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Excluding calibration standards: the 75% rule and the record behind it.

ICH M10 lets a laboratory reject a failing calibration standard and refit the curve, within firm limits. Each exclusion is only as defensible as the record behind it, and a pattern of exclusions is a finding in its own right.

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THE SHORT VERSION

- 01 ICH M10 lets a lab reject a failing calibration standard and refit the curve, as long as at least 75% of standards and six levels still pass, and it moves the run's LLOQ or ULOQ when an end standard is lost.
- 02 An exclusion holds up when a rule written before the run made it, and the original fit, the refit, the user, the reason and any reintegration all stay in the record.
- 03 The same level failing run after run usually points to the model, the weighting or the method, and calls for an investigation rather than another exclusion.

01 • WHAT M10 ALLOWS WHEN A STANDARD FAILS

What M10 allows when a standard fails.

ICH M10, final FDA guidance since November 2022, sets the calibration test for chromatographic study runs^{1,2}. Back-calculated standards should fall within $\pm 15\%$ of nominal, or $\pm 20\%$ at the LLOQ, for “at least 75% of the calibration standard concentrations, which should include a minimum of six concentration levels” (Sec. 3.3.2)¹.

With more than six levels, a failing standard “should be rejected” and the curve “re-evaluated and a new regression analysis performed” (Sec. 3.3.2). Eight levels in singlicate allow two exclusions per run; six allow none. If the LLOQ is rejected, the next lowest acceptable standard becomes the run's lower limit and “will retain its original acceptance criteria (i.e., $\pm 15\%$).” A rejected top standard makes the next acceptable one the ULOQ. The revised range should still cover the low, medium and high QCs, and study samples outside it are reanalyzed (Secs. 3.3.2, 3.3.4)¹.

If only one of the replicate LLOQ or ULOQ standards fails, the range is unchanged (Sec. 3.3.2). Validation is stricter: at least 50% of replicates per level should pass, an accuracy and precision run that loses every LLOQ or ULOQ replicate is rejected, and “if the next validation run also fails, then the method should be revised” (Sec. 3.2.4)¹.

Two rules sit behind every exclusion. The model and weighting are determined during validation (Sec. 3.2.4), and reinjecting standards “simply because the calibration standards or QCs failed,” with no identified analytical cause, “is not acceptable” (Sec. 3.3.5)¹.

02 • WHERE M10 PUTS THE EXCLUSION RECORD

Where M10 puts the exclusion record.

M10's documentation table has no row for excluded standards; the evidence is spread out. Section 8, Table 1 asks the bioanalytical report for a “table of all runs, status (accepted and failed), reason for failure, and analysis dates,” and the analytical site for run summary tables of accepted and failed runs “including calibration curve, regression, weighting function.” For validation reports, “Values outside the acceptance criteria should be clearly marked.” Section 3.3.6 adds a list of reintegrated chromatograms with reasons¹.

FDA's compliance program for analytical BA/BE inspections lets FDA investigators request run summary tables that can include "Failed calibrators," and tells them, "If chromatograms for QCs and calibrators were re-integrated, determine if run acceptance was affected" (Program 7348.004, Part III)³.

M10 RULE	SEC.	RECORD THAT SHOWS COMPLIANCE
Acceptance criteria defined in advance	3.3.2	The SOP version in force for the run
±15% of nominal, ±20% at the LLOQ	3.3.2	Back-calculated value, %RE and limit for every standard
Failed standard rejected and curve refit; 75% and six levels still pass	3.3.2	Original fit and refit, both retained, with the passing count
A rejected end standard moves the range, which still covers low, medium and high QCs	3.3.2, 3.3.4	The run's LLOQ and ULOQ, QCs inside them, reanalysis entries
No reinjection only because standards failed	3.3.5	Reinjection log naming the analytical cause
Reintegrations listed with reasons	3.3.6, Table 1	Reason, mode, both results and the audit trail
All runs reported with status and reason	8, Table 1	Run table with failed runs included

Sections are ICH M10¹. The right-hand column is WLTR's mapping.

03 · THE RULE HAS TO EXIST BEFORE THE RESULT

The rule has to exist before the result.

M10 makes these decisions rule-driven: acceptance criteria in the protocol, plan or SOP, reanalysis reasons predefined, and integration described a priori (Secs. 3.3.2, 3.3.4, 3.3.6)¹. Applying a criterion fixed before the run is the guideline working as written. Choosing an exclusion after seeing which combination passes is selection.

An exclusion is defensible when the rule existed before the result. The record has to show which came first.

In its 2018 CGMP data integrity guidance, FDA prohibits sampling and testing "with the goal of achieving a specific result or to overcome an unacceptable result," which it calls testing into compliance (Q13). Invalidating a CGMP result "requires a valid, documented, scientifically sound justification," with the original data kept (Q2), and reprocessed chromatography needs written procedures and "each result retained for review" (Q14)⁴. It covers drug manufacturing; WLTR reads its logic as applying equally to a calibration curve.

