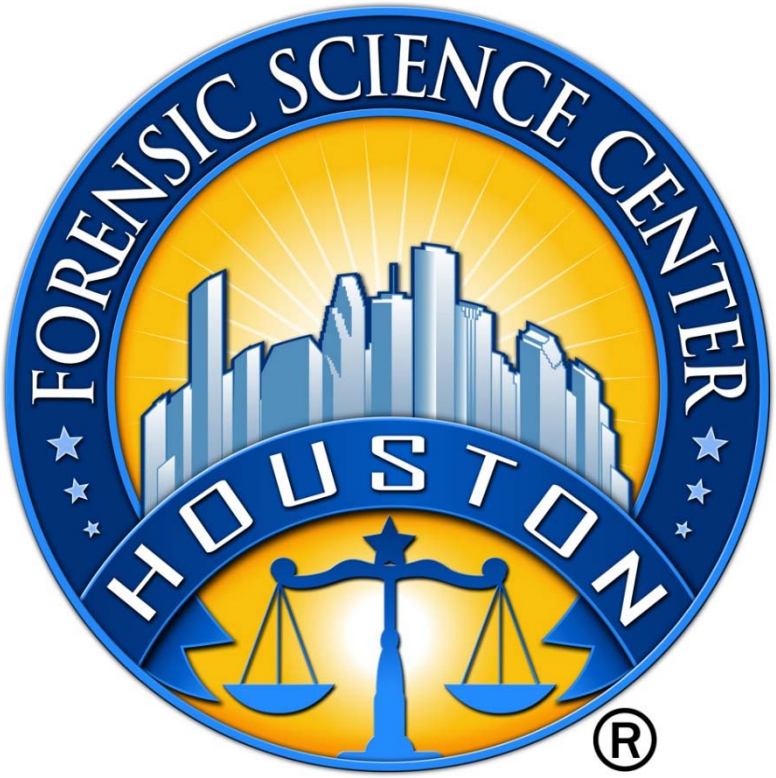


# Biology/DNA

# DNA Maintenance SOP

Biology/DNA Division

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# 1. Equipment Overview, Quality Control, and Maintenance

## 1.1. Purpose

1.1.1. In order to provide and maintain the quality of the work provided by the DNA section, it is necessary to ensure laboratory equipment is in good working order. Routine quality control and maintenance accomplishes this. The maintenance 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 shall be performed immediately.

1.1.2. Full records must be maintained and be readily available for inspection. Documentation must include the numerical result, date of maintenance, analyst's signature and/or initials, and any other relevant observations. The Operations and Training Supervisor is responsible for ensuring all systems are checked annually. Whenever practical, equipment that requires calibration shall be labeled with the date when last calibrated and the date or expiration criteria when recalibration is due.

1.1.3. The following equipment must be maintained and subjected to quality control measures: autoclaves, balances, DNA automation equipment, genetic analyzers, heat blocks, hoods, microcentrifuges, pipettes, thermal cyclers (including real-time PCR instruments), thermometers, and thermomixers. Maintenance and quality controls for these instruments are detailed in the HFSC Forensic Biology - Biology SOP Manual (autoclaves, pipettes, and thermometers), the HFSC Forensic Biology - DNA SOP Manual (DNA automation equipment, genetic analyzers, heat blocks, hoods, microcentrifuges, thermal cyclers (including real-time PCR instruments), and thermomixers) or the Quality Manual (balances, hoods, and pipettes).

1.1.4. Details for these instruments are provided below. User manuals may also be referenced for specific instruction.

1.1.5. 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.

## 1.2. Equipment

### 1.2.1. **Heat Blocks and Thermomixers**

1.2.1.1. Heat blocks and thermomixers are dedicated equipment whose temperature is routinely maintained at 56-70°C for DNA procedures.

1.2.1.2. Observed Temperature

1.2.1.2.1. Observe temperature reading 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 display. Displayed temperature must be within +/- 5°C of the targeted temperature. If not within the acceptable range, discontinue use on casework samples and use the control knobs to adjust the temperature to the acceptable range. Units shall not be used on casework until an acceptable temperature reading is obtained. If the temperature is not stable, repair or replace the heat block or thermomixer. Please refer to the equipment manual for specific instructions on temperature adjustment and maintenance.

1.2.1.3. Annual Heat Block Performance Checks

1.2.1.3.1. Using a NIST-traceable thermometer, measure the set temperature in each of the six regions.

1.2.1.3.2. The NIST-traceable thermometer should be within +/- 2°C of the set temperature for each reading.

1.2.1.3.3. If not within the acceptable range, repeat the temperature readings. If not within the acceptable range a second time, notify a Forensic Biology Supervisor or the Technical Leader. In addition to notifying the supervisor or Technical Leader, the heat block shall be marked out of service.

1.2.1.4. Annual Thermomixer Performance Checks

1.2.1.4.1. Using a NIST-traceable thermometer, measure the set temperature three times.

1.2.1.4.2. The NIST-traceable thermometer should be within +/- 2°C of the set temperature for each reading.

1.2.1.4.3. If not within the acceptable range, repeat the temperature readings. If not within the acceptable range a second time, notify a Forensic Biology Supervisor or the Technical Leader. In addition to notifying the supervisor or Technical Leader, the thermomixer shall be marked out of service.

### 1.2.2. **Microcentrifuges**

1.2.2.1. Microcentrifuges are bench top, unrefrigerated centrifuges that have been designed for centrifugation of microcentrifuge tubes, test tubes, Microcon tubes, and 96-well plates. These microcentrifuges are equipped with fixed angle rotors. The relative centrifugal force can be determined as outlined in the manufacturer's instructions, if required.

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

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

1.2.3. **Fume and Laminar Flow Hoods**

1.2.3.1. The laminar flow hood, when used with proper technique, is effective in reducing the potential for exposure of both product and personnel to airborne biological agents. The laminar flow hood contains a HEPA filter.

1.2.3.2. The fume hood, when used with proper technique, is effective in protecting personnel to exposure of chemicals.

1.2.3.3. The hood shall be re-certified at least once a year, after maintenance, or at the operator's discretion. A qualified technician must certify the cabinet.

1.2.3.4. Proper airflow may be checked periodically, as needed, by the safety specialist using a flow meter.

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

1.2.3.6. Hoods that have not been certified may be used for sample preparation or handling, as the contained space serves as protection for the samples from the environment.

