Milestone DMA-80, Tricell

for measurement of Total Hg [THg] in solid, liquid or gas samples without sample prep.*

(working range: 0.1ppb-30ppm or 0.0015ng-1500ng·Hg)

CEST Training Protocol

created August 2015, updated May 2021

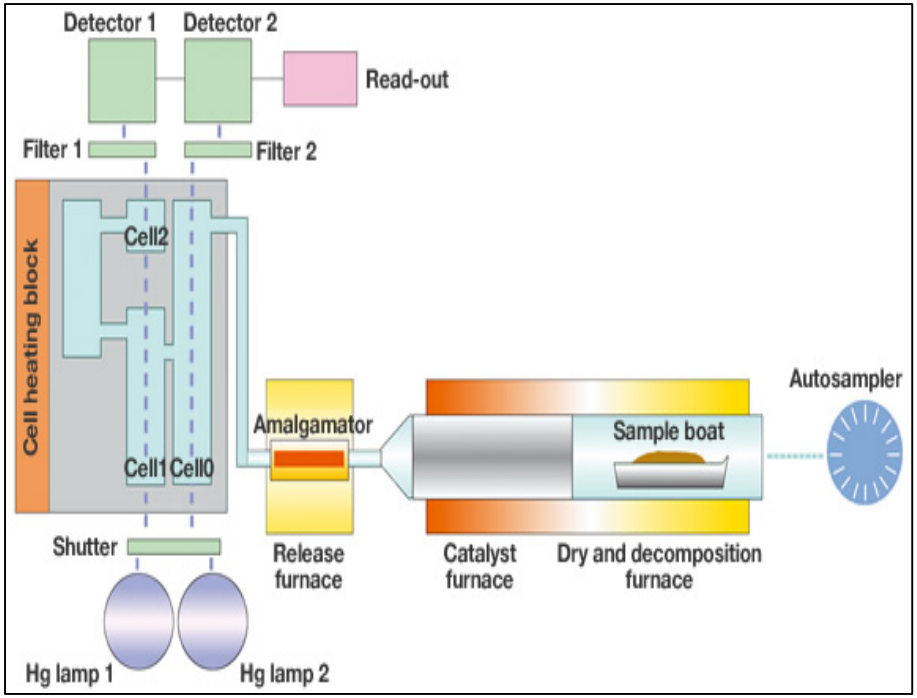
*Additional supplies required for this analytical method.

What exactly does this blue box do?

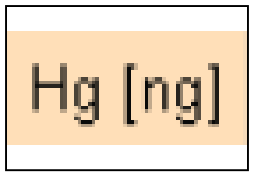
- I'm glad you asked. Take it away Milestone!
- Principles of Operation:
  - The DMA-80 combines the techniques of thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry.
  - Controlled heating stages are implemented to first dry and then thermally decompose a sample introduced into a quartz tube.
  - A continuous flow of oxygen (@CEST: air) carries the decomposition products through a catalyst bed where interferences are trapped.
  - All mercury species are reduced to elemental Hg and are then carried along to a gold amalgamator where the mercury is selectively trapped.
  - The system is purged and the amalgamator is subsequently heated which releases all mercury vapors to the single beam, fixed wavelength atomic absorption spectrophotometer.
  - Here, absorbance measured at 253.7 nm is proportional to mercury content in the sample.

- Basically, it analyzes in a similar manner as a traditional AAS instrument for THg analysis, but removes the error-prone and acid-heavy wet digestion steps. Also, you have the flexibility of analyzing multiple types of samples, nice!

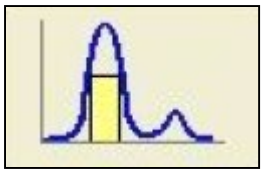
- Below is a lovely diagram of the guts in our tricell model.



Yes, I stretched this. Sorry about that. It fits a lot better, though!



Ok got it, what else?



- The DMA-80 uses a fairly specific notation, reporting back data as the absolute amount of Hg, as measured in nanograms.
- The end result is simply a concentration calculated in ng/g (ng of Hg detected/g of mass inputted), which can be converted to other units via the software interface.
- Commonly you will see trace element data reported as dry-weight concentrations ([DW]), but a lot of THg results are also presented in wet-weight concentrations ([WW]). It's very important to be aware of this difference in datasets.
- At the end of this document, we have formulas for going back and forth and for calculating % Moisture, which can also be estimated, depending on sample type.
- Solid reference material certified concentrations, are going to be presented in [DW] as a general rule.

This document won't cover liquid-based analyses, but the basics remain the same.

This image shows about $174 worth of nickel boats, hmph.

Analysis prep., I

This image shows something like $2140 worth of quartz boats!

- If working with solid materials, use only pre-ashed nickel boats. CEST has 80+ available to borrow. The instrument can clean them on a pre-set cycle, or just arrange them in a single layer in a foil-covered aluminum pan, in a muffle furnace for 2h @ 500°C.
    - It's a good idea to vigorously brush out your dirty nickel boats after a sample run. Also, don't bring that toothbrush near your mouth ever again.

- **<u>Please avoid using the quartz boats for solid sample analysis if possible!!</u>**
    - Quartz boats are "clean" to use again with liquid samples after being run through the instrument. Solid samples will require ash removal and elbow grease, as above.

Still awake? I flipped the pictures on ya!

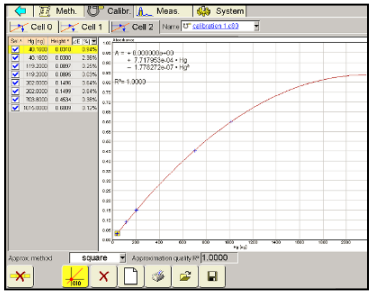
They won't stay this pretty, believe me.

Analysis prep., II

- Depending on sample type, weigh out between ***30-100mg** of your ideally ground and homogenized sample, on a laboratory balance using the stand.

- The nickel boats can accommodate up to 500mg of non-acidified samples, whereas the quartz boats hold 1500µL of acidified matrices.
  - Note: the instrument will only take weights to 0.0001g range, just like the balance nearby that CEST graciously provided.
  - Never fill the boats more than ¾ full, as they a) can be rather tippy and b) the autosampler moves juuust fast enough to spill material.
    - So, top-out nickel boats around 350mg and quartz boats around 1100µL . Also, the more material you weigh, the more ash you have to clean out, later!
    - *Milestone recommends starting at 5mg for unknowns, and increasing mass thereafter. A reference we talked to, who mostly uses this instrument for fish tissue and sediments, suggested the 30-100mg range. Other users start at 200mg in protocols we've seen (they apparently enjoy cleaning up ash piles).

"Square" fit curves are misleadingly named.



Analysis prep., III

- This instrument stores calibration curves (try not to freak out about it), but does require a daily calibration prior to running samples, in addition to QA/QC sprinkled through a sample run.

