# Perkin Elmer ICP Instructions

Before starting:

1. Check is hood is flowing (the tissue should be moving).
2. Check if there is enough Ar for analysis.
   a. If "T–cylinder" (large center tank) of liquid Ar does not seem heavy, make sure one of the reserve tanks is on and has sufficient Ar for analysis. Assume 300 psi/hr on reserve tank.
3. Make sure computer is turned on with WINLAB 32 loaded.
   a. If autosampler started moving into wash position when WINLAB turns on, press **F11** or **Analysis → Autosampler → Up**. You can then take out the sample probe and put it in DI water.
4. Check if the ICP is in standby mode.
   a. If not, proceed as usual.
   b. If so:
      i. Go to **System → Diagnostics → Spectrometer → Initialize**
      ii. Click **OK**
      iii. Chiller will turn on, and after 5 minutes the software will display an estimated time the ICP will be ready (approximately 15–75 minutes)
      iv. Return at that time and continue running ICP startup
5. Check that the compressed air supply is showing at least 58 psi on the right gauge (located on the back side of the center lab bench near the computer).
6. If using autosampler, check that there is sufficient wash water.
7. If running manually, rinse and refill DI water bottle using DI water at sink.
8. Check that torch and injector are not broken and there is no obvious salt buildup.
9. Check tubing and replace if:
   a. Black–black or green–orange tubes feel flattened when rolled between fingers.
   b. Red–red tube shows signs of blackness.
10. Snap tubing into place according to the following diagram. Pay attention that the red tube is opposite of the others and the output is flowing into the waste bin!
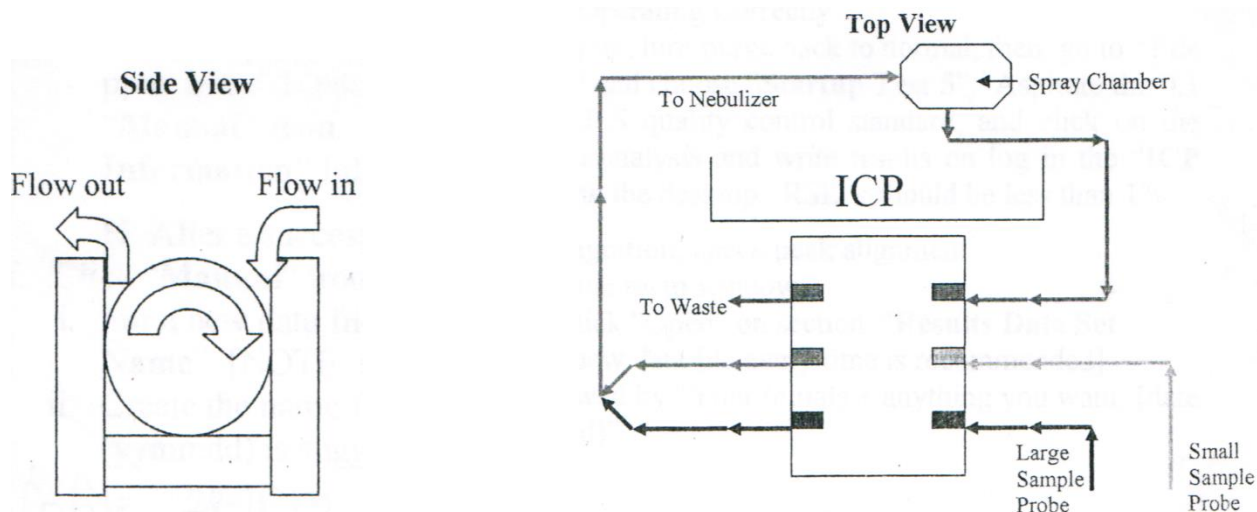
Startup procedure:

1. Click on **Plasma** icon.
2. Click on **Pump** icon to turn the pump.
3. Let pump make a few turns, then snap the pump tensioners into place around tubing.
4. Place both sampling rods into the wash water.
5. In the **Plasma Control** window, click on the switch to turn the plasma on.
   a. There is a 40 second delay, make sure to watch the plasma as it ignites. If the plasma is not steady or badly formed, immediately press the red button to the top right of the plasma window.
6. If you are running elements less than 195 nm (e.g. S, P), go to **Tools → Spectrometer Control →Purge Gas** and click on **High** to purge ICP for 15 minutes while plasma is warming up.
7. **Sign in your name and plasma ignition time in the binder behind you!**
8. Re–tension the tubing (Note: you do not need to tension the red waste tube on the far right):
   a. Take the rod of the tube you are tensioning out of the water (count "1–one thousand" as few times) to generate a flow of bubbles through the tube.
   b. Turn the knob on pump tensioner counterclockwise until the bubbles flowing through the tube stop.
   c. Turn the knob clockwise by half a turn at a time until you see the bubbles flowing smoothly through the tube.
   d. Turn the knob one additional turn clockwise to finish adjustment.
   e. Repeat for the other tube. Afterwards, there should be a smooth spray visible in the spray chamber.
9. Place the small sampling rod into the 50 ppm Sc solution (bottles are located at the end of the lab bench). If using small sampling rod for samples, place large rod into 5 ppm Sc solution.
10. There should not be any water droplets on the sides of the spray chamber (droplets on the top are OK).
11. You can now close the door after arranging the tubing. Don't squish the tubes as you do this!