1.2.4. **Thermal Cyclers**

1.2.4.1. 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.

1.2.4.1.1. **ProFlex™ PCR System**

The user interface consists of a touchscreen with a graphical display that shows the time, status, and temperature for each run. A touchscreen keypad allows you to enter information into fields on the display screen. The instrument has interchangeable blocks, and detects which module is present on the system automatically. Only the 1x96 Well Sample Block is used for current applications.

1.2.4.2. <u>Every 6 months</u>

- The sample block and exterior surfaces shall be cleaned at least once every 6 months.
- Block Verification Tests must be performed once every 6 months.
    - This includes Temperature verification, Temperature Non-uniformity, Heated Cover and Cycle Performance
    - The Proflex has established parameters for determining pass or fail; the machine reports a pass or fail result for all tests except Cycle Performance. The Cycle Performance will display the result but does not indicate pass. It will indicate a failing results.
    - Any variations outside of established parameters shall 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.

1.2.4.3. Implementation validation for new cyclers must include:

1.2.4.3.1. Temperature verification, Temperature Non-uniformity, Heated Cover and Cycle Performance. Different models of Thermal cyclers may have different names for these tests.

1.2.4.3.2. Amplification and analysis of the amplification kit positive control for concordance.

1.2.4.4. Any repair, service, or calibration of the thermal cyclers requires a performance check prior to reintroduction to casework analysis. At a minimum, the performance check of the thermal cyclers shall include a passing Block Verification Tests.

1.2.5. **Real-time PCR Thermal Cyclers (Applied Biosystems 7500 Real-Time PCR System)**

1.2.5.1. The 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. A tungsten-halogen lamp directs light to each well on the reaction plate. The light excites the fluorescent dyes in each well of the plate. During the run, the CCD camera detects the fluorescence emission. The SDS software obtains the fluorescence emission data from the CCD camera and applies data analysis algorithms.

1.2.5.2. Each month, while in use on case work, the Function test must be performed.

1.2.5.3. Every quarter, while in use on case work, the following maintenance shall be performed on the 7500:

- Background and Optical calibration
- Block cleaning per the manufacturer's instructions, as warranted by background calibration results
- Performance check post calibration (run a standard curve and negative sample)
- The hard drive should be defragmented if necessary

1.2.5.4. Every 6 months, a Region of Interest (ROI) calibration and the Pure Dye Calibration shall be performed.

1.2.5.5. The analyst shall 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.

1.2.5.6. The following maintenance must be performed after a bulb change:

- ROI Calibration
- Background and Optical Calibration
- Pure Dye Calibration
- Performance Check

1.2.5.7. Validation of new real-time PCR thermal cyclers shall include at a minimum:

1.2.5.7.1. 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.

1.2.5.7.2. A reproducibility study using the same series of DNA samples run on at least three different plate runs

1.2.5.8. Any repair, service, or calibration of the real-time PCR thermal cyclers shall require a performance check prior to reintroduction to casework analysis. At a minimum, the performance check shall include a run with acceptable standard controls.

1.2.5.9. Maintenance may be performed before its next scheduled period if there is a concern about the quality of the data.

1.2.5.10. The laboratory has a planned maintenance agreement with Thermo Fisher for the preventative maintenance of these instruments. This plan allows for 1 planned-maintenance visit per year by an Thermo Fisher Field Service Engineer.

### 1.2.6. DNA Automation Instruments

#### 1.2.6.1. QIAGEN BioRobot EZ1 Advanced XLs

Before the first run of the day

Open the EZ1 door and wipe the inside down with DNA Away followed by ethanol.

***DO NOT USE BLEACH ON THE EZ1!***

UV the inside of the EZ1 instrument:

- 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 and the door is closed
- In the main menu press "1" to select the UV light function
- Use the keys "0" through "9" to set the duration of the decontamination time. A 20-30 minute setting is sufficient
- 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

After each run

Clean the piercing unit by:

- Making sure that the sample-preparation waste is removed and discarded appropriately
- Close the EZ1 Advanced XL door
- Press "2" in the main menu to select the manual function
- Press "3" to choose the "clean" operation
- Press "Start" and the piercing unit lowers
- Open the EZ1 door and wipe the piercing unit with DNA Away followed by ethanol. The piercing unit is ***SHARP***, so wearing two pairs of gloves is recommended ***DO NOT USE BLEACH ON THE EZ1!***
- Close the EZ1 door and Press "ENT"; the piercing unit returns to its home position
- Press "ESC" to return to the main menu
- Record that the Piercing unit has been cleaned on the daily maintenance log

Open the EZ1 door and wipe the inside down with DNA Away followed by ethanol.

***DO NOT USE BLEACH ON THE EZ1!***

U.V. the inside of the EZ1 instrument:

- 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 and the door is closed
- In the main menu press "1" to select the UV light function

- Use the keys “0” through “9” to set the duration of the decontamination time. A 20-30 minute setting is sufficient
- 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 shall be performed every week and recorded as being performed on the weekly maintenance log. Every week each instrument must be ***shut down*** to cycle the instrument and then restarted. The ON/OFF switch is located on the back left of each instrument. Examine the drip pans and clean as needed.

Biweekly Maintenance

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

- Grease the O-rings by:
- Applying a small amount of silicon grease to the fingertips of clean gloves
- Apply the silicon grease to the surface of the O-rings, located in the back portion of the instrument
- Place the fingertip of glove with the grease onto the pipettor head and rotate on the pipettor head to distribute the grease evenly
- After applying the grease, take a Kimwipe and wipe below each O-ring to remove any excess grease that has accumulated
- Record that the O-rings have been greased on the maintenance log

Annual Maintenance

Preventative maintenance shall be performed once a year. Any repair, service, or calibration of the QIAGEN BioRobot EZ1 Advanced XLs shall require a performance check prior to reintroduction to casework analysis. At a minimum, the performance check shall consist of a run using a known source of DNA in each EZ1 position.

