Troubleshooting Automation

Automation has changed the way we do science – increasing assay throughput and speed. When your assay data does not look the way you would expect, do you know how to identify the source of the problem? Is it the liquid handler, detector, reagents, or assay design? This troubleshooting guide will help you work through the critical issues and find the source of liquid handling error.

1 OBSERVE

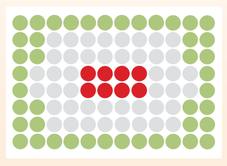
A careful look at your data, your instrument, and your labware is a good starting point.

Your data

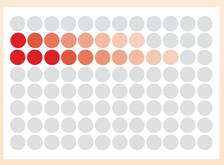
Is there a pattern to the bad data?

Specific plate-to-plate patterns of underdelivery/error can indicate issues with bent tips, clogged channels, loose or obstructed tubing, or environmental influences. Also, don't forget to check for problems with your source plate.

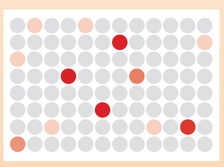
Column or row bias



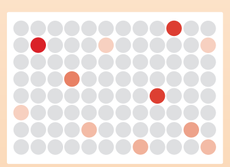
Edge effects

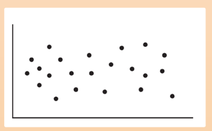


Non-linearity for serial dilutions

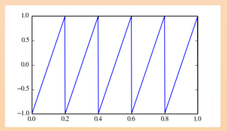


Active "hot" spots

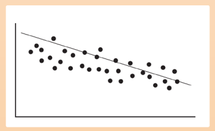




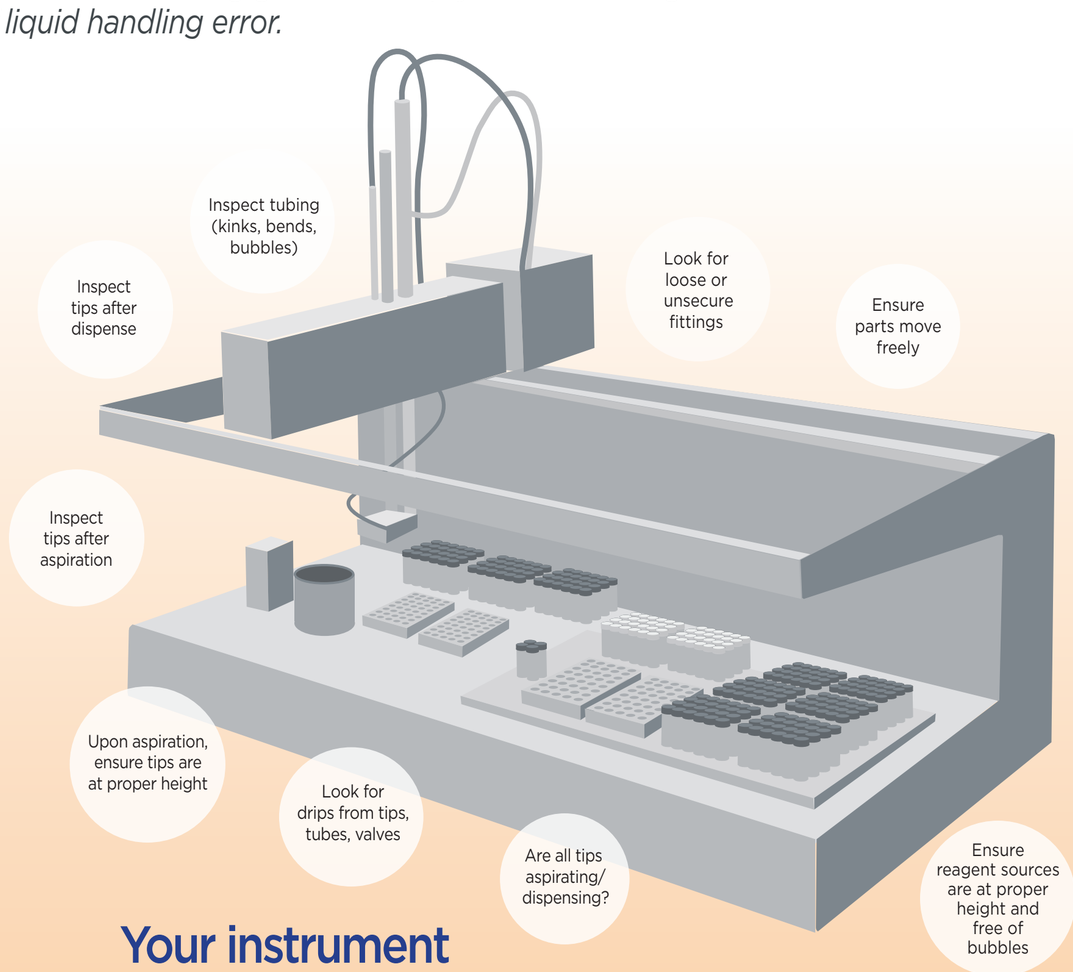
Random variability



Periodic variability



Loss of signal over time



Your instrument

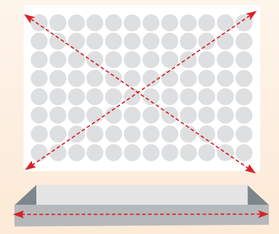
Looking closely at the instrument during a run, do you see anything that is not operating correctly?

