Agilent RapidFire High-throughput MS System

Troubleshooting Guide

Before you begin: Understand and interpret the flowpath diagrams 2
Symptom 1. RapidFire and MS computers are not communicating 10
Symptom 2. The RapidFire system does not seem to be sipping 11
Symptom 3. The sip sensor does not detect liquid sample 14
Symptom 4. A valve port or groove is clogged 15
Symptom 5. Pump 1 pressure is too high 17
Symptom 6. Pump 2 pressure is too high 20
Symptom 7. Pump 3 pressure is too high 21
Symptom 8. Pump pressure is too low 22
Symptom 9. The sipper guide needle crashed into the plate 23
Symptom 10. The mass spectrometer signal is very low 24
Interpreting Common Error Messages from the RapidFire Log 26
From AB Sciex Analyst 26
From National Instruments Boards 27
From Agilent Pumps 27

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

Agilent Technologies

Before you begin: Understand and interpret the flowpath diagrams

Use the following flow diagrams to help identify and resolve most problems that might arise on your Agilent RapidFire High-throughput MS System.

"State #1: Aspirate" on page 3

"State #2: Load/Wash" on page 4

"State #3: Extra Wash" on page 5

"State #4: Elute" on page 6

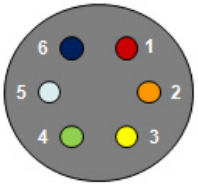
"State #5: Re-equilibrate" on page 7

"Flush the sipper tube" on page 8

"Physical colors of tubing (flush the sipper tube)" on page 9

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

State #1: Aspirate

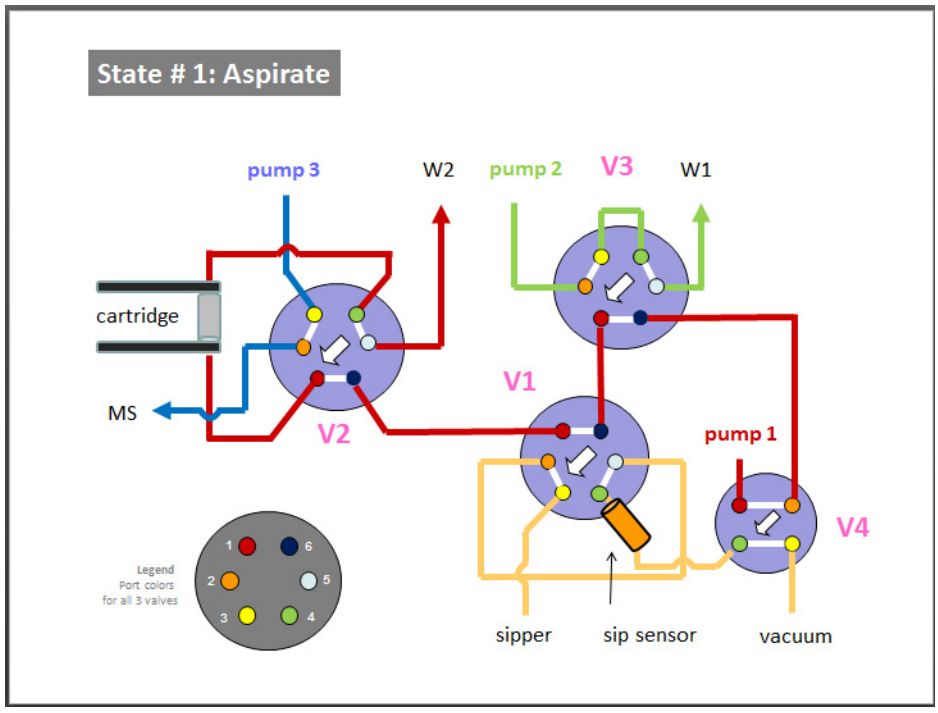


Figure 1 State #1: Aspirate

State #2: Load/Wash

