
NATIONAL CORAL REEF MONITORING PROGRAM

Standard Operating Protocol

for

Total Alkalinity (Climate)

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NOAA
CORAL REEF
CONSERVATION PROGRAM

National Coral Reef Monitoring Program Total Alkalinity (TA) Procedure

The AOML Coral Program team uses an Apollo SciTech, Model AS-ALK2 Total Alkalinity Titrator to measure the (TA) total alkalinity of seawater samples (Figure 1). The Apollo SciTech machine measures TA values in $\mu\text{mol/L}$, however our laboratory reports TA values in $\mu\text{mol/kg}$. Therefore, the density of every sample is measured in order to convert $\mu\text{mol/L}$ to $\mu\text{mol/kg}$. The TA machine needs to be calibrated every time samples are run. Calibrations are carried out using a (CRM) certified reference material. They can be new or recently opened CRM bottles. The following procedures detail how to calibrate and use the TA machine from start to finish. CRM readings must be within $\pm 10\mu\text{mol/L}$ of the expected TA value for that batch of CRM.

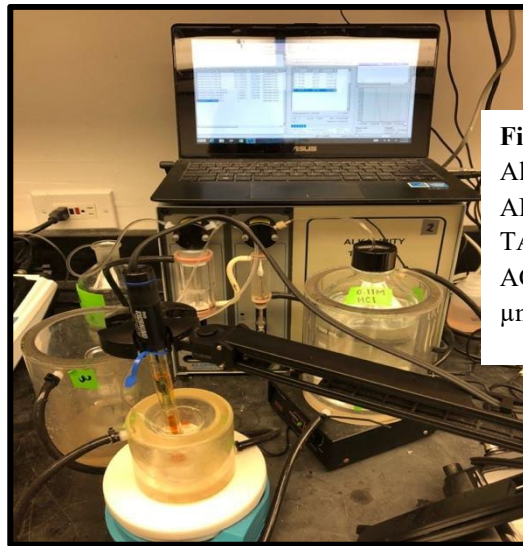


Figure 1. Apollo SciTech Total Alkalinity Titrator-Model AS-ALK2. This machine measures TA in $\mu\text{mol/L}$ however the ACCRETE team reports TA in $\mu\text{mol/kg}$.

TA Machine Directions

1. First turn on the water bath. Push the power button until the screen lights up, then hit the center arrow to turn on. Temperature should read around 25.0°C . Place a CRM in the water bath. This can be a fresh bottle or an open CRM bottle.
2. Turn on the TA machine. Green light is on when the machine is running (Figure 2).



Figure 2. Photo of the water bath on the left set to 25°C showing the appropriate water level. Photo of the TA machine on the right.

3. Remove pH probe from electrode solution. Clean with Kimwipe[®] and place in beaker of distilled water. Remove the blue plug from the top of the probe while in use.
4. If the interior reference filling solution is low, fill up to where the blue plug goes.
 - ****It is important to do this the day before using the probe, adding the electrode solution and immediately using the probe for analysis will result in inaccurate and noisy EMF readings. After using the machine and shutting it down, be mindful to check the level of solution in the probe and fill if needed for the next use.**
5. Turn on computer above TA machine. Open **TALK** program on desktop (Figure 3).

