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5.5 Verification and validation

1.0 Purpose

The aim of the procedure is to provide guidance on how Proteus Laboratories handle the verification/ validation of equipment and methods.

2.0 Scope

The scope covers the procedures used for the verification and validation of Laboratory methods/equipment and recording the results obtained i.e. Sensitivity, Specificity, Precision, Accuracy, Linearity and comparability. The SOP is applicable to all technical personnel.

3.0 Terms, Definitions and Abbreviations

3.1 Terms and Definitions

Validation-confirmation through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled.

Verification-confirmation through provision of objective evidence that specified requirements have been fulfilled

3.2 Abbreviations

- QM - Quality Manual
- SO - Safety Officer
- QO - Quality Officer
- SOP - Standard Operating Procedure
- NA - Not Applicable

• CLIA - Clinical Laboratory Improvement Amendments
• CI - Confidence Interval
• MGT- Management
• EQA - External Quality Control
• r - Correlation coefficient

Procedures

7.1 Selecting test procedure:

The Laboratory ensures that the manufacturer's guidelines are followed when selecting methods to be used for test method/equipment being verified. Validation of methods is done by the manufacturer; only onsite verification is done.

7.2 Verification of new equipment

New equipment installed by the manufacturer undergoes verification before putting to use. Installation and verification reports are filed in the equipment book of life. The verification is done by the lab manager or designee, with close consultation from the Lab consultants, during which the equipment is shown to comply with the required specifications and that the results generated are valid.

7.3 Verification of equipment/method already in use

The verification process is performed before the equipment is put back into service after a period of malfunction, service or maintenance. The Laboratory personnel are responsible for execution of these verifications.

The laboratory manager takes the initiative for verification. The lab manager documents the verification report including measurement results, statistical calculations, and the conclusion. Competent Laboratory personnel signs the verification reports for agreement. The laboratory manager is responsible for maintenance of the verification records.

7.4 Qualitative Assays Validations/Verifications

Qualitative tests are used for screening, diagnostic purposes, monitoring of patients or as confirmatory tests. The test's sensitivity and specificity determine its clinical use.

Verification for methods are conducted using a 2X2 contingency table:

● **Sensitivity** (also called the true positive rate) - the proportion of positives that are correctly identified as such (e.g. the percentage of sick people who are correctly identified as having the condition).

● **Specificity** (also called the true negative rate)- is the proportion of negatives that are correctly identified as such.

Table: 2x2

Contingency table

Method X

	Diagnostic accuracy criteria		
	Positive	**Negative**	**Total**
Positive	# true positive - TP	#false positive – FP	TP+FP
Negative	#false negative - FN	# true negative - TN	FN+TN
Total	TP+FN	FP+TN	N

Estimated sensitivity = 100x {TP/ (TP+FN)}

Estimated specificity = 100x {TN / (FP+TN)}

● If the ratio of true-positive rate/False positive is equivalent to Sensitivity/ 1-sensitivity), then test has no diagnostic value for the condition of interest.

● On the other hand, a test where both sensitivity and specificity are close to 100% has high diagnostic value.

● The laboratory sensitivity and specificity values are compared with the manufacturer's values provided on the inserts.

7.4.1. Procedure

o Sample:

 Patient or quality control

o Testing:

 Ensure there is enough reagent to perform at least 10 runs on each kit

 Run each sample at least 10 times, if possible, let the testing be conducted by more than one personnel.

o Analysis:

 Compute the sensitivity and specificity for the kits using the 2X2 contingency table.

Note:

The percentage sensitivity or specificity of the laboratory should be equal to or greater than the manufacturer claims for the test to be deemed as acceptable. For tests where there are no manufacturer claims, a percentage sensitivity and specificity of 100% is considered acceptable.

7.5 Comparability of Methods

To perform comparability studies, samples of known results/concentrations are run on both methods being compared at least ten times and the results obtained are then compared.

