

VALIDATION AND TOTAL ALLOWABLE ERROR

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What is Method Validation?



Comprehensive Definition

- **ISO 15189:** "Confirmation through objective evidence that the requirements for a specific intended use have been fulfilled."
- **CLSI EP15-A3:** "The process of proving that an analytical method is acceptable for its intended purpose."
- **Practical Meaning:** Ensuring a method reliably detects clinical conditions without errors.
- **Hematology Context:** Critical for tests where small errors impact clinical decisions (e.g., transfusion triggers, leukemia diagnosis).

Validation vs. Verification

Feature	Validation	Verification
Purpose	Prove clinical suitability for new/modified methods	Confirm manufacturer claims for approved devices
Scope	Comprehensive (all performance characteristics)	Limited (accuracy, precision, linearity)
Timeline	2-4 weeks	3-7 days
Documentation	Extensive protocol and report	Simplified checklist
Hematology Examples	LDTs for specialized coagulation tests	FDA-approved CBC analyzers

Regulatory Framework for Validation

Global Standards Overview

- **ISO 15189:2022:** Mandates validation for all laboratory-developed methods
- **CLSI Guidelines:**
 - EP15-A3: Verification of Precision and Trueness
 - EP09-A3: Measurement Procedure Comparison
 - EP06-ED1: Evaluation of Linearity
- **ICSH 2025:** Specific requirements for hematology LDTs in hemostasis
- **EFLM Working Group:** Harmonized approach across European laboratories

Practical Implication: *Laboratories must document compliance with relevant standards.*

Rationale for Validation in Laboratories

Why Validation is Non-Negotiable

- **Patient Safety:** Prevents misdiagnosis and inappropriate treatment
- **Regulatory Compliance:** Required for laboratory accreditation
- **Quality Assurance:** Ensures reliable patient results
- **Risk Management:** Identifies and mitigates potential errors
- **Clinical Credibility:** Builds trust with healthcare providers

Hematology-Specific Rationale: *Variable sample stability and complex pre-analytical factors necessitate thorough validation.*

Importance in Hematology

Critical Applications in Hematology

- **CBC Analysis:** Small errors impact transfusion decisions
- **Coagulation Testing:** INR accuracy critical for anticoagulation management
- **Flow Cytometry:** Requires high sensitivity for minimal residual disease
- **Hemoglobinopathy Screening:** Specificity essential for accurate diagnosis
- **Specialized Testing:** Thrombophilia workup, platelet function assays

Invalidated flow cytometry methods can miss 15-20% of MRD cases (ICSH 2025).

Risks of Invalidated Methods

Consequences and Case Examples

- **False Anemia Diagnosis:** Inappropriate iron therapy or transfusions
- **Missed Leukemia:** Delayed diagnosis due to insensitive blast detection
- **Incorrect Anticoagulation:** Thrombosis or bleeding from inaccurate INR
- **Laboratory Errors:** Increased repeat testing and costs
- **Legal Liability:** Malpractice claims from erroneous results

Prevention Strategy: *Comprehensive validation reduces these risks by 80-90%.*

Evolution of Validation Guidelines

Historical Development

- **1980s-1990s:** Basic precision and accuracy requirements
- **2000-2010:** Introduction of TEa and Six Sigma concepts
- **2010-2020:** Risk-based approaches and ISO 15189 emphasis
- **2020-Present:** AI integration and personalized medicine considerations

Current Trend: *Das (2011) demonstrated lean approaches reduce validation timelines by 30%.*

When is Validation Necessary?

New Method Implementation: Laboratory-developed tests

Method Modification: Changes in reagents, instrumentation, or procedures

Instrument Changes: New analyzer installation

Reagent Lot Changes: Significant formulation modifications

Clinical Need Expansion: New patient populations or clinical applications

Hematology Examples: *New flow cytometry panels, modified coagulation protocols, updated CBC flags.*

Step 1 - Planning Validation

Define Objectives: Based on clinical requirements and intended use

Risk Assessment: FMEA to identify potential failure points

Resource Allocation: Staff, equipment, samples, time

Acceptance Criteria: Pre-defined based on TEa and clinical needs

Timeline Development: Realistic schedule with milestones

Step 2 - Sample Selection

Strategic Sample Selection

- **Sample Types:**
 - Patient samples (n=40-100)
 - Commercial controls
 - Spiked samples for specific concentrations
- **Selection Criteria:**
 - Cover entire clinical range
 - Include pathological conditions
 - Represent patient demographics

Hematology Considerations:

- Fresh EDTA samples for CBC
- Citrated plasma for coagulation
- Viable cells for flow cytometry