- We've successfully calibrated using solid CRMs (certified reference materials = $$$), but resources are available for detail on calibrating using aqueous [THg] standards (a cheaper route).
  - Speaking of CRMs, you do have some that match your sample matrix, right? The Lamberti lab (in BIOS) has limited supplies of NRCC's DORM-4 (dogfish protein homogenate), IAEA-436 (tuna homogenate) and NIST's SRM-1515 (apple leaves). CEST has NIST's SRM-1547 (peach leaves) and possibly others, just ask what might be laying around – and go find its most recent certificate to see the certified range listed for THg.
  - Gary's lab created their own in-house lab control material (do this for your sample matrix too), also from tuna, but they bought it at Meijer, so it was a lot cheaper.

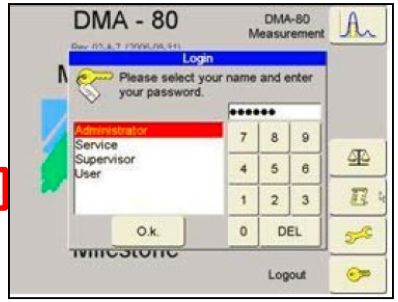
Milestone likes to say that a HS graduate with 1h of training can run this instrument, so what's your deal anyways?

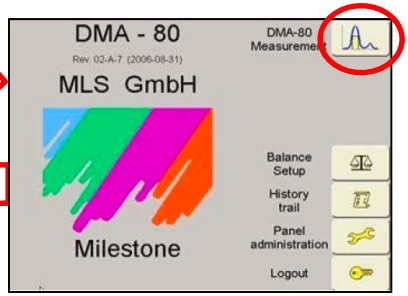
Start-up

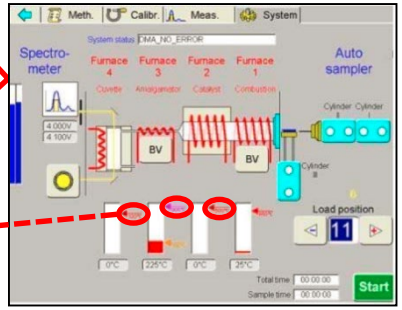
- Turn on the instrument, and allow at least 30min for warm-up, before running your daily calibration..
- The secondary valve on the air should cylinder be opened up next.
  - If you see this icon 

, the system is not ready. Hold your horses, sheesh!
- The login screen will ask for your user/password, enter: ***Admin/12345***
- The system screen will then change, and you need to select the ***DMA-80 Measurement*** icon at the upper right.
- Next, select the ***System*** tab 

while things warm-up. Furnaces that aren't ready will blink until they reach the starting setpoint (designated by red arrows). The first furnace only gets "ready" when a sample is about to be run.

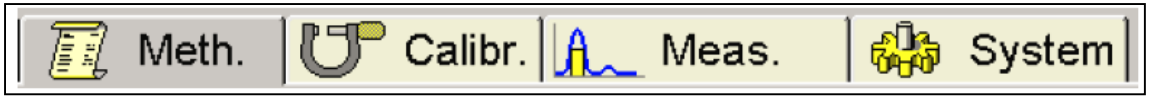






Software particulars, I

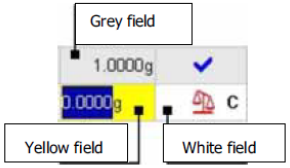
- The touchscreen interface has a stylus, but will also work with either a gentle finger caress or a firm tap.
- The four main tabs (Milestone calls them registers) are:



  - Methods: creation, modification of temperatures, drying times, heating intervals, decomposition, rinsing time of measurement program
  - Calibration: You can specify the regression curves for each cuvette (tricell = 3); administers calibration files.
  - Measurements: Where the magic happens™! You can see real time values or previous data here, statistics are also calculated.
  - System: Aren't you paying attention? Seriously there are some real guts here, you can see all the heating elements, details on the spectrometer and shift along the autosampler (which is really helpful when adding boats). Tread with caution.
- *Double-tapping* on any active input field will bring up a keyboard with letters AND numbers, woo-hoo!

Conditions valid for all tabs:

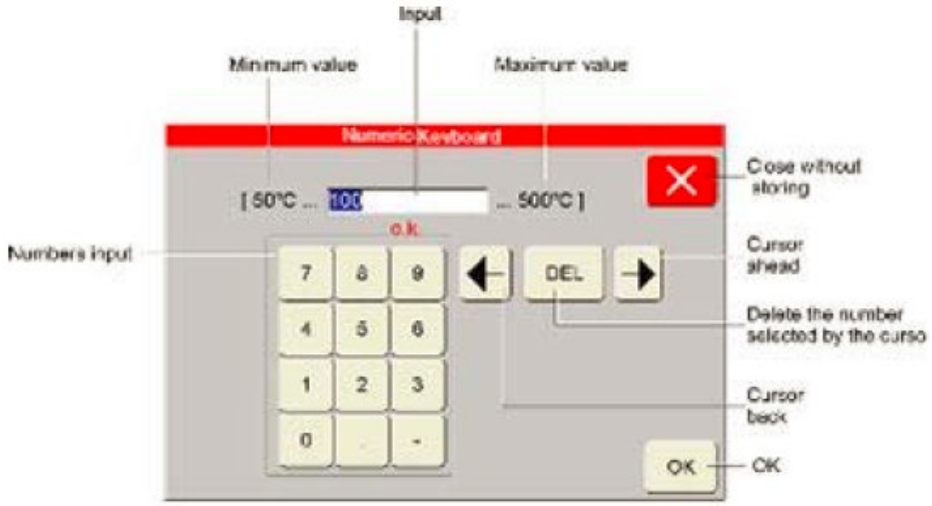
- only white fields can be worked on.
- grey fields cannot be worked on as the sample was already measured. It is possible however to add remarks and make statistics.
- yellow fields are marked and ready for input.

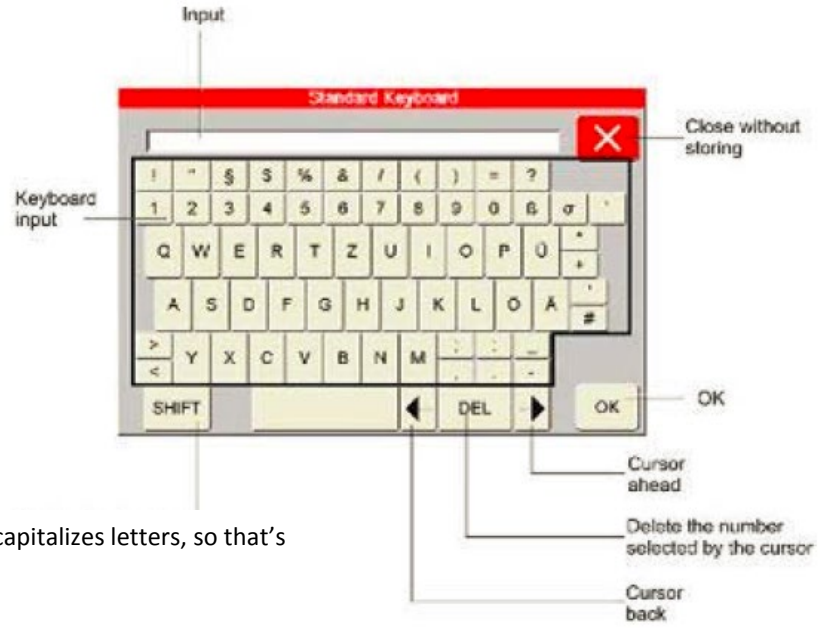


- You'll find that most of your time will be spent in the ***Measurement*** tab, but lets familiarize you with the others, too.

