Standard Operating Protocol for data handling of serum enriched in deuterium from LGR DLT-100 Liquid Water Isotope Analyzer

Modified by G. Gordon from template from Vince Debes (dated 180607) and [1-3]

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1. Sample Collection

   1.1. This protocol assumes samples are prepared as in the protocol "D_2O Plasma Handling" from Kavouras group. Blood samples were centrifuged to isolate the plasma, and then filtered through a 10 kDa filter to remove proteins before frozen storage.

2. Sample Measurement

   2.1. This protocol assumes samples are measured as described in "Stable Water Isotope Analysis in Serum Protocol using LGR DLT 100 LWIA" from METAL laboratory. Changes to this operation should be considered prior to using this technique.

3. Data extraction

   3.1. It is important to transfer all files soon after the run.

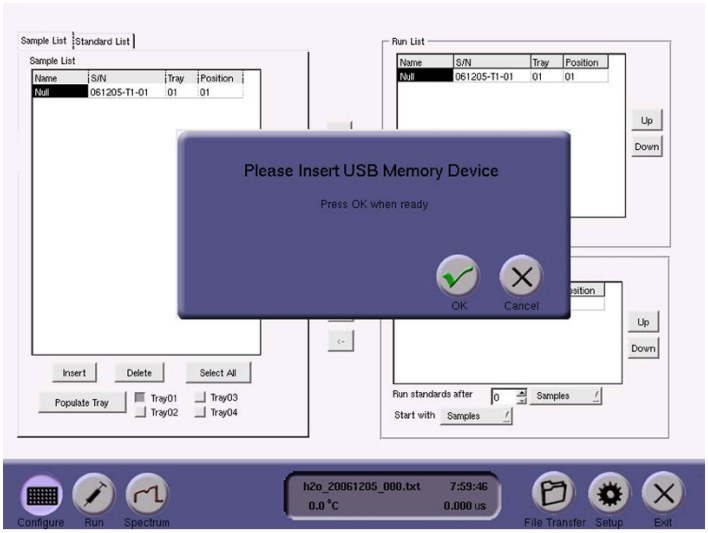
   3.2. Each sequence has three data file types of different types on the LGR instrument. If you have followed the recommended convention, they should look like:

      3.2.1. h2o_20241004_001_LIMS.csv. This is the most important one, and the only one you need for daily data analysis and reporting. In fact, you will only be using a few columns from this file but you will need to be monitoring and evaluating data quality from additional columns.

      3.2.2. h2o_20241004_001.txt.lgr. This is a file that can be sent to LGR for troubleshooting purposes as it has additional instrument configuration information. It is not needed for daily work but should be saved for reference later if issues arise.

      3.2.3. h2o_20241004_001.spectrum.txt.bz2. This is a file that can be sent to LGR for troubleshooting purposes but is not needed for typical data analysis.

3.3. Go to the Run Configuration screen and select “File Transfer” button. You will have a dialog box requesting that you insert a USB drive:



3.4. Insert a USB drive on the back of the instrument in the upper left corner as you are facing the front of the instrument. Some USB drives are not easily read, but there should be a USB drive marked as LGR that will work.

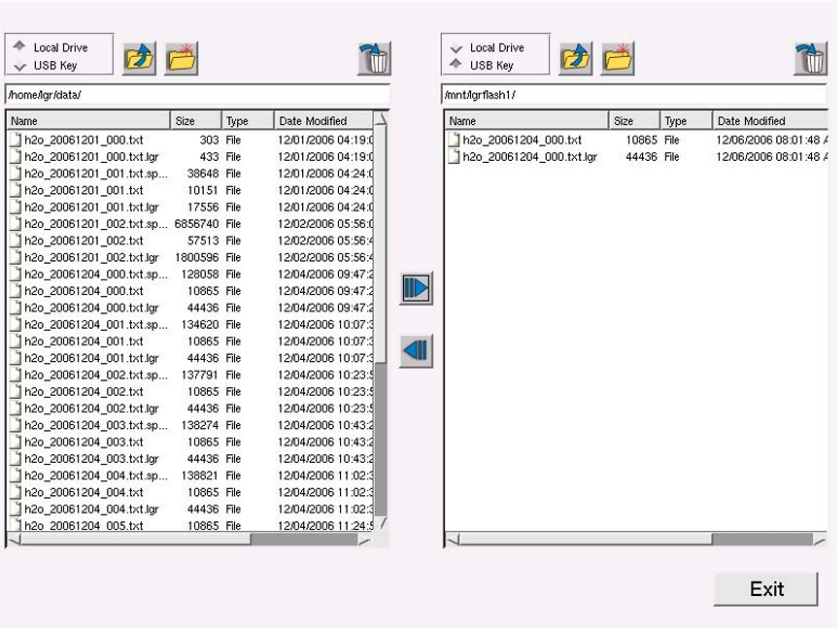
3.5. Press the green check mark once the USB drive has been inserted.