ICSH Recommendation: *Include 20% abnormal samples in validation sets.*

Step 3 - Precision Evaluation

Definitions:

- **Repeatability:** Same conditions, short time (CV%)
- **Intermediate Precision:** Varying conditions, operators, days

Protocol:

- 20 replicates for within-run precision
- 20 days for between-run precision
- Multiple operators and reagent lots

Acceptance Criteria:

- $CV\% \leq 1/4$ TEa for optimal performance
- $CV\% \leq 1/3$ TEa for acceptable performance

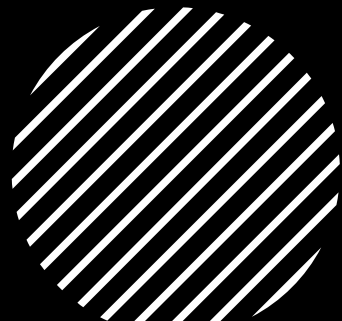
Hematology Example: *Hb CV% < 1.5%, PLT CV% < 5.0%*

Step 4 - Accuracy and Bias

- **Comparison to Reference Method:** Gold standard or reference laboratory
- **Recovery Studies:** Spiked samples with known additions
- **Proficiency Testing:** External quality assessment results
- **Bias Calculation:**
 - Bias (%) = $\frac{Mean_{test} - Mean_{reference}}{Mean_{reference}} \times 100$
 - **Acceptance:** $|Bias| \leq 1/2 TEa$



Step 5 - Linearity and Reportable Range



Linearity Assessment Protocol

- **Method:** Serial dilutions across claimed range
- **Points:** 5-7 concentrations covering clinical range
- **Statistical Analysis:**
 - Linear regression ($R^2 \geq 0.99$)
 - Residual analysis (< 5%)
 - Visual inspection for hooks
- **Hematology Ranges:**
 - Hb: 2-25 g/dL
 - WBC: $0.5-100 \times 10^9/L$
 - PLT: $20-2000 \times 10^9/L$

CLSI Criteria: *Slope 0.97-1.03, intercept clinically insignificant*

Step 6 - Limit of Detection (LOD) and Quantitation (LOQ)

Detection Capability Evaluation

- **LOD Definition:** Lowest concentration distinguishable from zero

$$\text{LOD} = \text{Mean}_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$$

- **LOQ Definition:** Lowest concentration measurable with acceptable precision

$$\text{LOQ} = \text{Mean}_{\text{blank}} + 10 \times \text{SD}_{\text{blank}} \quad \text{or} \quad \text{LOQ} = \text{Concentration where } CV \leq 20\%$$

- **Hematology Applications:**
 - MRD detection in flow cytometry
 - Low platelet counts
 - Minimal coagulation factor activity

Sensitivity Requirement: 0.01% for MRD detection in leukemia

Step 7 - Method Comparison

Sample Size: 40-100 patient samples

Statistical Methods:

- Passing-Bablok regression
- Correlation coefficients

Acceptance Criteria:

- Slope: 0.97-1.03
- Intercept: Clinically insignificant
- Correlation (r) > 0.975
- Bias within TEa limits

Visualization: *Scatter plots, regression lines*

Step 8 - Stability Testing

Conditions Tested:

- Room temperature (2-8 hours)
- Refrigerated (4°C, 24-72 hours)
- Frozen (-20°C, -80°C for long-term)

Parameters Monitored:

- Percentage change from baseline
- Clinical significance of changes

Hematology Specifics:

- CBC stability at room temperature
- Coagulation sample stability
- Cell viability for flow cytometry

Acceptance: *Change < 5% or within TEa*

Step 9 - Interference and Specificity

Common Interferents:

- Hemolysis, icterus, lipemia (HIL)
- Medications and metabolites
- Common diseases states

Testing Protocol:

- Spiking studies with interferents
- Comparison with baseline
- Multiple concentration levels

Hematology Examples:

- Hemolysis effect on potassium and LDH
- Lipemia effect on hemoglobinometry
- Drug effects on platelet function

Acceptance: *Interference < 10% or clinically insignificant*

Step 10 - Sensitivity and Specificity

Diagnostic Performance Characteristics

- **For Qualitative Tests:**
 - Sensitivity: True positive rate
 - Specificity: True negative rate
- **For Quantitative Tests:**
 - Clinical decision point analysis
 - Disease detection capability
- **Hematology Applications:**
 - Blast detection in CBC
 - Abnormal cell identification
 - Flagging accuracy