For BA/BE studies, FDA's April 2024 draft guidance recommends "SOPs with prespecified objective criteria" for repeat analysis, reintegration and reinjection (Sec. III.C.2.c) and audit trails that capture "when, by whom, and the reasons changes were made" (Sec. III.C.5). It names data "excluded without authorization or without detection" as a

risk (footnote 14)⁵. As of October 26, 2026, it remains a draft.

FDA's four Notifications on Data Integrity (2016 to 2025) concern falsified data or substituted samples; FDA declared affected studies unacceptable or required them to be repeated⁶. A 2021 untitled letter objected to Panexcell Clinical Lab's removal of subject data FDA identified as concerning, "because you provided no justification for the removal"⁷. In a 2019 FDA webinar, staff described a site that reported reinjected stability results under an SOP leaving reinjection "at the discretion of the bioanalytical Principal Investigator," without following its pre-established run acceptance criteria⁸.

04 • A RECURRING EXCLUSION IS A FINDING ABOUT THE METHOD

A recurring exclusion is a finding about the method.

Each exclusion is judged within its run; the pattern across runs says more. An unbiased LLOQ standard with a 10% CV, half its limit, fails by chance in about 4.6% of runs, so three or more failures in ten runs would happen about once in 113 sets of ten (WLTR's calculation). A level that keeps failing usually has a systematic cause: the model, the weighting or the method.

When exclusions cluster at the low end, suspect the weighting first: an unweighted fit lets the top standards set the intercept, so the lowest standards carry large errors that shift between runs. Gu and colleagues found that "the most stable curve could be obtained when the correct weighting factor was used," and that incorrect weighting significantly affected assay performance⁹. A one-sided bias at the LLOQ alone points instead to the standard itself, or to interference or carry-over, which M10 caps at 20% of the LLOQ response (Secs. 3.2.1, 3.2.6)¹.

M10 already reads patterns: a repeat validation failure means revising the method (Sec. 3.2.4), and failing between-run QC accuracy or precision triggers an investigation (Sec. 3.3.2)¹. For ISR failures and trends of concern, the M10 Q&A says the investigation "should be driven by a standard operating procedure (SOP)" (Q8)¹⁰. WLTR recommends the same discipline for calibration standards: trend exclusions and signed %RE by level, the intercept, slope and LLOQ response, the IS response (Sec. 3.3), and reintegrations of standards by analyst and instrument.

Set the trigger in the SOP before the study, for example the same level excluded in two of five consecutive runs, which a well-behaved LLOQ does by chance in about 1.9% of five-run sets (WLTR's calculation). If the cause is the weighting, the remedy is a method change: M10 settles the weighting in validation (Sec. 3.2.4) and treats a range change, such as raising the LLOQ, as partial validation (Sec. 3.3.3)¹. WLTR reads refitting finished runs until they pass as the reprocessing FDA's Q14 addresses⁴.

05 • TEN RUNS, ONE WEIGHTING DECISION

Ten runs, one weighting decision.

Illustrative. Consider an LC-MS/MS assay in human plasma with eight levels in singlicate (1, 2, 5, 20, 100, 400, 800 and 1,000 ng/mL) and QCs at 3, 400 and 800 ng/mL. Ten runs were simulated with 1% proportional scatter plus a small floor (4.9% CV at the LLOQ) and fit unweighted, the example method's model, and with $1/x^2$. The example

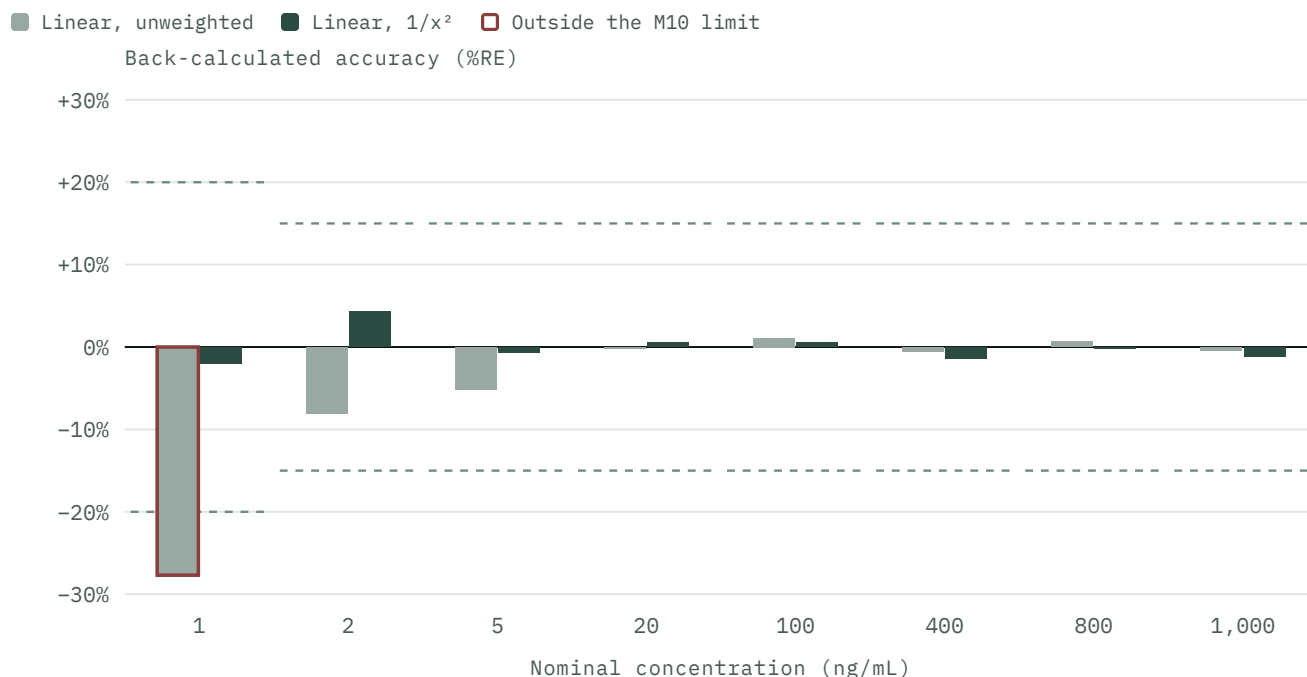
SOP excludes the worst failing standard and refits.

