

## 6 Equipment Quality Control and Maintenance

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#### 6.1 Purpose

In order to provide and maintain the quality of the work provided in the DNA section, it is necessary to ensure laboratory equipment is in good working order. Routine quality control and maintenance accomplishes this. The calibration intervals listed below are generally considered to be the minimum appropriate in each case, providing that the equipment is of good quality and of proven stability and the laboratory has both the equipment capability and expertise to perform adequate internal checks. More frequent checks are not discouraged. If there is any question concerning the reliability of an instrument or piece of equipment, a maintenance check should be performed immediately.

Full records must be maintained and be readily available for inspection. Documentation must include the numerical result, date of calibration, analyst's signature, and any other relevant observations. The section supervisor and QA Manager are responsible for ensuring all systems are checked annually.

The following equipment must be maintained and subjected to quality control measures: **water baths, dry baths**, microcentrifuges, thermometers, genetic analyzers, balances, thermal cyclers (including real-time PCR instruments), hoods, autoclaves, **and robots (including extraction, qPCR/PCR, and post-amp set-up automation)**. Maintenance and quality controls for these instruments is detailed in the HPD Crime Laboratory SOP – Biology or the Quality Manual (balances), except for **water and dry baths, microcentrifuges, hoods**, thermal cyclers, genetic analyzers, and **DNA automation instruments (QIAGEN BioRobot EZ1 Advanced XLs and TECAN workstations)**.

Details for these instruments are provided below.

**Each additional or modified critical instrument must be subjected to a performance check prior to its use in casework. Software upgrades without significant software modifications must also be subjected to a performance check prior to its use in casework.**

#### 6.2 Equipment

##### **Water Bath and Dry Bath**

**Critical water baths and dry baths are dedicated equipment whose temperature is routinely maintained at 37-100°C for DNA procedures.**

##### **Observed Temperature**

**Observe temperature reading on the thermometer and on the display. Each day for which a block is used on casework, a temperature must be recorded (both days should an incubation go overnight). Update the temperature log with the temperature from the bath reading plus or minus the calibration-based adjustment (that will be marked next to each individual block). Blocks used in incubations that incorporate Proteinase K should be between 55 and 60°C. If not within the acceptable range, discontinue with use on casework samples and use the control knobs to adjust the temperature to the acceptable range, or document the correct setting**

needed to obtain the correct temperature. Blocks should not be used on casework until an acceptable temperature reading is obtained. If the temperature is not stable, repair or replace the water bath or dry bath. Please refer to the equipment manual for specific instructions on temperature adjustment and bath maintenance.

#### Water Condition

For water baths, the water should be clear and clean with no evidence of bacterial/fungal growth or rust. If the water becomes dirty, discard and clean water bath. Replenish with water.

#### Maintenance Procedure

The following procedure should be performed as needed:

1. Decant and discard water.
2. Wash inside of water bath with detergent.
3. Rinse well with water.
4. Fill bath with the appropriate quantity of water. Clear bath or another algaecide may be used.

#### Microcentrifuges

Microcentrifuges are bench top, unrefrigerated centrifuges that have been designed for centrifugation of 1.5 ml tubes, test tubes, and Microcon tubes. These microcentrifuges are equipped with fixed angle rotors. The maximum speed is specified in the operations manual for each centrifuge. The relative centrifugal force can be determined as outlined in the manufacturer's instructions, if required.

The microcentrifuges will be cleaned at least one time per year or as needed. Please consult the appropriate equipment manual for specific instructions on maintenance and operation of the microcentrifuge.

Centrifuge housing, rotor chamber, and rotor accessories should be cleaned with neutral cleaning agents (pH 7.0), such as DNAway, at least one time per year. All parts must be dry prior to use.

#### Fume and Laminar Flow Hoods

The hood, when used with proper technique, is effective in reducing the potential for exposure of both product and personnel to airborne biological or particulate chemical agents. The laminar flow hood contains a HEPA filter. These hoods will be evaluated for proper airflow annually by an external vendor.

The hood must be re-certified at least once a year and after every filter change or maintenance action or at the operator's discretion.

A qualified technician must certify the cabinet.

No analysis should be performed on the interior of the cabinet unless the cabinet has been disinfected and expected to be biologically clean.

### Genetic Analyzers

The Applied Biosystems (AB) genetic analyzer AB 3130xl is a capillary electrophoresis instrument used to separate DNA fragments based upon size and fluorescent tags. The main parts of the instrument include the CCD camera, laser, pump block with automated polymer delivery, heat block, autosampler, and syringe(s). All of these parts must be working properly to ensure accurate and usable results are obtained. The laboratory has a Planned Maintenance agreement with Applied Biosystems for the maintenance of these instruments. This plan allows for 1 planned-maintenance visit per year by an Applied Biosystems Field Service Engineer.