Software particulars, II

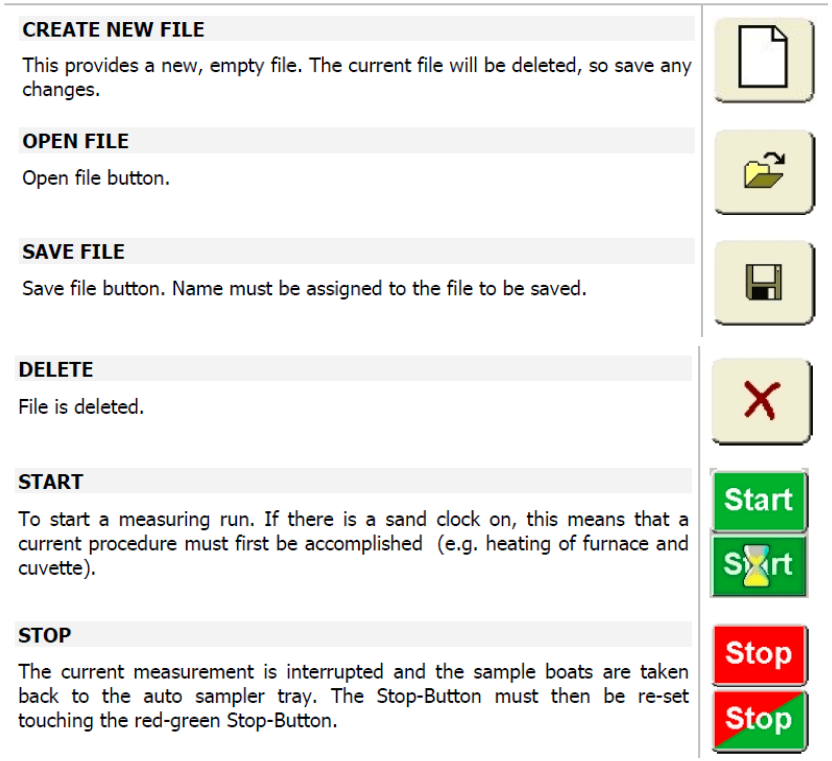
- The touchscreen interface does take a little bit of practice to master. The cursor part can be weird, as its not very PC or Mac-ish in its behavior - so learn to use the arrow buttons to direct the cursor.





Shift capitalizes letters, so that's nice.

Icons you should (probably) learn, I



- In terms of importance, the blank page, and 3.5” floppy (kudos if you remember 5.25s”) and the red X icons are among your most important here.
  - The ***new file*** button will delete anything not saved, so if you filled out a whole run of samples and then hit that – boom you’ll be sorry. After you can view an old run, then hit this to start a new one, essentially. Or when you screw up a sample list (we do this too, it’s ok).
  - You should be hitting that ***save*** button something like 100+ times a run, if you’re doing things right.
- Otherwise, the ***green start*** button is the next most common icon (guess what it does!). We were warned not to hit the ***red stop*** button much – so we don’t, especially while a boat is loaded.

- After each mass entered…
- After each new sample added…
- After each tweak of the calibration…
- Etc….

This is just a modest little reminder for you.

Icons you should (probably) learn, II

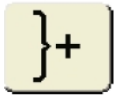
- When you see this, a sample has been successfully measured by the instrument.

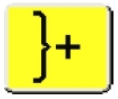
- A sample is actively being measured when this icon is present.

- These indicate whether a sample in the run sequence is ready for analysis (red=*nyet*), so enter a weight.

- Embrace your inner wizard, this is how you add a sample in the ***Sample***/***Result*** tabs

- This is how you delete a entire sample from several tabs; wield carefully. Use when you put sample #3 before sample #2.





- This is how you can concentrate a high sample across multiple boats, i.e. all the THg is trapped from said boats (low masses) and only thermally released from the gold amalgamator after the last boat is loaded. Yellow indicates the function is activated. *Please note, we've not used this function, yet.*

SIMPLE MODE

Each sample is measured individually. Each measurement has to be manually started.

1 Click on the sample to measure and start the measurement.

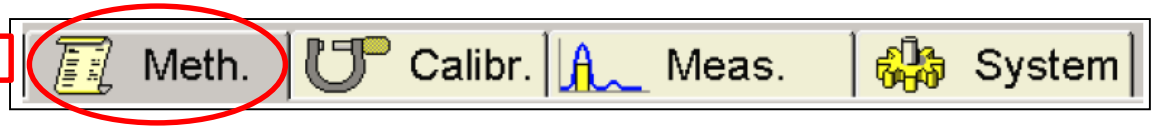
After measurement the rotating plate is turned to loading position of the next sample. However it is possible to click on and measure any sample.

AUTOMATIC MODE

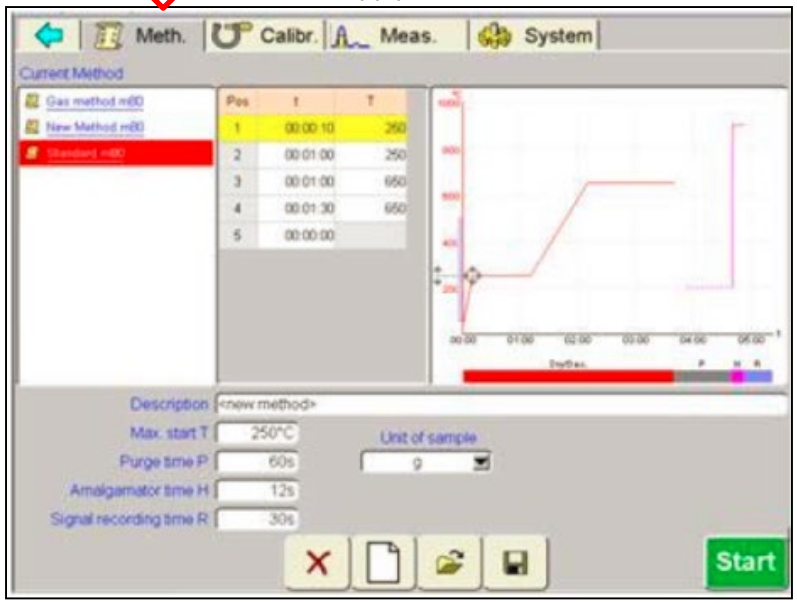
Samples are automatically measured in sequence one after the other. After measurement the rotating plate turns to loading position of the next sample, and the next measurement starts automatically.

- This is fairly self-explanatory, but if you are doing a calibration, its likely you'll want to use ***Simple Mode***, *aka* boat-at-a-time.
- For most typical runs, even for a daily calibration, ***Automatic Mode*** *is preferred,* as long as you set things up right.