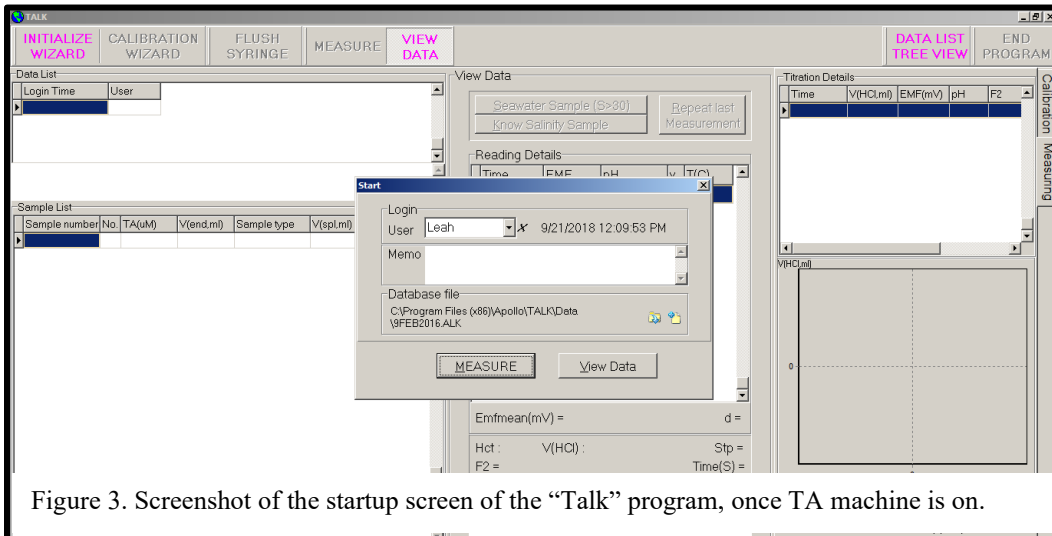


Figure 3. Screenshot of the startup screen of the “Talk” program, once TA machine is on.

6. To begin the program, select “**Measure**”. The program will begin to initialize pH meter, (Figure 4) select “**Next**”. Communication with 3star pH meter should begin, once that is finished select “**Next**” again. Initialization of digital pumps should appear, select “**Next**” again.

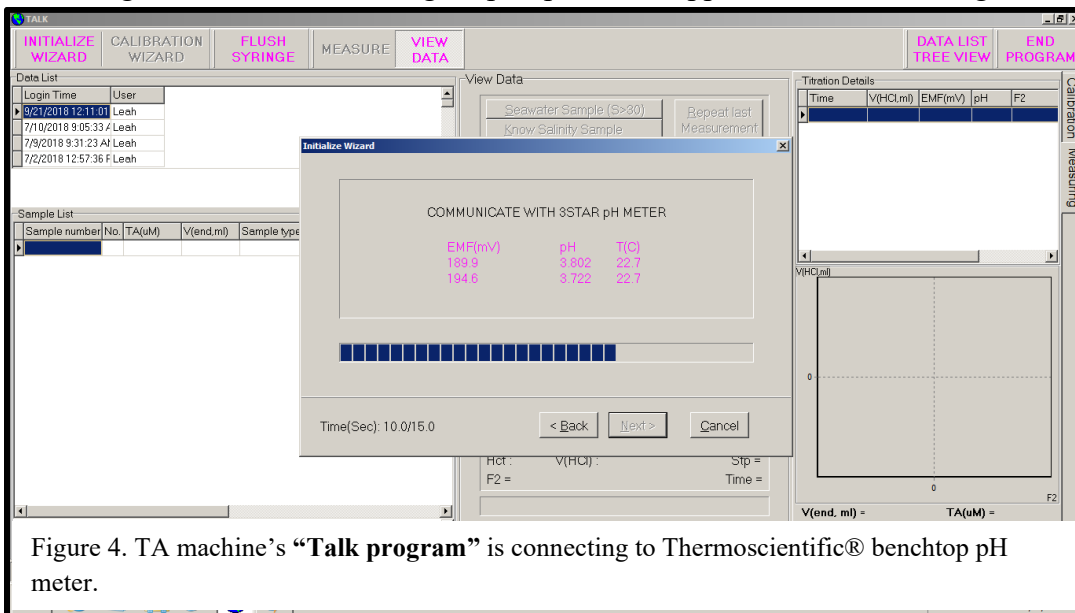


Figure 4. TA machine’s “Talk program” is connecting to Thermoscientific[®] benchtop pH meter.

7. Then the HCL pump will flush twice. **Make sure that hose B of the smaller pump is in the HCL and hose A is in the waste bottle** (Figure 5).

