National Standard Operating Procedure for Validation and verification in hematology laboratory

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Acronyms

SOP	Standard operative procedure
MOH	Ministry of Health
HPLC	High Performance Liquid Chromatography
QC	Quality Control
Hb	Hemoglobin
MCH	mean cell haemoglobin
MCV	mean cell volume
RBC	Red blood cell count
HCT	Hematocrit
PLT	Platelet
WBC	White blood cells
RET	Reticulocytes
CSF	Cerebrospinal fluid
LIS	Laboratory information system
AMM	Adjusted Male Median
AMR	Analytical Measurement Range
CBC	Complete Blood Count
CLSI	Clinical and Laboratory Standards Institute
CV	Coefficient of Variation
EDTA	Ethylenediaminetetraacetic Acid
ESR	Erythrocyte Sedimentation Rate
G6PD	Glucose-6-Phosphate Dehydrogenase
CE	Capillary electrophoresis
ICSH	International Council for Standardization in Haematology
IVD	In Vitro Diagnostic
LDT	Laboratory Developed Test
LLoD	Lower Limit of Detection
LoB	Limit of Blank
QC	Quality Control
SD	Standard Deviation
TEa	Total Allowable Error
WST	Westergren
HOD	Head of Department

1. **Purpose:**

   This SOP is to define a systematic, scientifically robust, and regulatory-compliant process for the validation and verification of automated analytical systems used in the Hematology Laboratory

2. **Scope:**

   This SOP is comprehensive in its coverage of the primary analytical activities within the modern hematology department. Its mandates apply to all laboratory personnel, including medical laboratory scientists, technicians, technical supervisors, and the overseeing hematopathologist.

3. **Definitions:**

- **Verification:** The confirmation, through the provision of objective evidence, that specified requirements have been fulfilled.
- **Validation:** The confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled.
- **Precision:** The closeness of agreement between independent test results. Divided into Repeatability (intra-run) and Reproducibility (inter-run/within-lab).
- **Trueness (Bias):** The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.
- **Total Allowable Error (TEa):** The maximum combined error (Bias + 1.65 × Imprecision) that can be tolerated without invalidating the medical usefulness of the result.
- **Comparability:** The degree of agreement between the new method and the current routine method or reference method.
- **Analytical Measurement Range (AMR):** is the range of analyte values that a method can measure directly without any pre-treatment, such as dilution or concentration.
- **Linearity**: is the mathematical relationship where the measured concentration is directly proportional to the actual concentration of the analyte. Establishing linearity is essential for ensuring that the instrument response is predictable across the entire reportable range.

- **Carryover:** is the systematic error introduced when a high-concentration sample contaminates the subsequent sample. This is a critical parameter for automated analyzers that use a single aspiration probe for multiple specimens.
- **Limit of blank (LOB)** is the highest measurement that should be obtained with a blank sample. The LOB is determined by running repeated measurements on multiple blank samples.
- **Reference range: are** a measured set of values determined to occur in a healthy non-disease population
- **Sensitivity:** Sensitivity refers to the ability of a laboratory test or method to correctly identify the presence of a specific analyte, even at very low concentrations. It is often expressed as the lowest amount of the substance that can be reliably detected, distinguishing true positives from false negatives.
- **Specificity:** Specificity refers to the ability of a laboratory test or method to correctly identify the absence of a specific analyte in samples that do not contain it. High specificity means the test minimizes false positives by accurately distinguishing true negatives, ensuring that individuals without the condition are not incorrectly identified as positive.

4. Procedure:

4.1. Clinical Background: Verification is the process of confirming, through objective evidence, that an instrument performs according to the manufacturer's established specifications when situated in the unique environment of the end-user laboratory. Validation, conversely, is required for laboratory-developed tests (LDTs) or when a manufacturer's method is modified significantly, requiring the laboratory to establish its own performance characteristics from first principles. By strictly following the recommendations of the International Council for Standardization in Haematology (ICSH), the laboratory harmonizes its practices with global standards, facilitating the comparability of results across different institutions and clinical trials.

The ultimate goal of these procedures is to minimize analytical error, which can be categorized into random and systematic components. Through precision studies, linearity assessments, and carryover evaluations, the laboratory defines its limits of detection and quantitation, ensuring that even at the extremes of physiological or pathological ranges, the data remains robust and clinically actionable.

4.2. Validation and verification procedures:

4.2.1 It is essential to prepare verification/ validation plan before performing any verification/ validation study. The hematopathologist / pathologist/ HOD to approve the plan before starting the study.