The standard deviations for the two methods are computed and the analysis is done as below;

Hypothesis:

Null Hypothesis (Ho): Results obtained from the two methods are the same (not statistically significant)

Ho=sd1=sd2

Alternative Hypothesis (HA): Results obtained from the two methods are not the same

Ha=sd1≠sd2

The standard deviations (SD) from the two methods are compared using the F distribution test. Acceptable results are when the calculated F value (F calculated) is less than the critical F value (F critical) i.e.

F calculated = (SD method one / SD method two) 2

F critical = obtained from F-table

F critical = (df1, df2)

where df = degrees of freedom given by (number of runs 1)

Decision rule:

- df1=K-1, where k= number of runs
- df2=N-1, where N= sample size
- F Critical value at (df1, df2) =xxxx [obtained from the F-distribution table at 95% CI]
- Reject H0 if Fcal≥ **critical value at** (df1, df2)

7.6 Quantitative Assay Validation/verification methods

At Proteus Laboratories the verification of test methods/equipment, precision, accuracy and linearity are determined at a minimum. Other verification methods are conducted as appropriate.

7.6.1 Precision

Precision is reproducibility – defined as the agreement of the measurements of replicate runs of the same sample. Replication experiments are performed to estimate the imprecision or random error of a given analytical method. Short term precision (within

run measurements) and long-term precision (between run measurements) are determined.

7.6.1.1 Short –Term precision (Within-run/Day)

A. Sample:

 Two levels (Low / High or Normal / Abnormal)

 Patient or quality control

B. Testing:

 Ensure there is enough reagent to perform all 20 tests.

 Run each sample at least 10 times on the same run, if possible, or at least within the same day.

C. Acceptability criteria:

 Calculate the coefficient of variation (CV) for each level using at least 20 data points.

 Compare the calculated CV to the manufacturer's stated precision claims found in the package insert or equipment manual.

 If manufacturer's precision cannot be met or available, it is acceptable to attain precision that is <25% of the CLIA Allowable Error.

 If short -term precision is unacceptable, document the occurrence in the non-conformity form.

 If unable to resolve issues with short-term precision, the service engineer will be contacted.

7.6.1.2 Long-Term (Between-run/between Day)

A. Sample:

 Two levels (Low and High or Normal and High)

 Patient or quality control serum.

Note: It is acceptable for the laboratory personnel to use the already available IQC (Internal Quality Control) data for long-term precision.

B. Testing:

 Run all levels of IQC 3X for 3 consecutive days.

C. Acceptability criteria:

 Calculate the CV for each level using at least 20 data points

 Compare to manufacturer's stated precision claims found in the package insert or equipment manual.

 If the manufacturer's precision cannot be met or unavailable, it is acceptable to attain precision that is < 25 % of the CLIA and RIQAS Allowable Error.

 If Long-Term precision is unacceptable, document the occurrence in the non-conformity form.

7.6.2 Accuracy

Accuracy is the closeness of agreement between the average of several replicates measured quantity value and a reference quantity value. The results of accuracy evaluation are expressed numerically as bias.

A reference measurement procedure is used if available for the analyte being verified. If a reference measurement procedure or traceability through standard reference materials is not available, then a comparative method is selected.

To select a comparison or reference method, the following are considered

 The ideal comparison method is a similar instrument/method.

 Samples with known values, such as proficiency testing samples or commercial standards, may be used as the reference method.

I. Sample Criteria

 A minimum of 20 samples covering the reportable range of the method are used

 Patient, quality control, and proficiency testing materials can be used.

II. Testing

 Run each sample on each instrument

1. Ideally, samples should be run within 8 hours of each other unless the analyte has a shorter stability.

 Analyse the replicates

 Retain the instrument printouts.

 Re-analyse any discrepant results between the test and comparative methods to confirm that the differences are real and not mistakes in recording the values or mix-ups of specimens.

 If an outlier is identified, then investigate the reason and take corrective action.

 Document the findings.

 Remove the outlier from the data set.