RUN	LLOQ %RE, UNWEIGHTED	2 NG/ML %RE, UNWEIGHTED	UNWEIGHTED OUTCOME	LLOQ %RE, 1/X ²
1	-72.1	-35.2	Not acceptable, third failure	-0.3
2	-27.7	-8.1	Accepted, LLOQ excluded	-2.0
3	+14.2	+12.5	Accepted	-2.3
4	-55.7	-30.0	Not acceptable, third failure	+0.7
5	+32.9	+17.4	Not acceptable, low QC outside range	-0.2
6	+115.8	+59.2	Not acceptable, third failure	-0.8
7	-18.1	-8.6	Accepted	-0.2
8	-50.2	-30.1	Not acceptable, third failure	+2.0
9	+65.9	+36.7	Not acceptable, third failure	-1.5
10	-81.9	-36.0	Not acceptable, third failure	-1.8

Illustrative data, computed by the accompanying script; %RE from the full-curve fit. Third failure: 1 and 2 ng/mL excluded, then 5 ng/mL failed. Under 1/x², no standard failed (largest |%RE| 4.3%).

The unweighted LLOQ was excluded in 8 of 10 runs, and all 15 unweighted exclusions fell at 1 and 2 ng/mL. Run 2 is the rule working as written: the LLOQ missed by -27.7% and was excluded, the refit put 2 ng/mL at -11.5% against its $\pm 15\%$ limit, and the run was accepted with a 2 to 1,000 ng/mL range, so its samples below 2 ng/mL go to reanalysis. The LLOQ error changed sign between runs, yet the 2 ng/mL error matched its sign in all ten at about half the size (median ratio 0.52), the signature of an intercept set by the high standards.

FIGURE 1 Run 2, the same responses under two weightings



Illustrative data. Only the unweighted LLOQ falls outside its band.

Over 10,000 simulated runs, the unweighted LLOQ failed 78.0% of the time and 72.7% of runs were not acceptable; under $1/x^2$ the LLOQ never failed. A method this fragile should not survive validation; the example exaggerates it so the pattern is easy to see. Refitting with $1/x^2$ diagnoses the cause. It does not rescue the runs.

06 • THE EXCLUSION RECORD CHECKLIST

The exclusion record checklist.

An auditor will ask for each of these.

- 01 **The rule, dated before the run.** The SOP or plan version setting the limits, exclusion procedure and minimums.
- 02 **The original fit.** Every standard included, with every back-calculated value.
- 03 **The failure.** Nominal and back-calculated values, %RE and the limit applied.
- 04 **Who, when and why.** The named user, time and reason, captured by the audit trail.

- 05 **The refit.** New parameters, back-calculated values and the count of passing standards and levels.
- 06 **The range consequence.** The run's LLOQ and ULOQ, the QCs inside them and samples sent for reanalysis.
- 07 **Integration and reinjection history.** Any reintegration or reinjection of standards, with reason and both results.
- 08 **The trend and the review.** Exclusions by level across the study, any triggered investigation, and QA review of the exclusion and audit trail before approval.

07 · WHERE WLTR FITS

Where WLTR fits.

WLTR is a verification layer for the calibration data your CDS already produces. It reads the raw instrument export directly, records a SHA-256 hash of the original file and evaluates every candidate model against the same acceptance criteria at once, including back-calculated accuracy at every level. The full evaluation is kept, so there is no curve to shop for and no failed attempt to hide¹¹.

Each exclusion is recorded with the user who made it and the reason given, and it travels with the record into QA approval. Method configurations are frozen once results exist, so the model cannot quietly change. The lab sets the acceptance value for each of 15 criteria per analyte, and WLTR can trend calibration performance across batches, instruments and sites, so recurring failures show. Your validated method and SOPs still decide the reported model. WLTR verifies. Your lab validates.

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