4.2.2 Precision: To determine the random error inherent in the instrument's measurement system (pipetting, counting statistics)

4.2.2.1 Within-run Precision: Select three patients samples representing low, normal and high range. If not available, the analyzer control can be used. Analyze each sample 10 to 20 times consecutively in a single run. Ensure the sample is mixed well between aspirations if the run takes time. Calculate Mean, Standard Deviation (SD), and Coefficient of Variation (CV%)

4.2.2.2 Between batch precision: Use stabilized commercial Quality Control material (3 levels: Low, Normal, High). Analyze each level three times daily, for a minimum of 5 days or analyze each level once or twice daily for 20 days. Calculate the total Within-Lab mean, SD and CV.

4.2.2.3 Please see appendix 1 for acceptable criteria.

4.2.3 Carryover: To verify that the analyzer's wash cycles are effective and that a high-concentration sample does not contaminate the subsequent sample. Select a High (H) sample and a Low (L) sample (Diluent or very low patient sample). Analyze in the sequence: H1, H2, H3, L1, L2, L3.
Calculation:

$$\text{Carryover } \% = \frac{L1 - L3}{H3 - L3} \times 100$$

The acceptable criteria: acceptable carryover varies according to the test methods. The percentage of carryover can range from < 1% to <10%.

4.2.4 Linearity (Analytical Measurement Range - AMR): To verify the range of values over which the analyzer reports results linearly without dilution. Use a commercial linearity kit (preferred) or create a dilution series from a very high patient sample (e.g., Buffy coat concentrate for WBC/PLT) using autologous plasma as the diluent to maintain viscosity. Prepare 5–7 concentration levels covering the manufacturer's stated AMR. Analyze each

level in triplicate. Plot Observed vs. Expected values. Perform polynomial regression. The non-linear coefficients should be statistically insignificant. The acceptable linearity is Slope 1.0 ± 0.10.

4.2.5 **Accuracy**: Accuracy or trueness can be verified using recovery of expected values from assayed reference materials such as commercial controls may also be used. The laboratory should set its own limits for the acceptable range.

4.2.6 **Method Comparison:** To demonstrate agreement with the laboratory's current reference method (or a "Gold Standard" if available). Minimum 40 samples are required for verification and at least 100- 300 samples for a full validation to capture sufficient pathological variety. Around 50% of samples should be normal. Analysis of data should contain scatter plot for visual assessment of correlation. Use Passing-Bablok regression (which assumes error in both methods and is robust to outliers). Do not use simple linear regression (OLS) as it assumes the comparator is error-free. Spearman rank correlation coefficient used to calculate correlation coefficient (r). (r) values of 0.8-1, 0.60-0.79, 0.4-0.59, <0.39 were considered as very strong, strong, moderate and weak correlation respectively. Use Bland-Altman difference plots. This is crucial for identifying concentration-dependent bias (e.g., analyzer reads lower at high Hb levels). Consider discordance Analysis: Any sample with a discrepancy > 10% (or exceeding TEa) must be investigated. Perform a manual slide review or re-run to rule out random error or sample integrity issues (clots).

4.2.7 **Reference Ranges:** To verify the clinical utility of an instrument for diagnosis, screening and monitoring of disease, it is necessary for the laboratory to establish a reference range.

- Reference ranges specific to the instrument, for all components of the test, should be calculated during the instrument evaluation.
- To verify a reference range claimed by the manufacture or from literature, published range a minimum of 20 healthy, non-diseased individuals for each subgroup of gender and age.
- The establishment of new reference range required at least 120 samples, from apparently healthy individuals, all normal values for the test, of each

sex, 60 from each, are tested within 4 h of venesection. Sex and ethnic groups should be examined where possible.

- Acceptability criteria: ranges are considered verified if 90% of values falls within the proposed range.

4.2.8 Sensitivity and Specificity:

4.2.8.1 Sensitivity: (Qualitative and Quantitative)

- It measures the lowest concentration of analyte that can be measured (lower limit of detection). For an FDA approval unmodified method, the manufacture's stated sensitivity will be used.
- Sensitivity studies are only required for those methods which have clinical relevance at values close to zero (0).
- Qualitative test: Sensitivity can be done by calculating the number of true-positive, and false-negative during comparison study

4.2.8.2 Specificity: (Quantitative and Qualitative)

- Determine the effect of interfering substance on the accuracy of the measured test.

4.2.8.3 Qualitative Tests verification calculations:

$$\text{Reactive Rate of Test Method} = \frac{\text{True Positives + False Positives}}{\text{Number of Samples Tested}} \times 100$$

$$\text{Specificity of Test Method} = \frac{\text{True Negatives}}{\text{True Negatives + False Positives}} \times 100$$

$$\text{Sensitivity of Test Method} = \frac{\text{True Positives}}{\text{True Positives + False Negatives}} \times 100$$

$$\text{Positive Predictive Value of Method} = \frac{\text{True Positives}}{\text{True Positives + False Positives}} \times 100$$

$$\text{Negative Predictive Value of Method} = \frac{\text{True Negatives}}{\text{True Negatives + False Negatives}} \times 100$$