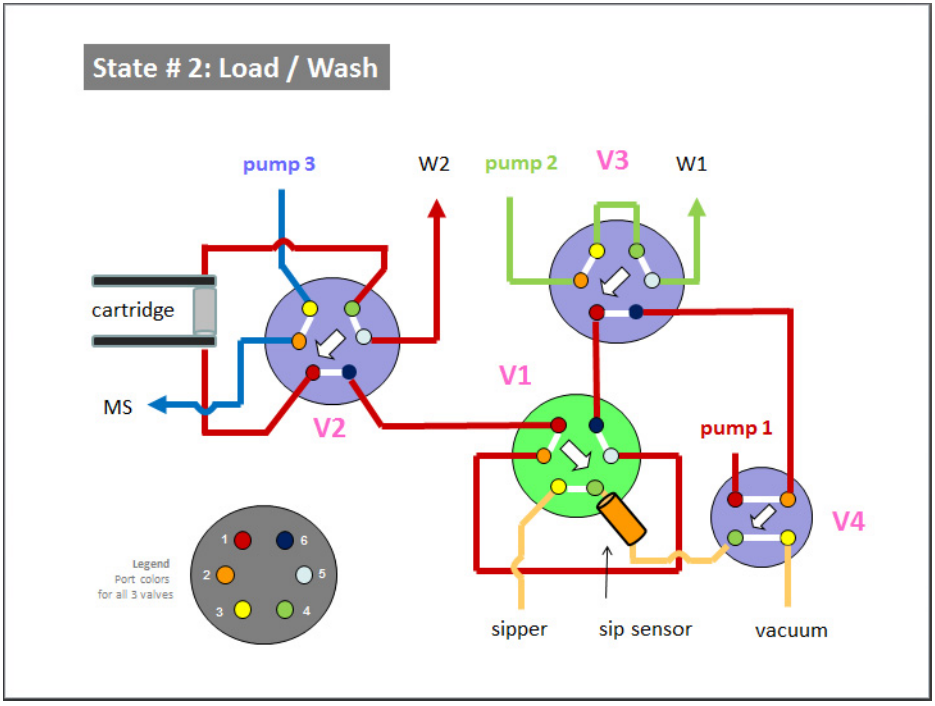


Figure 2 State #2: Load/Wash

State #3: Extra Wash

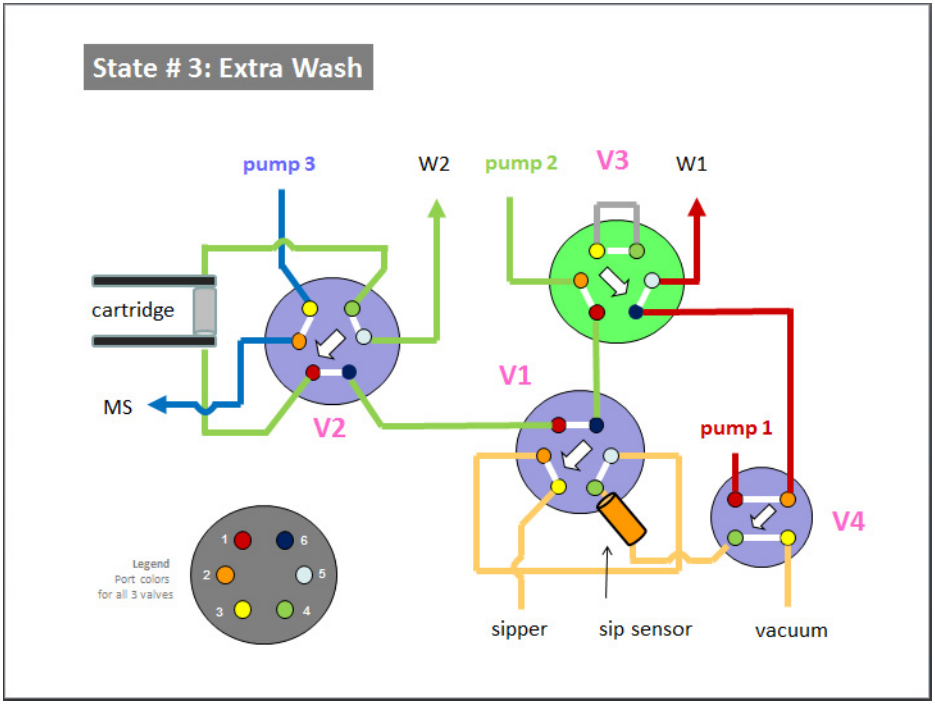


Figure 3 State #3: Extra Wash

State #4: Elute

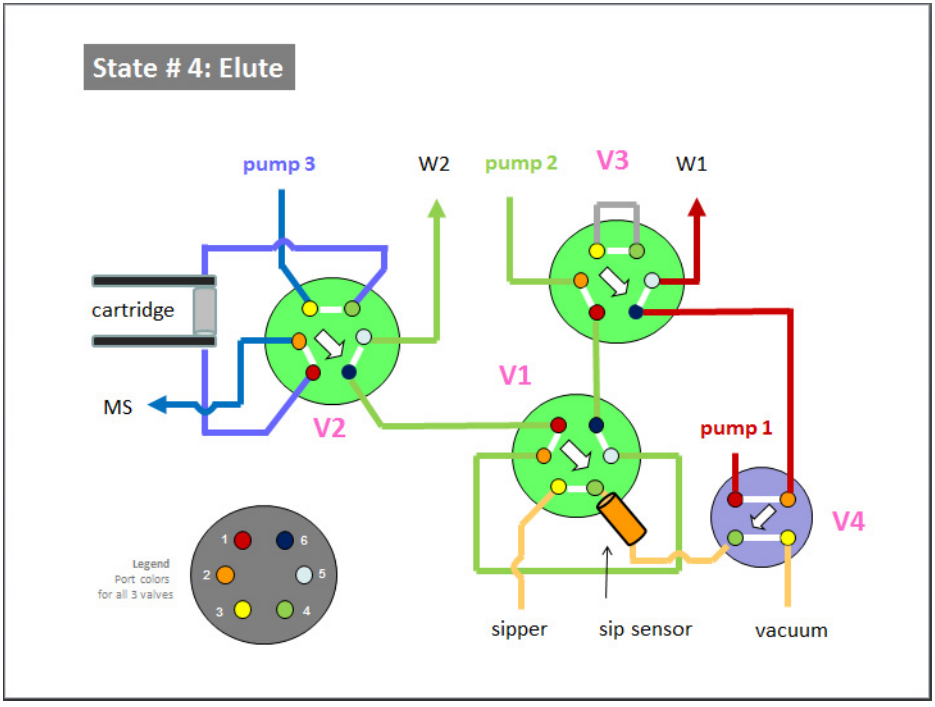


Figure 4 State #4: Elute

State #5: Re-equilibrate

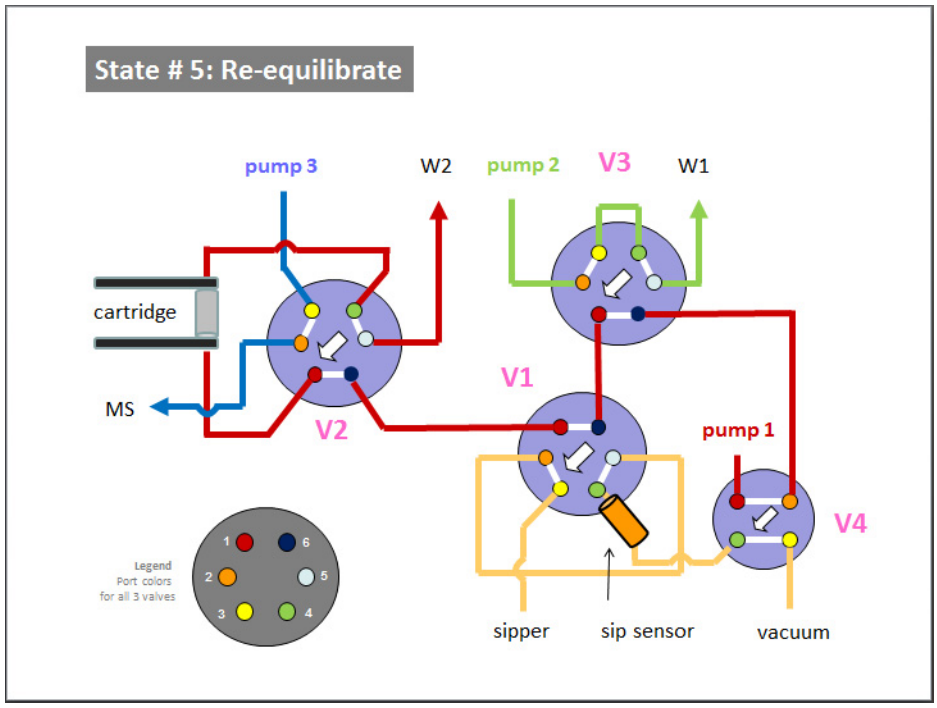


Figure 5 State #5: Re-equilibrate

Flush the sipper tube

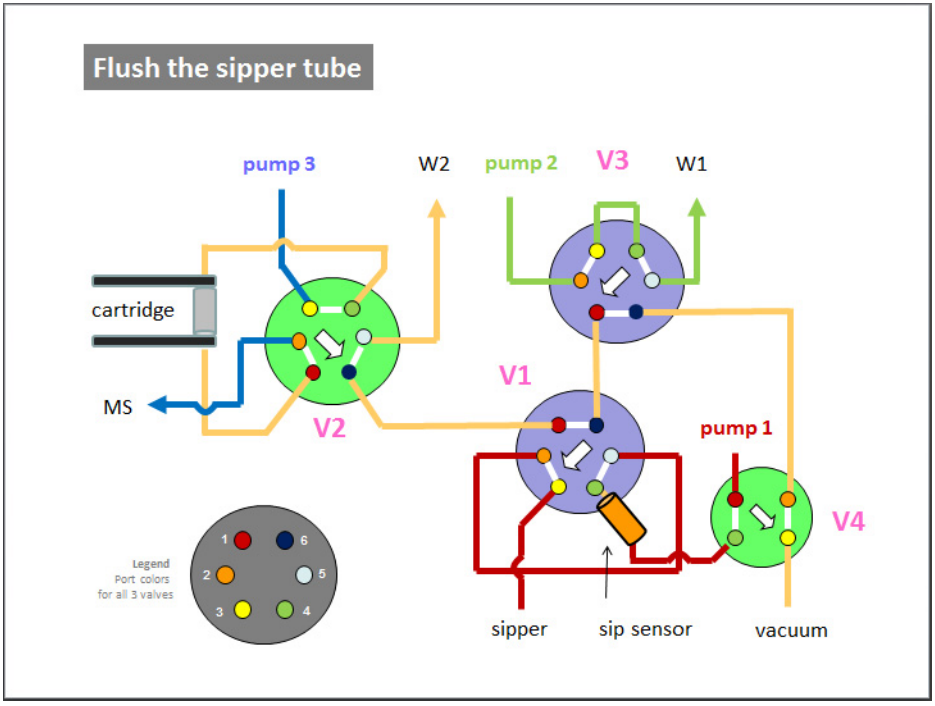


Figure 6 Flush the sipper tube

Physical colors of tubing (flush the sipper tube)

