AQUAREAD
water monitoring instruments

CALIBRATION GUIDE AP-2000

FLUORIDE

OBJECTIVE

Procedure for calibrating the Fluoride sensor used with the AP-2000 where a smaller volume is required. Principal is identical only difference is the Stock solution used for dilutions.

PREPARATORY STEPS

- **Tips before you start**

Preparation is everything when it comes to liquid handling and the dilution of concentrated stock solutions for calibration. The standard method for preparing solutions for multiple calibration points involves the preparation of the highest standard concentration first from a stock solution, then preparation of the second calibration point from the previously prepared highest standard concentration.

When preparing ISE calibration solutions Point 2 is the highest concentration we need for calibration so we prepare this point first, then as point 1 and point 3 are the same concentration (the only difference is that point 3 has to be 10°C lower than points 1 and 2) we prepare them in a larger vessel by diluting some of the point 2 we have prepared, this is to ensure point 1 and 3 are identical. Then we dispense the point 1/3 into two smaller bottles and put the point 3 in the fridge, point 1 and 2 can be kept at room temperature.

These preparation steps are not required if using purchased solutions at the correct concentrations. If you are using these please skip to the CALIBRATION section.

- **The basics**

Make sure all measurement vessels and storage vessels are clean. Mark up any pipettes so they are not accidentally used in the wrong solution. Keep a separate pipette for each solution, if possible, if using measuring cylinders make sure these are thoroughly rinsed between changing solutions with **deionized water** and thoroughly dried.

Account for the meniscus; if glass measuring cylinders are to be used to prepare standards, then it is very important to make sure you have accounted for the meniscus. A meniscus is the curve in the upper surface of a liquid close to the surface of the container that is caused by surface tension. When reading a depth scale on the side of an instrument filled with liquid, such as a measuring cylinder the meniscus must be taken into account in order to obtain an accurate measurement. Depth must be measured with the meniscus at eye level (to eliminate parallax error) and at the centre of the meniscus (Figure 1).

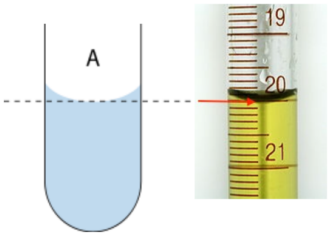

Figure 1, meniscus.

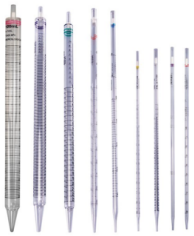
Figure 2, serological pipettes

Water adheres less to plastic than to glass, so the meniscus in a plastic measuring vessel is much smaller than the meniscus in a glass measuring vessel. For this reason, at Aquaread we use polystyrene measuring pipettes called 'stripettes' or 'serological pipettes' (Figure 2) to reduce parallax errors caused by the meniscus as these types of pipettes are more accurate than plastic measuring cylinders.

Use Deionised water for the preparation of ISE standard solutions. Deionised water is water that has had any free ions removed from it, it differs to what some people term distilled or purified water as these types of water have only had microbes eliminated, in some cases they may have also had their ions removed but always double check. Deionised water should typically have an EC of under 5µS. The reason we need to use deionised water to prepare standards is because tap water and mineral water can contain very high amounts of ions which can either interfere with the ISE electrode measurement or if the ion is the same as the one being measured, can give a false high reading once calibrated as the solutions used to calibrate have high levels of the ion in and are not the true ppm value required for calibration.

Keep a tally. If measuring large amounts of liquid from one vessel to another e.g 700ml using a 100ml pipette, keep a tally of how much you have put in just in case someone distracts you! It's easier than starting again and it ensures you have confidence in your standard solutions if there is a problem in calibration.

Slowly does it. When using serological pipettes, it is imperative that you move slowly. If you move quickly or tap the pipette you are at risk of losing some liquid out of the pipette and you will not have the correct volume anymore. If there is a droplet on the end of the pipette, gently wipe it on the neck of the vessel you are aspirating from and gently move the pipette over to the vessel you are dispensing into.