Analysis:

1. Have these windows opened in WINLAB: Manual Process Control (click on **Manual**), Spectra Display (click on **Spectra**), and **Results**.
2. After 15 minute warm–up period, turn purge back to normal if you were running a high purge.
3. Go to **File → Open → Method** and choose the latest version of "**Startup Test 5**" (as of 3/18/14 the latest version was entitled "sartup test 5").
4. Run analysis using the **Manual Process Window**.
5. Record the results on the log found in ICP Information folder located on the desktop. The RSDs should be less than 1%.
6. Open your method file by going back to **File → Open → Method**. If you have never used the ICP, or have a new set of analyses, create a new method file by following the instructions in **HOW TO CREATE A METHOD**.
7. In the Manual Process Window, click **Open** on the section **Results Data Set Name**. It is recommended to use a new data file every time.
8. Name the file "your initials" + date (yymmdd is recommended) + anything else you want.

9. Choose **Info File** (use **empty.sif** is none and aspirate to high standard).
10. Input your desired sample name in the **Sample ID** box for each sample.
11. Place the large sampling rod into your sample solution.
12. Click on **Analyze Sample**.
13. Place large sampling rod in the wash water for a few seconds between samples.
14. Repeat until all samples have been analyzed.

Post analysis:

1. In the **Plasma Control** window, click on the switch to turn the plasma **OFF**. Watch all the three gas flows until reaching 0 ml/min, then close the Ar tank.
2. Place both sampling rods into the wash water to run water through the system for a few minutes.
3. Take both sampler rods out of the water and place into a paper towel on the table.
4. **De–tension** all of the tubings so they do not flatten.
5. Leave the computer on with WINLAB32 running.
6. **Remember to sign out of the log book with your end time!**

How to Create a Method

File → New → Method

Starting Conditions:

Default
Plasma Conditions: Aqueous

Method Editor:

Spectrometer

Define Elements

Method Description:
- Describe your method however you would like (Elements, date recommended)

Wavelength Table:
- Select the elements you would like to analyze (all wavelengths).
- Select all wavelengths of Sc except line 424.
- Click Enter Selected Wavelengths in Method
- Close Wavelength Table popup box.
- For Sc lines, under Function heading, change Analyte to Int. Std.

Settings

Time:
- Min – 5sec
- Max – 20sec
- Delay Time – 30sec

Replicates:
- Usually **3** but depends on your sample volume available and desired precision

Sampler

Plasma

In the top row of the table (where is says All under Element) change:
- Nebulizer – 0.5
- Power – 1450 (lower if analyzing alkali or alkaline earth metals)

Peristaltic Pump

- Sample Flow Rate – 1 mL/min
- Flush Time – 15 sec

Autosampler

- Adjust if applicable

Process

Internal Standards

- Report – As a percent

File → Save As → Method
Save as YourInitials_yymmdd

Close Method Editor

Run your high standard and check optimal optical alignment:

1. Analyze your high standard
2. Click the **Examine** icon
3. Click **Data ▼**
   a. Select your data set and choose the data
4. Highlight your high standard → **Next** → Make sure all your elements are highlighted → **Finish**
5. Align the Peaks
   - Go through windows to see if the elements are aligned properly.
   - Look to see if peaks are centered within the highlighted region.
   - If not aligned, move the **yellow** <u>**triangle**</u> (not the yellow line) until the region is highlighted to our liking.
   - Sometimes too many points are used to draw the curve (> 2 points) so one of them can be eliminated.
     - Right click in the graph area and select **Update Method** (or press **M**)
     - Go to the bottom of window and change number of peaks to **2**
     - Go back to the Examine window and adjust the peak again.
     - Right click the area and select **Update Method**.
     - Choose **Update Method and Save**.
6. **File → Save → Method**