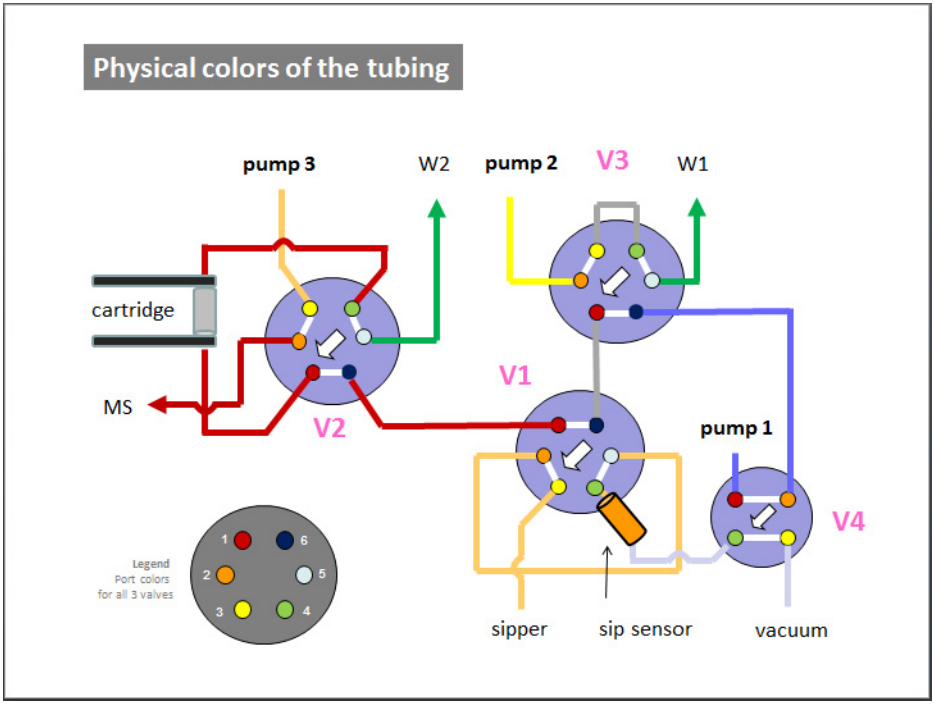
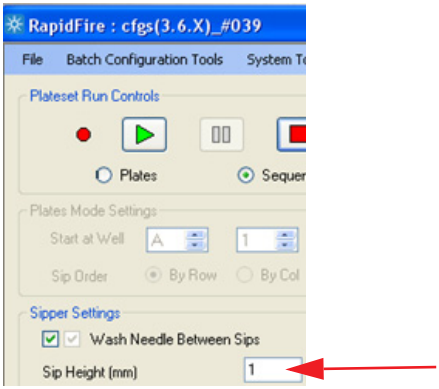


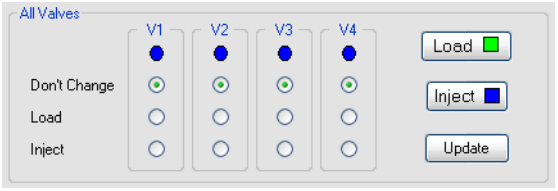
Figure 7 Physical colors of tubing (flush the sipper tube)

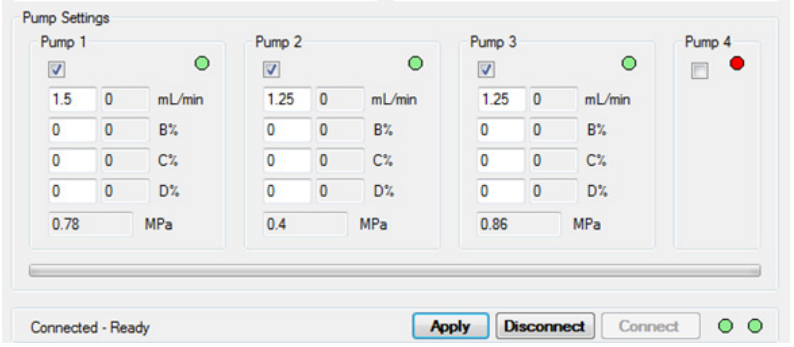
Symptom 1. RapidFire and MS computers are not communicating

Actions	Supporting actions	Comments or results
1 On the MS computer, make sure that two **RFxxx.EXE** programs appear in the list of Processes running in the Windows Task Manager.	If not, double-click on the RapidFire Communicator shortcut on the desktop, and ascertain their presence.	
2 In the RF software user interface, under **Systems Tools\ Network Settings**, verify that the IP addresses for the RF and MS computer correct (and distinct).		
3 On the RF computer, is the shared drive mapping still valid?	Check that you can browse and see files on the shared drive, usually either **M:\ or N:**.	Typically, the **M:** drive of the RF computer is mapped as the **D:** drive of the MS computer.
4 On the MS computer, are all firewalls turned off?		
5 Contact Agilent at 1-800-227-9770.		