### 1.2.6.2. **Tecan Freedom EVO®100 and Tecan Freedom EVO®150 Workstations**

General Maintenance

- As necessary, the machine surface can be cleaned with DNA Away followed by alcohol (70% ethanol or 100% isopropanol (2-Propanol)), water, or a weak detergent such as Liquinox. Strong detergents can dissolve carrier and worktable surface coatings. Upon cleaning, thoroughly dry the work area to prevent residual cleaner remaining on the surface.
- If the instrument is leaking, switch it off immediately and eliminate the source of leakage.

At the beginning of each day when the instrument is in operation

- Check the liquid system for leakage
- Check the tubing connections and tighten as necessary
- Check the syringes and plunger lock screws and tighten, if necessary
- Check the disposable tips (DiTi) cones for deposits and clean as necessary (Tecan Freedom EVO 150 only)
- Ensure DiTi gold cone is tight using TECAN wrench*(Tecan Freedom EVO 150 only)
- Check the system liquid container and fill with diH2O as necessary (allow diH2O to degas prior to use, overnight is preferred)
- Check the waste liquid container and empty as necessary
- Flush the liquid system and check for air bubbles
- Replenish DiTis (Tecan Freedom EVO 150 only)
- Ensure the worktable is clean and free of clutter

Prior to each application run

- Flush the liquid system and check for air bubbles
- Clean hardware, carriers, and racks, if needed
- Check the disposable tips (DiTi) cones for deposits and clean and tighten as necessary* (Tecan Freedom EVO 150 only)
- Check the DiTi waste bag and empty as necessary (Tecan Freedom EVO 150 only)
- Clean nested DiTi waste slide if necessary (Tecan Freedom EVO 150 only)
- Double-check the number of available DiTis and replace additional tip racks as necessary (Tecan Freedom EVO 150 only)
- Check system liquid level

**Check the DiTi cones are finger tight before each run. Use the wrench tool daily by loosening the DiTi cone and then finger tighten back. Then use wrench tool to turn the cone an additional one-quarter turn. It is imperative that you loosen and then re-tighten when doing this. Just using the wrench tool could result in over tightening.*

After each run

- Flush the system with system liquid and watch for bubbles and/or leaks
- Visually inspect all tubing, tubing connections, and syringes for leakage
- Clean the worktable, carriers, and racks as necessary

- Check the system liquid container and fill with diH2O as necessary
- Check the waste liquid container and empty as necessary
- Replenish DiTis (Tecan Freedom EVO 150 only)

Weekly

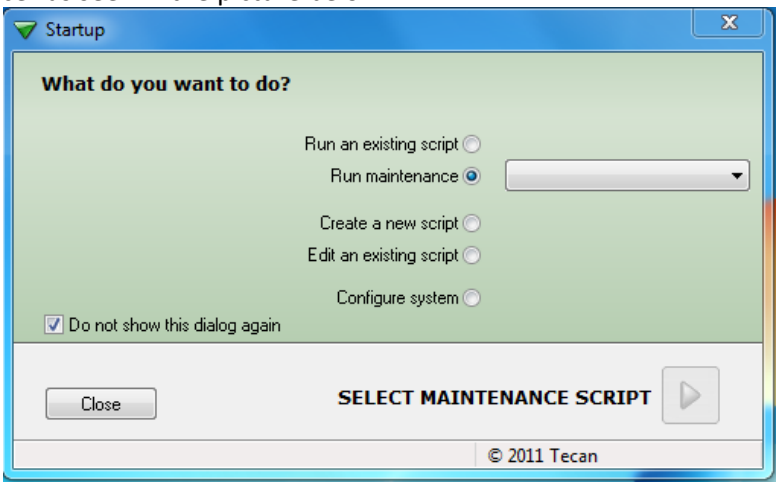
- Empty system liquid container and clean, as needed
- Empty waste container and clean, as needed
- Shut down/Restart instrument computer
- Run a weekly flush of the system if the system is not being used on a regular basis

Performance Check

Any repair, service, or calibration of the Tecan Freedom EVO®100 and Tecan Freedom EVO®150 Workstations shall require a performance check prior to reintroduction to casework analysis. At a minimum, for the Tecan Freedom EVO®150 Workstation, the performance check shall include a quantification set-up if the quant script is edited, an amplification set-up if the amp script is edited, and a quantification set-up for any servicing or changes that are not script-specific (e.g., z-max edits for tubes), including preventative maintenance. For the Tecan Freedom EVO®100, a CE plate must be set-up. All controls must yield acceptable results.

Tecan 150 Maintenance Scripts

To perform some of the maintenance tasks on the Tecan 150, the startup screen provides an option entitled "Run Maintenance" as seen in the picture below:

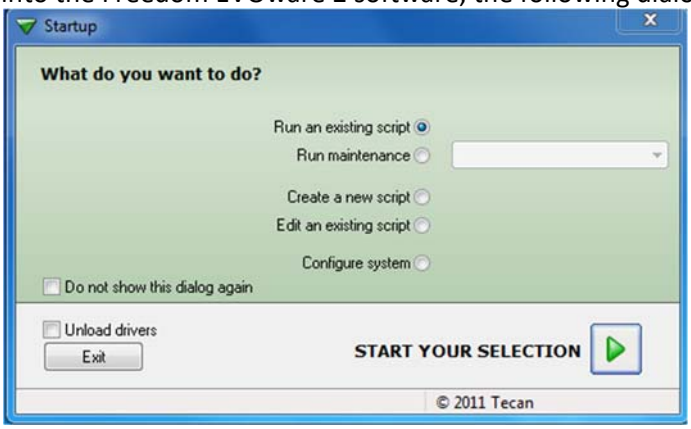



| Protocol | Action |
|---|---|
| Daily Start Up | Cycles through the system start up prompts, flushes the system, removes any DiTis attached to the arm, and moves the liquid handling arm to the home position |