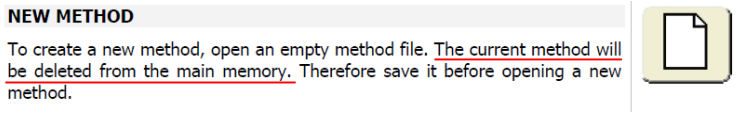
Breaking out the tabs: Methods



I resisted making any jokes about "Meth.," which is really hard as you know we are in Indiana….

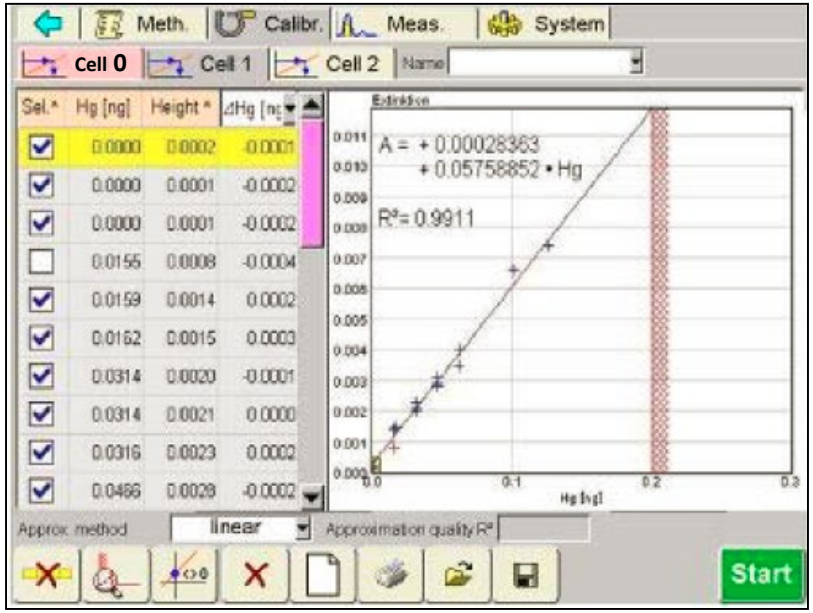


- In the ***Methods*** tab you can alter any system parameter for your desired type of samples.
  - Programming steps include: temperature, heating ramp, decomposition time, amalgam heating time (i.e. when to release Hg to the spec. cells) and system purge time.
- <u>Any digital file ending in *.m80 is a method file type</u>.
  - Analysis of wet or gas samples would likely require development of a new method, and Milestone has the relevant details for this.
  - The current method, ***Salmon.m80*** is tuned for analysis of solid dry organic materials (i.e. fish). Be careful, <u>don't modify/overwrite it (see @ lower left)!!</u>
  - There are also methods for cleaning used boats and for conditioning new catalysts, <u>don't modify them either!!</u>



- Multiple resources are available to tweak the programmable steps per sample type, see an exhaustive paper from Germany's Fraunhofer Institute, called: *Determination of Mercury in Environmental Samples by Direct Solid Analysis*.

Tabs, part deux: Calibration



- In the ***Calibration*** tab you can open, create or save calibration files.
  - Full calibrations are only required after a major change in the system (replacement of the catalyst, amalgamator, etc.) which could happen after ~500 injections or exhaustion of a consumable component.
  - However, we still run a daily calibration (details ahead) before an analytical sequence.

Any digital file ending in *.c80 is a calibration file type.

- The current calibration file, ***CRMs_calibration.c80*** is tuned by analysis of that CRM at varying weights (ng·Hg). Please don't modify/overwrite it!!
- We initially made an aqueous Hg calibration too, but it was spotty on the low end. We could make a much better stab at it now.

In a tricell model, there are three optical spec. cells (cuvettes) of different pathlengths. Each is tailored to a different range of ng·Hg content, and each is visible in a separate sub-tab.

- Cell 0: **0ng-10ng** (approx.)
  - For the solid curve above, we used: 0ng-5ng
- Cell 1: **10ng-20ng** (note, no "zero")
  - For the solid curve above, we used: 0.05ng-20ng
- Cell 2: **20ng-1200ng** (note, no "zero")
  - For the solid curve above, we used: 40ng-300ng

- The calibration process is similar to a regular run of samples; masses are entered in the ***Measurement*** tab and coded that they are calibration samples.

Measuring points are included in the calculation. Marked blue in the Table. — blue

Measuring points are included in the calculation, but new calibrated and not confirmed by the user (e.g. saving). — red

Measuring points are **not** included in the calculation. No marking in the Table. — grey

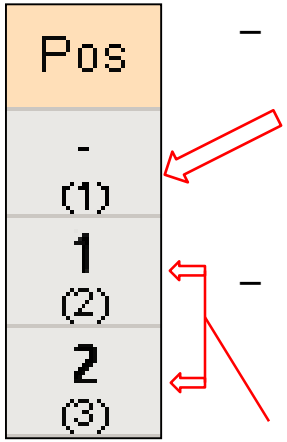
ZERO POINT SETTING

Here it is specified whether the calibration curve is to be placed across the zero point. The curve is placed across the zero point using the yellow button. Normally it makes sense to put the calibration curve across the zero point. An exception is represented by the Ultratrace analysis.

- Milestone recommends using a square fit for calibration curves. However if you did a good job, there won't be much difference between it and a linear fit.
  - Each cell requires a minimum of 3 points (in addition to zero for Cell 0), Milestone recommends 4-5, but making extra for the low Hg content cell is a good idea.

In depth: Calibration

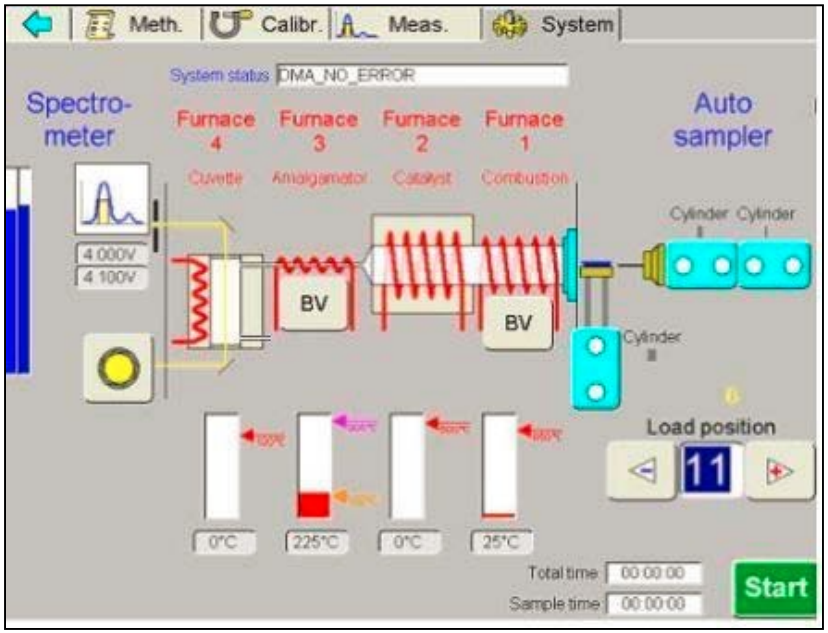
- Select [ ] under Calibr. tab.
- Select [ ], name it, [ ] to flash drive.
- Switch to Meas. tab, [ ] samples, blanks first.
- Give a nominal weight of 1.0000g for any blanks or enter as an arbitrary weight until you have an actual sample mass.
  - An instrument blank (*null boat*) is measuring the amount of Hg content in the drying chamber (where the boat goes!)
    - Add a sample and double-tap the position column, enter a "0." In the run sequence list it will show a **(1)** , meaning this measurement will be happen first, but with a " " above it, meaning there is no actual boat to move.
  - Next a pair of method blanks (*empty boat*) can be added just like a regular sample, give them a 1.0g mass and make sure the position, the number on top; matches where it resides on the autosampler.
  - Skip two positions after each boat you add, just so you have room to move stuff around.
  - Only add run sequence items in order as the software won't let you re-use a position, if you make a mistake, clear the board and start over.