Symptom 2. The RapidFire system does not seem to be sipping

Actions	Supporting actions	Comments or results
1 Check that the vacuum source is on.	• Verify the integrity of the connections to the 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.	• If necessary, adjust the **Sip Height** on the main RapidFire tab, shown below. • Make the value smaller to lower the position of the sipper tube in the wells. 	• The value you enter for **Sip Height** sets the distance in millimeters that the tip of the sipper tube is above the plate bottom.
	• If the optimal sipper position is not obtained by adjusting the Sip Height, then click the **Home** button under the Sipper Configuration Wizard and follow all of the calibration steps.	
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 20 - 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 4. A valve port or groove is clogged" on page 15.
5 If sipping still appears to be inefficient after you have 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. 	

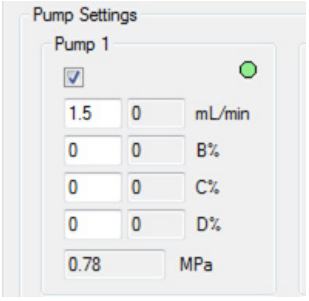
Actions	Supporting actions	Comments or results
	c Turn on pump 1 (P1) using the commands of the main window. • Set the flow rate to 1.5 mL/min. • Watch the backpressure of P1. • If the backpressure remains low, then ramp up the flow rate incrementally (1.5, then 3, then 5 mL/min). 	
	d Switch V1 to **Load** (green). **e** Observe the backpressure of P1.	• If P1 overpressures, a clog is likely to exist at V1P3, V1P4 or in the groove that connects the two ports. • See "Symptom 4. 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 3. 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 2. The RapidFire system does not seem to be sipping" on page 11.	
2 Check that a reasonable aspiration time is set in your RF method (**.rfcfg**).	With a sip sensor installed, set State # 1 or aspiration time 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 water 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). If not, contact Agilent for assistance.

Symptom 4. A valve port or groove is clogged

This section assumes that a clog in the V1P2-V1P3 groove (between the sipper tube and the injection loop) has been identified in the previous troubleshooting steps. The affected valve port or groove can 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. Place a beaker to catch the liquid before tuning on pump 1.

Actions	Supporting actions	Comments or results
	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) to 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.	• Disconnect one end of the loop from V1P2 and connect it instead into V1P3. Leave V1P2 open. • Do not 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.
2 Flush the groove in both directions with organic solvent.	Instead of pumping aqueous solvent through the OD 1/16" blue tubing that is normally connected to the front panel of P1, fill up this fluidic path with organic solvent.	
	a Disconnect the OD 1/16" blue tubing from the outlet of P1 and plug it into the outlet of P3 (temporarily remove the beige tubing from P3 first). **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.	• This procedure uses P3 to try to dissolve the V1P2-V1P3 clog.
	c If the back pressure of P3 remains low, ramp up its flow rate incrementally (1.5, then 3, then 5 mL/min). **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.	• This procedure can force the clogging material out of the groove.
3 If you think that the clog has been removed from your RapidFire High-throughput MS System, then resume your experiments.		Otherwise, call Agilent. The valve on your system may need to be replaced.

