A. PURPOSE:

This policy is intended to define the overview of the CT Division of Scientific Services' procedures for Performance Checks and Validations.
Validations are used to demonstrate the Division's ability to properly perform methods prior to putting them into use. The DSS will validate all methods used for casework whether they are published or developed at the DSS prior to implementation. When performing validations the DSS must consider if any changes made will meet the needs of its customers.
Performance checks are used to monitor the continued functionality of equipment.

B. SCOPE:

This policy is intended to be applied to all critical instruments and methods that can influence the results of testing.

C. RESPONSIBILITY:

1. Managers: Responsible to provide direction to subordinate staff under their purview as indicated by the organization chart.
2. DNA Technical Leader (DNA TL): responsible to ensure that components of validations and performance checks are compliant with the FBI-QAS requirements for DNA. Additionally, they are responsible to review all DNA validations for completeness.
3. Supervisors: Responsible to provide supervision to subordinate staff under their purview as indicated by the organization chart.
4. FSE2: responsible as a working lead to subordinate staff as indicated on the organizational chart.
5. FSE1 and Lab Assistants: Responsible to adhere to this procedure as it pertains to their Unit.
6. ECO: Responsible to adhere to this procedure as it pertains to their Unit.
7. Support Personnel (however titled): Responsible to adhere to this procedure as it pertains to their Unit.

D. PROCEDURE:

1. Performance Checks

A performance check is a quality assurance process to assess the functionality of laboratory instruments and processes 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 may be 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. GL-21 describes guidance on checks for some general equipment such as microscopes, pipettes and balances; the calibration schedule is maintained by the Quality Section through the Quality Management Software QMS). Units requiring performance checks beyond those listed in GL-21 will include this guidance in their Unit procedures. For DNA specific equipment, the schedule is on the DNA server.

Unit procedures describe needed performance checks and the acceptable performance criteria. A Performance Check does not require a plan. The conclusion of the performance check will be documented in a manner to demonstrate if the acceptable criteria were met or not. Additionally 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 an authorized analyst. The Unit Lead or Supervisor or applicable Manager will be apprised of performance check results that do not pass.

Performance Check Scenarios

1. Minor modification of a currently validated procedure (comparison study)
    i. When possible, 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. If the results compare favorably (equivalent or improved) a validation would not be required.

2. 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 as compared to the previously validated instrument.
   iii. Example: if a 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. If the data from the additional instrument is equal or better than the current, a validation would not be required.
   iv. If the laboratory purchases a different brand of general equipment such as an analytical balance, a performance check can be performed to show suitability based on how the equipment is used.
3. 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. If the service performed can potentially change or effect results produced from the instrument a performance check is required.
   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 Unit 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 critical instruments are maintained within each Unit; this may be part of the instrument maintenance logbook or electronically maintained (example: records for the DNA Unit are maintained on the DNA server).

1. The schedule for performance checks for general equipment such as microscopes, balances and pipettes is in GL-21 and maintained by the Quality Section through the QMS.
2. The individual Units utilizing the specific devices maintain records of service/repairs.

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".

4. Certain types of upgrades of previously validated software
i. A software upgrade that does not impact the analytical process will only require a performance check.
ii. 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.
iii. A software upgrade to the LIMS does require a verification or validation depending on the extent of the upgrade. Refer to GL-4 LIMS.
1. The term significant in this document applies to changes in methodologies/technologies, upgrades or changes to software/hardware or similar changes where the change can influence the outcome of a result.

2. 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 the same, only a performance check would be required. This performance check may include simulating casework and ensuring operability.

2. Validations

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 valid procedure.
- New methodology (i.e., new test kit, platform change, extraction method)
- New software or 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 validation will include data analysis and interpretation, and will establish the required method parameters to interpret data, report a result or opinion. The number and type of samples will depend of what is being validated. Documentation of a validation plan, results, and summary will be maintained for a period of no less than the life of the method plus 10 years (refer to GL-11).

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.

If worksheets or macros are developed that use information/data gained as part of the validation they will be included in the review process. These will be reviewed against the validation documentation for accuracy and to verify that there are no errors in data transfers.

Validation Process

a. Plan
    i. The Validation of a new procedure will generally be initiated by the Unit Lead, Supervisor or their designee. A validation plan will be provided to the section's Manager(s) and Director. The DNA TL will also approve validations in the DNA Unit. This shall include a description of the proposed validation, a summary of the experiments planned and criteria to evaluate the results.

ii. At a minimum, the Section Manager and Director must approve the proposal before the validation is initiated. In DNA the DNA TL must also approve the proposed plan. This approval can be documented through signing and dating the plan.
The General Laboratory Form "Laboratory Method Validation Summary Form" will be started to capture the approval of the plan by the appropriate parties. Minimally the header and "Plan Approved" box will be signed by the appropriate parties. This form can be found the "General Laboratory Forms" folder in the QMS.

iii. When implementing a validation plan, if a change to the plan is required, the changed plan will be approved by the Section Manager 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.
    1. This may include sample matrices, temperature variances, environmental factors or other based on the technique.
- For quantitative methods Limit of Detection (LOD), Limit of Quantitation (LOQ) and factors affecting Uncertainty will be addressed.
- Other requirements of validation experiments may be found in Unit specific SOPs as appropriate.

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 (swabbing, 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. <u>Summary of Results</u>

i. A validation summary will be completed and provided to the Section Manager and Director (and TL in DNA). The results will contain a table of contents and be organized 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
- Precision (reproducibility/repeatability)
- Linearity

- Range (e.g., limit of detection, upper and lower limit of quantitation etc…)
- Uncertainty of the results (measurement uncertainty will be evaluated for all methods providing a quantitative result such as a measurement or concentration)
- Selectivity/Specificity
- Robustness

It is noted that not all methods lend themselves to the above assessments and some methods may require other parameters to be assessed.

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 procedure where appropriate.

d. New Procedure

After approval of the validation, a new or modified procedure will be issued through the QMS following the normal approval and publishing process. This will be completed prior to its use in casework. A draft version of the procedure may be used for training purposes, however if changes are made to the draft prior to publishing, examiners will be notified of the changes. This procedure will be based on the results of the validation and include interpretation guideline if applicable.

e. Training

Prior to initiating casework personnel will be trained and authorized in the new method. Training will include a competency test to the level of participation for the individual. For the DNA Unit, the new method must be incorporated into the training program, prior to the training beginning. Upon successful completion of the training and competency test a 'Personnel Authorization' workflow will be initiated to obtain the authorization from the Director for the work.

For personnel intimately involved in the validation activities, the Supervisor or Section Manager or their designee may document that the validation activities served as demonstration of competency (or the TL in DNA). This will be documented in a competency memo and a 'Personnel Authorization' workflow will be initiated in the QMS to obtain the Directors approval for authorization of the employee for the task.

f. CT DSS Forms and Validation Documentation

When a validation is to be initiated, the General Laboratory form "Laboratory Method Validation Summary Form" will be completed to ensure all necessary information is captured. This form can be found in the "General Laboratory Forms" folder in the QMS.

The Director will be the final approver on this form. The completed form will be maintained with the validation documentation.

The validation documentation will be scanned and added to the QMS, in the Section specific folder within the Quality folder.