II. Evaluation of Data

A. Calculate the slope, Y-intercept, and r.

B. Evaluate the data using one of the options below: If	**Then**
r ≤ 0.975	● Data does not extend over acceptable range. ● More data must be evaluated over larger range.
r ≥0.975	● Proceed with Linear Regression Analysis to evaluate acceptability.

 Acceptability criteria for Linear Regression Analysis

C. Visually inspect the comparison plot for linearity and outliers

If an outlier is removed, then recalculate the regression statistics

D. Visually inspect the difference plot for constant scatter

 Compute the linear regression equation =y=mX+b; where b is y –intercept and m is the slope.

 For a perfect correlation the slope=1 while the y-intercept =0

III. Determine Bias or Difference between the Methods

A. Enter Decision Points (Xc)

 Using the linear regression equation, calculate the predicted Y value (Y') that corresponds to the concentration of Xc.

 Determine the bias (difference) by subtracting Y' from Xc

 Calculate the % bias (% difference) as bias/Xc * 100.

IV. Acceptance criteria should consider any other following

A. Bias should be less than that provided by the manufacturer

B. Bias should be less than TEA (Total Error Allowable) from CLIA and RIQAS

Total Allowable Error Approach may also be used where;

Precision (RE) + Accuracy (SE)≤ TEA

7.6.3 Linearity

Linearity studies are performed to determine the linear reportable range for an analyte. The linearity for each analyte is assessed by checking the performance of recovery throughout the manufacturer's stated range of the testing system. This is done using a set of standards/patient samples containing varying levels of an analyte in high enough and low enough concentrations to span the entire range of the test system.

7.6.3.1 Procedure

Linearity studies are performed as part of the procedure for verification for automated test methods in order to determine linear reportable range. For each analyte, a set of linearity standards/patient samples are tested in the same manner as patient samples.

i. **Sample Criteria**

▪ A minimum of 5 samples that cover the reportable range of the method are recommended.

▪ Quality control, calibrators or commercial linearity standards should be used and are preferable.

▪ If these are not available, utilise patient samples. Patient samples are diluted using a dilution factor to the lowest concentration

ii. Testing

▪ At a minimum, run

n each sample in duplicate. If one value deviates greatly from the others due to random error, it may be removed from the data analysis.

▪ When using patient samples, the original concentration (c1) is multiplied by the dilution factor to get the concentration for the subsequent tube (C2) and so on i.e. C3, C4,…..Cn. These concentrations become the expected values (X). The observed values are the values generated on the test reports when the concentrations (C1, C2, C3,……Cn) are run as samples **Validation, Verification of Examination Procedures and Equipment SOP** PRL-RMM-001

▪ The test results are graphed and statistically analysed as described below under "Evaluation of Linearity Study Data."

▪ Once a linearity study has been performed to determine the linear reportable range for a test method, it may be repeated as recommended by the manufacturer to verify continued acceptable performance of the analyser or analyte.

7.6.3.2 Evaluation of Linearity study data

● The data from the linearity study is recorded into an excel file.

● Values are plotted as observed values (Y axis) vs. expected values (X axis). Examine the raw data for obvious errors. If an analytical or technical problem is found, repeat the testing. Assessment is made by evaluating the data and statistics using the following guidelines.

● Review the linearity data against the manufacturer's stated acceptable performance.

7.6.3.3 Slope and y-Intercept

● Two key statistical values in determining linearity are:

Slope:

Ideally, the slope is equal to **1.0** Acceptable Range: **0.9-1.1**

if the slope is outside the acceptable range; examine the results of the highest standard first. It is possible that the test is nonlinear at its highest value.

Y-intercept:

Ideally, the Y-intercept is equal to **zero**. For clinical chemistry and other assays with results in high numerical values, the Y—intercept may be much higher with no clinical significance. The Y—intercept for assays with low numerical values should be 0.0 + /— 1.0.

7.6.4 Reportable Range

A reportable range is established for each analyte tested. The upper limit of the reportable range is set at the concentration of the highest standard tested which exhibited acceptable results for linearity, accuracy and

precision. This concentration, however, may not exceed the manufacturer's stated linear range.

