BIOGENIX

POLICY PROCEDURE FOR SELECTION, VERIFICATION, VALIDATION OF EXAMINATION PROCEDURE

	NAME	DESIGNATION	SIGNATURE	DATE
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1. REVISION HISTORY

#	Version	Date	Changes Made by	Reason for Changes	Clause Changed
1	1.0				

2. REVIEW HISTORY

#	Version	Date	Changes Made by	Reason for Changes	Clause Changed
1	1.0				

3. POLICY STATEMENT

3.1. Laboratory is validating analyzers after they are installed for testing according to the manufacturer's requirement.

3.2. This document provides instructions for a uniform process of validating methods in the laboratory.

3.3. All Laboratory tests are validated before being placed into routine use for testing and reporting of patient results.

3.4. All validation/verifications is approved, signed and dated by the Laboratory Services Section Director prior to use.

4. PURPOSE

4.1. The purpose of this procedure is to explain the changes in the test methods followed. The procedure is as per clause 5.5. of ISO 15189: 2012 Medical laboratories –requirement for quality and competence.

4.2. The purpose of method validation is to ensure sufficient analytical reproducibility and accuracy to meet the clinical requirements.

4.3. **Validation is performed**:

4.3.1. When a new test is being introduced;

4.3.2. During the purchase and before application of a new analytical measuring system in the laboratory;

4.3.3. When a perennial problem is shown by quality control;

5. SCOPE

5.1. This procedure applies to examination processes for all the tests.

5.2. Target Audience: All BIOGENIX Laboratory staff

6. DEFINITIONS:

6.1. **Accuracy**: A test method is said to be accurate when it measures what it is supposed to measure, i.e closeness of measured value to the "true" value – bias.

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

6.3. **Verification**: confirmation, through provision of objective evidence, that specified requirements have been fulfilled,

6.4. **Precision:** dispersion of repeated measurements about the mean – reproducibility

6.5. **Repeatability**: within-run variation (same sample, same conditions).

6.6. **Sensitivity**: is a measure of how well the test/method detects positive results,

6.7. **Linearity or** Analytical measurement range (AMR) – range of values that instrument can report directly.

6.8. **Specificity**: is the degree to which the method can quantify the target analyte in the presence of other analytes, matrices, or other potentially interfering materials.

6.9. **Limit of Detection (LOD):** is the smallest measured concentration of an analyte, which allows the presence of the analytes to be detected in the test sample.

6.10. **Limit of Quantification (LOQ):** represents the lowest concentration of analyte that can be determined with an acceptable level of uncertainty e.g. the lowest reportable result.

7. ACRONYMS

7.1. **Real time RT-PCR:** real-time reverse transcriptase polymerase chain reaction

7.2. **qPCR:** quantitative polymerase chain reaction

7.3. **BSC:** Biosafety Cabinet

7.4. **Ct value** (cycle threshold): The number of cycles required for the fluorescent signal to cross the threshold (i.e exceeds background level)

7.5. **VIC/HEX:** Human House-keeping gene β-actin for control with HEX/VIC channel

7.6. **FAM:** COVID 19 specific gene ORF-1ab for SARS-CoV-2 with FAM channel

7.7. **PBS:** Phosphate–buffered saline or solution

8. RESPONSIBILITIES

8.1. All BIOGENIX Laboratory **TECHNICAL STAFF**

8.2. It is the responsibility of the BIOGENIX Laboratory to perform the validation study following to the installation of the machine.

8.3. The validation analysis is repeated every 6 months

9. PROCEDURE

VERIFICATION OF EXAMINATION PROCEDURES:

9.1. Preferred procedures are those specified in the instructions for use of the instruments.

9.2. The validated examination procedures used are verified by the lab. before being introduced in to routine use.

9.2.1. The manufacturer gives the information on the performance characteristics.

9.2.2. Lab. verify through obtaining objective evidence that the performance claims for the examination procedure have been met.

9.2.3. Performance characteristics of an examination procedure includes:

9.2.3.1. Measurement Accuracy

9.2.3.2. Measurement precision

9.2.3.3. Measurement uncertainty

9.2.3.4. Reportable Range

9.3. All the records are reviewed and approved by laboratory director.

9.4. Verification of the method is done every 6months, which includes:

9.4.1. Linearity:

9.4.1.1. Linearity studies are performed in order to determine linear reportable range. For each analyte, a set of linearity standards are tested in the same manner as patient samples.

9.4.1.2. Testing is performed in triplicate, and at a minimum, in duplicate, when performed within a single run. If one value deviates greatly from the others due to random error, it may be removed from the data analysis and repeated.

9.4.1.3. The test results are statistically analyzed

9.4.1.4. Two key statistical values in determining linearity are:

Slope:

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

lf 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 enzyme determinations 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.

9.4.2. **Reportable Range:** A reportable range is established for each analyte tested. The upper limit of the reportable range will be 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. For analytes which have a lower limit of linearity, the lower limit of the reportable range will be 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 will be diluted with the appropriate diluents and retested, when the analyzer provides this capability. Samples with concentrations which are lower than the reportable range will be reported as "Less than (the lower limit)"

9.4.3. **Accuracy:** Review the linearity data for acceptable accuracy. Ideally, endpoint assays should be within 10% of the standard's stated value or peer group comparison value (comparison is done by comparing 30 patients samples) but at a minimum, manufacturer's stated tolerance limits should be met.