How to Export Data

1. Open the Data Manager program on the desktop.
2. Select your data set to be imported.
3. Click the Export icon.
4. Export Design
   - If you do not already have a design created, select Create New Design.
5. Samples to Export
   - Make sure all of the data points are highlighted.
6. Analytes to Export
   - Check that all desired analytes for analysis are in the selected analytes column (or that All Analytes box is checked).
7. Export Options
   - Change the directory to your personal directory at C:\guest\your initials
     - If you do not yet have a directory, create a folder in C:\guest named as your initials
8. Sample Parameters
   - Selected:
     - Sample ID (name)
9. Mean-Related Parameters
   - Selected:
     - Analyte Name
   - Add:
     - Date and Time
     - Element Wavelength
     - Intensity (corrected)
     - % RSD (corr. intensity)
     - SD (corr. intensity)
10. Replicate-Related Parameters
    - Selected:
      - Replicate Number
      - Date and Time
    - Add:
      - Intensity (corrected)
11. Export Data Set
    - **Save Design**
      - Save with your initials for later use
    - **Export Data**

Set-up Excel Spreadsheet:

1. Sort data sheet by analyte name.
2. Add a blank row between wavelengths for ease of reading data.
3. Freeze and bold your header row.

4. Reduce % RSDs to 2 decimal places.
5. Remove Intensity decimal places and add comma separators.

How to Draw a Calibration Curve

1. Move about 5 columns to the right of your data for a selected analyte.
2. Create 5 columns with the headings:
   - x
   - y
   - y = ax+b
   - a
   - b
3. In the x column, add the measured intensity of your high standard.
4. Below that, add the intensities of your medium standard, low standard, and blank.
5. In the y column first row, type the final concentration of the element in the high standard (the concentration that you created the high standard for).
6. Repeat for medium and high standards (should be 1/10th and 1/100th of high standard, respectively).
7. In the next row of the y column, type 0 since your blank should have a concentration of 0 for that element.
8. Your table should look like this:

| x	y	y = ax+ b	a	b
Intensity of high standard	Final concentration of high standard			
Intensity of medium standard	Final concentration of medium standard			
Intensity of low standard	Final concentration of low standard			
Intensity of blank	0			

9. Select a 2x4 box under the a and b columns.
10. Click in the function bar.
11. Type "**=LINEST(your y values, your x values, true, true)**"
12. Hold down CTRL, SHIFT, and then press ENTER.
13. Your table should now look something like this:

x	y	y=ax+b	a	b
100267.94	250		0.002498	-0.52774
10380.794	25		4.21E-06	0.212451
1102.0794	2.5		0.999994	0.353419
174.354	0		351302.5	2

14. In the column y=ax+b type "=(intensity of high std)*(a value)+(b value)"
15. Select all of the boxes that you just made and copy and paste them for all the other wavelengths.

16. Go back to the column with the y = ax+b function and place $ after the column name to lock the values: **"=(intensity of high std)*Column$Row(a value)+Column$Row (b value)"** e.g. "=B2*G$2 + H$2"
17. This final value will be the µg/mL concentration of that element in your samples.