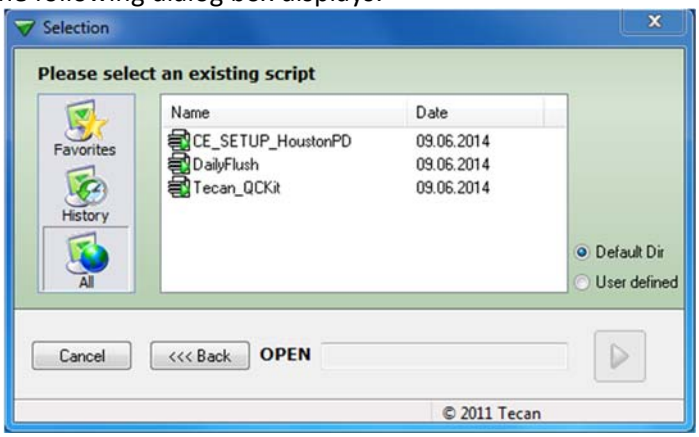
| | |
|---|---|
| Drop DiTis | Moves the arm to the DiTi disposal chute and removes any attached DiTis |
| Flush | Flushes the liquid system |
| Set 200 tip position | Repositions the 200 µL DiTi tip position via user input to the first available tip as its new starting point |
| Set 50 tip position | Repositions the 50 µL DiTi tip position via user input to the first available tip as its new starting point |

Tecan 100 Daily Flush Protocol Script

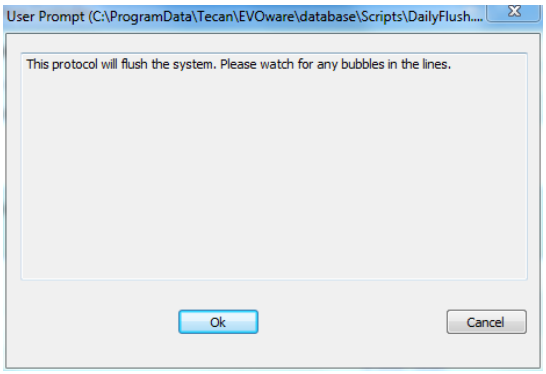
At the beginning of each day, a daily flush script must be run to check the system for leaks and/or bubbles. Upon logging into the Freedom EVOware 2 software, the following dialog box displays:



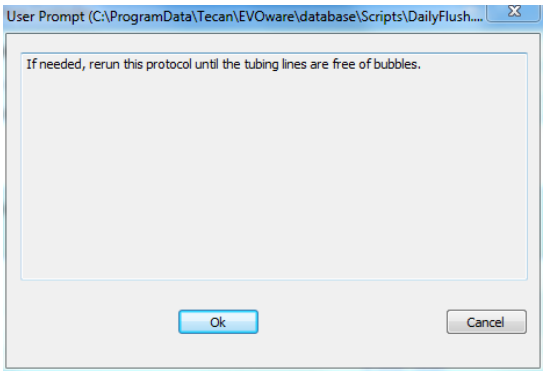

Choose the "Run an existing script" option and click the **MAKE YOUR SELECTION** green arrow in the bottom right corner. The following dialog box displays:




Select the "DailyFlush" script and click the green arrow to continue. A prompt displays showing the following message:




The instrument proceeds through several steps of flushing. Once complete, the following dialog box displays:




Click **OK** to return to the original script selection screen.

#### 1.2.6.2 QIAGEN QIAcube Robotic Workstation

Performed before the first run of the day

- Clean the following with DNA Away followed by ethanol:
    - Worktable
    - Shaker rack
    - Lab ware tray
    - Heating adapter
    - Reagent bottle rack
    - Waste drawer liner
    - Inside and outside of QIAcube (except for the door). Do not use alcohol or alcohol-base disinfectants to decontaminate the QIAcube door.
- Clean QIAcube door with laboratory grade water, taking care that no liquid runs down the touchscreen.
- To clean the touchscreen, moisten a soft lint-free cloth with 70% ethanol or a mild disinfectant and carefully wipe the display. Wipe dry with a paper towel.

Performed after every run

- Replace lids of reagent bottles and close tightly.
- Empty waste drawer contents into biohazard trash, and wipe with DNA Away followed by Laboratory grade water.
- Remove all used lab ware from worktable and dispose into biohazard trash.
- Clean the following with DNA Away followed by ethanol:
    - Worktable
    - Shaker rack
    - Lab ware tray
    - Heating adapter
    - Reagent bottle rack
    - Waste drawer liner
    - Inside and outside of QIAcube (except for the door). Do not use alcohol or alcohol-base disinfectants to decontaminate the QIAcube door.
- Clean QIAcube door with laboratory grade water, taking care that no liquid runs down the touchscreen.
- To clean the touchscreen, moisten a soft lint-free cloth with 70% ethanol or a mild disinfectant and carefully wipe the display. Wipe dry with a paper towel.

Weekly Maintenance

Every week each instrument must be ***shut down*** to cycle the instrument and then restarted. The ON/OFF switch is located on the back left of each instrument.

Monthly Maintenance

Clean optical sensor and tip adapter with a soft lint-free cloth moistened with laboratory grade water.

Bi-Annual Maintenance (Refer to the QIAcube® User Manual, version 1.2 or later)

- Clean the centrifuge rotor and buckets
- Perform tightness test to check pipetting system

Annual Maintenance

Preventative maintenance shall be performed once a year.

Performance Check

Any repair, service, or calibration of the QIAGEN QIAcube Robotic Workstations shall require a performance check prior to reintroduction to casework analysis. At a minimum, the performance check shall consist of a run using a known source of DNA and a reagent blank control.

#### 1.2.6.3 Hamilton ID STARlet/STAR

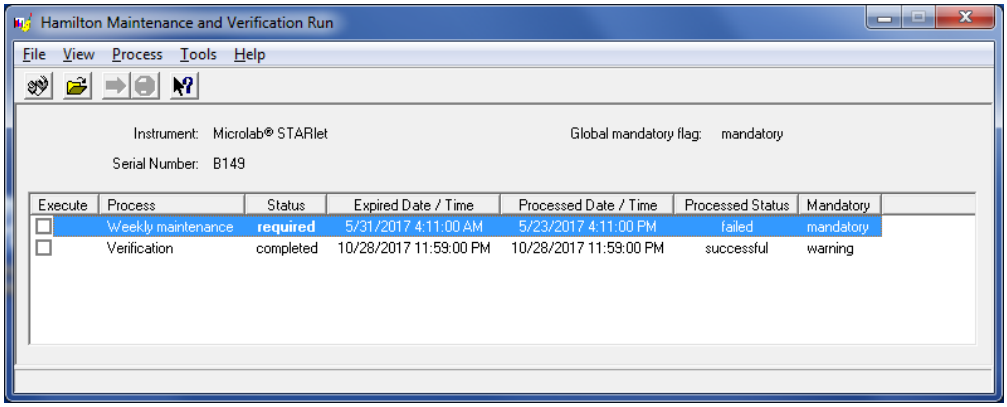
***DO NOT USE BLEACH ON THE HAMILTON ID STARlet/STAR!***

