

# Troubleshooting

This troubleshooting guide has also been placed on the desktop of all the FACS Facility computers.

In the event of a problem with the machine, please seek Geoff Lyon at TAC S617 or S631 for help. If he is not available please report your problem to him later via the form provided on top of the cytometers. You may also call Becton Dickinson for help. Their BD phone number is located on the side of Calibur S along with the serial numbers of all the machines in the facility. They can walk you through most any problem.

**NOTE:** The CellQuest Status window from Cytometer menu can be used to help diagnose STATUS problems.

## System Stays In NOT READY

**Check the following:**

- Sheath reservoir empty
- Waste reservoir full
- Initial 5-minute warm up is not complete
- Electrical connector on sheath probe is loose or disconnected

## System Stays In STANDBY (System Not Pressurized)

When the system is not pressurized, installation of a sample tube with the fluidics control button set to RUN will not change the status to READY it will remain in STANDBY. Pressure may be leaking from the reservoir caps or the vent valve, or the sample tube has not been pressurized properly. The sample either does not flow to the flow cell or flows poorly, and the FACSCalibur produces poor data.

### **Check the following:**

- **Air escaping from the sheath reservoir (tighten cap)**
- **Vent valve in clown position**
- **Cracked sample tube**
- **Bal seal worn out**
- **Blue connector for sheath reservoir not correctly seated**

### **Excess Noise (Bubbles In the Sheath Fluid)**

Bubbles are registered as events, producing spurious data. Bubbles can also cause alteration in the sample flow path, resulting in less than optimal data. The fluidic system should be re-primed. If the sheath reservoir was run dry, refill sheath and RUN system for 5 to 10 minutes with a test tube of distilled water before using for sample acquisition. This will remove bubbles and air from the sheath lines.

### **No Events on the Computer Screen**

#### **Check the following:**

- **If the system remains in STANDBY, check items under System Stays in STANDBY above.**
- **If the STATUS window displays READY, make sure sample concentration is adequate and properly mixed.**
- **Ensure instrument settings are correct for applications being run.**
- **Ensure threshold is not eliminating the populations by being set too high.**
- **Check the CellQuest Status window from Cytometer menu to see if readings are being updated. If the readings are not being updated, communication between the instrument and the computer is not present. Turn off the computer and the FACSCalibur, turn them back on, and resume where you left off.**

- **Prime the fluidics to remove air bubbles that may be trapped in the flow cell. These trapped air bubbles can deflect the sample stream away from the laser beam, causing no events to be detected.**

## **Sheath Fluid Dripping from the Sample Injection Tube**

**Check the following:**

- **Ensure that the droplet containment tube is seated as far up into the flow cell assembly as possible. Loosen the droplet containment tube retainer, push the tube up, and re-tighten the retainer.**
- **Replace the O-ring inside droplet containment tube retainer.**
- **Listen to hear if droplet containment system pump is rotating. If you cannot hear the pump with the tube support arm to the side, the pump may have stalled. Turn off the FACSCalibur and turn it on. If the motor is still not rotating, call BDIS Customer Support.**