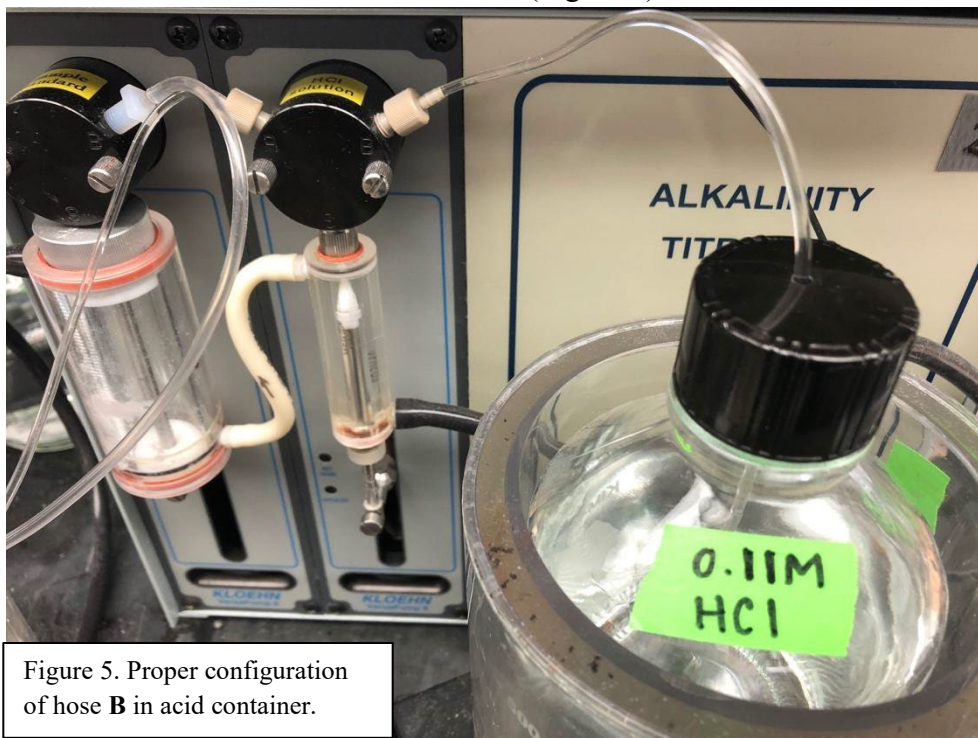


Figure 5. Proper configuration of hose **B** in acid container.

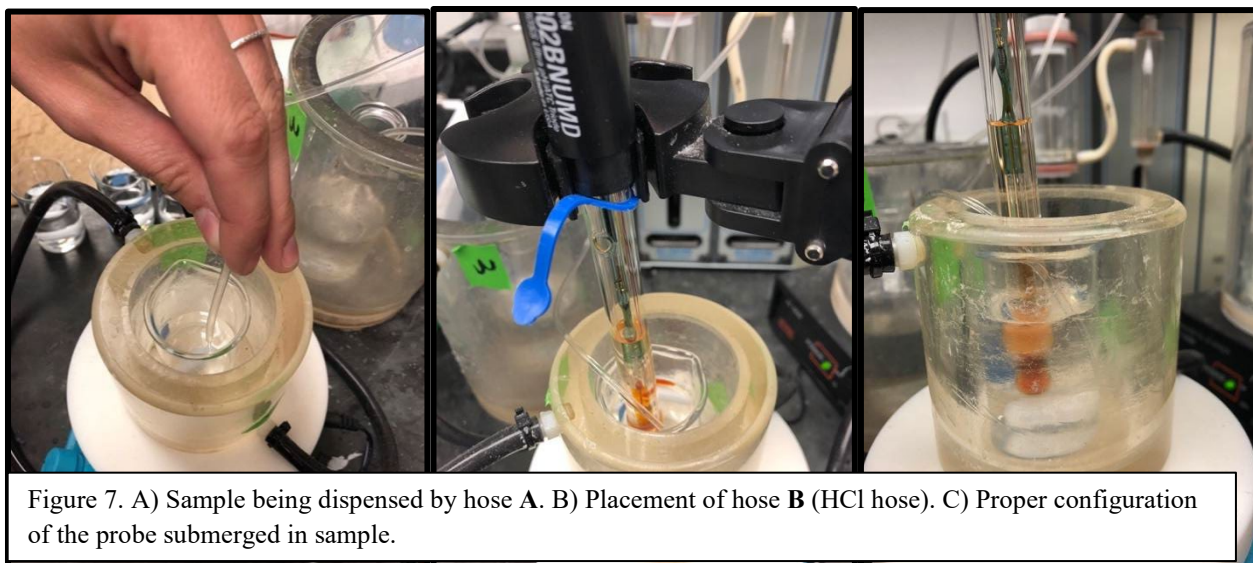
8. Once the system has been initialized the last calibration values will appear. It will ask if you want to accept these results, to begin a new daily calibration select “NO” and hit “Next”.
9. The temperature input should appear, select **OFF ATC** and input temperature at 25.0° C, select “Next”.
10. Then the prompt screen should have a place to input 3-point calibration results. This is where the calibration begins.