Daily Maintenance

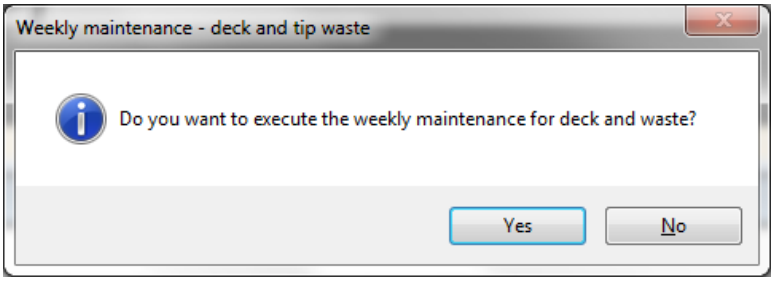
- Daily Before Run
  - Run Weekly Maintenance Method
    - Clean deck
    - Emptying the tip waste
    - Check the tightness of the 1000µl pipetting channel

Note: The Weekly Maintenance Methods contains all necessary tasks as the Daily Maintenance Method

Open the Microlab STAR Maintenance & Verification

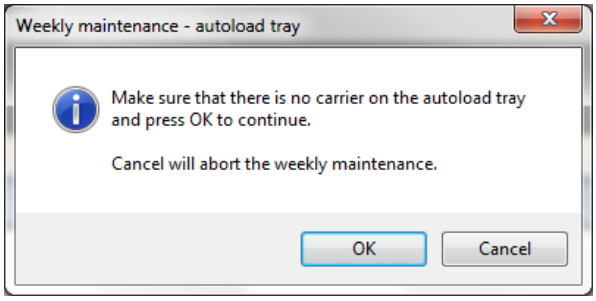



Select Weekly Maintenance, then Process and Run

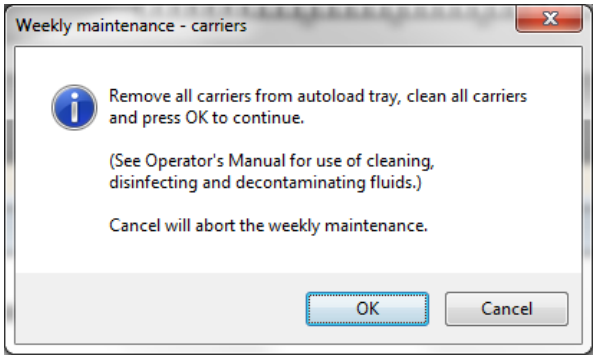



The front cover (the hinged Plexiglas window that shields the instrument in front) can be opened for user intervention.

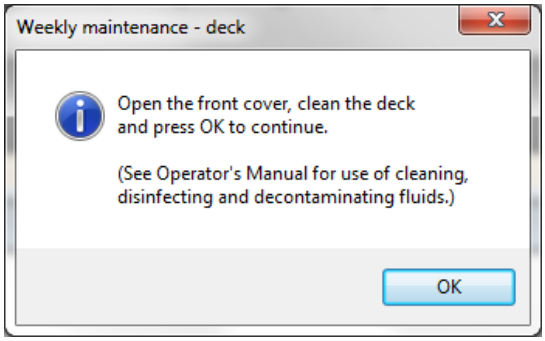
A series of prompts will direct you through the maintenance. Follow the window prompts and commands to complete the maintenance



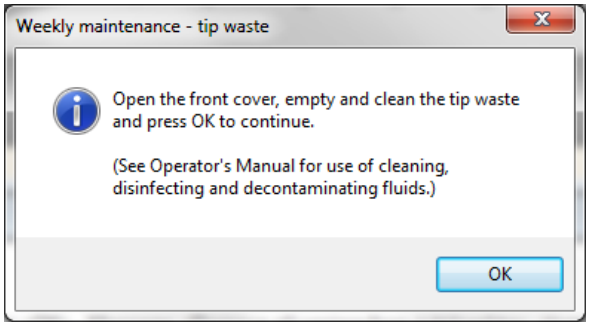

The instrument will unload all carriers on the instrument.




Clean the carriers with an alcohol wipe.

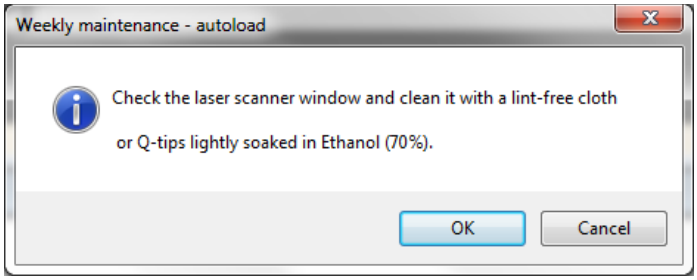



Remove the carriers and clean the deck as necessary.

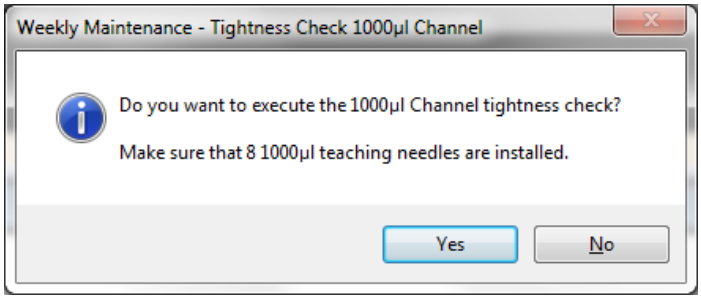



Remove the tip eject plate of the tip waste station and clean it with an alcohol wipe as necessary. Remove the frame that holds the plastic bag in place and discard the plastic in the laboratory's contaminated waste. Pull a new plastic bag over the frame and re-attach it. Put the clean tip eject plate back in place.

