Luminex® xPONENT® 3.1 Quick Guide

This guide outlines the basic procedures for routine use of the Luminex xPONENT 3.1 system. For detailed instructions, refer to the appropriate Luminex Hardware User Manual or the Luminex xPONENT3.1 Software User Manual.

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The Luminex 100/200 is a class 1(I) laser product.

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Open xPONENT 3.1

When you turn on the computer attached to your Luminex system, xPONENT software opens automatically. If it does not open, click the Luminex icon on the desktop, or:

1. Click **Start**.
2. Click **Programs**.
3. Click **Luminex**.
4. Click **xPONENT**.
5. Click **Luminex xPONENT**.

The System Login page appears. Type your user name, and if required, your password. The **Home** page appears. You can access most tasks from the **Home** page.

Start-up Indicators

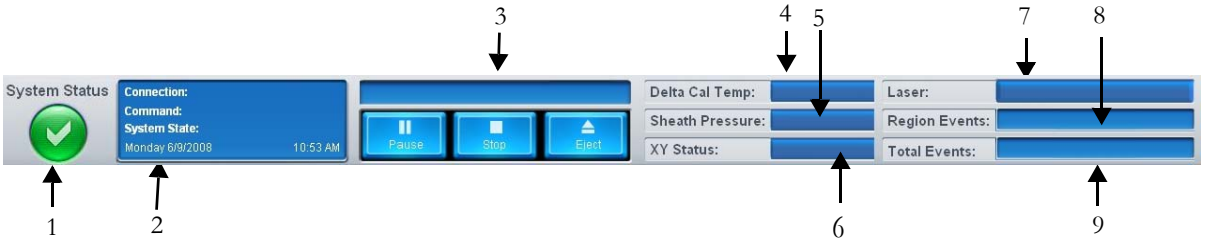
When you turn on the instrument, the following start-up indicators occur:

- The blue light on the analyzer, located above the sample probe, turns on.
- The blue light on the XY Platform (XYP™) turns on.
- The blue light on the Sheath Delivery (SD™) turns on.
- The compressor engages and emits a rumbling sound.
- Air and sheath pressure build in the system.
- Air blows from the fans at the back of the analyzer and the XYP.
- The syringe pump initializes and emits a high-pitched sound as the syringe plunger makes a full stroke.
- As the unit primes, sheath fluid drips into the waste bottle.

The System Monitor

The System Monitor, located at the bottom of the screen, displays continuous system status updates.

FIGURE 1. System Monitor



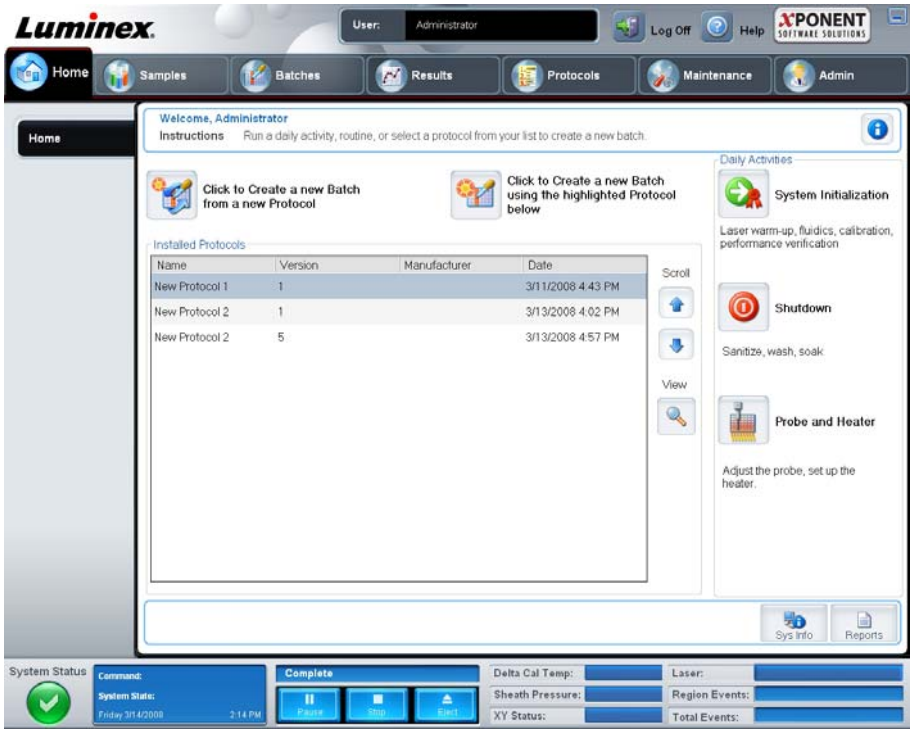
1 System Status button
2 Command display
3 Progress bar and buttons
4 Delta Cal Temp status button
5 Sheath Pressure status
6 XY status
7 Laser status
8 Region Events status
9 Total events status

