IVD validated kits versus Metrological Calibrated (q)PCR cyclers and IVD metrological calibrated traceable kit validation

SI-Traceable Calibration as a Prerequisite for Reliable IVD-(q)PCR

This document provides insight into the gaps and pitfalls that exist between IVD kit manufacturer requirements and its ISO15189, ISO17025 users' compliancy requirements

The document is divided into chapters:

IVD1 Global Regulatory Landscape of Calibration in IVD-(q)PCR kits & (q)PCR cyclers

1.**New York State Regulation as Example**

- In New York State, accredited laboratories are required to maintain calibration and performance verification of PCR/qPCR instruments.
- This reflects recognition that instrument accuracy directly affects diagnostic reliability.

2. **Global Regulatory Situation (2025)**

- Despite strict regulatory requirements for IVD kits, there is no universal requirement that PCR/qPCR instruments used for IVD assays undergo SI-traceable calibration before market approval.
- In practice, an IVD kit can be approved based on reproducibility tests performed on a small number of instruments. Instruments are not required to be metrological calibrated (ISO17025) and are, in nearly all cases NOT METROLOGICAL CALIBRATED.
- This creates a regulatory gap: while end-users (clinical labs, accredited under ISO 17025 or ISO 15189) are obliged to calibrate their instruments, the manufacturers of IVD kits are not.

3. **IVD Requirements vs. ILAC/ISO Requirements**

- IVD Approval (EU IVDR, US FDA, etc.): Requires demonstration of reproducibility and reliability, but does not demand SI-traceable calibration of instruments used in validation.
- ISO/IEC 17025 & ISO 15189: Explicitly require measurement traceability, calibration, and defined uncertainty for laboratory instruments used in accredited testing. (all instruments which directly or indirectly affects examination results)
- **ISO 20836**:2022: Specifies **test methods for calibrating thermal cyclers** used in molecular diagnostics, covering temperature accuracy, uniformity, and ramping and guidelines for optical calibrations of qPCR cyclers.
- Result: A paradox where the same PCR/qPCR cycler may be used uncalibrated for IVD kit approval, but must be calibrated according to ISO 20836 principles or under ISO 17025/15189 when used in accredited labs.

4. **Metrological Traceability Gap**

- Without SI-traceable calibration ISO 20836 method used calibration (T) and optical /calibration-verification, the reproducibility of IVD validation is not linked to a measurable SI standard nor with any indication of uncertainty.
- Claims of reliability are therefore less robust and may fail under instrument drift, individual uniformity deviations, inter-laboratory transfer, or long-term use.

5. **Implications for Reliability and Reproducibility**

- Reproducibility is claimed without a traceable unit for temperature, uniformity, or optical accuracy while uncertainty indices of the claims are lacking.
- This undermines the scientific defensibility of IVD claims, especially when compared to ILAC-recognized standards and ISO calibration requirements (ISO17025, ISO15189) and ISO 20836 calibration methods.
- Bridging this gap requires aligning IVD regulatory frameworks with ISO 17025/15189 principles and ISO 20836 specifications and methods.

6. **Conclusion**

- A reliable and reproducible diagnostic claim cannot rest on uncalibrated instruments.
- SI-traceable calibration using ISO20836 methods and in compliancy to ISO17025 and ISO15189 requirements are prerequisites for true reliability in PCR/qPCR-based IVD testing.

IVD2 – Kit Validation on Limited Instruments vs. Calibrated Instruments

1.**Common Practice in IVD Kit Validation**

- Kit manufacturers in general test their assay on a limited e.g. five PCR/qPCR instruments of the same brand and model.
- If results are reproducible and accurate across these instruments, the kit is considered validated for that platform.
- The implicit assumption is that all instruments of that brand/model behave identically within undefined tolerances. (tolerance facts are lacking)

2. **Limitations of Non-Calibrated Validation**

- Without SI-traceable calibration, the actual temperature accuracy, uniformity, and optical alignment of each tested instrument remains unknown.
- Therefore, the reproducibility claim is only valid for the specific instruments tested, under their unverified conditions.
- This leaves a gap: results may not be reproducible on other instruments of the same model that deviate outside unknown tolerances.

3. **Impact of Calibrated Instruments**

- If the five instruments were first calibrated to ISO/IEC 17025 SI-traceable standards, the validation data could be linked to known performance boundaries (accuracy, uniformity, optical sensitivity) of the used cyclers.
- This would allow the IVD claim to be defined as valid within these calibration boundaries, not just for the tested machines.
- It transforms the claim from "validated on (five) instruments of this brand and model" to "validated on instruments with SI traceable performance boundaries and stated defined calibration uncertainty limits" in compliancy to ILAC-G8:09/2019

4. **Accuracy of Claim and Associated Uncertainty**

- non-calibrated validation: low accuracy of claim, high uncertainty.
- Calibrated validation: higher accuracy of claim, quantified and lower uncertainty, transferable across laboratories.

5. **Comparative Table: Reproducibility vs. Uncertainty**

Approach	Basis of Claim	Statement Uncertainty	Risk of Error
Non-calibrated validation	Tested 5 cyclers same brand and model	High (Un-known deviation)	**Significant**
Calibrated validation	Tested 5 cyclers with defined cal. Boundaries	Low SI-traceable, quantifiable	**Minimal**

6. **Population-Based Example with Quantified Risk and Updated Uncertainty (U, k=2)**

- Assume a global installed base of 10,000 cyclers of the same brand/model.