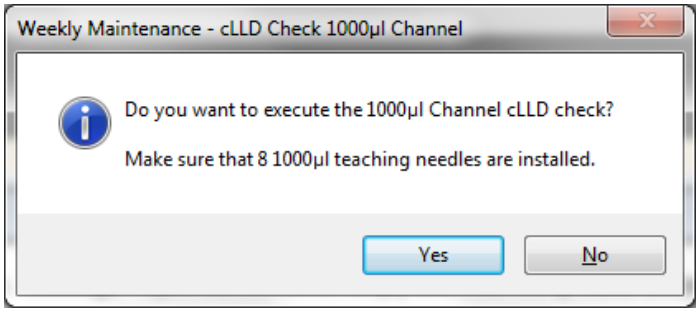
**ATTENTION**
*The tip waste is always to be regarded as contaminated.*



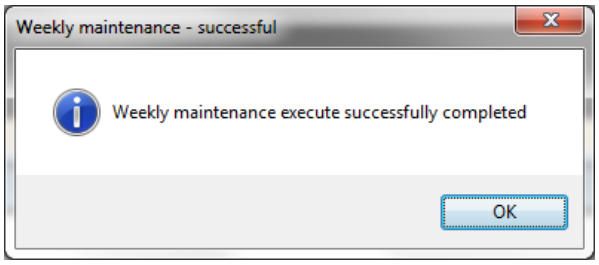

At least once a week the Barcode Reader should be cleaned with alcohol. Record on the maintenance log when the reader is cleaned.




The instrument will utilize the teaching needles to check the channel tightness.




The instrument will utilize the teaching needles to check the channel capacitive liquid level detection.




Daily After Run

- Cap resin tube
- Empty wash buffers
- Discard all used disposable labware
- Wipe down any obvious spills/drips

Weekly Maintenance

- Clean the barcode reader with EtOH
- Cleaning of the pipetting head: stop disk, O-ring, tip eject sleeve
- Cleaning of the covers and autoload protecting ribbon

**ATTENTION**
*Take care that no liquid gets inside the tip channel. Whenever it is necessary to move Channels on the X-Arm, move them gently by pushing close to their Y-slide. Never force them as this may lead to damage. If possible, turn on the instrument as this will result in a smoother motion when Channels have to be moved on the X-Arm.*

- Clean the front and side cover with an alcohol wipe and wipe dry.
- Clean the autoload protecting ribbon with an alcohol wipe. Wipe without exerting pressure.

**ATTENTION**
*Do not spray directly at the Autoload unit or at electrical boards or connectors.*

Monthly Maintenance
Clean the X-guide shaft behind the upper front cover with a dry cloth.

Annual Maintenance
Preventative maintenance shall be performed once a year.

Performance Check
Any repair, service, or calibration of the Hamilton ID STARlet/STAR shall require a performance check prior to reintroduction to casework analysis. At a minimum, the performance check shall consist of a run using eight known sources of DNA (one for each pipette head) and a reagent blank control.

## 1.3 Genetic Analyzers

1.3.1 The 3500xl 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, and autosampler. All of these parts must be working properly to ensure accurate and usable results are obtained. The laboratory has a planned maintenance agreement with Thermo Fisher for the preventative maintenance of these instruments. This plan allows for 1 planned-maintenance visit per year by an Thermo Fisher Field Service Engineer.

1.3.2 A new spectral calibration must be made once every 6 months or as needed for each instrument in the laboratory. If an array is used where some of the capillaries failed the spectral, a note must be attached to the 3130xl or 3500xl maintenance records indicating which capillary in the array is bad and samples must not be injected on that capillary.

1.3.3 The instrument pump block and syringe must be cleaned as needed. The Wizards for the 3130xl and 3500xl Collection Software can be consulted for useful information on how to maintain the instruments.

1.3.4 Any repair, service, or calibration of the genetic analyzers requires a performance check prior to reintroduction to casework analysis. At a minimum, the performance check shall include the injection of an allelic ladder, a positive control, and a negative control with the appropriate results.

1.3.5 Maintenance Calendar

1.3.5.1 The maintenance calendar is a monthly or daily view of the routine maintenance tasks scheduled for the instrument. When a task is due to be performed, it is listed in the Maintenance Notifications list in the Dashboard.

1.3.5.1.1 In the Dashboard click **Maintain Instrument** toggle key.

1.3.5.1.2 From the left-hand pane, under Planned Maintenance, click **Schedule.**

1.3.5.1.3 A set of AB recommended tasks are scheduled in the calendar. The priority of factory tasks can be changed, but they cannot be removed nor the frequency altered.

1.3.5.1.4 Create calendar entries.

1.3.5.1.4.1 Click **Create** and follow prompts.

1.3.5.2 Review the Maintenance Notifications log, which is a history of all notifications messages and the action taken for each task (completed or dismissed).

1.3.5.2.1 From the Dashboard, click **Maintain Instrument.**

1.3.5.2.2 From the left-hand pane, under Planned Maintenance, click **Notifications Log.**

1.3.6 Daily Preventive Maintenance on the 3500xl for DNA Fragment Analysis When the Instrument is in Use

1.3.6.1 Check and make sure that the maintenance log has been filled out for that day. If the daily maintenance log has not been completed, perform the required daily instrument maintenance. Daily maintenance should be performed by the first person using the 3500 xL Genetic Analyzer each day.

1.3.6.2 Clean the assemblies, anode buffer container, and cathode buffer container. Ensure that the outside of the assemblies is dry.
1.3.6.3 Check consumables on the Dashboard. Refer to the gauges on the Dashboard to see the status for anode buffer container, cathode buffer container, and polymer.
1.3.6.4 Changing the anode buffer container (ABC)
1.3.6.4.1 Remove ABC from storage and check for expiration date to make sure it is not expired prior to or during intended use.
1.3.6.4.2 Allow ABC to come to room temperature prior to first use.
1.3.6.4.3 Ensure that all buffer is moved to the larger side of the ABC prior to removing the seal. Verify that the buffer level is at or above the fill line and check that the seal is intact. Tilt the ABC slightly to make sure most of the buffer is in the larger side of the container. There should be less than 1 ml of buffer remaining in the smaller side of the container. Verify that the buffer is at the fill line.
1.3.6.4.4 Peel off the seal at the top of the ABC.
1.3.6.4.5 Place the ABC into the Anode end of the instrument, below the pump.
1.3.6.4.6 IMPORTANT: The RFID label must be facing the instrument (not you) to ensure that the RFID information is read accurately by the instrument
1.3.6.4.7 Close the instrument door to re-initialize.
1.3.6.4.8 Click **Refresh** from the Dashboard to update the screen and check the Quick View section of the Dashboard for updated status after changing the ABC.