Software Overview

xPONENT 3.1 software is organized into a series of pages, with a menu for each page. As you click each menu, applicable tabs appear on the left-hand side of the screen.

xPONENT opens to the **Home** page. You can access most tasks from the **Home** page.

FIGURE 2. The Home Page.



xPONENT 3.1 includes the following pages:

Home	Access most commonly used features
Samples	Import or enter sample data (optional)
Batches	Set up batches (experiments)
Results	Analyze previously acquired batches
Protocols	Set up protocols (called templates in IS 2.3) used to run batches
Maintenance	Calibrate, verify, and maintain the application
Admin	Perform administrative tasks
Log Off	Log out of software
Help	Access help file

Adjust the Sample Probe Height

Adjust the sample probe height if:

- You change the type or style of plate being used.
- You remove and replace the sample probe for cleaning or troubleshooting.
- You move the instrument.
- You observe slow or sporadic sample acquisition.

CAUTION: Correct sample probe height is critical to successful sample acquisition and calibration. Problems with the sample probe can lead to fluid leaks and pressurization problems, as well as inhibit sample acquisition. To adjust the height of the sample probe, follow the steps for your system. For detailed instructions, see the Luminex LX100 or LX200 manual that you received with your instrument.

To adjust the sample probe height:

1. On the **Home** page, click **Probe and Heater**. The **Probe & Heater** tab opens.
2. If you are using a filter-bottom plate, place 3 large alignment disks in a well. For other v-bottom plates, use one sphere; for a round-bottom plate, use 2 alignment disks; for a flat-bottom plate, use 2 large alignment disks.
3. Ensure that the well location is selected on the plate image, preferably A1 (a blue pin marks the location). To change the well location, click on a well in the plate image.
4. Verify that the microtiter plate is not warped. Warped plates can lead to incorrect probe height adjustment.
5. Click **Eject** to eject the plate holder.
6. Place the plate on the plate holder.
7. Click **Retract** to retract the plate holder.
8. Select the plate type from the **Plate Type** drop-down list.
9. Click **Sample Probe Up** to raise the sample probe.
10. Gently loosen the probe adjustment screw. Move the probe to its highest position.
11. Click **Move Probe Down**. Loosen the probe adjustment screw and move the probe down until it makes contact with the alignment disc or sphere. Tighten the adjustment screw.
12. Click **Sample Probe Up** to raise the sample probe.
13. Open the XY door. Click **Move Probe Down** and watch as the probe moves down. The black plate should not bounce when the probe reaches the down position.
14. Click **Sample Probe Up**.
15. Click **Eject** to eject the plate holder. Remove the alignment tools from the plate.
16. Replace the plastic shield that covers the sample probe area.