Problems with clogged or loose tips, faulty o-rings, or poorly optimized methods and liquid classes can sometimes be seen visually during a run.

Your labware

Does everything look the way it should?

Issues with your consumables may be visually noticeable. When setting up a run make sure labware meets your quality expectations.



Ensure plates are flat, squared and unobstructed

All tips should be straight and unclogged

Check variability in different lots of labware

2 TRY

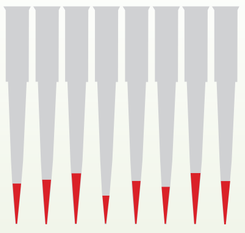
During aspiration and dispense, if you experience:

- Uneven liquid levels across wells

- Reagent sticking to tips or tubing

- Droplets at the end of tips and in wells

- Uneven liquid level in tips (which could indicate tips are touching bottom of well)



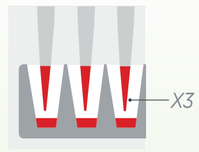
Try optimizing your liquid class parameters, such as:

- Aspirate and/or dispense speed
- Blowout speed and/or height
- Interwell speed

- Tip touch

- Aspirate or dispense height

- Single dispense vs. multi dispense



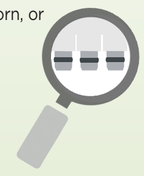
- Air gaps

Try inspecting liquid handler and consumables prior to operation:

- Check seal of tips, tip lot and tip size to ensure they are appropriate for the task. Also check for bent tips.

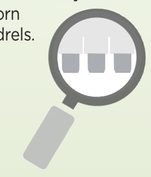
- Check syringes (if liquid displacement) to make sure they are tight. If using an air displacement system with an instrument connected to a compressor, ensure there is sufficient pressure for the instrument.

- Look for faulty, worn, or unseated o-rings.



- Inspect for clogs in tips, channels, or tubing, or air bubbles in fluid-filled tubing.

- Check for faulty or worn mandrels.



- Make sure all labware is correctly defined for your method and the robot is properly framed.
- Make sure all labware and hardware are level.

Other things to try:

- With viscous liquids, aspirate/dispense steps may need to slow down and/or include a pause. Consider reverse mode pipetting for viscous samples.

- Check for water uptake in the wells near the plate edges. Consider moving ALH or adding a protective shield to avoid evaporation.

- Review tip cleaning/rinse cycles when using reusable tips. Tips may need to be changed more frequently.

- Check for temperature or humidity gradients (next to window, next to heating element, etc).

3 IDENTIFY

If automation is not the source of the problem, a review of your biological process may help identify or eliminate other potential issues.

Experimental conditions

Are assay conditions optimized and implemented during a run?

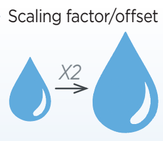
Liquid class parameters

- Aspirate/dispense speeds

- Aspirate/ dispense height

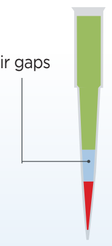
- Mixing - number of cycles, speed, volume and tip immersion depth

- Scaling factor/offset



- Blowout

- Air gaps



Reaction times

- Incubation temperatures
- On-deck storage temperatures

Methods

- Wet vs dry

- Mix vs no mix

- Dispense order

- Multi vs single dispense

- Forward vs reverse mode

Reagents

Are the reagents really what you think they are?

- Concentrations

- Storage buffers

- Storage temperature

- Purity

- Expired or new lots?

Environment

Are the environmental conditions of the lab, the instrument and the individual wells optimal and consistent?

Because of windows, heating ducts, forced air, and outside walls (to name just a few factors) the local environment of one instrument may not be consistent over time, or similar to another instrument in the same room. Temperature and humidity can have significant effects on data and data quality.

Control studies

Are you getting unexpected results?

In addition to positive and negative signal controls, you can design different studies to test for sources of biological, instrument, method, environment, and labware variation.

4 MANAGE

Keeping your liquid handler well-maintained and calibrated can go a long way toward maintaining good data quality.

Verify performance

Calibrate critical volumes by optimizing for precision and accuracy.

Maintain

Perform routine maintenance on movable parts and replace tubes, valves, pumps, seals and motors when needed.

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