9.4.3.1. **Inter-laboratory Comparison (Accuracy Studies)**

- Perform an inter-laboratory method (Biogenix Laboratory) with another laboratory's (such as SEHA Laboratory) method comparison.
- Obtain or share a minimum of 30 patient samples and results from another laboratory using the same instrumentation and testing methodology.
- Proceed with testing the samples as per the laboratory's standard SOP.
- Record the comparison results on the Inter-Laboratory method to method Correlation Data form in the column labeled Instrument X Result.
- Record the sample results as performed in Biogenix laboratory in the column labeled Instrument Y
  i. For COVID PCR:
  qPCR readings (for both Biogenix and other laboratories) of the Ct values for FAM and VIC/HEX is be plotted in the regression graph for analysis. Ct values should agree within 5% of each other.
  ii. For other methods:
  readings (for both Biogenix and other laboratories) of the analyte is plotted in the regression graph for analysis. Values should be agree within total allowable error.
- Subtract Y value from X value. Record in the column labeled X minus Y (pos or neg); this column demonstrates if there is a pos or negative bias seen when compared to the reference instrument (X) the results of the Biogenix Laboratory instrument (Y) results.
- Calculate the % method/analyte deviation: [(X value) minus (Y value)]/ (X value) multiplied by 100 %].
- Record Y or N in the column Deviation Acceptable (Y or N) Record the initials of the individual performing the % deviation calculations in the last column.
- Forward Inter-laboratory comparison data to the Laboratory Section Supervisor/Designee for review.

9.4.4. **Precision:** Coefficient of Variation, which is a measure of precision, and is the standard deviation expressed as a percentage of the mean, ideally should also be less than 10%, or at a minimum, remain within the threshold of the manufacturer's stated acceptable performance. Precision is done by processing of Quality Controls as samples.

It is ultimately the responsibility of the laboratory director to determine acceptability of this data and the validity of analyzer results with respect to accuracy and precision.

- **COVID PCR**

a. Known 5 positive and 5 negative patient samples will be run for 5 times on the same plate and using the same MGISP-960 and Gene-9660 instruments.
b. Intra Run Precision Studies: Know 5 positive and 5 negative patient samples will be run every day for 7 days. Precision statistics for the test including standard deviation (S.D.) and/or coefficient of variation (C.V.) will be calculated and determined.

- For other methods: Core lab-
  a. Inter Run Precision: 10 patient samples (5 with high value and 5 with normal values) to be run 5times in same time.
  b. Intra Run Precision: 10 patient samples (5 with high value and 5 with normal values) to be run every day for 5 days.

9.4.5. **Carry Over Studies.**

9.4.5.1. Perform extraction and qPCR for 5 high positive samples (i.e Ct value <14 at FAM Channel) and 5 confirmed negative samples.

9.4.5.2. Apply the positive samples into the first two rows of the extraction plate and apply the negative samples into the next two rows.

9.4.5.3. Add the positive and negative controls.

9.4.5.4. Following to reading the plate, use the below equation to assess the carryover percentage :-

$$Carryover\ \% = B1 - B2 \div A2 - B2 \times 100$$

9.5. We don't validate examination procedures as we don't use any procedure derived from the following sources:

a. non-standard methods;
b. laboratory designed or developed methods;
c. standard methods used outside their intended scope;
d. Validated methods subsequently modified.

9.6. Measurement uncertainty of measured quantity values: Refer to SOP of Measurement of uncertainty of measured quantitative values.

9.7. Biological reference intervals or clinical decision values:

9.7.1. The laboratory defines the biological reference intervals or clinical decision values, document the basis for the reference intervals or decision values and communicate this information and any associated changes to the users.

9.7.2. The basis of the reference intervals or decision values is from the text books, manufacturer defined i.e. from the kit insert or from the associations e.g. diabetes association.

9.8. **Documentation of examination procedures:** Examination procedures are documented and are written in English which is commonly understood by all staff in the laboratory and is available in appropriate locations.

SOP Template (Examination Procedure)

a) Purpose of examination
b) Principle and method of the procedure used for examinations;
c) Performance characteristics;
d) Type of sample;
e) Patient preparation;
f) Type of container and additives;
g) Required equipment and reagents;
h) Environmental and safety controls;
i) Calibration procedures;
j) Procedural steps
k) Quality Control process;
l) Interferences
m) Calculation;
n) Reference Range;
o) Reportable interval of examination results;
p) Instructions for determining quantitative results;
q) Alert / critical value;
r) Laboratory clinical interpretation;
s) Potential sources of variation;
t) References

9.9. If there is any change in existing examination procedure such that results or their interpretations could be significantly different, the information is transferred to the users.

10. CROSS REFERENCE

10.1. ISO 15189 :2012 Medical laboratories – Requirements for Quality and Competence.;
10.2. DoH standard for clinical laboratories.
10.3. CLSI Guidelines
10.4. EP evaluator: www.datainnovations.com/ep-evaluator

11. RELEVANT DOCUMENTS & RECORDS

11.1. **BG/SOP/EXAM/001** Procedure for Measurement Uncertainty
11.2. **BG/REC/EXAM/002** Linearity Summary
11.3. **BG/REC/EXAM/003** Instrument Verification summary