The analyte which have a lower limit of linearity, the lower limit of the reportable range is set at the lowest standard tested which exhibits acceptable results, however, this concentration may not exceed the manufacturer's lower limit.

Patient samples with concentrations which exceed the reportable range are diluted with the appropriate diluents and retested, when the analyser provides this capability. Samples with concentrations which are lower than the reportable range will be reported as "Less than (the lower limit)".

8.0 References/Related Documents

- Quality Manual
- Equipment Operators Manual

https://www.westgard.com/mltirule.htm

ISO 15189:2012

9. 0 Attachments/Annexes

- Appendix 1: Verification reports, Methods and Equipment
- Annex 1: SOP Attestation Form

Appendix 1: Verification reports, Methods and Equipment

Equipment / Method	
Performed by	
Date of Verification	
Date of Report	
Reviewed by	
Approved by	
Date Approved	
Comments	

Introduction
Results
Statistical analysis
Limitations of the method
Conclusion
Literature

Measurement Uncertainty

1.0 Purpose

The Laboratory estimates the measurement of uncertainty because a measurement only gives an approximation of the true value of the quantity to be measured. The Laboratory also uses MU to monitor the performance of the quality management system. The purpose of the SOP is to determine measurement uncertainty for each measurement procedure.

2.0 Scope

The SOP covers the estimation, application, and communication of measurement of uncertainty. This SOP applies to all technical personnel involved in performing measurement procedures in the laboratory. Measurement uncertainty is performed during initial verification and reviewed annually.

3.0 Terms, Definitions and Abbreviations

3.1 Terms and Definitions

 Analyte - the substance or constituent of interest that is the subject of measurement. However, a substance can have several properties, some or all of which can be utilized to quantify the substance in an appropriate measuring system.

 Measurand - the quantifiable property of the analyte used in the measuring system.

 Uncertainty - a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand.

 Traceability - Property of the result of a measurement or the value of a standard, whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.

 Repeatability is "a measure of variability derived under specified repeatability conditions", i.e. independent test results are obtained with the

same method on identical test items in the same laboratory by the same analyst using the same equipment, batch of culture media and diluents, and tested within short intervals of time.

 Reproducibility is "a measure of precision derived under reproducibility conditions" i.e. test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment. A valid statement of reproducibility requires specification of the conditions used.

3.2 Abbreviations

 QM Quality Manual
 SOP Standard Operating Procedure
 SO Safety Officer
 QO Quality Officer

7.0 Procedures

7.1. Identify the measurand

 for example, venous blood haemoglobin concentration, vB – haemoglobin mass concentration.

7.2. Set an analytical goal that the laboratory should achieve

The Laboratory utilizes the values in the database on the Westgard website. thus, for haemoglobin the goal might be: 0.5 CVI = 1.4% (from database http://www.westgard.com/biodatabase1.htm).

If these values are not available, then the laboratory utilizes the total allowable errors as the analytical goals for each of the measured analytes.

7.3. Identify all measurement uncertainties

a. Imprecision can be calculated from the laboratory's own internal QC or obtained from the performance specification reports for the laboratory. This is also known as CVA% and is derived from the internal QC data of a control sample. Data should be from many consecutive determinations. *For example, CVA% = 1.1% for 'control X' (n = 20 samples)*.

b. Uncertainty of calibrator. If the manufacturer provides traceability to a reference standard, these data is used in the determination of combined uncertainty

C. Bias. Can be calculated from the laboratory EQA results or obtained from the performance specification reports. For example, uncertainty CV for haemoglobin (mean of the last five cycles) = 1.2%

7.4. Determine the Relative combined uncertainty

Relative combined uncertainty (uC) is computed using the formula below i.e. For example

$uC = ([(CV1)^2 + (CV2)^2 \ldots.])0.5$

7.5. Determine the Relative expanded uncertainty

The Relative expanded uncertainty is computed as a product of the relative combined uncertainty (uC) and the constant K,

where k is at a confidence interval (k=1.5 at 68% CI, k=2 at 95% CI, k=3 at 99.7% CI)

For example,

$U = uC \times k$

7.5.1 Acceptance criteria

1. The MU is accepted if it's less than the % CV as per CLIA limits.
2. In case the MU is greater than the % CV, verify the steps
3. If verification confirms that the MU is greater than the % CV, perform a root cause analysis and corrective action.

