Calibration and Maintenance of DNA Equipment

Table of Contents

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1 INTRODUCTION

This document establishes the procedures used to maintain the performance of laboratory equipment, including software, to ensure the accuracy and reliability of the data generated for forensic DNA examinations and DNA databasing.

2 SCOPE

These procedures apply to all DNA personnel that are responsible for ensuring the reliability of equipment that have an effect on the quality of forensic DNA examinations (i.e., body fluid identification, nuclear DNA, and mitochondrial DNA) and DNA databasing (i.e., individual characteristic databasing).

3 RESPONSIBILITIES

Personnel with responsibilities related to ensuring the reliability of equipment will execute the responsibilities identified in the appropriate FBI Laboratory level 1 documents (i.e., LAB-100, and LAB-200) and those identified below.

A. The DNA Unit Chiefs will ensure that adequate resources (e.g., personnel, funding, materials) are provided for the calibration, performance verification (PV), preventative maintenance (PM), and/or repair of DNA equipment.
B. The DNA Quality Assurance/Quality Control (QA/QC) personnel and additional DNA Unit personnel, as appropriate, will:
    - Coordinate, manage, perform and/or facilitate the calibration, PV, PM, general maintenance, and/or repair of laboratory equipment.
    - Ensure equipment that requires calibration, PM, and/or PV is entered in the appropriate Sample Tracking and Control Software (STACS).
    - Ensure that instructions for PV and general maintenance are maintained.
    - Maintain calibration, PM, PV, and repair records for laboratory equipment.
    - Advise the Technical Leader of current activities or problems related to these procedures.
C. DNA personnel are responsible for the day-to-day operation and control of the equipment present in their laboratory work area(s). DNA personnel will perform the following functions as necessary:
    - Conduct and record PV and/or general maintenance of equipment as required or as needed.
    - Clearly identify equipment as "out of service" when an item cannot be performance verified, successfully calibrated, the calibration due date has been exceeded, and/or essential maintenance is required.
    - Notify the QA/QC personnel whenever an instrument or item of equipment is “out of service” and/or requires calibration, PV, maintenance, and/or repair.

4 PROCEDURES

A. DNA personnel will comply with the requirements in the FBI Laboratory *Quality Assurance Manual* (QAM/LAB-100) and the *Laboratory Operations Manual* (LOM/LAB-200) for all equipment used for casework and DNA databasing.

B. Calibration, PM, and PV intervals are based upon manufacturers' operating guidelines, historical performance experience, and/or the performance check requirements of the *Quality Assurance Standards for Forensic DNA Testing Laboratories* or *Quality Assurance Standards for DNA Databasing Laboratories* (QAS), as applicable. Calibration, PM, and PV of equipment will be routinely conducted at the minimum frequency listed in Appendix A.
    1. Calibration, maintenance (i.e., PM, general maintenance), and PV records will be maintained in accordance with the level 1 documents except that DNA discipline records are generally retained on BASNet, not UNet.
    2. PV and general maintenance procedures performed by DNA personnel will be maintained, generally within the relevant DNA procedures or within the appropriate STACS.
    3. Equipment maintained at an offsite location outside of DNA unit permanent control may be used so infrequently that calibration, PM, or PV at the minimum frequency defined in Appendix A may not be practicable. In these instances, the performance of critical equipment will be verified to ensure accurate functionality prior to the use of the equipment in forensic examinations or DNA databasing. DNA personnel will ensure appropriate performance check records are retained for each piece of equipment to be used.

C. Software having an effect on the accuracy or validity of DNA examinations and/or DNA databasing will be considered equipment and must meet the applicable requirements of the level 1 documents.
    1. Additional software testing requirements prior to using the software for casework or DNA databasing are listed in section 4.5.

D. Critical equipment are those whose accurate functionality directly affects the results of the analysis and requires calibration, certification, or performance check prior to use and periodically thereafter. Equipment that is deemed critical:
    - Traceable Thermometer used for PVs
    - Balance
    - Incubator/Thermal Shaker used in casework or databasing analysis
    - Thermal Cycler Temperature Verification System
    - Thermal Cyclers
    - Rapid DNA Instruments/System
    - Real Time PCR Instruments (aka Sequence Detection Systems)
    - Robotic Workstations/Robotic Systems
    - Capillary Electrophoresis Instruments
    - Mechanical Pipettes
    - Expert systems software approved for use at NDIS

E. An item of equipment will be clearly identified as "out of service" if successful calibration cannot be achieved, the minimum frequency since the last successful calibration, PM, or PV has been exceeded, and/or maintenance or repair is required.
    1. Equipment deemed “out of service” will not be used in casework examinations or DNA databasing until it successfully undergoes calibration, PM, and/or PV, as applicable. The “out of service” status may be indicated by use of a physical sign and/or by placing the equipment in a maintenance status in STACS.