3.6. You should get a new dialog box instructing you to wait until being prompted before removing the USB drive. If you do not get this message, the USB drive is not being read by the LGR. Try a different drive or remove and reinsert the drive until you get this message.

3.7. Press the green check to acknowledge this message.

3.8. You should see two file directory windows. On the left is the files on the LGR. Find the files you wish to copy. Highlight them and select the right arrow to

put them in the list to copy. An example is below.



3.9. Once you have selected the proper files, hit the Exit button. It may take several seconds to complete this file transfer. When it is complete, you should get a message that it is safe to remove the USB drive. Click the green arrow to acknowledge.

4. Data storage

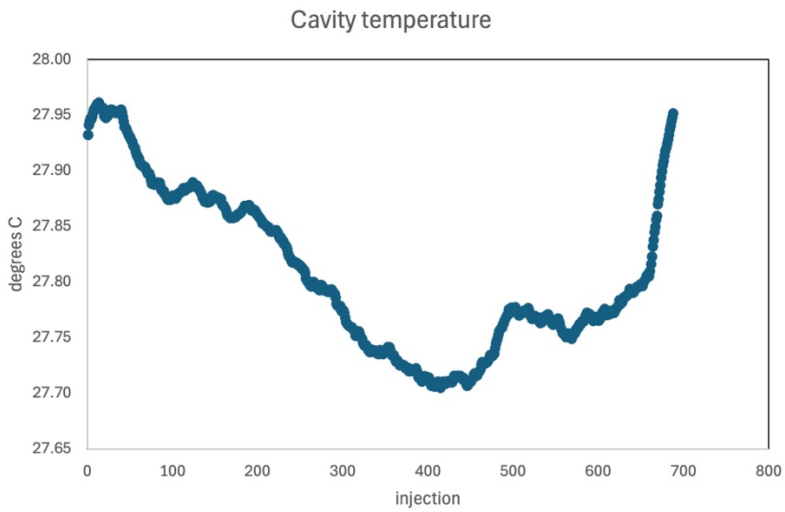
4.1. Always keep unedited versions of the files in a separate location. Best practice is to make sure there are several locations where raw files are stored, including one place that is off-site such as cloud storage.

4.2. Clearly label the raw files and store in a separate folder from files that have been processed. You should ALWAYS be able to go back to the original file and reprocess the file in case of questions.

4.3. For standard processing, you will only need the h2o_20241004_001_LIMS.csv file. However, to do the processing to get reportable values, you will need to

save in a different format such as .xlsx. In an ideal world, we would have R-code to automatically process the data. This is not yet an ideal world.

5. File overview
   5.1. There are 29 columns of data. For the most basic data processing of enriched deuterium values in serum, we only need a few of these columns. These include:
      5.1.1. "Peak Nr." (column B) is the number of injections for the sample. For enriched biological samples, we will exclude the first 9 of the 12 samples and average only the final three.
      5.1.2. "Identifier 1" (column F)
      5.1.3. We can ignore any column for oxygen unless specifically requested for the project
6. As an overview of the data process, we must:
   6.1. Evaluate data for QA/QC due to instrument flags and carryover
   6.2. Cull the data to remove the first 9 lines for samples/standards and remove the between sample DI rinses
   6.3. Calculate the correction for any drift over the analytical sequence
   6.4. Normalize the data to the VSMOW scale
   6.5. Evaluate the accuracy and precision of the overall run from the values of the drift and check standards
   6.6. Correct the samples for any required dilution to bring the samples on scale
   6.7. Convert the sample data from $\delta^{2}H$ to D_{ppm} as needed.
7. Data evaluation for instrument flags
   7.1. Duplicate the raw data into a new sheet in the same workbook, and label it "Plots". Insert two rows below the header. This is where you will do some very basic calculations to evaluate data quality. See example file.
   7.2. Laboratory temperature must be stable for reliable results. You can evaluate this in columns N ("Temp_Celsius"). Acceptable conditions are when the gas temperature inside the cavity changes <0.3°C/hour and show minimal oscillations during the run. This corresponds to a lab temperature change of <2-3°C per hour. Here is a plot of temperature over a 25 hour run with a total range of 0.25°C:



To easily check this calculate the range for column N using an equation like =max(N2:Nxxx)-min(N2:Nxxx) where xxx is the last row there is data. Include this calculation at the top of the column of data. You can also plot the data as shown above to quickly evaluate the range and frequency of variations.

7.3. The temperature must be stable within each injection. This can be determined by column O (stdev_Celsius). These should typically be <0.004°C. A value of 0.01°C indicates unacceptable temperature variations. To quickly check this, calculate the maximum value for this column using =max(O2:Oxxx) where xxx is the last row of data. Include this calculation at the top of the column of data.

7.4. The water molecule density, column P (H2O_N/cm3) should be high (~2-4 x 10^{16} molecules/cm^3) and stable. It should not fluctuate more than 2-4% over the entire run. If it is low or fluctuating, it indicates leaking or clogging. Check all fittings. Calculate the percent variation = stdev(P3:Pxxx)/average(P3:Pxxx) where xxx indicates the last row of data. Include this calculation at the top of the column of data. If you notice substantial variation in water density, you can use conditional formatting to highlight low or high values. When water density varies, data is likely to be unreliable.

7.5. Include plots for Temperature, Water density, delta D/H, and delta 18O/16O over top the data. This allows other analysts to rapidly look at the data.

8. We now want to rapidly get an idea of the amount of carryover and drift during the run. The easiest way to do this is to look at the rinses between samples only.

8.1. Duplicate the raw data into a new tab and call it “rinse only”.

8.2. Sort by Identifier 1. This will be easiest if you have named your rinses distinct from all your samples. Check the peak number for your selected rinse name.

You should have a maximum of 6 peaks for a rinse. If you have more, than this was a rinse run as a sample.

8.3. Delete all lines that are not your rinse solution (typically in the vial with no septa in Tray 1, position 1. Resort by TimeCode to make sure you have everything in order. Ideally, your rinse will come back down to the initial value, and the baseline will remain constant throughout the run. Typical errors on d2H measurements are around 2 permil. Drift standards can correct for a certain amount of drift, but if your rinses are not returning within 10 permil of the initial values at the beginning of the run, you may need to modify your sequence, your sample dilution, or another factor to reduce the amount of drift.

9. Copy your data into a new tab and label it “last 4 injections”. Sort by Identifier1 and delete all the rinses. Sort again by Time Code. We will remove the first 6 injections that have memory.

9.1. Freeze panes so you can see the first header row and the Identifier 1 column (*i.e.*, sample name). Scroll to the end of the columns with data. Add the headers “d2H raw average”, “d2H raw st dev”, and “n injections”. Give these green fill to indicate that they are calculated values.

9.2. In the row for Peak number 1 for each sample, calculate the average, standard deviation, and count of values of the last four injections for each sample.

9.3. Check the standard deviations. These should all be below 2 permil. If the standard deviation is larger than this, the sample may need to be rerun. Look at the pattern of the variation – is it still increasing at the last injection? If the sample is isotopically very enriched, it will likely need to be diluted more. Also check the water density to make sure there aren’t outliers (increased variation, low or high values).

10. Duplicate the sheet and label the tab “results”. Highlight all the cells and paste as values.

10.1. Sort the sheet by Peak Number. If you put the equations in the correct row, all your average, standard deviation, and number of injection information will be at the top of the sheet. Delete all peak numbers >1.

11. Drift correction

11.1. Insert a column to the right, call it “raw d2H for drift correction”, and put all the d2H raw average for only the drift standards in this column.

11.2. Create a plot for all the drift standards (don’t include normalization standards or samples). Your x-axis should be line number, while the y-axis is the d2H value. Label your plot “Drift correction” and label your axes. Add a

trendline and put the equation for the line on the plot. If your drift sample is varying more than 5-10 permil, there is too much drift and you may need to rerun the sequence for more accurate and precise results. For biological samples, this occurs when VOCs condense on the inside of the transfer line, causing long-term memory. Examine the transfer line, particularly close to the injection block.