Symptom 5. Pump 1 pressure is too high

Pump 1 (P1) can overpressure in either of the following scenarios, which correspond to the valve position combinations during RapidFire cycles:

- P1 overpressures with the valves positions as in State # 1: Aspirate (all three valves in their Inject (blue) positions).
  See Table 1 for troubleshooting steps for this scenario.
- P1 overpressures with the valves positions as in State # 4: Elute (all three 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 Change the cartridge.	**a** Turn off P1. **b** Select the **Load** position from the **System Tools > Cartridge Changer** menu, then click **Go To**. **c** Take out the cartridge and insert a new one. **d** Select the position of the new cartridge from the **System Tools > Cartridge Changer** menu, then click **Go To**. Use a cartridge 0 if available. **e** If the back pressure of P1 returns to normal, continue normal operation.	For State #1: Aspirate flow diagram, see "State #1: Aspirate" on page 3.
2 If P1 is still overpressuring with the V1P1-V2P6 red tubing disconnected at V1, then Re-home 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 the RapidFire user interface, under System Tools, under the Valve Tuner window, click on the **Find** button for all three valves. **f** With all valves homed and in the Inject (blue) position, if the back pressure of P1 is still high, then the clog is further up the lines coming from P1.	If green disks are not shown next to the Home section of the Valve Configuration utility for all three nanovalves, then call Agilent for assistance.

Table 1 Scenario 1: Valve Positions as in State #1: Aspirate (continued)

Actions	Supporting actions	Comments or results
3 Replace the OD 1/32" red tubing between V2P1 and the bottom of the cartridge changer.	**a** With the cartridge 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.	
4 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.	
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. **c** Disconnect the blue tubing from pump 1 to make sure that the purge valve is not the cause of the back pressure.	• If you deduce that the clog is within a valve (at a port or in a groove), see "Symptom 4. A valve port or groove is clogged" on page 15.
6 Replace the OD 1/32-inch red tubing between the top of the cartridge changer and V2P4.	• If the back pressure of P1 is low with *no* cartridge in the cartridge holder but becomes high as soon as *any* cartridge gets inserted, then the problem lies downstream of the cartridge on the aqueous flow path. • Disconnect the cartridge changer-V2P4 red tubing at V2P4. • If P1 still overpressures, then this tubing is at fault. Replace it.	

Table 2 Scenario 3: Valve as in State #4: Elute

Actions	Supporting actions	Comments or results
1 Re-home 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 the RapidFire user interface, under the Valve Tuner window, click on the **Find** button for all three valves. **f** If green disks are not present next to the Home section of the Valve Configuration utility for all three nanovalves, 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 #4: Elute flow diagram, see "State #4: Elute" on page 6.
2 Disconnect the green waste line at V3P5.	**a** If the back pressure of P1 comes down, then the flow is being obstructed by one of the following: • the adapter at V3P5, *or* • the OD 1/16" green tubing itself, examine the openings of the tubing and make sure that they are rounded and open. **b** Replace the culprit.	
3 Disconnect V4P2-V3P6 at V3 if the back pressure of P1 remains high.	• If the back pressure of P1 is still too high, 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 4. A valve port or groove is clogged" on page 15.

Symptom 6. Pump 2 pressure is too high

Actions Follow essentially the same troubleshooting instructions as described in "Symptom 5. Pump 1 pressure is too high" 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 7. Pump 3 pressure is too high

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 Change the MS probe.	**a** Disconnect the red tubing from MS inlet. If the pressure drops, then the MS probe is clogged. **b** Gently replace the ESI or APCI injection probe of your MS.	
2 Change the cartridge.	**a** Turn off all pumps and actuate all three nanovalves to the **Load** position (green). **b** Select the **Load** position from the **System Tools > Cartridge Changer** menu, then click **Go To**. **c** Take out the cartridge and insert a new one. Use cartridge 0, if available. **d** Select the position of the new cartridge from the **System Tools > Cartridge 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 remains low, both when the valves are in **Load** position (green) and when they are in **Inject** position (blue).	
3 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.	

Symptom 8. Pump pressure is too low

Actions	Supporting actions	Comments or results
1 Check that the purge valve (black knob on the front panel of the pump) is properly closed (finger-tight only).		
2 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 that all nuts and ferrules are tightly in place and hold the PEEK tubing firmly. • Make sure that 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.
3 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 5 minutes at a flow rate of 5 mL/min with the diversionary valves open. • Close the purge valve after priming the solutions. • Resume your RapidFire run.