4.1 External Calibration

A. DNA personnel do not perform calibration services. When required, calibrations will be performed by external vendors.
    1. The DNA units do not have measuring equipment whose measurement uncertainty affects the validity of the DNA results or that require metrological traceability of results; therefore, calibration is used as a form of performance check.
    2. The DNA units will use calibration laboratories accredited to ISO/IEC 17025, when possible. However, since the measurement equipment is not used to establish or maintain metrological traceability, the calibration report is not required to include the pre and post adjustment/repair data.
        i. When applicable, accreditation certificates will be reviewed to ensure the services appear on the provider’s scope of accreditation. The approval of these external service providers will be recorded in STACS and/or in the FBI Laboratory’s records for externally provided products and services.

B. The types of equipment that undergo external calibration as the method for ensuring accurate functionality are:
    - Balance
    - Electronic Temperature Monitoring Component(s)
    - Multichannel Verification System (MVS) Calibrator Plate
    - Handheld pipette
    - Thermal Cycler Temperature Verification System (if used)

C. Newly acquired or repaired equipment that require calibration but are not accompanied by current calibration records will be calibrated or performance verified prior to being initially placed into service or returning to service for casework or databasing.

4.2 Maintenance

A. General maintenance (e.g., routine cleaning, greasing O-rings, replacing reagents and consumables) of instruments and equipment is generally performed by QA/QC or appropriately trained personnel. Equipment does not require PV after general maintenance.

B. PM services will be performed by external vendors according to manufacturer recommendations or specifications.
C. The types of critical equipment that undergo regular PM are:
    - Rapid DNA Instruments/System
    - Robotic System (e.g., EZ1)
    - Robotic Workstation (e.g., Tecan)
    - Real Time PCR Instruments (aka Sequence Detection Systems)
    - Capillary Electrophoresis Instruments
D. Critical equipment requires a PV following PM. (See section 4.3.)
E. Newly acquired critical equipment will undergo PM during the next full cycle (i.e., calendar year) after the date the equipment is placed into service. Equipment under a service warranty may be exempt from PM requirements during the term of the warranty.
F. The types of equipment that are not considered critical but may undergo regular PM to ensure proper performance are:
    - Biological Cabinet (hood)
    - Microscope
    - Refrigerator / Freezer
    - Punch Instrumentation
    - Artel MVS plate reader
    - Agilent Bravo Liquid Handler
    - Plate Sealer

4.3 Performance Verification

A. PV may be performed by DNA personnel or an external vendor.
B. PV refers to those methods used to assess the functionality of laboratory instruments, equipment, or reagents that affect the accuracy and/or validity of forensic sample analysis and may be accomplished in several ways which include but are not limited to:
    - Running a known control or reference sample through the equipment/process as detailed in the applicable technical procedure
    - Performing a diagnostic check on the equipment
    - Performing a self-test
    - Temperature verification or temperature monitoring

C. The types of instruments and equipment that will undergo PV prior to being initially placed into use for forensic casework examinations or DNA databasing, following annual PM (if applicable), repair, or service and prior to returning to use in forensic casework examinations or DNA databasing are:
    - Incubator/Thermal Shaker used in an analytical procedure
    - Rapid DNA Instruments/System
    - Robotic System (e.g., EZ1)
    - Robotic Workstation (e.g., Tecan)
    - Real Time PCR Instruments (aka Sequence Detection Systems)

- Thermal Cycler
- Capillary Electrophoresis Instruments

D. PV procedures for critical equipment are generally maintained in the relevant technical procedure. However, the method of PV following maintenance or repair should be commensurate with the repair conducted and therefore, may be simplified, if appropriate.
E. Transport of critical equipment will be done in accordance with manufacturer recommendations. If transported, DNA personnel will ensure proper functioning of the critical equipment, which may include a PV, prior to use in forensic examinations or DNA databasing.
F. If the PV indicates that the item is not performing as expected, the instrument or piece of equipment will be clearly identified as "out of service" and not used in forensic examinations or DNA databasing until it can be demonstrated that it is performing as expected.
G. Equipment that is not in regular use may be put into storage in accordance with any manufacturer recommendations. If equipment in storage misses a required calibration, PM, or PV, the performance of critical equipment will be verified to ensure accurate functionality prior to the use of the equipment in forensic examinations or DNA databasing.