To ensure the Genetic Analyzers are working properly after repairs, an allelic ladder must be run and analyzed under normal conditions to ensure all peaks are being called appropriately as indicated by the manufacturer. The GeneMapper ID™ data from this run must be printed and placed in the appropriate logbook. If the ladder does not contain all of the appropriate alleles after several injections, the technical leader will be notified and a service call scheduled with ABI. Casework samples cannot be run on the instrument during the time in which a valid allelic ladder cannot be generated.

A new spectral calibration must be made once every 6 months or as needed for each instrument in the laboratory. Follow the manufacturer's guidelines for making the matrix and or spectral and verifying its accuracy. These guidelines can be found in the operations guides located in the post-amp room. Additionally, the User Bulletin for the AB 3130xl dated January 2003 (or an updated version) should be consulted; this bulletin is maintained with the operations guides. The AB 3130xl may be run using the current array if 14 or more of the capillaries pass the spectral. If an array is used where some of the capillaries failed the spectral, a note should be attached to the 3130xl indicating which capillary in the array is bad and samples should not be injected on that capillary.

The instrument pump block and syringe should be cleaned as needed. Please consult the operations guides for each instrument for specific instructions on how to clean the instrument. Additionally, the Wizards for the 3130xl Collection Software can be consulted for useful information on how to maintain the 3130xl.

Implementation validation for any new genetic analyzers must include:

1. Precision study comparing 10 positive control injections from the same run and preparing a summary report to include justification of the +/- 0.5 bp window.
2. Sensitivity (dilution) study ranging from 0.156 ng to 2 ng of template DNA and prepare a summary report.
3. Accuracy study using a CTS (or other commercial) proficiency test with published results OR using a NIST-SRM.

Any repair, service, or calibration of the Genetic Analyzers will require a performance check prior to reintroduction to casework analysis.

## Thermal Cyclers

Thermal Cyclers automate the polymerase chain reaction (PCR) for amplifying DNA. The cycler contains a programmable heating and cooling block that performs repeated temperature cycling profiles on samples contained within the block.

The sample block and exterior surfaces should be cleaned at least once every 6 months. Temperature calibration, temperature uniformity, and diagnostic tests must be performed once every 6 months. Follow manufacturer's instructions for performing these tests. The cyclers have established parameters for determining pass or fail; the machine will report a pass or fail result at the end of every test. Any variations outside of established parameters will necessitate recalibration or repair of the instrument by the manufacturer or a qualified service technician. If the cycler is damaged or not functioning, either the manufacturer or a qualified service technician may repair the instrument.

Implementation validation for new cyclers must include:

1. Temperature calibration, temperature uniformity, and diagnostic tests.
2. Amplification and analysis of the **amplification** kit positive control for concordance.

**Any repair, service, or calibration of the Thermal Cyclers will require a performance check prior to reintroduction to casework analysis.**

### Real-time PCR Thermal Cyclers

The 7000 and 7500 are specialized Thermal Cycler units used to detect amplified product in real-time. These units contain a programmable heating and cooling block, several filters, and a halogen lamp. The units are used in conjunction with quantification kits to estimate the amount of DNA in a given sample.

**Each month, while in use on case work, the 7000 and 7500 should be subjected to the following maintenance:**

- function test
- block cleaning per the manufacturer's instructions
- Background calibration
- Performance check post calibration (run a standard curve and negative sample)

Additionally, the hard drive should be defragmented quarterly and a Region of Interest (ROI) calibration should be performed for the instrument every 6 months.

The analyst will change the bulb if it is determined to have burned out or weakened. Remember to always wear gloves and avoid direct contact when handling the bulb.

**Refer to the individual instrument maintenance guides located in the post-amplification room for instructions on proper instrument maintenance.**

Validation of new real-time PCR thermal cyclers should include at a minimum:

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1. A precision study determining the quantity of the same DNA sample at least two times on the same plate. Several plate runs may be used to add data points.
2. A reproducibility study using the same series of DNA samples run on at least three different plate runs

Any repair, service, or calibration of the Real-Time PCR Thermal Cyclers will require a performance check prior to reintroduction to casework analysis.

### DNA Automation Instruments

#### QIAGEN BioRobot EZ1 Advanced XLs

##### Regular Maintenance

This type of maintenance is required after each run on the EZ1 Advanced XL and should be recorded as being performed on the maintenance log.

