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Calibration standards from a liquid handler: how to verify what the robot made.

A liquid handler repeats the same transfer the same way every time. That is its strength, and it is why one wrong liquid class can bend every curve the system prepares.

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SUMMARY

Automated liquid handlers such as the Hamilton Microlab STAR, NIMBUS, Prep and VANTAGE now prepare calibration series in many CRO and environmental labs. This paper explains why the main risk moves from random error to repeatable bias, works an illustrative serial series prepared with a liquid class that does not suit the solvent, and shows that the resulting curve passes while the low end is far off. It sets out how ISO 23783 and gravimetric checks verify the system, and why an independently prepared QC remains the check that catches what verification missed.

THE SHORT VERSION

01 A liquid handler turns small transfer errors into repeatable bias, and in a serial series the bias compounds.

02 A curve built from biased standards can pass every acceptance test while its low end is far from the truth.

03 Verify each liquid class gravimetrically at the volumes and solvents you use, and keep an independently prepared QC.

The robot made the same mistake eight times.

The method moved to the liquid handler in the spring. Precision improved overnight. Replicate standards agree to a fraction of a percent, and analysts have their afternoons back.

The low QC, still made by hand, has started reading high. Not every run. Most runs. The robot is not making random mistakes. It is making the same one, very precisely, at every transfer.

From random error to repeatable bias.

Hand pipetting fails in ways that scatter. A liquid handler fails in ways that repeat. Its pipetting follows a liquid class: draw and dispense speeds, air gaps, blowout and settle times. On Hamilton systems, for example, liquid classes for specific liquids are set up in the VENUS software [1].

A liquid class tuned for water behaves differently in methanol or acetonitrile. Volatile solvents tend to drip and to lose volume into the air in the tip. If the class does not suit the solvent, every transfer delivers a little less than it should. The same little less, every time.

In a serial series, that bias multiplies. Snow showed that each added dilution step adds to the uncertainty of the result [2]. A fixed bias is worse than random error, because it does not partly cancel. It builds up in one direction.

A serial series with the wrong liquid class.

Here is an illustrative two-fold serial series from 128 to 1 ng/mL in methanol. With a liquid class that does not suit the solvent, each transfer delivers 2.5% low. With a class verified for methanol, 0.3% low.

LABELLED (NG/ML)	TRANSFERS	ACTUAL VS LABEL, UNSUITED CLASS	ACTUAL VS LABEL, VERIFIED CLASS	REFIT ERROR ON THE ROBOT CURVE	
128	1	−2.5%	−0.3%	+5.3%	PASS
64	2	−4.9%	−0.6%	+2.7%	PASS
32	3	−7.3%	−0.9%	+0.4%	PASS
16	4	−9.6%	−1.2%	−1.6%	PASS
8	5	−11.9%	−1.5%	−3.2%	PASS
4	6	−14.1%	−1.8%	−3.7%	PASS
2	7	−16.2%	−2.1%	−2.4%	PASS
1	8	−18.3%	−2.4%	+2.6%	PASS

Illustrative calculation, not instrument data. Responses are taken as proportional to the actual concentration. The curve is fitted linear with $1/x^2$ against the labelled concentrations. Limits are ±15%, and ±20% at the lowest level.

−18.3%
LOWEST STANDARD VS ITS LABEL

6.2%
RF RSD OF THE ROBOT CURVE

+22.3%
MANUAL QC AT THE LLOQ, 1 NG/ML

The robot curve passes. RF RSD is 6.2%, and every standard refits inside its limit. Yet the lowest standard holds 18% less analyte than its label. A real sample at 1 ng/mL reads +22.5%.

The bias is smooth, so the curve soaks it up. A smooth error looks like a slightly curved detector, not a prep fault.

The robot is not making random mistakes. It is making the same one, very precisely, at every transfer.

Verifying the system: ISO 23783 and gravimetry.

ISO 23783 is the standard for automated liquid handling systems. Part 2 specifies how to measure volumetric performance, including traceability and measurement uncertainty. Part 3 covers how to specify, track and report it [3].

Gravimetric checks are the direct way to see a delivery bias. Hamilton's Liquid Verification Kit, for example, weighs dispenses on the deck with 10 µg resolution. It is meant for tuning liquid classes, including for volatile solvents, and lets the data be kept in quality records [1].

Verify each liquid class with the solvent it will move, at the volumes the method uses. A class verified with water at 100 µL says little about methanol at 20 µL.

The independent QC is still the last word.

ICH M10 says calibration standards and QCs should be prepared from separate stock solutions [4]. With a liquid handler, separate preparation matters as much as a separate stock.

In the example, a manually prepared QC at the LLOQ, 1 ng/mL, reads +22.3% on the robot curve and fails a ±20% limit. A low QC at 3 ng/mL reads +12.7% and only just passes ±15%. The high QC reads +8.0%.

That pattern, small at the top and growing toward the bottom, is the signature of a compounding preparation bias. It also shows why a QC near the LLOQ earns its place: it is the one that sees the problem first.

If the QCs are made by the same robot with the same liquid class, they carry the same bias. They will agree with the curve, and the run will pass while every low result is high.

A liquid handler verification checklist.

01 **Match the liquid class to the solvent.**
Water classes do not suit methanol or acetonitrile.

02 **Verify gravimetrically.**
Each class, at the volumes and solvents the method uses.

03 **Keep the verification records.**
With the date, class version and acceptance criteria.

04 **Shorten serial chains.**
Use intermediate stocks so bias cannot compound for long.

05 **Prepare QCs independently.**
Separate stock and, where possible, a separate route.

06 **Read the QC pattern.**
High at the bottom and fine at the top points to preparation.

07 **Re-verify after changes.**
New tips, new solvent, new class or a service visit.

Where WLTR fits.

Automation makes preparation precise, and precision makes a bias easy to miss. Seeing every standard back-calculated under every model, run after run, alongside independent QCs, is what separates a curved detector from a curved preparation.

WLTR is calibration data integrity infrastructure for regulated analytical laboratories. For each analyte, it derives nine calibration regression models: average response factor, plus linear and quadratic fits across four weightings each. It scores every model against fifteen ICAL parameters, including RSE. All of it is computed orthogonally to the CDS, under U.S. Patent 12,038,448 B2 [P].

CALIBRATION VERIFICATION

See every calibration standard back-calculated under every regression model and weighting, computed orthogonally to your CDS.

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SOURCES

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