1.3.7 Changing the cathode buffer container (CBC)
1.3.7.1 Remove CBC from storage and check for expiration date to make sure it is not expired prior to or during intended use.
1.3.7.2 Allow CBC to come to room temperature prior to first use.
1.3.7.3 Wipe away condensation on the CBC exterior with a lint-free lab cloth.
1.3.7.4 Verify that the buffer level is at or above the fill line and check that seal is intact.
1.3.7.5 Tilt the CBC back and forth gently and carefully to ensure that the buffer is evenly distributed across the top of the baffles.
1.3.7.6 Verify that the buffer is at or above the fill line.
1.3.7.7 When ready to install CBC, place the container on a flat surface and peel off the seal.
1.3.7.8 Wipe off any buffer on top of the CBC with a lint-free cloth. Ensure the top of the container is dry.
1.3.7.9 Place the appropriate septa on both sides of the CBC.
1.3.7.10 Install CBC on the autosampler.
1.3.7.11 Close the instrument door to re-initialize.
1.3.7.12 Click **Refresh** from the Dashboard to update the screen and check the Quick View section of the Dashboard for updated status after changing the CBC.

1.3.8 Replenish Polymer
1.3.8.1 In the Maintenance Wizards screen, click "**Replenish Polymer"**

1.3.8.2 Follow the prompts in the "Replenish Polymer" wizard window
1.3.8.3 Click Refresh from the Dashboard to update the screen and check the Quick View section of the Dashboard for updated status after replenishing the polymer

1.3.9 Fill capillary array with fresh polymer
1.3.9.1 The filling of the capillary array with fresh polymer is dictated by the instrument wizards
1.3.9.1.1 To fill the capillary array with fresh polymer (same type of polymer), click Fill the Array with fresh Polymer.
1.3.9.1.2 Follow the prompts in the Fill Array Wizard window
1.3.9.2 Click Refresh from the Dashboard to update the screen and check the Quick View section of the Dashboard for updated status after filling of the Capillary Array with fresh polymer

1.3.10 Use the Conditioning Reagent
1.3.10.1 The use of the conditioning reagent is dictated by the instrument wizards. Install the pouch only when requested to do so by the wizard
1.3.10.1.1 Check for expiration date on the label to make sure it is not expired prior to use
1.3.10.1.2 Peel off the seal at the top of the conditioning reagent pouch fitment
1.3.10.1.3 Insert the pouch fitment on to the slot of the pump lever mechanism. Push the lever up to snap the pouch into the connector end of the instrument pump.
1.3.10.1.4 Follow the wizard for further instructions
1.3.10.2 Click Refresh from the Dashboard to update the screen and check the Quick View section of the Dashboard for updated status after changing the Conditioning Reagent
1.3.10.3 Visually inspect the level of fluid inside the anode buffer container and the cathode buffer container. The fluid must line up with the fill line.
1.3.10.4 Ensure that the plate assemblies are properly assembled.
1.3.10.5 IMPORTANT: Align the holes in the plate retainer with the holes in the septa to avoid damaging capillary tips.
1.3.10.6 Ensure that the plate assemblies and the cathode buffer container are positioned on the plate deck properly. They should sit securely on the deck.
1.3.10.7 Ensure the array locking lever on the capillary array is secured.
1.3.10.8 Check for bubbles in the pump block and channels.
1.3.10.9 Use the Remove Bubble wizard to remove bubbles. Click **Remove Bubbles.** The Bubble Remove Wizard takes 5-15 minutes to complete
1.3.10.10 Follow the prompts in the Bubble Remove Wizard window.
1.3.10.11 Check the Quick View section of the Dashboard for updated status of the polymer pouch after removing bubbles from the polymer pump fluid path.
1.3.10.12 Check the loading-end header to ensure that the capillary tips are not crushed or damaged.

1.3.10.13 Ensure that the pump block is in pushed back position.
1.3.10.14 Clean the instrument surfaces of dried residue, spilled buffer, or dirt.
1.3.10.15 Wipe off any liquid on or around the autosampler using a lint-free tissue
1.3.10.16 Clean off any polymer crystals on the instrument, including the capillary tips, with deionized water and lint-free tissue
1.3.10.17 Clean the array plug
1.3.10.18 Clean out the drip trays with deionized water, or ethanol (absolute), and lint free tissue
1.3.10.19 Check for leaks and dried residue around the Buffer-Pin Valve, check valve, and array locking lever.

1.3.11 Weekly, Monthly and Quarterly Preventive Maintenance of the 3500xl When the Instrument is in Use
1.3.11.1 Weekly Maintenance Tasks
1.3.11.1.1 Check the storage conditions of the used arrays to ensure the array tip is covered in the reservoir
1.3.11.1.2 Run the Wash Pump and Channels wizard
1.3.11.1.2.1 From the Maintenance Wizards screen, click Wash Pump and Channels.
1.3.11.1.2.2 Follow the prompts in the Wash Wizard window.
- The Wash Pump and Channels Wizard takes ~40 minutes to complete

1.3.11.1.3 Use a lab wipe to clean the anode buffer container valve pin assembly on the polymer delivery pump
1.3.11.1.4 Restart the computer and instrument

1.3.12 Monthly Maintenance Tasks
1.3.12.1 Flush the pump trap
1.3.12.2 Fill the supplied 20ml, all-plastic Luer lock syringe with distilled or ionized water. Expel any bubbled from the syringe.
1.3.12.3 Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.
1.3.12.4 Open the Luer fitting by grasping the body of the fitting and turning it to loosen. Attached syringe and turn counterclockwise approximately one-half turn
1.3.12.5 Because the water trap is approximately 325ul, a relatively small volume of water is adequate for complete flushing. However, a larger volume only improves flushing as long as force and flow rate are kept within limits
1.3.12.6 Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand
1.3.12.7 Close the Luer fitting by lightly turning clockwise until the fitting seals against the block
1.3.12.8 Empty the condensation container and the water trap waste container. The waste container is to the right of the pump block.