7.5.2 Application of MU

For every measurand whose result is generated by the laboratory is compared to the respective uncertainty within which the true value lies.

8.0 References/Related Documents

 SANAS TR 28: criteria for validation, uncertainty of measurement and quality assurance in microbiological and molecular testing.

CLIA guidelines.

Appendix 1: Measurement of Uncertainty Report

Equipment / Method	
Performed by	
Date of report	
Reviewed by	
Approved by	
Date approved	
Remarks	

Procedure

Analyte	
Test Principle	
Units of measurement	
Test Limitations	
Interferences	
Analytical Goal (% CVI) Biological variation obtained from Westgard database	
Calibrator traceability uncertainty	
Analytical Bias	
Analytical Impression	
Combined relative uncertainty	

5.6 Ensuring the quality of examination results

1.0 Purpose

This SOP provides a means of determining the performance of the testing procedure to ensure the results obtained are accurate, reliable and obtained the right way.

2.0 Scope

This procedure is used at all stages of testing (pre-examination, examination and post examination) for maintaining and improving quality performance standards.

3.0 Terms, Abbreviations, Definitions

3.1 Definitions

Quality control: refers to those measures that must be included in each assay to verify that the test is working properly.

Quality Assurance: the overall program that ensures that the results reported by the laboratory are as correct and accurate as possible

3.2 Abbreviations

- EQA External Quality Assurance
- IQC Internal Quality Control
- QM Quality Manual
- QA Quality Assurance
- QC Quality Control

6.0 QA & QC

7.0 Procedure

7.1 Preparation of in-house quality control material.

 In-house quality control materials are prepared for qualitative assays without commercially supplied controls. These controls are prepared from known samples previously analysed by the laboratory or confirmed using gold standard laboratory methods if appropriate.

 The laboratory personnel ensure that the specimen acceptability criterion as defined in the clinician's handbook for each test performance area is met before in-house quality control materials are prepared.
 The quality control containers/vials are labelled with **date prepared, control name** and **expiry date.**

7.2 Analytical quality Control

 All new control reagents lots are verified, prior to being used.
 The Laboratory uses commercially prepared control materials for quantitative tests performed while for qualitative test, in-house control materials are used if no commercial controls are available.

7.3 Frequency of performing IQC

 The frequency for performing internal quality Control (IQC) depends on the type of tests' procedures and manufacturer's instructions.

 IQCs are performed before running patient samples for quantitative and qualitative tests.
 IQCs are also used when verifying reagents and consumables.

7.3.1 Qualitative tests

 Run controls for each reagent or test kit, following manufacturer's instructions.
 Known 'positive' or 'Negative' patient sample/ in house controls are used in the absence of commercial controls.

7.3.1.1 Acceptability Criteria

 Known 'positive Control' must be positive and a known 'negative Control' must be negative for acceptable test performance.
 Commercial controls must react as described in the manufacturer's user instruction

7.3.2 Quantitative tests

 Control materials for all analysers are run daily **or** whenever ***samples to be processed are available.*** Every analyte tested has at least two control levels or values run per analytical run.

 Manufacturer's ranges are used for all examination procedures for internal quality controls.

 Review controls acceptability against expected limits and file the records.

The following Westgard rules are used in the analysis of IQC results;

o **12s-**referred to as the control rule that is commonly used with a Levey-Jennings chart when the control limits are set as the mean plus/minus 2s. This rule is used as a warning rule to trigger careful inspection of the control data by the following rejection rules at Proteus Laboratory

o **22s** - reject when 2 consecutive control measurements exceed the same mean plus 2s or the same mean minus 2s control limit.

o **10x** - reject when 10 consecutive control measurements fall on one side of the mean.

