General Maintenance

1. Purpose

To describe the general maintenance of the Laboratory and the instruments used within.

2. Summary

The Laboratory and the instruments used require maintenance to assure proper function, accuracy, and cleanliness. Decontamination, calibration, and individual instrument maintenance is addressed. The Maintenance schedule is listed for each instrument/equipment.

3. Procedure

1. Documentation of the completion of annual, biennial, and biannual maintenance, as well as dates of the most recent calibrations and dates of annual performance checks will be in the Equipment Log for each site.
2. Documentation of the completion of as used, daily, weekly, and monthly maintenance will be maintained in the DOC-336 Instrument Maintenance and Decontamination Log, or its equivalent.
    a. Completed DOC-336 Instrument Maintenance and Decontamination Log, or its equivalent document used, will be maintained for 2 accreditation cycles.
3. Maintenance will be completed by an assigned staff member.
    a. Maintenance will be scheduled by the DNA Technical Leader and communicated to the assigned individual and their reporting manager.
4. Equipment/Instruments that are out of service must be clearly marked to indicate they may not be used for casework and to indicate their period of validity after calibration or maintenance is completed.
5. Critical instruments as applicable to the FBI QAS are identified in the Equipment Log and are listed below:
    a. Pipettes
    b. NIST Traceable Thermometer
    c. Incubator/Heat blocks used in analytical procedures
    d. Extraction Robots
        i. EZ1 Advanced XLs
        ii. QIACube Connect
    e. Thermal Cyclers
        i. ProFlex
    f. Benchmark
    g. 3500 Genetic Analyzer
    h. MiSeq FGx
6. Equipment/Instruments may be moved within their designated room without requirement for a performance check prior to resuming casework.
    a. Significant movement within the post amplification room for the ABI 3500 and MiSeq FGX may require a performance check before resuming operations.
        i. Extent of performance check is at the designation of the DNA Technical Leader.

b. Any equipment removed from the laboratory (sent out for service) and returned to the laboratory requires a performance check before resuming casework.

7. Any instruments/equipment that are to be removed from the Identification Lab must be decontaminated thoroughly with bleach prior to removal from this area.

Equipment Maintenance

1. Pipettes
    a. Calibrate pipettes annually or as needed.
        i. The external vendor used for calibration MUST be ISO/IEC 17025 accredited for calibration of volume measurements within the volume ranges required by IMF pipettes.
        ii. Pipettes may be shipped to a qualified vendor in increments to minimize impact on casework.
            1. Calibration will be scheduled by the DNA Technical Leader, or their designee
            2. Shipment and receipt of the pipettes will be performed by an assigned Forensic Molecular Biologist, Laboratory Manager or Forensic Genetic Genealogy Analyst.
        iii. The following elements may be included in the calibration:
            1. As Found and As Left values must be provided.
            2. Evaluation at 3 points (high, mid, and low ranges) for all pipettes
            3. Passing calibration will be based on the manufacturer specifications.
        iv. Certificates will be reviewed by the DNA Technical Leader, or their designee, prior to the pipettes being returned to use on casework.
            1. Certificates will be stored in the Quality Management System folder of the shared drive, in the Instrument Tracking folder.
            2. All pipettes not passing calibration will be removed from service.
        v. Pipettes will be checked to ensure proper function prior to being returned to casework if they are shipped to another location for calibration.
            1. The check will be documented on DOC-338 Pipette Post Calibration Check Spreadsheet
            2. The spreadsheet for each year will be saved in the file for the year the calibration was performed.
            3. The parameters that will be checked include:
                a. The pipette's ability to seat and eject an appropriately sized pipette tip.
                b. The pipette's ability aspirate and dispense liquid at various volumes within the pipette volume range (low, mid, high).
            4. If the pipette fails to meet the expected criteria, it will not be put in to service and the DNA Technical Leader must be notified immediately so they can initiate the appropriate corrective action.
    b. Gravimetric interim checks may be performed as necessary to assess the functionality of a pipette.
        i. Accuracy can be checked by weighing water: 1mL=1g.
            1. Low, mid, high (Example ranges)
                a. 10: 1ul, 5ul, 10ul
                b. 100: 10ul, 50ul, 100ul

c. 1000: 100ul, 500ul, 1000ul
2. Repeat a minimum of 3 times.
3. Evaluate results for %CV and/or Standard Deviation against manufacturer specifications.
    a. A folder with manufacturer specifications for the pipettes in use at IMF is maintained in the in the Quality Management System Folder of the shared drive, in the Instrument Tracking folder, for reference for these values.

ii. Documentation of the gravimetric check will be maintained in a separate spreadsheet in the Quality Management System Folder of the shared drive, in the Instrument Tracking folder when performed.