FIGURE 3. Move the Probe Up and Down

Initialize the System

xPONENT 3.1 enables you to customize system initialization using one of these three options:

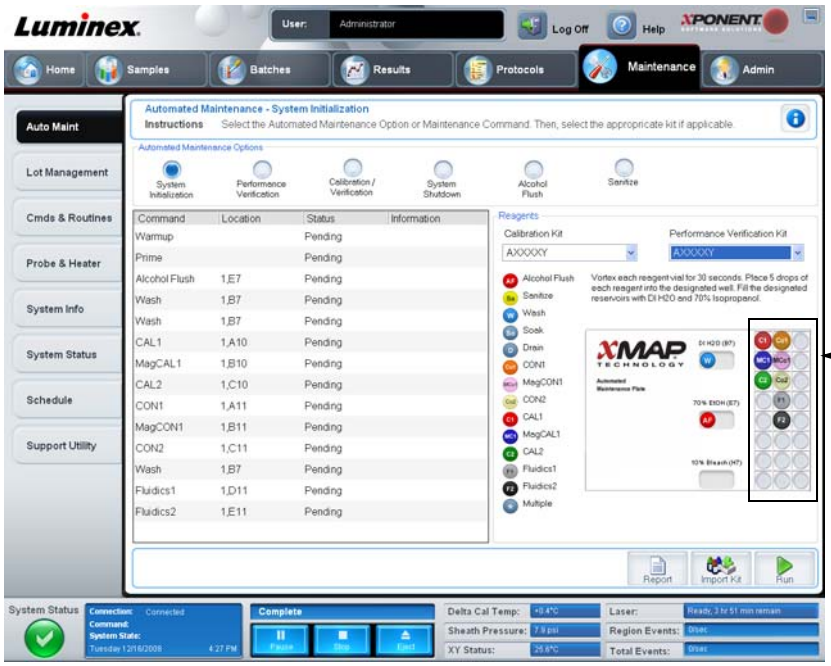
- Warmup and fluidics.
- Warmup, fluidics, and verification.
- Warmup, fluidics, verification, and calibration.

NOTE: Perform a verification daily to check system integrity and to ensure that calibration is still valid.

To run the system initialization routine:

1. On the **Home** page, click **System Initialization**.
2. Select the performance verification kit from the **Performance Verification Kit** list.
3. Select the calibration kit from the **Calibration Kit** list.
4. Vortex the xMAP calibrator, verification, and fluidics containers to ensure homogeneity. *Do not dilute xMAP calibrator or verification agents*.
5. Click **Eject** on the status bar.
6. Place the Automated Maintenance Plate (AMP) on the plate holder.
7. Load the plate with at least five drops of the beads in the Calibration and Performance Verification Kit, as indicated on the plate image and in the kit insert instructions.
8. Click **Retract**.
9. Click **Run**.

FIGURE 4. System Initialization Routine



Commands selected for the System Initialization Routine

Calibrate the Instrument

Calibrate the instrument weekly. You should re-calibrate if:

- The delta calibration temperature exceeds ±3 degrees Celsius.
- You move the system.
- You perform maintenance on the system.
- System acquisition is low or sporadic.

To calibrate the system, click the **Maintenance** menu. The **Maintenance** page opens. Click the **Auto Maint** tab. Select the routine you want to execute and click **Run**.

Create a Protocol

You can create four types of protocols using xPONENT 3.1:

- Quantitative - Used to analyze unknown data generated with a standard curve.
- Qualitative - Used to analyze unknown data generated with cutoff ranges based on one standard.
- Allele Call - Used to analyze data generated as either heterozygotes or homozygotes, based on specific cutoff ratios.
- None - Used when analysis of the data will be carried out in another program. (xPONENT 3.1 reports are unavailable for data acquired with a protocol of type “none.”)

