp up its flow rate incrementally (1.5, then 3, then 5 mL/min). This may force the clogging material out of the groove. **h** If the clog fails to disappear, flush the groove with organic fluid as described in Step 2.	• Remove one end of the loop from V1P2 and connect it instead into V1P3. Leave V1P2 open. • Don't hesitate to remove the center post if it is in the way. The set screw that holds it in place is accessible from the back of the RapidFire platform, on the pyramidal bracket between V1 and V3.

Actions	Supporting actions	Comments or Results
2 Flush the groove in both directions with organic solvent.	Instead of feeding aqueous solvent through the OD 1/16" blue tubing normally connected to P1's front panel, fill this fluidic path up with organic solvent.	
	a Disconnect the OD 1/16" blue tubing from P1's outlet and plug it instead into P3's outlet (after having temporarily removed P3's beige tubing).	• Doing this uses P3 to try and dissolve the V1P2-V1P3 clog.
	b With the sipper tube disconnected and the loop in its usual location between V1P5 and V1P2: ◦ Turn on P3 only using the commands of the Pump Settings section. ◦ Keep its flow rate at 1.5 mL/min at first.	
	c If the back pressure of P3 remains low, ramp up its flow rate incrementally (1.5, then 3, then 5 mL/min).	• This may force the clogging material out of the groove.
	d If the clog fails to disappear, now link the loop to V1P3, leaving V1P2 open for a backflush of the groove.	
	e Turn on P3 again and ramp up its flow rate if possible.	
3 If you think that the clog has been removed from your RapidFire High-throughput MS System, then resume your experiments.		Otherwise, call Agilent as the valve on your system may need to be replaced.

Tip: Use a spare adapter 10-32-F-to-6-32-M to redirect a "thick" (OD 1/16") tubing into any valve port of your choosing can be very helpful in flushing grooves with both types of solvents, in both directions.

Symptom 6. Pump 1 is overpressuring

Pump 1 (P1) can overpressure in any of the following three scenarios, which correspond to the three valve position combinations during a RapidFire cycle:

- P1 overpressures with the valves positions as in State # 1: Aspirate (all 3 valves in their **Inject** (blue) positions).
  See Table 1 *below* for troubleshooting steps for this scenario.
- P1 overpressures with the valves positions as in State # 2: Wash / Load (V1 in **Load** (green), V2 and V3 in **Inject** (blue)). In this case, follow the same steps outlined in Table 1 (valves configuration as in State # 1), but be aware that the clog could be in the V1P2-V1P5 beige loop.
- P1 overpressures with the valves positions as in State # 3: Elute (all 3 valves in their **Load** (green) positions).
  See *Table 2 on page 19* for troubleshooting steps for this scenario.

Table 1 Scenario 1: Valve Positions as in State # 1: Aspirate

Actions	Supporting actions	Comments or Results
1 You may need to change the cartridge.	**a** Turn off P1. **b** Select the **Load** position from the **System Tools > Column Changer** menu, then click **Go To**. **c** Take out the cartridge and insert a new one. **d** Select the position of the new column from the **System Tools > Column Changer** menu, then click **Go To**. **e** If the back pressure of P1 returns to normal, continue normal operation.	For State #1: Aspirate flow diagram, see Figure 1 on page 3.
2 You may need to replace the OD 1/32″ red tubing between V2P1 and the bottom of the column changer.	**a** With the column changer disconnected, if P1 is still overpressuring, then the clog is upstream of the SPE cartridge on the aqueous flow path. **b** Disconnect the red tubing at V2P1. **c** If pressure becomes low, the red tubing is clogged. Replace it.	
3 You may need to replace the V1P1-V2P6 OD 1/32″ red tubing.	**a** Disconnect the V1P1-V2P6 red tubing at Valve 1. **b** If pressure becomes low, the red tubing is clogged. Replace it.	

Table 1 Scenario 1: Valve Positions as in State # 1: Aspirate

Actions	Supporting actions	Comments or Results
4 You may need to rehome the valves, if, with the V1P1-V2P6 red tubing disconnected at V1, P1 is still overpressuring.	**a** Turn off the pumps. **b** Push the E-STOP button. **c** Manually, rotate the valve couplings in the counter-clockwise direction or towards the Inject position as far as possible **d** Release the E-STOP button. **e** In RF-MS, under the Valve Tuner window, click on the **Find** button for all three valves. **f** All three nanovalves should thereafter show green disks next the Home section of the Valve Configuration utility. If they do not, call Agilent for assistance. **g** With all valves homed and in Inject (blue) position, if the back pressure of P1 is still high, then the clog is further up the lines coming from P1.	
5 Disconnect the pieces of tubing that lead step-by-step to P1.	**a** Disconnect the tubing in the following order: • Start with V3P1-V1P6. • Then V4P2-V3P6. • Then finally P1-V4P1. **b** Evaluate the results to determine where the clog is located.	• If you deduce that the clog is within a valve (at a port or in a groove), see "Symptom 5. A valve port or groove is clogged" on page 15.
6 You may need to replace the OD 1/32" red tubing between the top of the column changer and V2P4.	• If the back pressure of P1 is low with *no* cartridge in the column holder but becomes high as soon as *any* cartridge gets inserted, then the problem lies downstream of the column on the aqueous flow path. • Disconnect the column changer-V2P4 red tubing at V2P4. • If P1 still overpressures, then this tubing is at fault. Replace it.	
7 Be aware that clogs can occur inside the beige adapters (10-32 to 6-32 threads).		