Minimum Standards: *Sensitivity > 95%,
Specificity > 90%*

Step 11 - Carryover and Contamination

Carryover Assessment

- **Testing Protocol:** High-low sample sequence

- **Calculation:**

$$\text{Carryover (\%)} = \frac{LOW_1 - LOW_3}{HIGH_3 - LOW_3} \times 100$$

- **Acceptance Criteria:** < 0.5% for most parameters
- **Hematology Concerns:**
 - High WBC samples affecting subsequent samples
 - Radioactive samples in specialized testing
 - Contamination in molecular hematology

Mitigation: *Proper washing cycles and sample sequencing*

Step 12 - Final Approval and Documentation

Required Elements:

- Validation protocol
- Raw data and calculations
- Statistical analysis
- Conclusion and recommendations
- Approval signatures

Retention Period: 2-10 years based on regulations

Quality System Integration:

- SOP development
- Staff training records
- Ongoing monitoring plan

EFLM Recommendation: *Risk-based documentation approach*

Hematology Example - CBC Validation

Complete Blood Count Validation

- **Scope:** All CBC parameters and indices
- **Sample Requirements:** 100 EDTA-anticoagulated samples
- **Key Parameters:**
 - WBC, RBC, Hb, HCT, PLT
 - Red cell indices (MCV, MCH, MCHC)
 - Differential counts
 - Reticulocyte count (if applicable)
- **Reference Method:** Manual counts and reference analyzer

Validation Focus: *Accuracy in pathological samples*

CBC Performance Data

Parameter	Within-run CV%	Between-run CV%	Bias vs. Reference	Status
WBC	1.8%	2.3%	+2.1%	Acceptable
RBC	1.2%	1.5%	-0.8%	Excellent
Hb	0.9%	1.2%	+0.5%	Excellent
PLT	3.5%	4.2%	-4.2%	Investigate

Action Required: *Platelet accuracy needs improvement*

Linearity and Intelligent Flagging

Linearity Results:

- WBC: 0.5-100 $\times 10^9/L$, $R^2 = 0.998$
- Hb: 2-25 g/dL, $R^2 = 0.997$
- PLT: 20-1000 $\times 10^9/L$, $R^2 = 0.995$

Flag Validation:

- Blast flag: 92% sensitivity, 96% specificity
- NRBC flag: 88% sensitivity, 98% specificity
- Platelet clump: 95% sensitivity, 99% specificity

Improvement Area: *NRBC detection sensitivity*

Flow Cytometry Validation

Flow Cytometry Validation Framework

- **Application Areas:**
 - Leukemia immunophenotyping
 - Lymphoma classification
 - Minimal residual disease
 - PNH and immunodeficiency
- **Validation Parameters:**
 - Sensitivity and specificity
 - Reproducibility between operators
 - Sample stability
 - Panel performance

Gold Standard: *Morphology and genetic studies*

Sensitivity and Gating

MRD Detection Validation

- **Sensitivity:** 0.01% (1 in 10,000 cells)
- **Precision:** CV < 15% at 0.1% level
- **Gating Strategy:**
 - Consistent lymphocyte gating
 - Clear separation of populations
 - Minimal background noise
- **Concordance:** 96% with molecular methods

Clinical Utility: *Accurate treatment response monitoring*

Total Allowable Error (TEa)

- **Definition:** Maximum error that does not affect clinical decisions
- **Components:** Bias + 1.96 × CV (at 95% confidence)
- **Formula:**

$$\text{TEa (\%)} = | \text{Bias} | + 1.96 \times \text{CV\%}$$

- **Clinical Basis:** Derived from biological variation and clinical outcomes

Application: *Sets acceptance criteria for validation*

TEa Benchmarks in Hematology

Note: Adapt based on local clinical requirements

Analyte	TEa (%)	Source	Clinical Rationale
WBC	15%	CLIA	Adequate for infection detection
Hb	7%	CLIA	Critical for transfusion decisions
PLT	15-25%	ICSH	Higher due to biological variation
INR	15%	WFH	Safe anticoagulation management
Reticulocyte	20%	CLSI	High biological variability

TEa in Flow Cytometry

Flow Cytometry Quality Goals

- **Cell Population %:** $\leq 10\%$ TEa
- **Absolute Counts:** $\leq 15\%$ TEa
- **Marker Expression:** $\leq 20\%$ TEa (MFI)
- **MRD Detection:** Special criteria (0.01% sensitivity)

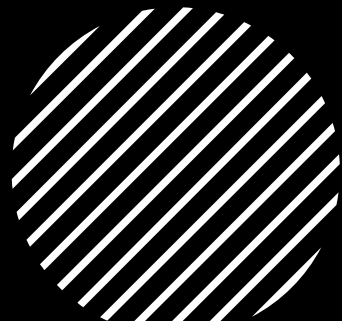
Validation Approach:

- Use stabilized control cells
- Multiple operators and instruments
- Longitudinal performance monitoring

Standard: *ICSH 2025 flow cytometry guidelines*



Challenges in Validation



Pre-Analytical Considerations

- **Sample Collection:**
 - Proper anticoagulant mixing
 - Correct fill volume
 - Minimal tourniquet time
- **Transport and Storage:**
 - Temperature control
 - Time limits
 - Protection from light
- **Hematology Specifics:**
 - EDTA effects on morphology
 - Platelet clumping
 - Cold agglutinins

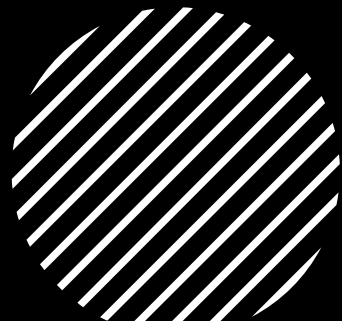
Mitigation: *Standardized procedures and staff training*



Reagent and Supply Considerations

- **Lot-to-Lot Variation:**
 - Validate new reagent lots
 - Monitor performance shifts
 - Maintain inventory for consistency
- **Storage and Handling:**
 - Temperature monitoring
 - Stability testing
 - Proper reconstitution
- **Supplier Issues:**
 - Multiple sourcing strategies
 - Quality audits
 - Backup plans

Best Practice: *Partial validation for new lots*



Managing High Workload Validation

- **Resource Constraints:**
 - Prioritize critical tests
 - Staged validation approach
 - Efficient sample utilization
- **Time Management:**
 - Parallel testing where possible
 - Automated data analysis
 - Clear timelines and milestones
- **Quality Maintenance:**
 - Ongoing quality control
 - Staff competency assessment
 - Continuous improvement

Solution: *Lean validation approaches*

Best Practices - Risk Assessment

FMEA in Method Validation

- **Process Steps:**
 - Identify potential failure modes
 - Assess severity, occurrence, detection
 - Calculate risk priority numbers (RPN)
 - Implement mitigation strategies
- **Hematology Application:**
 - Sample misidentification
 - Instrument calibration drift
 - Reagent degradation
 - Operator error

Outcome: *Focused validation on high-risk areas*

Post-Validation Quality Control

Statistical QC:

- Westgard rules implementation
- Levey-Jennings chart monitoring
- Moving average programs (Bull algorithm)

Process Control:

- Delta checks for patient results
- Proficiency testing participation
- Equipment maintenance logs

Continuous Monitoring:

- Trend analysis
- Alert and action limits
- Regular review meetings

Goal: *Sustained method performance*

CBC Validation Failure

Problem Scenario

- **Issue:** New analyzer showing 8% CV for platelets (target <5%)
- **Investigation:**
 - Pre-analytical clumping identified
 - Inadequate sample mixing
 - Variable EDTA concentrations

Resolution:

- Updated sample collection SOP
- Implemented automated mixers
- Staff retraining program

Outcome: CV reduced to 2.5%

Lesson: *Validate entire testing process*

Post-Validation Monitoring

Tools and Methods:

- Monthly precision verification
- Semi-annual accuracy assessment
- Annual comprehensive review
- Proficiency testing performance

Triggers for Re-validation:

- Instrument major service
- Reagent formulation changes
- Clinical complaints
- QC rule violations

Lean Approaches in Validation

Efficient Validation Strategies

- **Lean Principles:**
 - Value stream mapping
 - Waste elimination
 - Standardized work
 - Continuous improvement

- **Application Examples:**

- Reduced validation timeline from 4 to 2 weeks
- 25% cost savings through efficient resource use
- Improved staff engagement and competency

- **Tools:**

- 5S methodology
- Kaizen events
- Visual management

Summary of Validation Process

1. **Planning:** Define scope and acceptance criteria
2. **Execution:** Comprehensive performance assessment
3. **Analysis:** Statistical evaluation against standards
4. **Documentation:** Complete records and reports
5. **Implementation:** Staff training and SOP development
6. **Monitoring:** Ongoing quality assessment

Future Trends in Validation

Digital Transformation:

AI-assisted validation protocols

Automated data analysis

Electronic validation documentation

Personalized Medicine:

Validation for targeted therapies

Companion diagnostic development

Pharmacogenomic testing

Regulatory Evolution:

Harmonized global standards

Risk-based approaches

Real-world evidence incorporation