1. Clean the Piercing unit by:
  - a. Making sure that the sample-preparation waste is removed and discarded appropriately.
  - b. Close the EZ1 Advanced XL door.
  - c. Press “2” in the main menu to select the manual function.
  - d. Press “3” to choose the “clean” operation.
  - e. Press “Start” and the piercing unit will lower.
  - f. Open the EZ1 door and wipe the piercing unit with a Kimwipe moistened with 70% Ethanol. The piercing unit is ***SHARP***, so wearing two pairs of gloves is recommended. ***\*DO NOT USE BLEACH ON THE EZ1!\****
  - g. Follow by wiping the piercing unit with a Kimwipe moistened with diH<sub>2</sub>O.
  - h. Close the EZ1 door and Press “ENT”; the piercing unit will return to its home position.
  - i. Press “ESC” to return to the main menu.
  - j. Record that the Piercing unit has been cleaned on the daily maintenance log.
2. Open the EZ1 door and wipe the inside down with a Kimwipe moistened with 70% ethanol and repeat with diH<sub>2</sub>O. ***\*DO NOT USE BLEACH ON THE EZ1!\****
3. U.V. the inside of the EZ1 instrument by:
  - a. At the end of a run the option for starting a U.V. decontamination appears. Make sure that the above steps have been accomplished before starting a U.V. run.
  - b. Make sure the door is closed.
  - c. In the main menu press “1” to select the UV light function.
  - d. Use the keys “0” through “9” to set the duration of the decontamination time. A 20-30 minute setting will be sufficient.
  - e. Press “Start” to turn on the “UV” lamp. The door may not be opened until the UV lamp has been shut off and cooled for ~3 minutes.

##### Weekly Maintenance

This should be performed at the end of every week and recorded as being performed on the weekly maintenance log.

1. At the end of every week each instrument should be **SHUT DOWN** to cycle the instrument and then restarted before the next run the following week. The ON/OFF switch is located on the back left of each instrument.

#### Additional Maintenance

This should be performed at least twice a month (weekly if the EZ1 is running at maximum capacity) and be recorded as being performed on the maintenance log.

1. Grease the O-rings by:
  - a. Applying a small amount of silicon grease to the larger opening of a filter-tip with the smaller tip cut off
  - b. Apply the silicon grease to the surface of the O-rings, located in the back portion of the instrument, by placing the top of the filter-tip with the grease onto the pipettor head, and rotate the tip on the pipettor head to distribute the grease evenly. Note: The filter-tips should sit flush against the upper white plastic bar if the O-rings are properly greased. There should not be a gap. Excess or insufficient grease can affect the performance of the EZ1 Advanced XL.
  - c. After applying the grease, take a Kimwipe and wipe below each O-ring to remove any excess grease that has accumulated.
  - d. Record that the O-rings have been greased on the monthly maintenance log.

#### Annual Maintenance

Preventative maintenance will be performed once a year on or around the anniversary of the installation of the instruments, which for HPD is 06/24/09. After annual preventative maintenance but before a reintroduction into casework, the EZ1 Advanced XLs will be subjected to a performance check that establishes proper extraction in a contamination free environment.

Any repair, service, or calibration of the QIAGEN BioRobot EZ1 Advanced XLs will require a performance check prior to reintroduction to casework analysis.

#### **TECAN Freedom EVO<sup>®</sup>100 and TECAN Freedom EVO<sup>®</sup>150 Workstations**

Refer to the quantification (#8), amplification (#9), and Genetic Analyzer Sample preparation (#10) SOPs for routine TECAN Freedom EVO<sup>®</sup>100 and TECAN Freedom EVO<sup>®</sup>150 Workstation maintenance. In addition to the daily and weekly maintenance, annual preventative maintenance by Tecan will be performed. After annual preventative maintenance but before a reintroduction into casework, the TECAN Freedom EVO<sup>®</sup>100 and TECAN Freedom EVO<sup>®</sup>150 Workstation will be subjected to a performance check that establishes proper sample handling in a contamination free environment.

Any repair, service, or calibration of the TECAN Freedom EVO<sup>®</sup>100 and TECAN Freedom EVO<sup>®</sup>150 Workstations will require a performance check prior to reintroduction to casework analysis.

### 6.3 Quality Check of the DNA analysis system through NIST-SRM

A NIST-SRM or a NIST-traceable stain must be extracted, quantified, amplified, and analyzed one time per year, **or whenever substantial changes are made to a procedure**, to verify the **DNA section's** system of analysis as required in the QAS. This sample **is to be** verified to have typed correctly by comparison with the known DNA profile. If the correct profile is not obtained from the NIST-SRM or NIST-traceable stain, the **DNA** technical leader **will** be informed and the analyst should work backwards to determine where any deficiencies in the system exist. Analysis of casework samples in the **DNA section will** cease until **the** NIST-SRM or NIST-traceable stain can be typed correctly.