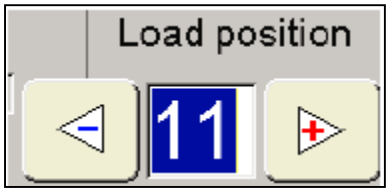
- Once your run sequence layout is filled out to your liking (with all weights entered), remember to save it!! Visit the Links sub-tab, and ensure the correct method & cal. curve is chosen.
- It's suggested that after running two blanks for a calibration curve, to drop the first blank at the beginning of the calibration (so as to keep the Hg content low).
- When populating a run sequence enter the actual weight for working standards *after* entering the "ideal" weight of a standard.
  - Say you ideally wanted a 5ng standard, but you got 5.8 because your weights are a tad off, so just make sure the correct ng·Hg is seen under this column Hg [ng] .
  - Choose the correct calibration state so you can "enter" the correct ng of Hg, the next column over should change to show your STD's [Hg] in correct units.
  - You might need to give the zero a nominal amount of Hg too, like 0.00001ng.
- When adding low standards, you can still change the weight of the next sample until the previous boat is ejected, or as the result peaks are being drawn. After those events, the weight is locked.
  - If doing an aqueous curve, its useful to wait until the last few minutes of a previous sample's run before weighing out the next standard. *Evaporation when you're pipetting mere µLs is a problem!*
  - *You can alternate between simple and automatic sample mode at any time – which could be handy here.*

Tabs, tabs, everywhere there's tabs: System

- We already mentioned this tab when you turned the instrument on, and unless you're way more qualified than the rest of us (as if!) you probably shouldn't be messing around in here much.



- However this



does come in very handy.

- Otherwise, as long as you see

, just leave well enough alone.

It's the final tab…doo-doo-doo, doo, doo-doo-doo-doo-doo: Measurement

- If you're following along at home, you might wonder why we haven't gotten to talking about that pesky old **Measurement** tab just yet.
  - Well, its because you will spend the most time there, and with its many sub-tabs things are…complicated*.
  - Hey, you've been saving everything as you go along, right? We pity the fool that doesn't.

- Tap the **Measurement** tab, then, go to the **Sample** sub-tab or **Result** sub-tab and clear any old results by tapping our old friend; if the previous user didn't save data – it's now their problem.
- Here's a good place to mention that functionally the only difference between the **Sample** and **Result** tab is that you can enter "Remarks" in the former and not in the latter. The gist: you might as well work in **Results**.

*insert joke about Facebook status, if still relevant in 5 years. Psst, did you know I can make red rectangles – because *I* can.

The Measurement strikes back: Daily Calibration

- Now that you've started a new analytical sequence, you're going to run the daily calibration first.

- Our general plan can be found in a copy of our *Run Sheet*, but briefly we run:
  - (2) Instrument Blanks, (2) Method Blanks, (1) Low CRM, (1) High CRM, & (2) Method Blanks.
  - So a daily warm-up would require 6 boats, with varying masses of CRM. The empty boats just help make sure the system is clean post-measurement. These blank absorbances should all be in the neighborhood of 0.0030 or lower.
    - CRM values: Low = ~5ng & High = ~125ng, going above 150ng might trigger an Auto-BV.
  - *The range we chose is based on a specific type of samples (fish homogenates) and should be adjusted as necessary.* Our range of values comes from other protocols and Milestone references (some use as many as four: 5ng, 20ng, 100ng & **300ng, yikes!**).

- <u>Any digital file ending in *.d80 is a measurement file type, meaning it contains actual data</u>.
  - We generally name our daily calibration files for the current date, which is also referenced in the name of our subsequent unknown measurements.
    - » **daily_cal_08_02_2015.d80** is therefore chrono-linked to **sample_sequence_08_02_2015.d80**, and they both are data files.

- There only 40 slots on the autosampler tray and we want to conserve them mostly for actual samples, so post-daily calibration save the results, clear the run sequence and remove all boats from the tray.

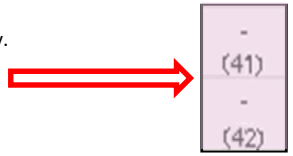
Measurement: (I) Run (so far away) Sequence

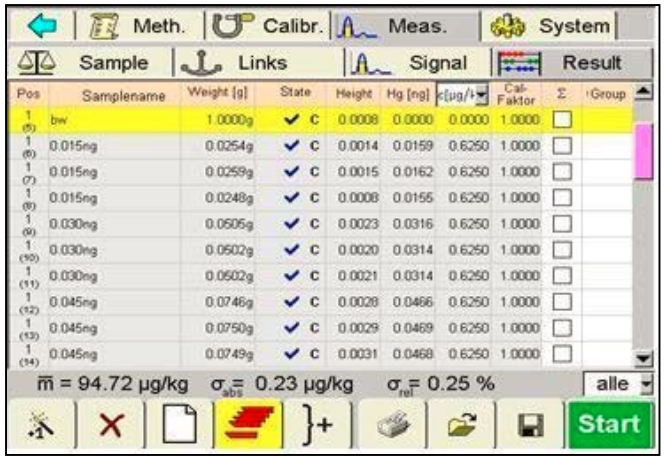
- We've sort of alluded to what this looks like, but essentially using the icons you've already learned, you need to tell the instrument what samples to analyze and in what order.
- Unless the positions are wonky, this should simply be a top to bottom order, like the diagram shown at right; this shows that the boat in slot one will be run first, the boat in slot two will be run second, etc.

- one sample at a time, enter the mass (⟹) and pick the units you want µg/kg, mg/kg, etc.
- Fill out the sample table until you reach position 40.
- To round out your sample run, add two instrument blanks (null boats) to clean out the system before you're done for the day.

If using the attached Mettler balance, highlight the sample weight field on the DMA and press the balance's "PRINT" button to input to the active field.

  - The system will pop-up a warning message, when you try to add these line items, telling you something to the effect of "Hey, I don't have that many slots?!? WAAHH, I'm confused."
  - Press **No/Cancel** and make sure they appear like this:
    - It highlights them in pink for who knows why.