Document all QC problems and corrective actions in the non-conformity form.

7.4 Post analytical

The laboratory manager reviews all results in order to detect significant clerical and analytical errors for timely action.

Data Collection and analysis to evaluate/monitor laboratory performance

Westgard rules and Levy-Jennings charts are used to monitor variations of quality control test performance for acceptability and corrective action. Other statistical and non-statistical methods may be adopted for evaluation of laboratory performance.

7.5.1 Troubleshoot/Corrective action on unacceptable results

All control failures are investigated and resolved before running and releasing patient results. In case QC was not run or was unacceptable and patient

results were reported, immediately initiate corrective action, recall all results and rerun samples since the last successful IQC run.

7.5.2 Review of IQCs

Laboratory Staff performing tests verifies that results of controls are acceptable before releasing results. On a weekly basis, the laboratory manager reviews all quality control results and related documentation for trends, biases and preventive actions.

The Equipment and IQC action log form will be used to record any IQC outliers, and the action taken.

Equipment and IQC Action Log

Name: _____ SIN: _____

Date	Problem	Action Taken	Resolved (Y/N)	Sign

7.5.3 communication to users

In case of any discrepancies that may arise from results comparison, both the users of the equipment and the Clinicians are informed. An action plan is developed to monitor the root cause and corrective action put in place for its effectiveness. Where need be, the results are re-called/withheld until the nonconformity is resolved.

7.6 External Quality Assurance

7.6.1 Participation in EQA/ ILC

Laboratory management identifies organizations that provide External Quality Assessment schemes for each test the laboratory performs. Where practicable the Quality Officer obtains a schedule reflecting the opening dates and closing dates of all surveys in a year (see PT plan) and the contact details of individuals to contact in case shipments are delayed or not delivered.

7.6.2 Receiving of EQA/ILC Samples

The Laboratory personnel receives samples in accordance with the procedure for sample Reception. The laboratory personnel notify other laboratory personnel of all received EQA/ILC samples and sends samples and accompanying documentation to relevant testing sections.

The Laboratory personnel performs requested tests in accordance with relevant testing procedures. EQA/ILC samples are treated in the same way as patient samples. Transcribe results to relevant EQA/ILC report worksheets in accordance with the instructions given by the EQA/ILC provider. A competent designee reviews the completed EQA/ILC reporting work sheets before submitting the results. Provide evidence of results submission to the Quality manager.

7.6.4 Review of EQA/ILC Feedback

The Laboratory personnel receives report from EQA/ILC provider and reviews. Analyse the report by comparing obtained results with the results from other

laboratories or assigned values by EQA/ILC provider. The acceptable performance is when all reported tests are within the acceptable limits as recommended by the EQA/ILC provider or as set by the laboratory management. If the results are unacceptable, investigate the unacceptable performance and no returns using the non-conformity form. The results from the EQA/ILC are communicated during the quarterly meetings.
ILC report will comply with the SANAS R80 guidelines.

7.6.5 Investigate Unacceptable Performance and No Returns

Fill in the non-conformity form for all unacceptable performances. Process the request in accordance with the procedure for identification and control of nonconformities as well as guidelines given on the EQA/ILC performance. No return EQA/ILC sample should be analysed and SDI calculated and compared to EQA/ILC report.

8.0 References

- Quality Manual **(PRL MAN 001)**

9.0 Attachments/Annexes

- Appendix 1- Manual test worksheet QC log
- Appendix 2-Internal QC preparation
- Appendix 3: Annual EQA/PT

Version review history table

Version No	Date of next review	Date reviewed	Reviewed by	Action taken/Remarks

ACKNOWLEDGEMENT OF READING FORM

Title……

My signature confirms that I have read and understood the contents of this document

Name	Signature	Date