Figure 6. The three ranges of pH buffers used to calibrate the TA probe.

11. There are 3 different calibration values used, pH 4 (pink), pH 7 (yellow), pH 10 (blue). Grab the three different pH standards from the cabinet above the TA machine. (Figure 6).
 - o Note: fresh buffer should be replaced in calibration bottles every month.
12. Select the first pH standard value.
 - a. This should say 4. Next select the EMF (mV) box next to the pH 4.

- i. This will still have the last calibrations value in it.
 - ii. This is the number that will be changed, EMF readings should be close if not the same to the previous values.
 - a. Take pH probe out of DI water, dry with a fresh Kimwipe[®], and place into the pH 4 standard, the ThermoScientific[®] benchtop beside the TA machine should be reading numbers around 170mV for pH 4 (-170 for pH 10 and around 0 for pH 7)
 - i. Allow the reading to stabilize-
 - 1. This number is the pH reading in mV that should be input in the box next to pH 4.
 - b. Repeat these steps for pH 7 and 10
 - iii. **Note: The negative sign on the thermoScientific[®] is easy to miss, pay attention when inputting mV values for pH 7 because it can be both positive and negative**
 - i. Once all mV values have been entered select “Next”
13. Once the pH values have been entered the HCl concentration needs to be determined.
14. Select “**Determine by standard solution**” and hit “Next”. The next prompt asks for the data from the CRM enter this info into the system
- a. Volume of **ALK** standard should be 25ml
 - b. Name of the standard is whatever **CRM** batch is being used Ex. CRM 138
 - c. “**Alkalinity value**” of the standard only changes when the CRM standard is changed
 - d. Estimated concentration of **HCL** should be 110Mm (unless using different concentration)
 - e. Once all the data is entered select “**Ok**”
15. Remove CRM from the water bath and place it in the large plastic bath connected to the TA machine. Now a screen will appear giving direction on how to perform the titration
- a. Place the hose **B** of the larger sample pump in the CRM
 - b. Place hose **A** of the same pump into the waste bottle
 - Select “**Ok**”
16. The pump should now rinse once, after the pump is rinsed it will take a sample and leave it in the pump. This is the sample that will be titrated. Take a clean small glass container and stir bar and place into the smaller plastic bath on the stir plate.



17. The system will now ask you to place standard solution into titration vessel (the small glass container). Before hitting “Ok” make sure that hose A is ready to dispense into the sample beaker (titration vessel). (Figure 7).
18. When ready click “Ok”. The pump will drain the sample into the 30ml beaker. Remove hose when pump is finished. Wipe pH probe from DI container with a fresh Kimwipe® and place into the sample beaker.
19. The red tip of the probe should be completely submerged without touching the stir bar or the glass 30ml beaker. This ensures the junction potential interface at the probe membrane is in full contact with the solution, and not the air.
20. Once the pH probe is in the sample. Take the small HCl hose “A” and place it into the sample beaker. Snake it along the sides of the beaker to ensure it lies near the bottom, but not interfering with the stirbar. Be sure to not let the tubing touch the probe. The system should then prompt “Turn on the stir bar”, once the pH probe and HCL hose are in the sample beaker hit “ok”. The titration will begin. (Each sample takes about 5-7 minutes.)
21. **Note: make sure the sample hose B is always submerged in the CRM while calibrating, because during calibration samples are drawn up from the CRM automatically until appropriate calibration results are achieved.**
22. When titration of the sample is finished the sample pump (in the CRM) will automatically take in another sample. The system will have directions to place “standard or CRM” in titration vessel again.
23. Before hitting “Ok” the old sample needs to be dumped out. A new/clean 30ml beaker should be used along with a clean stir bar.
 - To properly clean beakers: rinse beaker and stirbar with tap water twice, then once with DI water. Dry thoroughly with a paper towel, and then wipe once more with a clean Kimwipe® to remove any dust or lint residue. Any water left or debris left in the beaker will give an inaccurate TA reading.
 - There are several 30ml beakers to help with efficiency of this step.