11.3. Calculate the slope of the drift correction line (=SLOPE(AH:AH,E:E)). Also calculate the standard deviation for the drift standards.

11.4. Put a new header “d2H drift corrected” to the right of the column “raw d2H for drift correction”. Correct the values for drift by taking the raw value and subtracting the line number times the slope of the drift correction line. The equation should look something like: = AH2-(AN$18*E2), if the slope of the drift correction line is in cell AN$18.

11.5. Calculate the standard deviation of the drift corrected drift standards. This should be less than the standard deviation for the raw values.

11.6. Make another column that calculates the drift correction for all the analyses, not just the drift standards.

12. Normalization

12.1. We will now need to normalize the data to the VSMOW scale. It is best practice to have low and high isotope standards bracketing your samples. If you don’t have normalization standards in your sequence, you can skip this section. Examples of when you can skip normalization is when you are only looking at reproducibility, or differences between samples.

12.2. Make a column and label it “VSMOW calibration raw”. Copy the final drift corrected value of only the normalized standards into this column.

12.3. Make another column, “VSMOW calibration defined”. Put the known or calibrated values for the normalization standards in this column.These will be listed in the file, “Water Isotope Standard Values” in the SOP folder in the METAL instruments shared drive.

12.4. Plot the raw versus known values for the standards, add a trendline, and put the equation and r^2 on the plot. Label the plot and axes.

12.5. Label a cell for the slope of the measured vs known.

12.6. Label a second cell for the intercept of the measured vs known.

12.7. Label a column as “VSMOW normalized.” Correct all the data by taking the drift corrected d2H value for all samples and standards, multiply it by the

slope of the normalization line, and add the intercept of the normalization line: =(drift corrected value*slope)+intercept.

13. Evaluation of quality of analytical run

13.1. Copy the final drift corrected and normalized values and sample names and paste as values into a new tab labelled “Standards”.

13.2. Sort by Identifier 1 and separate into normalization standards, drift standards, check standards (if used), and samples. Calculate the average and standard deviation for anything measured >2 times.

13.3. This tab is important because it will be needed when you want to calculate the expanded bias and uncertainty for a project as in Szpak et al [4]. Doing these summary sheets that have all the standard values clearly labeled and sorted will save you a huge amount of time when writing up a project.

14. Import the tab “dilution correction” from the sequence template into your analytical file.

14.1. Fill in any cells that have blue fill. This will include the gravimetric weights determined when originally diluting your samples, the trial number, and the isotope composition of the water you used for dilution (likely the d2H near zero water).

14.2. Any cells with grey fill are either calculations or constants. Do not edit them without consulting with staff.

14.3. To convert from δ^{2}H values to mass fractions of deuterium, D_{ppm} (which is the preferred nomenclature in metabolic literature for enriched samples)[1]:

$$D_{ppm} = \frac{(\delta^{2}H_{VSMOW\text{-}SLAP} + 1000)}{\left[(\delta^{2}H_{VSMOW\text{-}SLAP} + 1000) + \left({}^{1000}/_{0.00015576}\right)\right] * 10^{6}}$$

which is equivalent to

$$D_{ppm} = \left(\frac{\delta^{2}H_{VSMOW\text{-}SLAP}}{1000} + 1\right) * 155.76$$

14.4. You can copy the sample names and the “spiked sample – D/H (ppm)” values and paste into a final summary file. These are the values that will be reported. For error calculations, review the Szpak et al paper and then consult with staff.

References

1. Wassenaar, L.I., et al., *Measurement of extremely (2) H-enriched water samples by laser spectrometry: application to batch electrolytic concentration of environmental tritium samples*. Rapid Commun Mass Spectrom, 2016. **30**(3): p. 415-22.
2. *DLT-100 Liquid-Water Isotope Analyzer*. Report Los Gatos Research. 36 p.
3. *Laser Spectroscopic Analysis of Liquid Water Samples for Stable Hydrogen and Oxygen Isotopes*. 2009. Report ISSN 1018–5518. International Atomic Energy Agency, Vienna. 49 p.
4. Szpak, P., J.Z. Metcalfe, and R.A. Macdonald, *Best practices for calibrating and reporting stable isotope measurements in archaeology*. Journal of Archaeological Science: Reports, 2017. **13**: p. 609-616.