1.3.12.9 Replace cathode buffer container septa
1.3.12.10 Clean the autosampler
1.3.12.11 Clean the drip tray

1.3.13 Personal Computer (PC) and AB Prism® 3500xl Genetic Analyzer
1.3.13.1 The computer and 3500xl Genetic Analyzer should be re-booted when a fatal error has occurred as indicated by the red light status. Optional: The computer can be re-booted once a week, preferably on the day of the weekly maintenance.

1.3.14 Personal Computer (PC) Maintenance
1.3.14.1 Defragmenting the hard drive
Frequency: As needed when the instrument is in use
1.3.14.1.1 From Windows desktop, right click on **My Computer**
1.3.14.1.2 Select **Manage**
**1.3.14.1.3** Click **Computer Management (Local) > Disk Defragmenter**
1.3.14.1.4 Select **E Drive**
1.3.14.1.5 Click **Defragment**

1.3.14.2 Check Available Disk Space
Frequency: As needed when the instrument is in use.
1.3.14.2.1 From tree pane of **Data Collection software > GA Instruments > Database Manager**.
1.3.14.2.2 Click on **Disc Space Status Window bar** to reveal available space.
1.3.14.2.3 Archive data and delete archived data as needed.

1.3.14.3 Delete Archived Data from Hard Drive
1.3.14.3.1 In tree pane of Data Collection Software, select GA Instrument > Database manager.
1.3.14.3.2 Select Cleanup Processed Plates.
1.3.14.3.3 Select OK after reading dialog box.

1.3.15 Annual Preventive Maintenance of the 3500xl When the Instrument is in Use
**1.3.15.1** An annual calibration performed by an outside vendor is required for the 3500*xl* Genetic Analyzer. After the calibration is performed, a performance check is required before release back into casework.

1.3.16 Installing and Removing the Capillary Array on the 3500xL
1.3.16.1 From the Maintenance Wizards screen, click **Install Capillary Array.** The Install Capillary Array Wizard takes 15-45 minutes to complete.
1.3.16.2 Follow the prompts in the Install Capillary Array Wizard window.
1.3.16.3 Check the Quick View section of the Dashboard for updated status of the capillary array. Communications with the manufacturer indicate the capillary expiration date is truly a warranty date, usage beyond which the manufacturer does

not guarantee quality. Capillaries may be used beyond the "expiration date", provided the quality of the CE data continues to be acceptable. The best indicator of the expiration of the array is the data quality, more specifically, loss of resolution, spikes, and other CE artifacts.

1.3.17 Running a Spatial Calibration on the 3500xl

1.3.17.1 A spatial calibration maps the location of each capillary position on the camera. A spatial calibration must be performed every time the capillary array is installed or replaced, removed from the detection window, and if the instrument is moved. A spatial may also be run if the data has low resolution.

1.3.17.2 Access the Spatial Calibration screen by clicking **Maintenance>Spatial Calibration** in the navigation pane.

1.3.17.3 Select **Fill** or **No Fill.**

**Note:** if there is a recent run on the instrument then select the No Fill option. The capillary array should already be full of polymer. If the instrument has been sitting for an extended period of time, select the Fill option to fill the capillary array with polymer.

1.3.17.4 Select **Perform QC Checks** if you want the system to check each capillary against the specified range for spacing and intensity (recommended).

1.3.17.5 Click **Start Calibration.**

1.3.17.6 When the spatial calibration run is complete, check results to ensure you see one sharp peak for each capillary (small shoulders are acceptable), one (+) marker at the apex of every peak (no off-apex markers), and all peaks are about the same height.

1.3.17.7 If the results meet these criteria, click **Accept Results.**

1.3.17.8 If the results do not meet these criteria, click **Reject Results.** Re-try running spatial calibration.

1.3.18 Performing Spectral Calibration on the 3500xl

1.3.18.1 A spectral calibration is used to remove spectral overlap from the dyes. A spectral is performed when you need to do the following: use a dye set not previously calibrated, change the capillary array, change the polymer type, have a service engineer perform an optical service procedure (such as realigning or replacing the laser or CCD camera or mirrors on the instrument), or see a decrease in spectral separation in the raw or analyzed data.

1.3.18.1.1 Dyes contained within the dye set, J6, are:

1.3.18.1.1.1 6-FAM- blue

1.3.18.1.1.2 VIC- green

1.3.18.1.1.3 NED- yellow

1.3.18.1.1.4 TAZ- red

1.3.18.1.1.5 SID- purple

1.3.18.1.1.6 LIZ-orange

1.3.18.2 Preheat oven and detection cell to 60°C by clicking **Start Pre-heat,** and wait at least 30 minutes.

1.3.18.3 Prepare the spectral standard according to the product insert.

1.3.18.4 Load the specified volume of standard matrix in injection position 1 in the spectral calibration plate (the first three columns: A1-H1, A2-H2, A3-H3)

1.3.18.5 Briefly centrifuge the plate and verify that each sample is positioned correctly in the bottom of its well.

1.3.18.6 Prepare plate assembly and load plate onto instrument.

1.3.18.7 Access the Spectral Calibration screen by clicking **Maintenance>Spectral Calibration.**

1.3.18.8 Select the number of wells in the spectral calibration plate and specify the plate location in the instrument.

1.3.18.9 Select the chemistry standard and dye set.

1.3.18.10 Select **Allow Borrowing** to automatically replace information from adjacent capillaries with high quality values.

1.3.18.11 Click **Start Run.** The system automatically sets up three injections in case the first run doesn't pass.

1.3.18.12 When a spectral calibration completes successfully, the Overall row displays green, red, or yellow results.

1.3.18.13 Individual capillaries can be selected. Each capillary should be reviewed. For each capillary: Click a capillary to display the spectral and raw data. Check that the data meet the following criteria: no extraneous peaks in the raw data profile, no gross overlaps, dips, or other irregular peak morphology in the spectral profile, and peaks separate and distinct in the spectral profile.

1.3.18.14 If the data for all capillaries meet the criteria above, click **Accept Results.**

1.3.18.15 If any capillary data does not meet the criteria above, click **Reject Results,** then re-try.

1.3.18.16 If the spectral does not pass after the three attempts, prepare a new spectral plate and run the calibration again. If the second spectral does not pass, then call the Service Engineer. This indicates there is a problem with the instrument.