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 that the sipper tube bottoms out in V1P3 and is tightly held in place.	• Pull on the sipper tube to make sure that it does not fall out. • Trim the tube to the desired length.	
6 Configure the sipper tube for all 96-well and 384-well types of plates.	Click the **Home** button under the Sipper Configuration Wizard and follow all of the calibration steps.	

Symptom 10. 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 2. The RapidFire system does not seem to be sipping" on page 11.	
2 Check that all pumps are turned on.	• In particular, verify that P3 is on. • Be sure the peristaltic pump (P4) is on.	
3 Check the back pressure of the pumps.	• If the pressures are *lower than normal*, Make sure that the fluidics lines are not leaking. Look for large air bubbles and get rid of them (see "Symptom 8. Pump pressure is too low" on page 22). • If the pressures are *higher than normal*, look for the location of the clogs.	
4 Check if the MS methods that you are using are adequate for your assay.		
5 Check that the MS source is clean.	If needed, remove the face plate from the MS head and wipe it gently with methanol and clean lint-free cloth wipe.	Contact your Agilent LC/MS specialist partners for more advice on how to maintain your mass spectrometer.
6 Check that you have inserted the cartridge adapted to your specific experiment.		Affinity of the cartridge 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.		Additives and ion pairing reagents can cause significant changes in MS signal quality and intensity.

Actions	Supporting actions	Comments or results
8 Consider changing method parameters.	• Be sure an adequate wash/load time is set (State # 2 duration in the RapidFire Cycle Durations section of the RF-MS software page). • Check the corresponding flow rate of P1. • Be sure the settings for elution time (State # 4) and the flow rate of P3 are adequate. • Add **Blank Injections Between Wells** (on main RF-MS software page) to reduce carryover. • Widen the resolution of quadrupoles Q1 and Q3.	
9 Make sure that the long red tubing is securely tightened into the MS inlet.	Be sure that no liquid is found around the inlet of the MS.	

Interpreting Common Error Messages from the RapidFire Log

From AB Sciex Analyst

Error code	Meaning	Comment
EW 0x20000049 DF0 0	Parameter DF0 value 0 below the MIN limit	
EW 0x20000001 TEM	Cannot set online device TEM	This happens when the TEM parameter is being set to a specific value, but is online. Set it to *offline* first.
EW 0x20000001 IHT	Cannot set online device IHT	This happens when the IHT parameter is being set to a specific value, but is online. Set it to *offline* first.
EW 0x20001058	User command received in wrong state	This can happen if you try set the instrument state to ready and the instrument is not in idle state.
EW 0x2000100c	Fault Set: Ion Source Temperature Control	This happens when the TEM actual value does not match the TEM set value for a specific time.
EW 0x2000102c	Fault Cleared: Ion Source Temperature Control	This happens when the fault above is cleared, that is, when the TEM actual value matches the TEM set value.
EW 0x20000044	Idle Ready Instrument state changed from Idle to Ready	This indicates an instrument state change, that is, the instrument goes to idle when set to standby, it goes to ready for acquisition.

From National Instruments Boards

Error code	Meaning	Comment
PCI7344 -70136	NIMC_eventTimeoutError	A wait operation timed out or a read function timed out.
motor killed, 22596 motor killed, 30732 motor killed, 30796 motor killed, 30813 motor killed, 55366 motor killed, 55374 motor killed, 63510	These can be due to one of the following causes: • sip sensor triggering prematurely • excessive stage velocity or acceleration, or • a problem with the limit switch or encoder	

From Agilent Pumps

Error code	Meaning	Comment
EE 0062	timeout getting ready	A wait operation timed out or a read function timed out.
EE 0063	error (for example, leak) in other module	Other pump connected via CAN cable
EE 0064	leak in this module	
EE 2014	overpressure	
EE 2015	underpressure	

www.agilent.com

Printed in USA
Revision B, December 2013

G9314-90002

Agilent Technologies