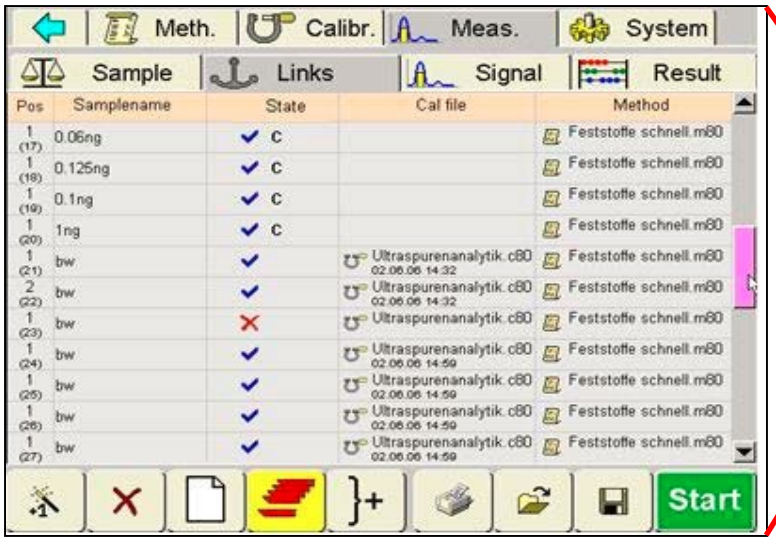


- If you're doing all this right, you're almost ready to highlight the first position and tap Start. But first you need to take care of the **Links** sub-tab!

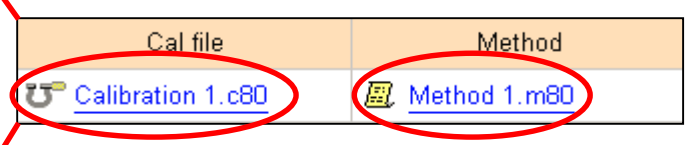
Measurement: Links (insert Zelda-joke)

- This sub-tab is fairly easy to understand.
  - In order to properly analyze samples you must ensure the correct calibration file and method file have been chosen ***for each sample*** in your run sequence. Tap a cell and a drop-down arrow will appear; pick a pair for each line item. It may apply what you've picked for position 1 to 2-40 automatically, don't be alarmed. Just make sure they're all correct.

  - That's it. Pick them, [💾] and then ignore away. *Now you can actually start the analysis*. Tap [Start].



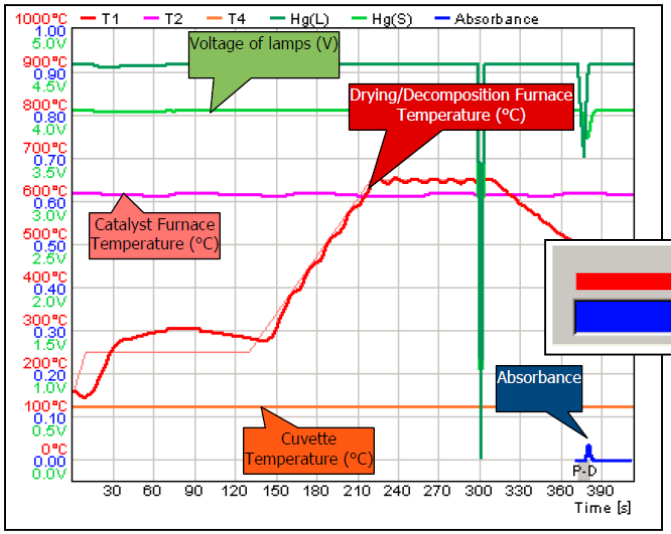
You should pick the most recent/applicable curve for your samples.

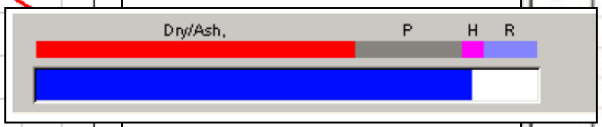


You should pick the most applicable method for your samples.

Measurement: Signal (plot along, yo)

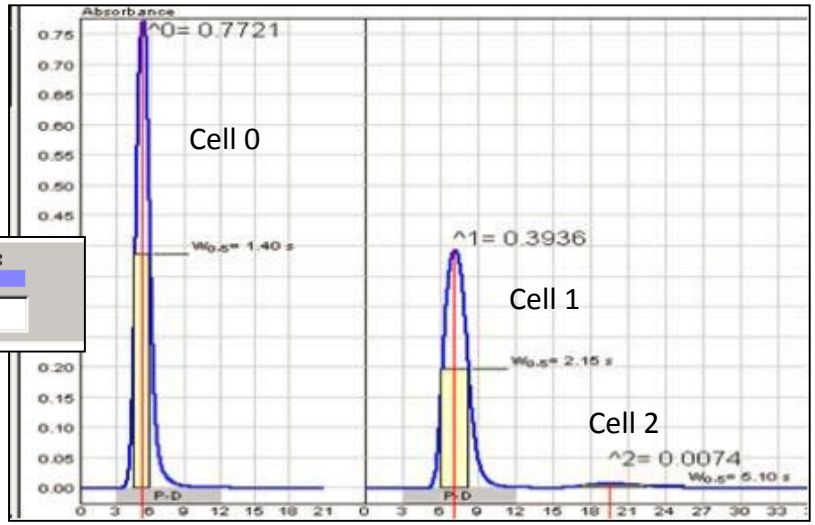
- Ok, so you started analysis – some congrats are in order! Now, you should keep your eye on what's happening in that blue paperweight in front of you.
  - This tab will show a series of lines progressing along, finally ending in a spectrograph of three peaks.
  - Pressing this  will alternate between the final peak(s) and the temp. gradients your sample encountered.





Sample Analysis Time

These status bars monitor your sample's progress during analysis.

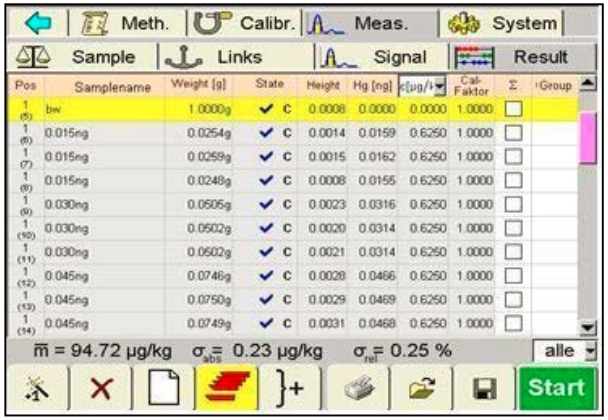


These graphs monitor important variables affecting instrument performance. The voltage lamps (green lines) may be worth scrutinizing! Ours are usually quite close together.

Behold! You have Hg in these samples. The peaks will now be calculated into sample concentrations, based on inputted weights.

Measurement: Results

- At last, your run is done,  your data!
- I hope the results/sample tabs are relatively familiar at this point, but let's just make sure.
- Verify your QA/QC samples at this point; CRMs should be in the acceptable range as provided by the item's distributed certificate.