4.2.9 Specimen Stability: (as applicable, qualitative and quantitative)

- Sample analyte stability should be checked if the condition of handling and storage permits the measurement and reporting of a clinically relevant result.
- Verify the samples at various storage temperatures over period of time as well as specimen freeze/thaw cycles if the samples are stored frozen.
- Analyse samples (at least 10 samples) at various days (1-7 days), record and compare the results.
- Compare samples stored frozen as well as refrigerated and room temperature.
- Analyze aliquots that have gone through 1, 2 and 3 freeze/thaw cycles if applicable

4.2.10 LIS verification:

Is the process of confirming that the Laboratory Information System is correctly configured, functioning accurately, and reliably processing orders, results, and data exchanges according to laboratory requirements before use or after any system change.

4.3. Specific considerations:

4.3.1 Automated Hematology Analyzers (CBC):

4.3.1.1 Validation:

- Use precision, carryover, linearity, accuracy and comparison methods
- Use minimal 100-300 samples for comparison studies.
- Minimal CBC parameters to be included in validation study: white cell count (WBC) and their differential, red cells count (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), hematocrit (HCT), platelet count (PLT), reticulocytes absolute count (RET) if available in the analyzer.

4.3.1.2 Verification:

- Use precision and comparison methods
- Use minimal 40 samples
- Only measurable parameters (can be differ according to the analyzer) to be included in verification study: white cell count (WBC) and their differential, red cells count (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), hematocrit (HCT), platelet count (PLT) and reticulocytes absolute count (RET) if available in the analyzer.

4.3.2 Verification of Body Fluid Analysis: Validating an analyzer for blood does not validate it for body fluids (CSF, pleural, etc.). The matrix is different (low protein, low cellularity).

4.3.2.1 Run the body fluid specific "Background Check" (diluent blank) to determine the Limit of Blank (LoB). This is critical for CSF where 5 cells/µL is clinically significant.

4.3.2.2 Compare automated counts against Manual Chamber Counting (Hemocytometer). Note: Manual counting is the reference but has high imprecision (CV ~20-30%). Discrepancies are common.

4.3.2.3 Verify stability of cells in body fluids, which degrade faster than in blood.

4.3.2.4 The laboratory must verify that the manufacturer's suggested reference intervals (or the lab's existing ones) are appropriate for the new analyzer.

4.3.3 ESR Analyzers: The validation of ESR is unique because the "analyte" is a physical phenomenon (sedimentation), not a substance. The guideline categorizes methods into Westergren (Gold Standard), Modified Westergren (EDTA, different tube), and Alternate Methods (photometry, centrifugation, rheology). Most modern automated analyzers fall into the latter two categories.

4.3.3.1 Reference Method: The manual Westergren method. Other alternative methods can be used for validation.

4.3.3.2 Use precision, carryover, linearity, accuracy and comparison methods for validation.

4.3.3.3 Sample Size: Minimum 60 samples for validation.

- Sample Distribution:
  - Low Range (< 20 mm/h): ~20 samples.
  - Medium Range (20–80 mm/h): ~20 samples.
  - High Range (> 80 mm/h): ~20 samples.

4.3.3.4 Use precision and comparison methods. Minimal sample size of 30 can be used for verification.

4.3.4 G6PD Assays:

4.3.4.1 Classification of Assay:

- Qualitative (Screening): Fluorescent Spot Test (FST)
- Quantitative (Confirmatory): Spectrophotometric Kinetic Assay (340 nm). Output: U/g Hb.

4.3.4.2 Validation: Use precision, carryover, linearity, accuracy and comparison methods.

4.3.4.3 Verification: Use precision and comparison methods

4.3.4.4 Establishment of the "100%" Value (Adjusted Male Median) for reference interval:

- Determine the Median G6PD activity of this male cohort. This value represents "100% Activity" for the local population. Setting Thresholds:
  - Deficient (<30%)
  - Intermediate (30–80%)
  - Normal (>80%)

4.3.4.5 Validation of Qualitative (Screening) Tests: Must be compared against a Quantitative Reference Method. Sensitivity: Probability of correctly identifying a Deficient patient. Target: > 95%. (A false normal lead to hemolysis). Specificity: Probability of correctly identifying a normal patient. Target: > 85%.

4.3.5 HPLC, Capillary Electrophoresis (CE):

4.3.5.1 The "Two-Method" Rule: for clinical diagnosis, the laboratory must verify that it can perform identification using two complementary techniques (e.g., HPLC as primary screening and CE or Gel Electrophoresis for confirmation).

4.3.5.2 Use precision, carryover, linearity, accuracy and comparison methods for validation.

4.3.5.3 Accuracy: use calibrator or Certified Reference Materials (CRM) for HbA2.

4.3.5.4 Compare new HPLC/CE results against the laboratory's current reference method using at least 40–100 samples, including:

- Normal adults (HbA2 2.0–3.3%).
- Thalassemia traits (HbA2 > 3.5%).
- Common variants (HbS, HbC, HbE, HbD).