You can create each quantitative, qualitative, and allele call protocol using one product composed of a definable number of standards and controls

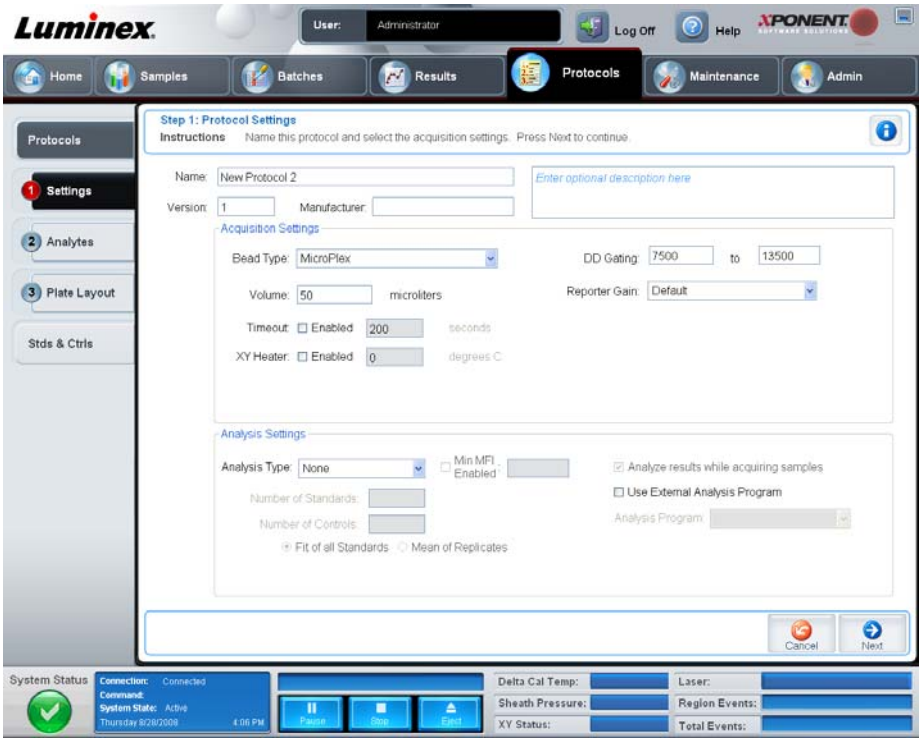
There are three steps to creating a protocol. All three are accessible from the **Protocols** menu, **Protocols** tab. The three steps are:

1. Define settings.
2. Select the analysis type.
3. Define plate layout.

To create a protocol:

1. Open the **Protocols** page.
2. Click **Create New Protocol**. The **Settings** tab opens.

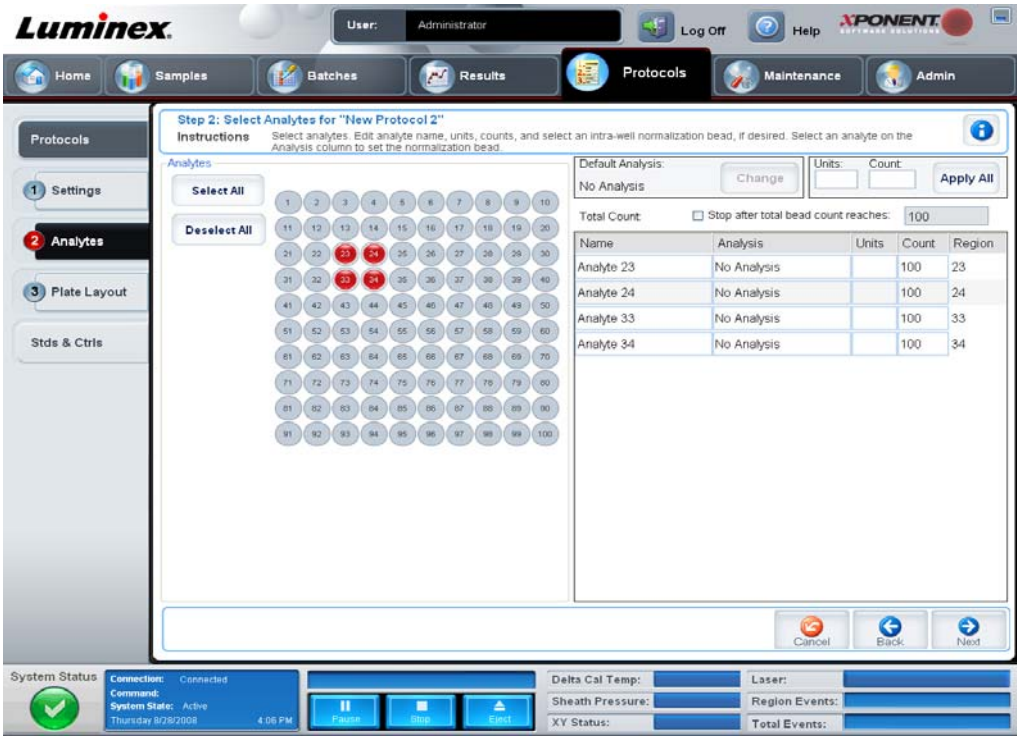
FIGURE 5. Settings Tab on Protocols Page