2. Thermomixers/heat block
    a. Check temperature annually or as needed using a NIST Traceable Thermometer:
        i. Place the probe of the NIST Traceable Thermometer inside the thermomixer/heat block
        ii. Temperature ±3 degrees is allowed.
        iii. Document the results of the performance check in the Equipment Log

3. NIST Traceable Thermometer
    a. Calibration by an external vendor is required once per year after the expiration of the initial manufacturer calibration.
        i. In lieu of calibration: A new NIST Traceable thermometer may be purchased when the calibration of the current thermometer lapses.

4. Refrigerator/Freezer
    a. Temperatures can be viewed at https://temperaturestick.com/sensors/dashboard.
        i. Refrigerator temperature range should be between 0°C and 10°C.
        ii. Freezer should not go higher than 0°C.
        iii. Alerts are set to notify a responsible party when the temperature is out of the expected range or if the sensor is offline for more than 3 hours.
    b. TempSticks will be performance checked annually using the NIST traceable thermometer.
        i. The check will be assigned by the DNA Technical Leader and will require the NIST Traceable thermometer probe to be placed in the refrigerator/freezer for a minimum of 30 minutes before the reading is taken. Corrective action must be taken for any TempSticks that do not report ±3°C for refrigerators or ±5°C for freezers.
        ii. The results will be documented in the Equipment Log.

Instrument Maintenance

1. Instrument maintenance procedures are provided by the instrument manufacturer. Refer to each instrument maintenance Standard Operating Procedure.

2. QIAcube Connect
    a. MNT-205 Qiagen QIAcube Connect Maintenance
        i. Run Daily Maintenance protocol at the end of each day of use
        ii. Run Monthly Maintenance protocol each month

3. EZ1 Advanced XL
    a. Refer to MNT-201 Qiagen EZ1 Advanced XL Instrument Maintenance
        i. Clean trays and piercing unit after each run
        ii. Wipe O-rings and Perform UV each day of use
        iii. Grease O-rings as necessary
        iv. Perform volume verification using gravimetric check of final elution volume, if necessary

4. Quant Studio 5
    a. Refer to MNT-204 ABI Quant Studio 5 Maintenance
        i. Decontaminate wells quarterly or as needed.
        ii. Complete Region of Interest (ROI), Background, and dye calibration biennially (every 2 years)
        iii. Performance Check: Run an Rnase P plate or process a NIST standard reference material or NIST traceable standards.

5. Thermal Cycler(s)
    a. Refer to MNT-202 ProFlex Thermal Cycler Maintenance
        i. Decontaminate wells quarterly or as needed.
        ii. Complete a Self-Verification Test annually.
        iii. Perform a performance check of the instrument as outlined in the annual performance check procedure below. A calibrated temperature verification unit may be used, if available, to satisfy the temperature verification element of a performance check, as required by the FBI's QAS.

    b. Refer to MNT-206 Benchmark Thermal Cycler Maintenance
        i. Decontaminate wells quarterly or as needed.
        ii. Annually
            i. Perform a performance check of the instrument as outlined in the annual performance check procedure below. A calibrated temperature verification unit may be used, if available, to satisfy the temperature verification element of a performance check, as required by the FBI's QAS.

6. 3500 Genetic Analyzer
    a. Refer to MNT-203 ABI 3500 Genetic Analyzer Maintenance
        i. Check dashboard before each run.
        ii. Weekly
            1. Check Stored Capillary Arrays
            2. Run Wash Pump and Channels wizard.
            3. Restart computer and instrument
        iii. Monthly
            1. Flush the Pump Trap
            2. Empty the condensation container and water trap waste container.
            3. Run Performance check.
            4. Clean the Autosampler and Drip Tray.
            5. Check Disk Space.

