A. <u>Purpose:</u> This policy is intended to define the overview of the CT Division of Scientific Services' procedures for Performance Checks and Validations.

B. <u>Scope:</u> This policy is intended to be applied to all critical instruments and methods that can influence the results of testing.

C. <u>Procedure:</u>

1. Performance Check

A performance check is a quality assurance process to assess the functionality of laboratory instruments and procedures that affect the accuracy and/or validity of the analysis. Performance checks are typically required after any of the following events:

- Repair, service or calibration of a previously validated critical instrument prior to their use in casework.
- Additional purchases of previously validated critical instruments.
- A minor modification of a currently validated procedure.
- For certain types of upgrades of previously validated critical instruments.
- Annual performance checks on critical instruments per FBI DNA QAS.

Performance Checks are generally not required after routine in-house maintenance. Unit specific SOPs will address when a performance check will be done. A schedule is kept of all required checks. Examples: For overall laboratory, the schedule is on the ADMIN server. For DNA specific, the schedule is on the DNA server.

A Performance Check is much less than a validation. Therefore, a plan for the performance check is not needed. However, the conclusion of the performance check will be documented. The acceptable parameters must be described. There will be guidance in the unit's SOPs of what to do if unacceptable results occur. The data collected during the performance check must be reviewed to determine the effects, if any, of the modification and/or the suitability of the new or repaired analysis instrument. This information must be reviewed and approved by the technically responsible person or their designee. An applicable manager will be apprised of performance check results.

Performance Check Scenarios

a. Minor modification of a currently validated procedure (comparison study)
    i. Perform the modified procedure in parallel with the original procedure.
    ii. Evaluate the results to show whether the change has an effect on the end results.
    iii. Example: if an incubation temperature is increased, samples can be processed using both this increased temperature and the original temperature, and then the end results compared.

b. Additional Purchases of Previously Validated Critical Instruments
    i. If the laboratory currently uses one instrument and adds another of the same make and model, the performance of the new instrument must be evaluated.
    ii. The components of the performance check will depend on the instrument's function and application. The performance check should demonstrate the results are reproducible on the new instrument and that values from the in-house validation can be compared.
    iii. Example: if the new instrument is used for quantitation, a dilution series can be created and analyzed on both the new and current instrument and the end results compared.

c. Repair, Service or Calibration of a Validated Critical Instrument
    i. The extent of the performance check will depend on what type of service was performed on the instrument.
    ii. Specific Unit SOPS will define the parameters of this type of performance check.
    iii. At a minimum per FBI DNA QAS, the following DNA critical instruments need a performance check following repair, service, or calibration:
        1. Electrophoresis Detection System.
        2. Robotic Systems.
        3. Genetic Analyzers.
        4. Thermal cycler, including quantitative PCR.
    iv. The schedule for and records of all repairs, service, and/or calibration for the critical instruments is maintained on a spreadsheet and folder on the admin drive (DNA server for DNA) or may also be in the respective maintenance binder for the instrument.
    v. Time thresholds used for scheduled service/checks are as follows:
        1. Monthly: +/- 7 days.
        2. Weekly: +/- 3 days.
        3. Annually: Once each calendar year+/- 30 days.
        4. Semi-Annually: Two times a calendar year within 6 months +/- 30 days.

vi. Records are maintained per record retention policy found in GL 11 "Control of Records".

d. Certain types of upgrades of previously validated software

i. New software or significant software changes that impact interpretation or the analytical process and significant instrument upgrades shall require a validation prior to implementation into casework.

ii. A software upgrade that does not impact the analytical process will only require a performance check.

iii. Example: If the new version of software only has superficial changes, e.g., the addition of more user-friendly features, but the analytical algorithms are kept the same, a performance check and not a validation would be required. This performance check may include for example, analyzing the same data with the old and new versions.

2. Validation

A validation is the process of performing a set of experiments to demonstrate the efficacy and reliability of a procedure in the laboratory. Validation studies are typically required for any of the following events:

- A major modification of a currently validation procedure.
- New methodology (i.e. new test kit, platform change, extraction method)
- An upgrade to software that impacts the interpretation, the analytical process, or sizing algorithms.
- A new instrument that has not been previously validated.
- New or significant software modifications.