3. Type the protocol name, version, and manufacturer.
4. Type the following information in the **Acquisition Settings** panel:
   - Bead type (MicroPlex or MagPlex)
   - Sample volume (20-200µL)
   - Timeout (1-250 seconds)
   - XY heater temperature (35-60°C)
   - DD gate (buffer dependent)
   - Reporter gain (Default or High PMT)
5. Select the analysis type (**Qualitative**, **Quantitative**, **Allele Call**, or **None**), and enter the number of standards and controls.
6. Select **Fit of all Standards** or **Mean of Replicates**.
7. Select **Analyze results while acquiring samples** if you want to view real-time analysis.

8. Click **Next** to open the **Select Analytes** tab. Select analytes by clicking the bead number. The table allows you to name the analyte and to select the analysis type, the units to be measured, and the count.

FIGURE 6. Select Analytes



9. Click **Change** to change the analysis settings.
10. Click **Next**. The Plate Layout Tab displays.
11. Add standards, controls, unknowns, and maintenance steps to the plate layout.
12. Click **Save** to save the Protocol.

FIGURE 7. Plate Layout Tab

Create a Kit

To calculate values for unknowns in your assay, enter specific values for standards and controls when you set up your qualitative or quantitative analysis protocol. Because standard and control values can change from lot to lot from the same manufacturer, enter these values separately from the protocol, as either lots or kits. If you initially enter standards and controls as kits and then save the lots individually, you can reuse the lots individually in later applications.

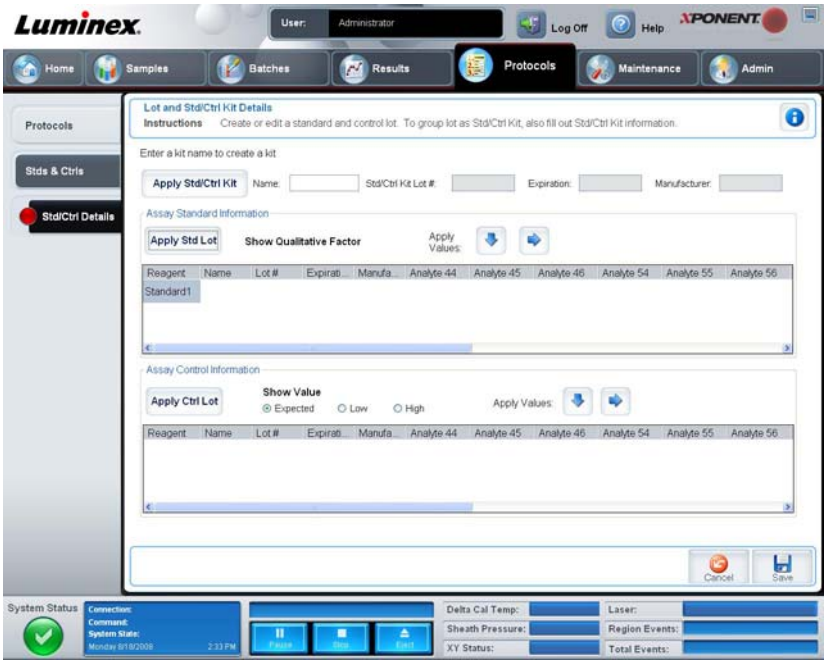
To create a kit:

1. On the **Protocols** page, click the **Stds & Crtls** tab.
2. Click **Create New Std/Ctrl Lots**. The Std/Ctrls Details page appears.
3. Select the appropriate protocol and click **OK**.
4. In the **Lot Details** panel, enter the following information:
   - Kit name, Std/Ctrl lot number, expiration date, and manufacturer
   - Assay standard or control information such as name, lot number expiration, manufacturer, and standard or control values for each analyte

NOTE: You can enter expected, high, or low values for control information. Click the appropriate button to make your selection.

Once kits and lots are created, they can be used with other protocols that use the same analytes.

FIGURE 8. Lot and Std/Ctrl Details



Create a Batch

You can run batches with or without protocols. There are three batch creation options:

- Create a New Batch from an Existing Protocol (uses a protocol; described in this section).
- Create a New Batch from a New Protocol (does not use a protocol).
- Create a New Multi-batch (runs several batches).

To create a new batch from an existing protocol:

1. Open the **Batches** page.
2. Click **Create a New Batch from an Existing Protocol**.
3. Type the batch name in the **Batch Name** box.
4. Click the protocol you want to use in the **Select a Protocol** list. If the protocol you select uses standards and/or controls, the active reagents are shown on the bottom of the screen. Verify that these are the correct standards and controls.
5. Click **Next**.
   - *If the protocol you selected uses standards and controls*, the **Stds & Ctrls** tab appears. View the details of the active reagents and verify that they are correct; apply different assay standards and controls, or manually enter new information. Click **Next**. The **Plate Layout** tab appears. Go to step 6.
   - *If the protocol you selected does not use standards and/or controls*, the **Plate Layout** tab appears. Continue with step 6.
6. On the **Plate Layout** tab, assign well commands for this batch. Add samples to the plate layout if needed.
7. Click **Run Batch** to begin batch acquisition, or click **Save** to save the batch information to the Pending Batch list, to be run at a later time.

NOTE: If the batch spans more than one plate, the tray ejects automatically when all defined wells have been acquired. A dialog box prompts you to insert the next plate.

TIP: Consider using the Single Step option when running batches. Most errors will be apparent in the first sample, and you can correct them without losing samples. You can observe real-time data collection on the **Results** page.

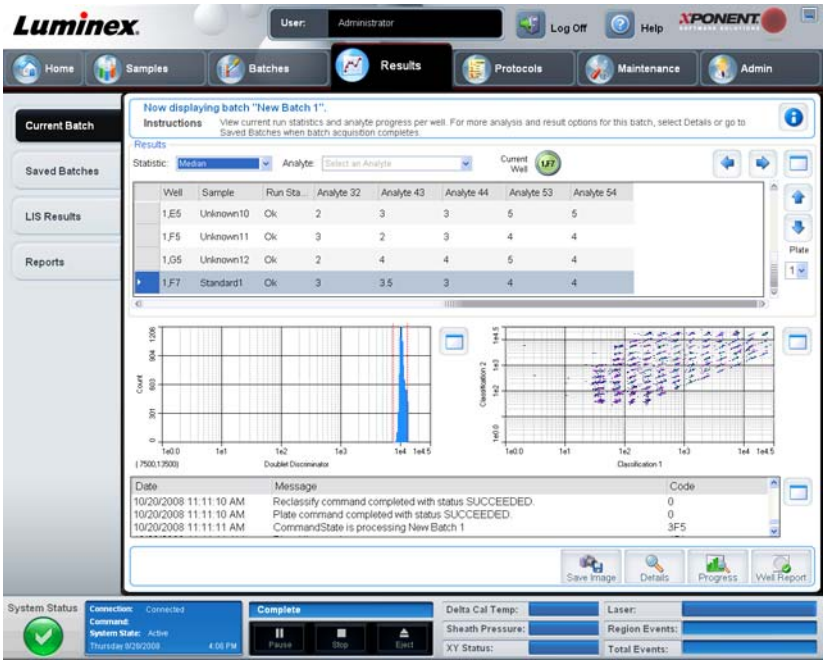
View Batches

You can observe and analyze current and previously run batches on the **Results** page. Batches run with a quantitative, qualitative, or allele call protocol can be analyzed (in other words, if you selected “none” as your protocol, you will not be able to analyze data).