Look after your measurement devices. When using serological pipettes do not allow liquid to be sucked up too high and come into contact with the cotton bung at the top of the pipette (Figure 3).

Figure 3, cotton bung in serological pipette

If this does occur the pipette will not be able to be used until the cotton bung has dried out. The bung is there to prevent liquid being sucked into device used to create suction in the pipette in order to aspirate or dispense liquid. At Aquaread we use handheld motorised pipette controllers in combination with the serological pipettes (Figure 4).

If the cotton bung is wet then the motorised controller cannot create enough suction to hold liquid in the pipette therefore gravity will cause the liquid to fall out of the pipette before you have had a chance to dispense it into the relevant container. Therefore if the bung gets wet, put the pipette to one side and use a fresh pipette!

Figure 4, handheld motorised pipette unit with serological pipette attached

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Avoid cross contamination. The biggest error when calibrating probes comes from cross contamination of solutions. A small droplet of a highly ionic solution into a 10ppm calibration solution can distort calibration values considerably. Always wash the probe tip in DEIONISED water, remove sleeve and dry thoroughly with a clean tissue or cloth. Check for any remaining droplets of water, especially between electrodes or on the combi-electrode tip and on the sleeve itself. Replace your deionised rinse solution often as it will rapidly become full of ions from rinsing!

Don't blind your electrode. When calibrating ISE electrodes it is important to remember that if you are doing a full probe calibration and using highly ionic solutions such as pH4, 10, 7 or to a lesser extent RapidCal then you must keep the ISE tip free of these solutions by placing a dry red cap over the ISE's before exposure of the probe to these solutions. Exposure to high ionic buffers will cause blinding of the ISE electrode (the electrode will not be able to detect the ions you are trying to measure), and it will require soaking in the relevant ISE buffer before calibration and use. If the ISE's are to be soaked prior to calibration, source some extra red caps, mark each one clearly with the electrode type and fill the red cap with the relevant soak solutions. I recommend removing the wet caps before commencing the other calibrations on the probe and replacing with dry caps in case the solutions from the red caps leak out into the other calibration solutions upon calibration. Remember to red cap any ISEs not being calibrated at the present time to avoid interfering ions contaminating the other ISEs. I.E. if you have a probe fitted with Amm and Nit electrodes and you want to calibrate Amm, red cap the Nit electrode whilst calibration is performed on the AMM electrode, then when you want to calibrate the Nit sensor remove the red cap from the Nit electrode and put it on the Amm sensor whilst Nit calibration is performed. Don't forget to remove red caps before using the probe for sample measurement. Try to store ISE's dry as much as possible, a dry ISE electrode has a much longer lifespan than one that is kept wet. I recommend removing all red caps when the probe is being stored to avoid moisture being kept at the tip of the ISE electrode. Overnight soaking of the electrode in relevant solutions can be performed before calibrations.

Don't use a sleeve when calibrating ISE electrodes. We do not use the probe sleeve simply because when we try to reduce the temperature of the probe for the third point calibration (the cold point), it takes a lot longer with a sleeve as there is more metal mass present. We may also remove the AP-7000 wiper as that again slows down the time to cool the probe.

Ensure your combi-electrode is functioning before ISE calibration, as it is used as a reference by the ISE. If you have a filthy combi, chances are it won't be suitable as a reference electrode…but remember before checking whether the combi-el is functioning correctly in pH4, 7 and 10 buffers fit a red cap to the ISE so as not to blind it.

Use the smallest possible stripette/pipette for the most accurate measurement. Using a 100ml pipette to measure 5ml for example will increase error in measurement due to the resolution on a 100ml pipette being a lot smaller than on a 5ml pipette for instance. Various sizes of stripette are available; 1ml, 2ml, 5ml, 10ml, 25ml, 50ml, 100ml. The smaller the pipette the greater the resolution, and the more accurate the liquid measurement.