Calibration Curve:
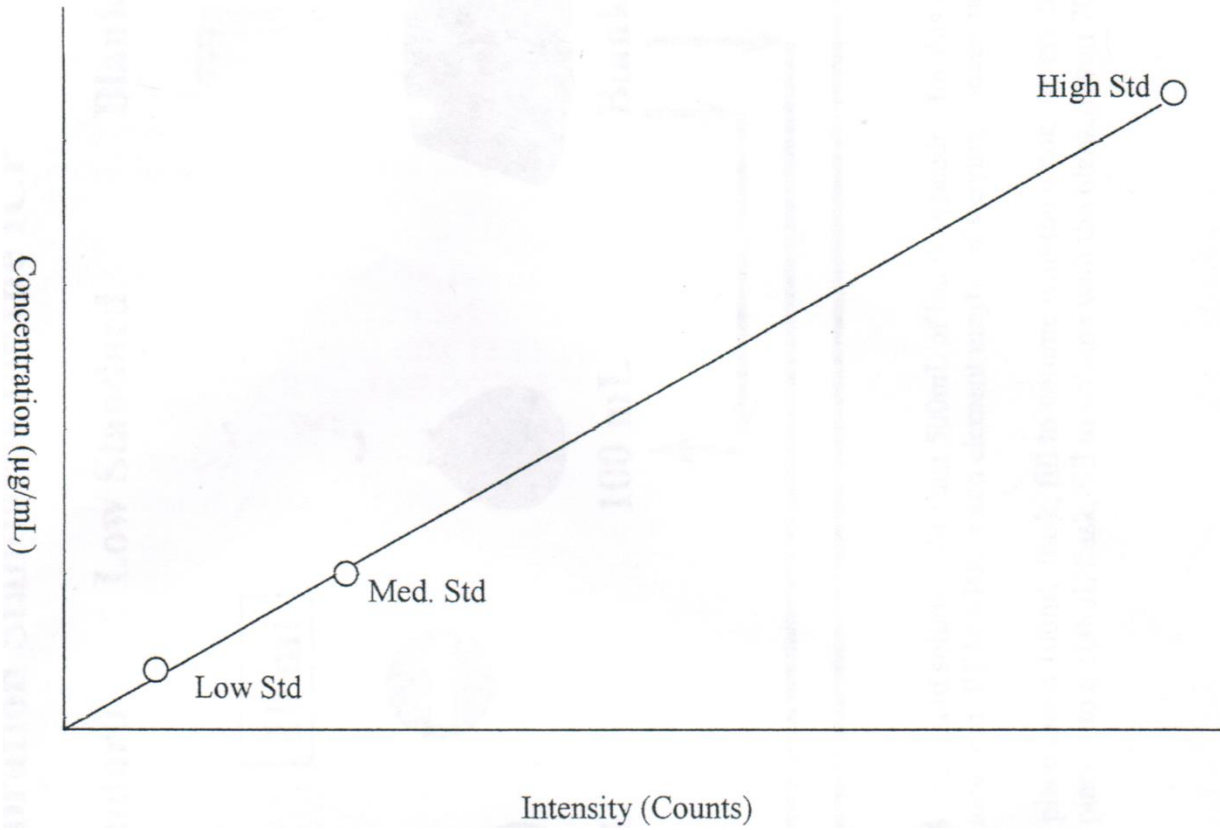
ICP Standard Calculations

Purpose:

The purpose of these calculations is to create a high standard so that a standard curve maybe drawn. The standard curve will then relate the intensities measured by the ICP to actual concentrations.

Step 1:

Estimate the range for all on the elements you think are in your sample in [µg/mL]

Step 2:

Multiply the estimated range by two so that the high standard will include all data points within the standard curve.

Step 3:

Use **Eq.1** to calculate the amount of stock solution needed to create the high standard for each element. [mL]

*Note: These calculations can easily be obtained using a spreadsheet. One is available on the website.

D = Desired final concentration (µg/mL)

W = Concentration of elemental standard (mL/µg)

Y = Volume of flask (mL)

Z = Amount of elemental standard to add (mL)

(1)
$$(D)\left[\frac{\mu g}{mL}\right] \times (W)\left[\frac{mL}{\mu g}\right] \times (Y)\left[mL\right] = (Z)[mL]$$

*Note: Round the amount of stock solution to manageable amounts. (i.e. 4.76mL → 5ml)

Step 4:

Use **Eq. 2** to calculate the actual concentration of each element in the high. These numbers will be used in the standard curve.

Z = Amount of elemental standard added (mL)

W = Concentration of elemental standard (mL/µg)

Y = Volume of flask (mL)

F = Actual concentration of final solution

(2)
$$(Z)\left[mL\right] \times (W)\left[\frac{\mu g}{mL}\right] \times (Y)\left[\frac{1}{mL}\right] = (F)\left[\frac{\mu g}{mL}\right]$$

Keep these calculations for future references

How to Create the Standards

Things you should know:
- Wear protective gear:
  - Safety glasses
  - Lab coat
  - Gloves
- You should know the approximate range of concentrations of the elements you wish to analyze.
- You should know the matrix your sample was digested in.
- You will be creating 3 standards (high, medium, and low) and a blank.
- Always clearly mark the container before adding the any liquid.
- Always add acid to liquid/water, never the other way around!
- Never place pipettes directly into the stock solutions, always pour them into a separate container!

Materials needed:
- 1 – 1000 mL flask or plastic container
- 1 – 250 mL flask
- 1 – 100 mL flask

Blank:

Create a blank that has the same matrix as your samples. You should make enough so that you can fill two 100 mL and one 250 mL flasks and have at least 100 mL left over for the analysis (about 500 mL in total). E.g. if you are in a 2% HNO_3 matrix, the blank should have a 2% HNO_3 matrix.