To view batch data:

1. Open the **Results** page.
2. Select the **Current Batch** or **Saved Batches** tab.
3. If you want to open a saved batch, highlight the batch and click **Open**.

FIGURE 9. Viewing Current Batch Results



Run, Save, and Print Reports

xPONENT 3.1 can provide information in three different report types:

- Batch
- Protocol

- Advanced (User)

It can also format your batch or multibatch results in a variety of export formats.

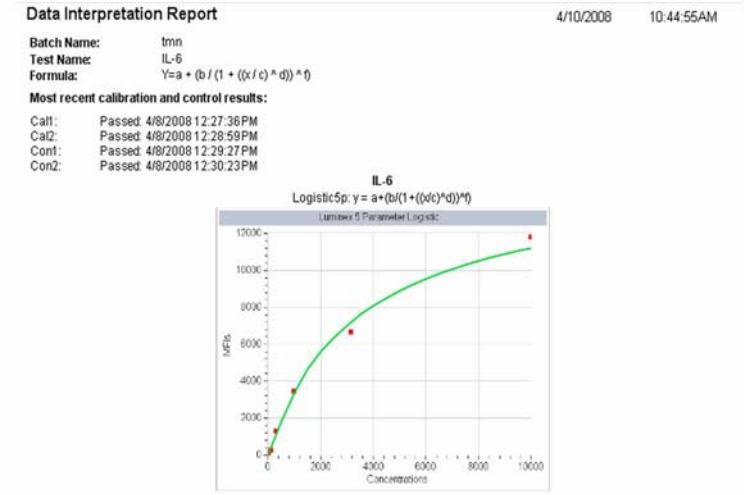
To run, print, and/or save a report:

1. Click the **Results** page.
2. Click the **Reports** tab.
3. In the **Report** list, click the report you want to view.
4. In the **Type** list, select the type of report you want to view.
5. Select the item for which you want to generate the report. If you are creating a Batch Report, select the analytes to include in the report.
6. Click **Generate**. The report appears in the lower part of the **Reports** tab.
7. Click **Print** to print the report, or **Save** to save the report as a PDF file.

To view a data interpretation report:

1. Open the **Results** page.
2. Select the **Reports** tab.
3. Select **New Report**, then **Batch Report**, then **Data Interpretation**.
4. Select a previously run batch.
5. Click **Generate Report.**
6. Click **Print** to print the report, or **Save** to save the report as a PDF file.

