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

Executive Summary

Current IVD regulatory frameworks (FDA, IVDR, etc.) allow qPCR-based assays to be validated on uncalibrated instruments, relying on internal reproducibility rather than SI-traceable measurement. By contrast, clinical laboratories operating under ISO 17025 or ISO 15189 must calibrate their PCR/qPCR instruments to SI standards. This mismatch creates a traceability gap that undermines reproducibility, scientific defensibility, and clinical reliability.

Using population-based modeling, non-calibrated validation is shown to carry ~45% failure probability across 10,000 instruments, versus ~1–2% when SI-traceable calibration is applied. This document outlines the regulatory paradox, quantifies the risk, and defines how ISO 20836 calibration methods and ISO/IEC 17025 traceability close the compliance gap.

IVD1 – Global Regulatory Landscape for Calibration in IVD (q)PCR Kits and Cyclers

- New York State Regulation: Accredited labs must maintain calibration and performance verification of PCR/qPCR instruments, recognizing accuracy as critical for diagnostics.
- Global Regulatory Situation (2025): IVD kits can be approved without SI-traceable calibrated cyclers. Manufacturers validate on limited instruments, while labs must calibrate under ISO 17025/15189.
- IVD Requirements vs. ILAC/ISO Requirements: IVD approval requires reproducibility but not calibration, whereas ISO 17025/15189 require SI-traceable calibration and ISO 20836 prescribes methods for thermal/optical accuracy.
- Metrological Traceability Gap: Without calibration, reproducibility claims lack measurable standards or uncertainty.
- Implications: Weakens transferability, comparability, and long-term reproducibility.
- Conclusion: SI-traceable calibration (ISO 20836 + ISO 17025/15189) is a prerequisite for defensible IVD reproducibility.

IVD2 – Kit Validation on Limited Instruments vs. Calibrated Instruments

- Common Practice: Validation on five non-calibrated instruments of one brand/model, assuming reproducibility.
- Limitations: Accuracy, uniformity, and optical alignment remain unknown, claims non-transferable.

- Impact of Calibrated Instruments: SI-traceable calibration links validation to performance boundaries.
- Accuracy of Claim: Non-calibrated = high uncertainty; Calibrated = quantified and transferable.

Comparative Table: Reproducibility vs. Uncertainty

Approach	Basis of Claim	Statement Uncertainty	Risk of Error
Non-calibrated	5 cyclers, same brand/model	High, undefined	Significant
Calibrated	5 cyclers with SI-traceable boundaries	Low, quantified	Minimal

IVD3 – Protocol Limits for Calibration and Normalization

- Normalization Boundaries: SI-traceable calibration values define application acceptance windows.
- Heating/Cooling Rates: Define ramp rates (e.g., 1.0 °C/s ±0.05).
- Absolute Run Time: Deviation signals drift or manipulation.
- Cycler Drift Detection: Compare calibration histories, use run-time monitoring.
- Implications: Strengthens IVD approval and ensures ISO 17025/15189 compliance.

IVD4 – Calibration Boundaries, Protocol Integrity, and Failure Risks

- Probability of Failure: Non-calibrated ≈45% vs Calibrated ≈1–2%; with active boundary adjustment <0.02%.
- Scenario Comparison: See table below.

Scenario Comparison

Cycler Status	Kit Status	Reproducibility	Failure Probability	Clinical Impact
Non-calibrated	Validated kit (5 cyclers)	Very Low	≈45% (~4,500)	Invalid diagnostics
Non-calibrated	Calibration-validated kit	Low	≈30% (~3,000)	Insufficient claim

Calibrated (ISO 17025)	Non-calibrated kit	Moderate	10–15% (~1,000–1,500)	Systemic bias
Calibrated (ISO 17025, U=0.40°C)	Calibration-validated kit	High	1–2% (≤200)	Reliable diagnostics
Calibrated + active boundary policy	Calibration-validated kit	Very High	<0.02% (<2)	Superior diagnostics

Closing Statement

True reliability of IVD-PCR cannot rest on uncalibrated platforms. Only SI-traceable calibration closes the gap between regulatory approval and accredited laboratory compliance, ensuring reproducibility, clinical safety, and defensible diagnostics.