4.4 Temperature Monitoring

A. The types of equipment that undergo regular temperature monitoring as a method of PV are:
    - Refrigerator / Freezer
    - Incubator (including Thermal Shaker)
B. Temperature monitoring will be performed by an electronic temperature monitoring system or by DNA personnel utilizing digital thermometers. Thermometers used for temperature monitoring will be traceable and therefore appropriately calibrated and/or undergo PV at the required intervals.
C. The temperature ranges for incubators (including thermal shakers), refrigerators, and freezers are as follows:
    - Incubators (as indicated in the applicable SOP +/-3°C)
    - Refrigerators (4°C +/-3°C)
    - Freezers (-20°C +/-10°C)
    - Ultracold freezers (-80°C +/-10°C)
D. When temperature monitoring is performed by an electronic temperature monitoring system, the temperature will be recorded at least once per day by the system. The electronic temperature monitoring system will collect and maintain this information.
    1. If the temperature of the equipment goes outside of the acceptable range, DNA personnel will be notified (typically via email by the ViewLinc software system).
    2. During normal business hours, QA/QC personnel will check the piece of equipment and determine what action, if any, is necessary.

3. Additional notifications may occur outside of normal business hours. When practicable, notified DNA personnel will take action necessary to mitigate any potential detrimental impact to evidence, samples, or reagents.

E. If a temperature reading is outside the acceptable range, the settings may be adjusted as needed.
    1. If an appropriate temperature can be reestablished, the records will reflect at least the final temperature observed.
    2. If the appropriate temperature cannot be established or maintained, the records will reflect at least the final temperature observed and QA/QC personnel will be notified.

4.5 Software

A. Software having a direct effect on the quality of DNA examinations and DNA databasing will be checked to ensure it meets the specifications (i.e., Biometrics Analysis Section DNA Network [BASNet] baseline configuration document(s) and applicable SOP requirements) prior to being placed into or returning to service. Checking the baseline configuration documents(s) is typically done by or in conjunction with a unit's information technology (IT) technical point of contact (TPOC) or the DNA IT support contractors.

B. The lab's IT support team (i.e., Information Technology & Strategy Unit [ITSU] and IT contractors) ensures the BASNet is protected from unauthorized access, safeguarded against tampering and loss, operated in an environment that complies with laboratory specifications, and maintained in a manner that ensures the integrity of the data and information. A System Security Plan is maintained by the IT support team.
    1. System failures are recorded to include the appropriate immediate and corrective actions. Generally, the IT helpdesk software (i.e., Ivanti) is used to record system failures and the actions and resolution to address the failures.

C. Where practicable, software permissions will be restricted to prevent unintended adjustments from invalidating test results.

D. New software or new modules of existing software and modifications to software that will be used for forensic examinations or DNA databasing will be evaluated to assess the suitability of the software for its intended use in the laboratory and to determine the necessity of validation studies or software testing. This evaluation will include the determination of which studies will and will not be conducted and will be documented as part of the software validation or testing summaries or records.
    1. New software or new modules of existing software that are used as a component of instrumentation, used for the analysis and/or interpretation of DNA data, or used for statistical calculations will be appropriately validated in accordance with the QAS. (See Appendix B)
    2. New software used for forensic examinations or DNA databasing but that do not impact the analytical process, interpretation, or statistical calculations will be tested for functionality.

3. Modifications to software used as a component of instrumentation, for the analysis and/or interpretation of DNA data, or for statistical calculations will be evaluated to determine if the modifications result in a major or minor revision to the software and, therefore, what testing is necessary prior to use for forensic examinations or DNA databasing.
    i. A major revision requires validation in accordance with the QAS prior to implementation.
    ii. A minor revision that does not impact the analytical process, interpretation, or statistical algorithms requires at a minimum, a functional test.
4. Modifications to STACS or other information management systems used for the collection, processing, recording, reporting, storage, or retrieval of data will be authorized, recorded, and validated/tested before implementation.