High Standard:

Create a high standard that contains **all** of the elements that you are searching for. Using the equations from **ICP STANDARD CALCULATIONS**, you should have determined the amount of stock solution that will be added to the standard. NOTE: the concentrations should be varying depending on what you believe the desired range will be! Pour the desired amount of stock solution into a clearly marked beaker or test tube. Then pipette out the desired amount of the standard into your 250 mL flask. Fill the flask to volume using your blank solution. Cap and invert 20 times.

Medium Standard:

From the high standard, draw 10 mL and place into a 100 mL flask. Then fill the flask to volume with your blank solution. Cap and invert 20 times.

Low Standard:

From the medium standard, draw 10 mL and place into another 100 mL flask. Then fill the flask to volume with your blank solution. Cap and invert 20 times.
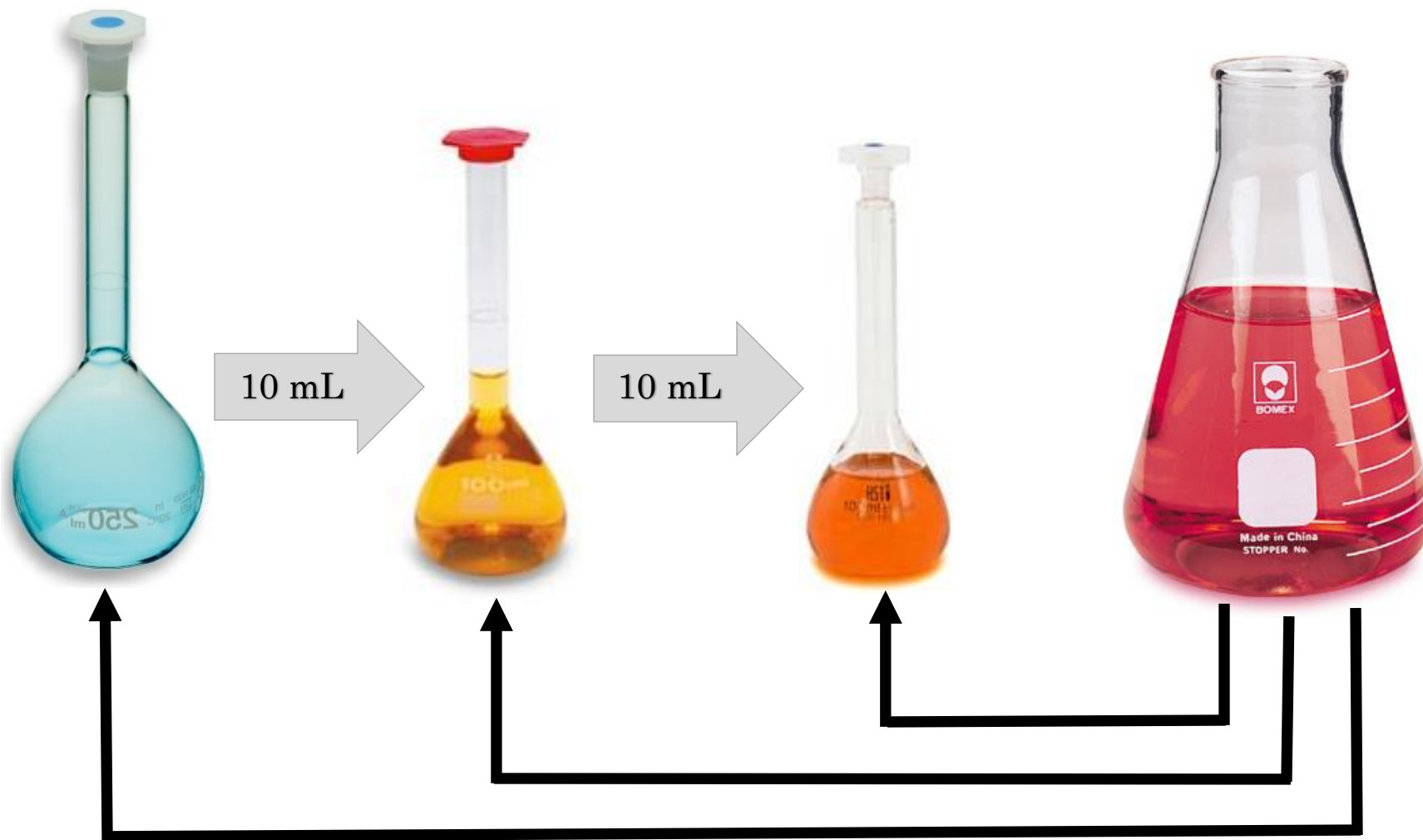
How to Make Calibration Standards for the ICP

High Standard   Medium Standard   Low Standard   Blank

10 mL   10 mL