6. Defragment the Hard Drive.

iv. Annually

1. Perform a performance check of the instrument as outlined in the annual performance check procedure below.

7. MiSeq FGX
   a. Maintenance is required within the processing for the MiSeq - Refer to the MiSeq FGX processing procedure for direction.
   b. Annually
      i. Perform a performance check of the instrument as outlined in the annual performance check procedure below.

8. Tapestation 4200
   a. Refer to the Tapestation User guide for instructions on the preventative maintenance.
   b. Annual Preventative Maintenance includes:
      i. Needle replacement
      ii. Electrophoresis probe block replacement
      iii. Firmware and software update, if applicable
      iv. General cleaning of instrument
      v. Execution of functional hardware test
   c. The TapeStation 4200 Controller software will actively flag the due date based on the instruments' throughput and time elapsed.

9. Qubit Fluorometer
   a. Regular maintenance is not required per the user manual. Functionality of the instrumentation will be monitored through the use of process controls.

10. NovaSeq 6000
    a. The performance of the instrument will be tracked through the continual evaluation of the performance metrics established in the validation and will include:
       i. %Q30 – Must be greater than 75% of bases at 2 x 150 bp
       ii. Yield – The number of bases generated in the run should be greater than 2400-3000 Gb per run.
       iii. % Pass Filter – The number should be greater than 75%.
       iv. Cluster Count: Value should be greater than 16-20 billion ($16 – 20 \times 10^9$) per run
       v. Phasing/Prephasing: Values should be > 0.1/0.1 for read 1 and Read 4 to ensure the reagents are performing well and the temperatures are not varying.
       vi. % Occupied by %Pass Filter: Indicates if the run is over or underloaded to help assess the library pools that are being created
       vii. Q Score Distribution: Graphical capture of the Q30 scores. Should be greater than 75% of bases greater than or equal to Q30.

Annual Performance Check

1. The Annual Performance Check is required every calendar year.
   a. The annual performance check will be planned and scheduled by the DNA Technical Leader
      i. The annual performance check will be planned and initiated in the last quarter of the calendar year

   b. The annual performance check will be completed by the assigned individual(s)
      i. The assigned individual(s) must be authorized to perform work on the instrument being performance checked
2. The annual performance check will be utilized to assess the performance of the following items for continuing quality output.
   a. Policies, Procedures and Processes.
   b. Equipment.
3. During this performance check, the laboratory will run NIST-traceable standard reference materials or samples with known profiles (such as previously completed proficiency test samples or validation samples) through the following processes using the prescribed reagents for the following instrumentation:
   a. DNA Extraction.
      i. EZ1 Advanced XL
      ii. QIACube Connect
   b. DNA Quantification
      i. QuantStudio 5
   c. DNA Amplification
      i. Thermal Cyclers
         1. Note: If the thermal cycler has multiple blocks, samples MUST be placed in each block to ensure the proper assessment of the entire thermalcycler.
   d. DNA characterization
      i. MiSeq FGx
      ii. 3500 Genetic Analyzer
4. This performance check should include running all appropriate controls.
5. The annual performance check should demonstrate the results indicated below and those results will be reviewed by the DNA Technical Leader.
   a. Amplification/Load should obtain usable DNA profile that must be 100% concordant to the expected profile.
   b. All controls must function appropriately (positive control, negative control/reagent blank, and ladder).
6. The file associated with the performance check will be stored in the following location: Laboratory - Files\Quality Management System\Equipment Tracking\Annual Performance Check in a folder specific to the year of the performance check.
   a. Files include:
      i. Worksheets
      ii. Raw data files
      iii. Electropherograms/allele bar charts for all samples and controls
      iv. Allele Summary Tables
      v. Reference documentation for the expected results
         1. If the expected results are included in a NIST Certificate of Analysis, validation file, or proficiency test documentation, and the samples are clearly marked to indicate their source, then the files are not required to also be stored in the performance check folder but can remain in their original folder.
      vi. Documentation to support the review of the results by the DNA Technical Leader
   b. The files will be stored in this location starting at the initiation of the performance check, to ensure access to the files as the process is on-going.

4. References

MiSeq System Guide
Applied Biosystems® 3500/3500xl User Manual
ProFlex™ PCR System User Guide
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance
Qubit® 3.0 Fluorometer User Guide
Agilent 4200 TapeStation System Manual

5. Definitions

N/A