4.3.6 Microscopic and Digital Morphology:

4.3.6.1 Manual Microscopic Review:

4.3.6.1.1 Inter-Observer Variability: Minimal two observers review 10 identical slides (normal and abnormal). Compare differential counts and morphology grading (e.g., 2+ vs 3+).

4.3.6.1.2 Observers must achieve > 85% concordance with the "Expert Consensus" for cell identification.

4.3.6.2 Digital Morphology (DM) Analyzers:

4.3.6.2.1 Pre-classification Accuracy: Compare the instrument's automated pre-classification against the final re-classification by a skilled morphologist.

4.3.6.2.2 Sensitivity/Specificity: Assess the DM system's ability to identify critical "flags" such as:

4.3.6.2.2.1 Blasts: High sensitivity required.

4.3.6.2.2.2 Schistocytes: Must verify 1+ grading (< 1%) vs 2+ (> 1%) thresholds for microangiopathy.

4.3.6.2.2.3 RBC Morphology: Verify the two-tiered grading system (2+ moderate, 3+ many) for anisocytosis, macrocytes, and hypochromia.

4.3.6.2.3 Slide Quality and Staining: Compare automated slide makers/stainers against manual wedges. Smear quality must be acceptable in > 95% of cases, covering two-thirds of the slide with an oval feathered end.

4.4. Reporting and Approval:

4.4.1 Upon completion of all experimental protocols, a comprehensive Validation Report must be generated.

4.4.2 Report Components

4.4.2.1 Executive Summary: A concise statement of "Pass" or "Fail".

4.4.2.2 Material Listing: Serial numbers of instruments, lot numbers of reagents/calibrators/QC.

4.4.2.3 Raw Data: Appendices containing all measured values.

4.4.2.4 Statistical Output: Regression graphs, Bland-Altman plots, Precision tables.

4.4.2.5 Discrepancy Log: A line-by-line listing of all samples where the new method disagreed with the reference, including the "Referee" result (e.g., manual slide review) and final conclusion.

4.4.2.6 Conclusion: A formal statement declared by the Technical Supervisor regarding the system's "Fitness for Purpose".

4.4.3 The final report to be approved by the hematopathologist / pathologist. A copy of the report is to be submitted to laboratory in-charge and biomedical department.

5. Responsibilities:

5.1 Laboratory Technologist:

- Performs instrument calibration, maintenance, and sample analysis.
- Ensures accurate transcription of results and proper sample handling (mixing, temperature).
- Adheres to universal precautions during sample manipulation.

5.2 Haematology Supervisor/ laboratory in-charge:

- Defines clinical decision limits and TEa.
- Approves the final Validation Plan and Report.
- Determining if a method requires Validation or Verification.
- Defines clinical decision limits and TEa.
- Approves the final Validation Plan and Report.
- Supervises daily validation activities and ensures instrument QC is acceptable prior to testing.
- Investigate outliers and discrepancies.

5.3 Quality Manager/Officer:

- Ensures protocol adherence to ICSH/CLSI/ISO standards.
- Ensures protocol adherence to ICSH/CLSI/ISO standards.
- Manages the retention of raw data, statistical outputs, and signed reports.
- Conducts internal audits of the verification and validation process.

5.4 Hematopathologist/ pathologist:

- Advises on the clinical impact of observed biases or limitations.
- Reviews discordant samples (e.g., slide review for CBC differences).
- Drafts the specific validation protocol (sample size, selection criteria).
- Sign off the final report of the verification or validation.

6. Documentation and Reporting

Version	Description	Review Date
01		August 2031

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8. Appendix 1 : Acceptance Criteria Summary:

Parameter	With-in run precision %CV
WBC (10^9/L)	5.73%
RBC (10^{12}/L)	1.60%
HGB (g/dL)	1.43%
MCV (fL)	0.70%
PLT (10^9/L)	4.6%
RET (10^9/L)	10%
HCT (L/L)	1.35%
G6PD	3.75%
ESR	15 % or SD=3
HbF	5%
HbA2	2%

Parameter	Between batch precision %CV
WBC (10^9/L)	5.73%
RBC (10^{12}/L)	1.60%
HGB (g/dL)	1.43%
MCV (fL)	0.70%
PLT (10^9/L)	4.6%
RET (10^9/L)	10%
HCT (L/L)	1.35%
Neutrophils (10^9/L)	8.55%
Lymphocytes (10^9/L)	5.10%
Monocytes (10^9/L)	8.9%
Eosinophils (10^9/L)	10.5%
Basophils (10^9/L)	14.0%
G6PD	3.75%
ESR	15% or SD= 3
HbA2	7.7%