Height = Absorbance

It's the maximum height of the evaluated peak.
Extinction = lg (100 % Transmission / X % Transmission)
or area of the peak.

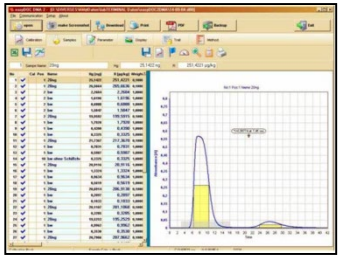
Hg [ng]

Absolute quanty of Hg in the sample (calculated from active calibration)

c[µg/kg] or c[mg/kg]

Concentration of Hg in the sample (calculated from active calibration and sample weight).

- Move your calibration or data files (*.c80, *.d80) via the memory stick to a computer that has Milestone's easyDOC3 DMA software (CEST has it).
- You can view these files and then export as *.xls, *.PDF or as *.JPGs. There is some capacity for making reports too. Snapshots of the peak signals are super nice for presentations.
- We've been exporting and working up our data in Excel and are pleased with that. The main reason to do so, is that taking a raw file straight from the DMA-80 and opening in Excel, may not properly populate all fields.



SAVE RESULT TABLE (CHART) AS EXCEL FILE

The file is exported as Excel file.

SAVE RESULT TABLE (CHART) AS ASCII

The file is exported as ASCII-file; or the diagram will be exported as graphic file.

Calculations & Conversions, I

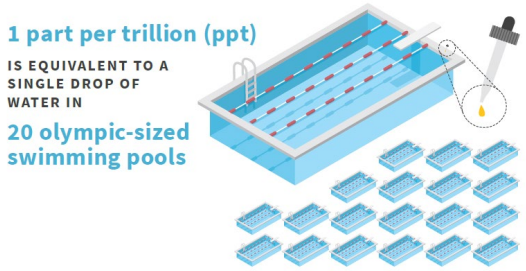
- Percent Moisture = [(wet-weight, g – dry-weight, g) / (wet-weight, g)]*100

- assume
  Unless otherwise noted, ~~a~~ trace-element concentrations in biological samples are in dry weight. Dry-weight and wet-weight concentrations in biological tissue can be converted from one to the other by using these equations:
  Dry-weight concentration = (wet-weight concentration) / [1 — (percent moisture/100)]
  Wet-weight concentration = (dry-weight concentration) x [1 — (percent moisture/100)]
  (from USGS 1655)

[SI unit]	Alternate Equivalent	[Parts-per Notation]	[Aqueous mass]	Alternate Equivalent
mg/kg	µg/g	ppm	mg/L	µg/mL
µg/kg	ng/g*	ppb	µg/L	ng/mL
ng/kg	pg/g	ppt	ng/L	pg/mL

1 ppm = 10^3 ppb (1,000 ppb) = 10^6 ppt (1,000,000 ppt)
10^{-3} ppm = 1 ppb = 10^3 ppt
10^{-6} ppm = 10^{-3} ppb = 1 ppt

*Again, this is what Milestone uses in most literature.



- CRMs will report [dry-weight THg], but often you will need to calculate the nanograms of Hg in a unit of tissue; therefore, simply input **X** & **Y** to get **Z**.

X ng (Hg)	1g	*Y* mg (tissue)		*Z* ng of Hg
g (tissue)	1000mg			

e.g., 50mg of DORM-4, with a [DW-THg] of 412ppb, will have ~20.6 ng·Hg.

Calculations & Conversions, II

STANDARD DEVIATION

The Volunteer Creek Monitoring Project wants to determine the precision of its temperature assessment procedure. They have taken 4 replicate samples:

Replicate 1 (X_1) = 21.1^0 C
Replicate 2 (X_2) = 21.1^0 C
Replicate 3 (X_3) = 20.5^0 C
Replicate 4 (X_4) = 20.0^0 C

To determine the **Standard Deviation (s)**, use the following formula:

$$s = \sqrt{\sum_{i=1}^{n} \frac{(X_1 - \overline{X})^2}{n-1}}$$

where x_i = measured value of the replicate, $\bar{x}$ = mean of replicate measurements, n = number of replicates, $\sum$ = the sum of the calculations for each measurement value--in this case, X_1 through X_4

First, figure out the mean, or average of the sample measurements. Mean = $(X_1 + X_2 + X_3 + X_4) \div 4$. In this example, the mean is equal to 20.68^0 C.

Then, for each sample measurement (X_1 through X_4), calculate the next part of the formula. For X_1 and X_2, the calculation would look like this:

$$\frac{(21.1 - 20.68)^2}{4-1} = \frac{(-0.42)^2}{3} = \frac{0.1764}{3} = 0.0588$$

For X_3 the calculation would be 0.0108; and for X_4 it would be 0.1541

Finally, add together the calculations for each measurement and find the square root of the sum: 0.0588 + 0.0588 + 0.0108 + 0.1541 = 0.2825. The square root of 0.2825 is 0.5315.

So, the standard deviation for temperature is 0.532 (rounded off).

The standard deviation (SD) indicates the range of variation observed in measurements.

(from EPA: *The Volunteer Monitor's Guide to Quality Assurance Project Plans*)

RELATIVE STANDARD DEVIATION

If we use the same replicate measurements as above in the standard deviation example, we can determine the **Relative Standard Deviation (RSD)**, or coefficient of variation, using the following formula:

$$RSD = \frac{s}{\overline{X}} \times 100$$

where s = standard deviation and $\bar{x}$ = mean of replicate samples.

We know s = 0.5315 and that $\bar{x}$ = 20.68. So, the RSD = 2.57. This means that our measurements deviate by about 2.57%.

The RSD or coefficient of variation expresses the SD as a percentage. Smaller values = more precision.

RELATIVE PERCENT DIFFERENCE

If the Volunteer Creek project had only two replicates (21.1^0 C and 20.5^0 C) they would use **Relative Percent Difference (RPD)** to determine precision.

$$RPD = \frac{(X_1 - X_2) \times 100}{(X_1 + X_2) \div 2}$$

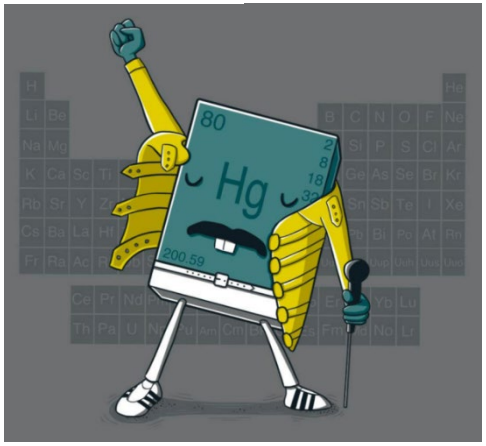
where X_1 = the larger of the two values and X_2 = the smaller of the two values. In this example, X_1 = 21.1^0 and X_2 = 20.5^0.

$$RPD = \frac{(21.1\text{-}20.5) \times 100}{(21.1+20.5) \div 2} = \frac{60.00}{20.8} = 2.88$$

So, in this example, the RPD between our sample measurements is 2.88%.