FIGURE 10. Data Interpretation Report



Shut Down the Instrument

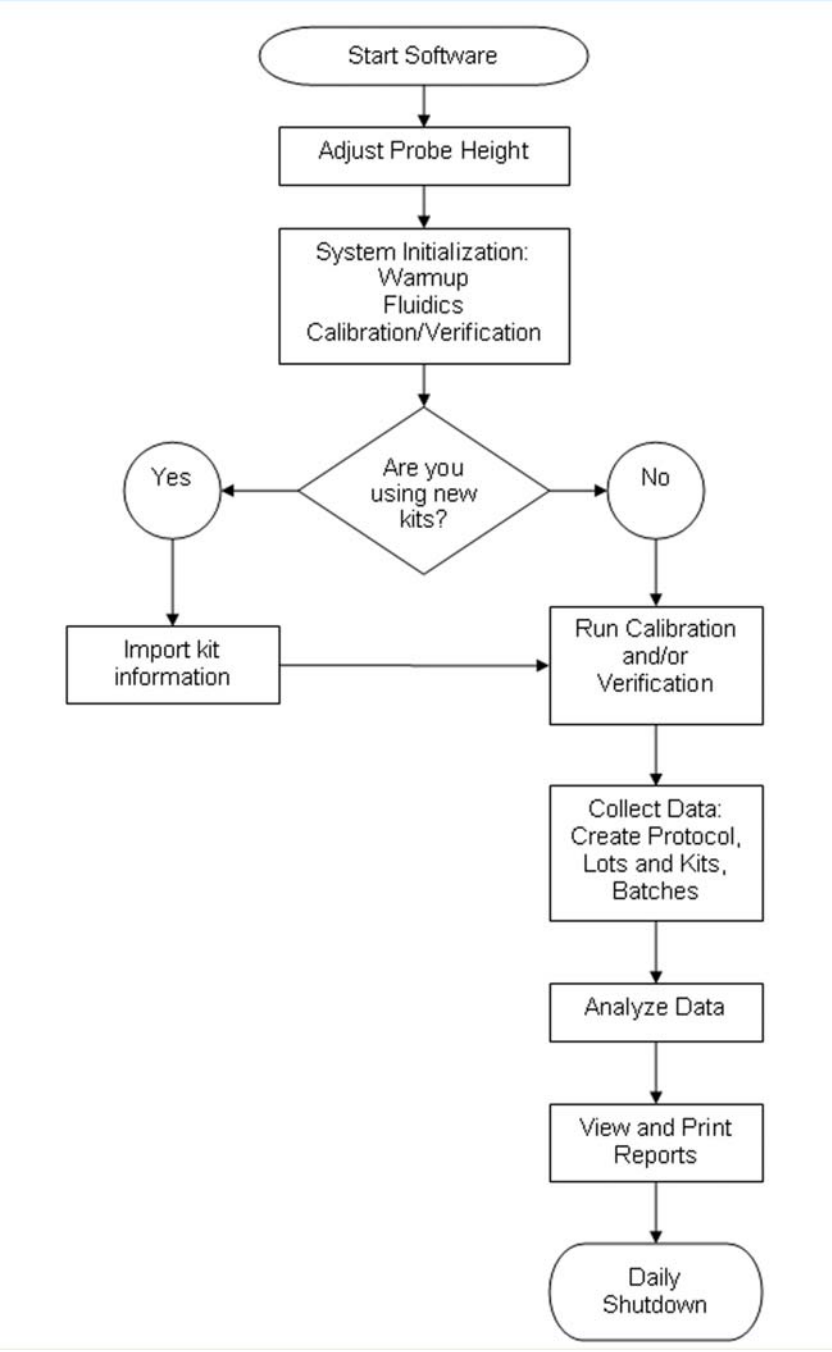
Run the daily shutdown routine to prevent clogs and crystallization of salt in the sample probe. Shut down the system properly to ensure system integrity.

To shut down the instrument:

1. On the **Home** page, click **Shutdown**. The **Auto Maint** tab opens, with **System Shutdown** selected.
2. Fill the labelled area of the AMP with a 10-20% household bleach and water solution.
3. Add deionized water to the labelled area of the AMP.
4. Click **Run**.

Appendix A: Common Daily Tasks

This flowchart illustrates the flow of common daily tasks. For more information about any task, see the hardware manual or xPONENT 3.1 Software User Manual.



Appendix B: Technical Support

On the Web

For more information, visit the Luminex FAQ page at *http://www.luminexcorp.com/support*

You can access the Technical Support website using a user name and password at *http://www.luminexcorp.com/support*

By Phone

Inside the U.S. and Canada

Phone: 1-877-785-BEAD (-2323)

Fax: 512-219-0544

Outside the U.S. and Canada

Phone: +1 512-381-4397

Email

Email questions to support@luminexcorp.com

Luminex xPONENT 3.1 Software User Manual

For detailed information about the functions, features, and use of Luminex xPONENT 3.1, see the *Luminex xPONENT 3.1 Software User Manual*, available for download at *http://www.luminexcorp.com/support*