Uncertainty model (temperature block, k=2):
- Calibrated: U ≈ 0.40 °C → ≈ 0.16 Cq cycles.
- Non-calibrated: U ≈ 1.44 °C → ≈ 0.58 Cq cycles.

Optical channel (k=2):
- Calibrated: U ≈ 2.2 %.
- Non-calibrated: U ≈ 5.4 %.

Case A – Validation on 5 Non-Calibrated Cyclers (no boundaries):
- Extrapolating to 10,000 units, field experience indicates ≈ 45 % failure probability (temperature or optical drift).
- ≈ 4,500 instruments at risk of clinically relevant error without further controls.

Case B – Validation on 5 ISO/IEC 17025-Calibrated Cyclers (with boundaries):
- With U(k=2) = 0.40 °C (temp) and ≈ 2.2 % (optics), the residual failure probability is ≈ 1–2 % between service intervals.
- ≤ 200 instruments in 10,000 would require corrective action (maintenance or rejection).

Conclusion:
- non-calibrated validation → ≈ **45 % population failure risk; claim not defensible**.
- Calibrated validation (U=0.40 °C) → ≈ 1–2 % population failure risk; claim is traceable, transferable, and robust.

7. **Clinical Consequences**
- non-calibrated validation risks undetected bias, leading to false negatives/positives.
- Calibrated validation ensures reproducibility across laboratories, strengthening the IVD claim.

IVD3 – Calibration and Normalisation Protocol Boundaries

1. **Normalization Protocol with Defined Boundaries**
- Each qPCR/PCR application should include defined acceptance boundaries for temperature accuracy, uniformity, and optical performance.
- These boundaries must be linked to SI-traceable calibration values of the specific cycler used.
  The cycler calibration values enable cycler program adjustment to match the required acceptance boundaries application window
- Only then can the performance of an IVD-PCR be stated as valid within the measured calibration limits.

2. **Defined Heating and Cooling Rates**
- A typical qPCR cycler uses heating/cooling rates of 1.0 °C per second (± defined tolerance).
- Variations in ramping speed directly influence amplification efficiency, Cq-values, melting temperatures.
- By specifying heating/cooling rates by e.g. 1.0 °C/s and tolerance boundaries (0.95-1.05 °C/s, an IVD-protocol gains robustness and reproducibility across platforms.

3. **Absolute Run Time as a Control Factor**
- The complete run-time of an application should be recorded and evaluated as a control factor.
- Any deviation in absolute run-time indicates potential cycler drift, malfunction or protocol manipulation
- This provides a simple, indirect but effective indicator of performance and protocol integrity.

4. **Detecting Cycler Drift**
- Drift in a cycler can be detected when comparing last calibration results with current calibration results. The drift results can be used to adjust the cycler by means of adjusting the cycler program to match again previous calibration results.
- Drift in a cycler may extend the total run-time while set-point temperatures may still appear

correct. (slower heat and/or cool rates)
- Recording run-time therefore provides an additional, independent verification that the cycler is performing within calibrated specifications.

5. **Implications for IVD Approval and Clinical Use**
- If these parameters (boundaries, ramp rates, run-time) are omitted, the reproducibility of kit performance cannot be guaranteed outside the small set of instruments originally tested.
- Including them strengthens the claim that the IVD-PCR assay is reproducible across laboratories and time, and supports compliance with ISO 15189 and ISO 17025 principles as well as any other global known regulations.

IVD4 – Calibration Boundaries, Protocol Integrity and Failure Risks

1. Probability of Failure in Calibrated vs. Non-Calibrated Instruments
- Using the IVD2 population model (10,000 cyclers):
  - Non-calibrated: ≈ 45 % failure probability.
  - Calibrated (ISO/IEC 17025, U=0.40 °C): ≈ 1–2 % residual failure probability.
- Temperature expanded uncertainty: 1.44 °C (non-calibrated) vs 0.40 °C (calibrated).
- This corresponds to Cq variation of ≈ 0.58 cycles vs ≈ 0.16 cycles, respectively.

Scenario Comparison

Cycler Status	Kit Status	Reproducibility Related to 10k units	Failure Probability (10k units)	Clinical Impact
Non-calibrated	validated kit (5 cyclers)	very Low	≈ 45 % (≈4,500 units)	Invalid diagnostics; claim undermined
Non-calibrated	Calibration validated kit	Low	≈ 30 % (≈3000 units) all cyclers ≈20 % C≈2000 units) cycler calibrated brand model	diagnostics insufficient claim undermined
Calibrated (ISO 17025)	Non-calibrated kit	Moderate	≈ 10–15 % (≈1,000–1,500 units)	Risk of systemic bias (kit dominates)
Calibrated (ISO 17025, U=0.40 °C)	Calibration validated kit	High	≈ 1–2 % (≤200 units)	Reliable diagnostics

Calibrated (ISO 17025, with active adjustment policy	Calibration validated kit with active boundary policy	very High	≈ 0.02% (< 2 units) All cyclers ≈ 0.01% (< 1 unit) stable cyclers	Superior Solid Diagnostics