The RPD can be used when you only have two replicate samples.

Percent Recovery = $\frac{\text{Amount of Substance you Actually Collected}}{\text{Amount of Substance you were Supposed to Collect}}$ x 100



Miscellaneous nuggets

- Your DMA-80 responsibilities are similar to those for any other asset:
  - Sign up using Google Calendars
  - Only use instruments you have been trained on by CEST Staff
  - Clean up after yourself (especially important here, with all the ash)
  - Clean and return CEST's boats in a timely fashion
  - **Detail all use** in the logbook, including the <u>total number of injections per run</u> (this includes all forms of blanks, CRMs, samples, etc.)

- Blank values should be <0.0030 ABS/Height
- A good calibration curve (using a square fit) should have an r2>0.995.
- Keep an eye on the back of the unit, we don't want this exhaust tube to get sliced or have any cracks in it! This would be bad.

Helpful references

- Canada, U. of Windsor, GLIER Metals Lab SOP 01-007: *Analysis of Total Mercury in Biological Tissues using a Direct Mercury Analyzer (DMA-80)*, March 2012.
- U.S.A., U.S. EPA Protocol: *Method 7473: Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry*, February 2007.
- Germany, Fraunhofer Institute for Molecular Biology and Applied Ecology, Guidelines for Chemical Analysis: *Determination of Mercury in Environmental Samples by Direct Solid Analysis*, February 2011.
- U.S.A., U.S. Geological Survey: *OFR 02-223-O: Mercury in plants and animal tissue by thermal decomposition-atomic spectrometry,* May 2002.
- Italy, Milestone SRL, *DMA-80 User Manual*, MA122-004
- Italy, Milestone SRL, *Tips and Techniques for DMA-80 Optimization*, September 2009
- Additional literature available from the Usernet:
  http://www.milestonesci.com/support.html

Feeling handy?

- In case anyone else wants to make a new boat holding tray:
  - Quartz boats are ~7mm wide and 34mm long
  - Nickel boats are ~8.4mm wide and 34mm long
    - Again, these are somewhat loose measurements as the width of the boat narrows, but these should allow the boat to “hang” properly in place on something homemade.
    - We made a few extra “weighed sample organizational stands” out of soft plastic container lids that we tediously cut many slots into.
- Milestone’s weighing stand (at right, below) is 7.5mm wide, but not as long as a boat.

- Finally, please find a copy of what our DMA-80 run sheet (instrument sequence) looks like.
- Feel free to adapt as you see fit.

DMA-80 *Total Hg* [THg] Analysis

Date:_____ **Sample Type:**_____ **User:**_____

Daily Calibration

Sample Type	ID	Weight (g)	Autosampler position (slot#)	Notes	
Instrument blank	null boat	1.0000	1		
Method blank	empty boat	1.0000	2		
Method blank	empty boat	1.0000	3		**DMA-80**
CRMs - "low"	NRCC DORM-4	_____	4	*~12.2mg is 5ng·Hg*	**Daily**
CRMs - "high"	IAEA-436	_____	5	*~29.8mg is 125ng·Hg*	**Warm-up**
Method blank	empty boat	1.0000	6		
Method blank	empty boat	1.0000	7		
Instrument blank	null boat	1.0000	1		

↑ *remove from tray, while confirming daily calibration* ↑

Quality Assurance/Quality Control *Remember, ng/g = µg/kg = ppb*

Certified Reference Materials	[CRM]	Acceptable recoveries for CRMs	
NRCC **DORM-4***	412 ± 36 ng/g	376-448 ng/g	(%recovery = 91-109%)
IAEA-**436**	4190 ± 360 ng/g	3830-4550 ng/g	(%recovery = 91-108%)

30 samples analyzed per analytical run

2	blanks (but including daily cal., should be ok too)	*Blanks should be under <0.0030 ABS/Height*
3	duplicates	
2	CRMs	*CRMs should be matrix-matched*
1	lab control sample	
1	matrix spike	
1	matrix spike duplicate	

Acceptance Criteria for QC Samples

% Recovery of CRMs	(Determined value of CRM/certified value of CRM)*100 Should be within ±10% certified value
Method blanks and Detection	The mean blanks should be <10% of the lowest sample [Hg] Calculate SD of the [blanks] Limit of instrument detection = 3*(SD of instrument blanks) Limit of method detection = 3*(SD of method blanks) Reporting detection limit = 2*(higher value of either above)
Duplicate samples	Mean should be within ±10% relative percent difference (RPD)
Matrix spike / MSDups	Should be duplicated at ±20% of MS for precision and ≤ 20 RPD limit 20% should be the limit of max deviation for % Recovery and RPD

Analytical Run *try (0.020g - 0.200g) for low/high)*

Sample Type	ID	Weight (g)	(slot#)	Notes	
Fish homogenate	_____	_____	1		**Analytical batch = 10 field samples**
Fish homogenate	_____	_____	2		
Fish homogenate	_____	_____	3		
Fish homogenate	_____	_____	4		
Fish homogenate	_____	_____	5		
Fish homogenate	_____	_____	6		
Fish homogenate	_____	_____	7		
Fish homogenate	_____	_____	8		
Fish homogenate	_____	_____	9		
Fish homogenate	_____	_____	10		
Duplicate ↻			11		**QA/QC**
Method blank	empty boat	1.0000	12		
CRMs (~___ng)	NRCC DORM-4		13	*matrix std.*	
Lab Control Sample	Ground Tuna		14	*weigh as unknowns*	
Fish homogenate	_____	_____	15		**Analytical batch = 10 field samples**
Fish homogenate	_____	_____	16		
Fish homogenate	_____	_____	17		
Fish homogenate	_____	_____	18		
Fish homogenate	_____	_____	19		
Fish homogenate	_____	_____	20		
Fish homogenate	_____	_____	21		
Fish homogenate	_____	_____	22		
Fish homogenate	_____	_____	23		
Fish homogenate	_____	_____	24		
Duplicate ↻			25		**QA/QC**
CRMs (~___ng)	IAEA-436		26	*matrix std.*	
Matrix Spike	G. Tuna +50mg DORM-4		27	*50mg should add ~20.6ng·Hg*	
Fish homogenate	_____	_____	28		**Analytical batch = 10 field samples**
Fish homogenate	_____	_____	29		
Fish homogenate	_____	_____	30		
Fish homogenate	_____	_____	31		
Fish homogenate	_____	_____	32		
Fish homogenate	_____	_____	33		
Fish homogenate	_____	_____	34		
Fish homogenate	_____	_____	35		
Fish homogenate	_____	_____	36		
Fish homogenate	_____	_____	37		
Duplicate ↻			38		**QA/QC**
Matrix Spike Duplicate	G. Tuna +50mg DORM-4		39	*50mg should add ~20.6ng·Hg*	
Method blank	empty boat	1.0000	40		
Instrument blank	null boat	1.0000	1	*(after removing used boat)*	**System Shutdown**
Instrument blank	null boat	1.0000	1	*(after removing used boat)*	

Save your run's data! ***Clean up after yourself!*** ***Document all use in the logbook!***