24. Now the next sample is ready to be processed. Place the sample hose A into sample container and hit “Ok” to dispense the next sample into the 30ml beaker (titration vessel) Repeat same steps above to get titration ready. Once ready, hit “Ok”. The system will run titrations three times per calibration.
25. After the third titration is completed a screen that says “**Standard titration**” will appear containing the three titration values that were just completed. The calibration titration values are aid in determining the acid concentration. On the bottom of the screen there should be an **RSD value**, this value needs to be **0.05% or less to accept calibration results**.
26. If this value is higher than **0.05%** then continue titrations with the CRM until the correct **RSD value** is displayed.
27. If it is not an acceptable value then the system will take up another CRM sample. Set up another titration clean 30ml beaker and.
28. Once this titration is complete the same screen will come up. If the **RSD value** is still too high, select continue and repeat the last steps. Continue to repeat until an acceptable **RSD value** is achieved.
29. Once the **RSD value is 0.05%** or lower select “**Accept**”. This accepts the measured HCL concentration determined from titrating the CRMs (Note: the HCL concentration should be close to the estimated value ~0.11M).
30. Once the calibration is completed and the HCL concentration has been accepted two CRM titrations need to be run. The same CRM used to calibrate can be used as long as there is enough left in the bottle.
31. To start a new titration, select “**Sea water sample**” at the top right
 - a. **Note: this is the same process that will be performed for all sample titrations. Every sample must be titrated twice after the machine has been calibrated.**
 - b. A screen will appear asking for the name of the sample
 - This will happen every time a new sample is started
32. Once the “**sample name**” is entered the screen will instruct to place the sample pump hose **B** into the bottle to be sampled and hose **A** into the waste bottle
 - The pump takes up a sample from the bottle, rinse and then prepare the next sample.
 - Then place hose **A** in the clean sample beaker with stir bar and select “**ok**”.
 - Once sample has been dispensed, place the probe and the HCL hose in the proper position and when ready select “**Ok**”
33. When the sample is finished it will not be automatically sampled again.
 - A small box will appear saying measurement complete click “**Ok**”
 - **Record the temperature of the sample using the pH probe, the temperature of the sample is located at the top left of the ThermoScientific probe. Write the temperature value in the TA log book.**
 - **Write the calculated pH of the sample (not completely accurate) in the TA logbook. pH of sample will be the first value in the top right of the program, when 0.00mL of acid was added.**

- Write the measured TA value (umol/L) in the TA logbook. TA value is bolded in the bottom right of the measurement screen in the program.
 - Then you must run the sample TWICE. Select repeat measure at the top right to begin another titration of the same sample.
 - Then the system will not rinse and will take up another sample.
 - Repeat steps (31. a-d) above to set up the 2nd titration.
34. **Note: each sample needs to be titrated twice, if the two titration values are more than 5umol/L apart a third titration needs to be done, and so on. The titration value will appear under the TA (uM) column in the machine. The two matching numbers should be recorded in the TA log book along with the sample name, temperature when being titrated and pH. The pH appears at the top right above the graph, this is the pH before any acid has been added and should be on the first line.**
35. After all samples are run, turn the system off by putting the pH probe back in the electrode storage solution. Be sure to replace the blue plug.



Figure 8. Proper storage of the pH probe when not TA machine is turned off and probe is not in use.

36. Take both pump hoses (**B**) and place into a larger beaker with DI water. Then place both pump hoses (**A**) in the waste bottle and then select “**End Program**” at the top right. A screen will appear asking to flush with DI water, the hoses should be ready then select “**yes**” and “**Ok**”.
37. Once the system is finished the program will close
- a. Replace HCL bottle cap to reduce evaporation
 - b. Turn off TA machine
 - c. Turn off water bath

Last Reviewed: April 21, 2023