Table 2 Scenario 3: Valve Positions as in State # 3: Elute

Actions	Supporting actions	Comments or Results
1 You may need to rehome the valves.	**a** Turn off the pumps. **b** Push the E-STOP button. **c** Manually, rotate the valve couplings in the counter-clockwise direction or towards the **Inject** position as far as possible **d** Release the E-STOP button. **e** In RF-MS, under the Valve Tuner window, click on the **Find** button for all three valves. **f** All three nanovalves should thereafter show green disks next the Home section of the Valve Configuration utility. If they do not, call Agilent for assistance. **g** With all valves homed and in **Load** (green) position, if the back pressure of P1 is still high, then the clog is between P1 and waste.	For State #3: Elute flow diagram, see Figure 3 on page 5.
2 Disconnect the green waste line at V3P5.	**a** If the back pressure of P1 comes down, then the flow is being obstucted by one of the following: • the adapter at V3P5, *or* • the OD 1/16" green tubing itself **b** Replace the culprit.	
3 Disconnect V4P2-V3P6 at V3 if the back pressure of P1 remains high.	• If P1 still overpressures, then move up the lines and disconnect V4P2-V3P6 at V4. • If P1 becomes low, then you can assume that the V3P6-V3P5 groove is clogged.	• See "Symptom 5. A valve port or groove is clogged" on page 15.

Symptom 7. Pump 2 is overpressuring

Actions Follow essentially the same troubleshooting instructions as desribed in "Symptom 6. Pump 1 is overpressuring" on page 17, but focus your examination on the organic flow path downstream of P2 (instead of P1).

Keep in mind that the lines that most commonly clog are:

- the V1P1-V2P6 red tubing
- the V1P2-V1P5 beige loop

Symptom 8. Pump 3 is overpressuring

This troubleshooting procedure consists of disconnecting one by one the segments of tubing downstream of the overpressuring P3 until the position of a clog can be determined.

Actions	Supporting actions	Comments or Results
1 You may need to change the cartridge.	**a** Turn off P3 and actuate all three nanovalves to the **Load** position (green). **b** Select the **Load** position from the **System Tools > Column Changer** menu, then click **Go To**. **c** Take out the cartridge and insert a new one. **d** Select the position of the new column from the **System Tools > Column Changer** menu, then click **Go To**. **e** If the back pressure of P3 returns to normal, continue normal operation. **f** Check that the back pressure of all three pumps remain low, both when the valves are in **Load** position (green) and when they are in **Inject** position (blue).	
2 You may need to replace the V2P2-MS OD 1/32" red tubing.	**a** Disconnect the V2P2-MS red tubing at MS input. **b** Turn on P3. **c** If the pressure of P3 is still high, the red tubing is clogged. Replace it.	
3 You may need to replace the MS input – MS inject OD 1/16" red tubing.	**a** Disconnect the red tubing at MS inject. **b** Turn on P3. **c** If the pressure of P3 is still high, the thick red tubing is clogged. Replace it.	
4 You need to change the MS probe.	• Gently replace the ESI or APCI injection probe of your MS.	

Symptom 9. The sipper guide needle crashed into the plate

Actions	Supporting actions	Comments or Results
1 Press the E-STOP button and clear up the platform.		
2 Repower the stages (by lifting the E-STOP).		
3 Unscrew the damaged pink sipper guide needle (luer lock) as well as the beige PEEK sipper tube that runs through it. 		The tubing is most likely severely kinked.
4 Screw in a new sipper guide needle and, from the bottom, slide in a new sipper tube.	With the help of tweezers if necessary, cap the sipper tube with a red ferrule and screw it into V1P3.	
5 Make sure the sipper tube bottoms out in V1P3 and is tightly held in place.	Pull on the sipper tube to make sure that it doesn't fall out.	
6 Re-home the sipper tube for both 96 and 384-well types of plates.	Click the **Home** button under the Sipper Configuration Wizard and follow all of the calibration steps.	

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In this book

This guide has troubleshooting information for common problems that may arise while using the Agilent RapidFire High-throughput MS System.

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