During a validation, known samples of those typically encountered in casework shall be examined to demonstrate any potential limitations of the procedure and to determine if the procedure generates acceptable results. The number and type of samples will depend of what is being validated. Documentation of a validation plan, results, and summary will be maintained.

DNA methods that require internal validation must have developmental validation completed and the citation and or publications referencing that validation must be available and accessible to support the underlying scientific basis.

If there is a need to change a validated method, the influence of such changes shall be determined and where they are found to affect the original validation, a new method validation shall be performed. This will also include data interpretation.

Validation Process

a. Plan
   i. The Validation of a new procedure will generally be initiated by the technically responsible person or designee. A validation plan will be provided to the appropriate Section Deputy and Director. The DNA TL will also approve validations in DNA. This proposal shall include a description of what the validation is, and a summary of the experiments planned to evaluate the new method.
   ii. At a minimum, the Section Deputy and Director (also TL in DNA) must approve the proposal before the validation is to be initiated. This approval can be documented through signing and dating the plan.
   iii. When implementing a validation plan, if a change to the plan is required, the change plan will be updated and approved by the Deputy Director and/or Director (or DNA TL if in DNA).

b. Experiments
   i. The validation experiments will include (where applicable):
      - The analysis of reference standards or reference materials
      - The testing of known samples designed to resemble actual casework samples
      - A complete assessment of the factors influencing the results
      - Other requirements of validation experiments will be found in Unit specific SOPs.
   ii. The FBI DNA QAS for validation require other experiments to be performed.

      The DNA validation experiments should include at a minimum:
      - Known and non-probative evidence samples or mock evidence samples.
        1. Use of known samples to show the method will give accurate results.
        2. Use of mock evidence representing the types of samples the method will be used on (swabbings, cuttings, semen, blood, saliva, hair)
      - Reproducibility and precision.

1. Use of known samples processed multiple times to show the robustness of the results.
2. Use of known samples to show precision of the method. Example; samples with multiple micro-variants.

- Sensitivity and stochastic studies.
  1. To evaluate the limitations of the method.
  2. Use of dilution series to determine how low and high concentrations of DNA will affect results.
  3. Process multiple known samples at different concentrations to determine at what RFU value sister alleles of heterozygous pairs drop out.
- Mixture studies.
  1. Effects of mixture samples on interpretation guidelines.
  2. Mixture studies will represent the full spectrum of mixtures that will be analyzed (example; 2-person, 3-person, 4-person).
- Contamination assessment.

  Negative controls and reagent controls will be evaluated for contamination and drop-in.

c. Summary of Results

i. A validation summary will be completed and provided to the Section Deputy and Director (TL in DNA). The results will contain a table of contents and be organized/separated in a way that is easy to read and be able to follow what was completed.

ii. The validation summary will contain
- The experiments used for the validation
- The results of these experiments
- Appropriate literature references
- A statement as to whether the procedure is suitable for its intended use

iii. The validation summary should also include an assessment of the following when applicable:
- Accuracy
- Range
- Uncertainty of the results
- Limit of detection
- Selectivity/Specificity
- Linearity

- Precision (reproducibility)
- Robustness

iv. Quality assurance parameters and interpretational guidelines will be defined in the summaries pursuant to the results of the internal validation. The parameters will also be included in the applicable SOP or policy where appropriate.

d. New SOP

After approval of the completion of the validation, a new or modified written SOP in Qualtrax will be approved and published prior to its use in casework. A draft version of the SOP may be used for training purposes, however if changes are made to the draft prior to publishing, examiners will be notified. This procedure will be based on the results of the validation and include interpretation guideline if applicable. The Director approves all SOPs in the Laboratory.

e. Training

Prior to initiating casework with any new validated method, the unit must ensure that staff have been trained and have successfully completed a competency test to their extent of their participation in casework.

For personnel intimately involved in the validation activities, the technically responsible person may document that the validation activities served as demonstration of competency.

For DNA, the new method must be incorporated into the training program prior to training beginning.

All Competency memos will be used for recommendation to the Director for authorization. This authorization process can be found in the General Training GL-14.

f. CT DSS Forms

At the completion of a validation, General Laboratory Form "Laboratory Method Validation Summary Form" will be filled out to ensure all necessary information is captured. This form can be found the "General Laboratory Forms" folder in Qualtrax. The Director will be the final approver on this form.