E. Software validation studies and software testing may be shared by all laboratory locations. The summary of the applicable shared validation data will be accessible at each location. Site-specific reliability testing will be conducted at each laboratory location.
F. Expert system software approved for use at NDIS will be subject to recertification in accordance with the NDIS Operational Procedures and the frequency requirements in Appendix A.
G. Software testing and validation studies will be recorded and records retained.
H. QAS required software testing and validation records will be reviewed and approved by the Technical Leader prior to implementation.

5 REFERENCES

Federal Bureau of Investigation, *Quality Assurance Standards for DNA Databasing Laboratories*, latest revision.

Federal Bureau of Investigation, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, latest revision.

National DNA Index System (NDIS) Operational Procedures Manual, latest revision.

6 REVISION HISTORY

Revision	Issued	Changes
00	09/15/22	Reformatted DNA 608-12 into new template and assigned new Doc ID. Minor modifications throughout. Edits to software section to reflect QAS. Added appendix B.
01	06/30/23	Added exception for calibration records stored on BASNet not Unet. Added review and approval of external service providers is recorded in STACS. Changed 3730 PV to semiannual.
02	12/16/24	Additions for NGS implementation. Accreditation approvals can be in lab records. TVS is no longer used but keeping listed in 4.1 as (if used). Removed liquid nitrogen generator and added Bravo and Plate Sealer to non-critical PM list. Removed Cyberscend contractor and added ITSU. Removed 3130 and liquid nitrogen generator from App A. Added MiSeq FGx Control Software, CLC, GeneMarker and EmPop to App B.

7 APPENDIX A: CALIBRATION, PM, AND PV FREQUENCY REQUIREMENTS

Type of Instrument / Equipment	Required Action	Minimum Frequency
Balance	Calibration	Annually
Capillary Electrophoresis (CE) Instrument [i.e., 3500xL Genetic Analyzer, 3730 DNA Analyzer]	PV	3500: Annually 3730: Semi-Annually
	PM	Annually
Electronic Temperature Monitoring Component	Calibration	Annually
Electrophoresis Detection Instrument [i.e., Agilent 2100 Bioanalyzer]	PV	Annually
Expert System Software (approved for use at NDIS)	PV	Quarterly
Incubator / Thermal Shaker	PV	Quarterly
Microscope	PM	Annually
Multichannel Verification System (MVS) Calibrator Plate	Calibration	Annually
Pipette	Calibration	Annually
Punch Instrument	PM	Annually
Rapid DNA Instrument	PM	Annually
	PV	Quarterly
Real Time PCR Instruments	PV	Annually
	PM	Annually
Refrigerator / Freezer	PV	Daily (work days)
Robotic System (for Extraction) [i.e., QIAcube, EZ1]	PV	Annually
	PM	Annually
Robotic Workstation (for Quant/Amp Set-up and NGS bead clean-up & normalization) [i.e., Tecan]	PV	Quarterly
	PM	Annually
Robotic Workstation (for CE Set-up) [i.e., Agilent Bravo]	PV	Annually
Thermal Cycler	PV	Annually
Thermal Cycler Temperature Verification System	Calibration	Annually
Thermometer	PV	Annually
UV Crosslinker	PV	Annually

PM = Preventative Maintenance PV = Performance Verification

Quarterly will occur 4 times per year, generally every 3 months.
Semi-annually will occur twice per year, generally every 6 months.
Annually will occur within a calendar year.

8 APPENDIX B: QAS SOFTWARE CATEGORIES

In general, the software or software that operates the equipment listed below will be validated or tested as appropriate in accordance with QAS for the applicable category.

A. Software used as a Component of Instrumentation
   - EZ1
   - QIACube
   - 7500 HID
   - Tecan – EvoWare, Fluent Control
   - Agilent – Vworks (Bravo)
   - Agilent Bioanalyzer
   - Data Collection
   - BSD punch
   - Artel – MVS Data Manager
   - MiSeq FGx Control Software

B. Software used for the Analysis and/or Interpretation of DNA data
   - Agilent Bioanalyzer
   - 7500 HID
   - Gene Mapper ID-X
   - Sequencher
   - STRmix
   - CLC Genomics Workbench
   - GeneMarker

C. Software used for Statistical Calculations
   - STRmix
   - PopStats
   - Y-HRD
   - EmPOP