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- **Required Items**

- FLU-CAL calibration solution (1000ppm Fluoride solution)
- De-Ionised water
- **Or** use the optional calibration solutions at the correct concentration to save making dilutions: FLU-CAL-0.5 and FLU-CAL-5

- **Preparation**

The probe should be left on the bench overnight, the point 1 and 2 solutions at 0.5ppm and 5ppm should be warmed in a water bath to around 25 degrees C. The third point at 10ppm should be stored in the fridge so that it is at least 10 degrees colder than points 1 and 2.

- **Equipment**

- Aquaprobe.
- Calibration instrument; Aquameter / Bluelink / Aquacal PC software.
- Purchased FLU-CAL solutions or your diluted solutions.
- Water bath
- Motorised pipette (for dilutions)
- Stripettes (for dilutions)
- 250ml and 500ml bottles (for dilutions)
- De-Ionised water (for dilutions)

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CALIBRATION

Calibration guidance will assume you are using an Aquameter, procedure will be similar when using Bluelink or AquaCal.

Required solutions:

Point 1 = 0.5ppm (room temperature ideally between 20-25°C)
Point 2 = 5ppm (room temperature ideally between 20-25°C)
Point 3 = 0.5ppm (cold point, ideally between 5-10°C)

Preparation:

- Lay out 1 x 250ml bottles, mark each one 50ppm Flu.
- Lay out 3 x 250ml bottles, mark each one pt1-Flu, pt2-Flu, pt3-Flu. Date each one. Ensure clean lids are available for each bottle.
- Lay out 1 x 500ml bottle and mark pt1 and pt3 Flu.
- Fill a 1L bottle with **deionised water,** make sure you label the bottle deionised water to avoid confusion later on.
- Have the 1000ppm Fluoride stock solution to hand.
- Have the electronic pipette and the correct serological pipettes to hand, all labelled with the correct liquid they will be used for.

Make a 50ppm stock solution:

1. Take the pipette marked deionised water and measure out 114ml Deionised water into the 250ml bottle marked 50ppm Fluoride.
2. Change the pipette to the one marked Fluoride and add 6ml of the **1000ppm Fluoride stock solution** to the 114ml of deionised water. Dispense the liquid into the 50ppm bottle, then suck up and blow out the liquid a few times to ensure no 1000ppm solution remains in the pipette.
3. **This creates a 50ppm solution from which we can prepare pt-2**.

Dilution- Point 2 preparation (5ppm point):

1. Take the 100ml pipette marked deionised water and measure out 225ml of deionised water into the 250ml bottle marked pt 2.
2. Change the pipette to the one marked Fluoride and add 25ml of the **50ppm Fluoride solution prepared in the first step** to the 225ml of deionised water in pt-2 bottle. Dispense the liquid into the point 2 pot, then suck up and blow out the liquid a few times to ensure no 50ppm solution remains in the pipette.
3. Put a lid on the bottle and invert a few times to mix the solution.
4. This is now pt2 prepared.

Dilution- Point 1 and 3 preparation (0.5ppm points):

1. Take the 100ml pipette marked deionised water and measure out 360ml of deionised water into the 500ml bottle marked pt 1 and pt 3
2. Change the pipette to the one marked Fluoride and add 40ml of the **pt-2 solution** to the 360ml of deionised water in pt-1/3 bottle.
3. Put a lid on the bottle and invert a few times to mix the solution.

4. This is pt 1/3 prepared. It can now be dispensed equally into the bottles marked pt-1 Fluoride and pt-3 Fluoride. Put pt-3 in the fridge to create the cold point.

(Dilution notes, the first step dilution is a simple 1 in 20 dilution, this is comprised of 1 part Fluoride stock solution and 19 parts deionised water. To prepare points 2 and then 1/3 the dilutions performed are a simple 1 in 10 dilution, this is comprised of 1 part relevant Fluoride solution and 9 parts deionised water)

During three-point calibration, the Aquaprobe and Aquameter must remain switched on. If the Aquameter is switched off between points, the calibration process will be aborted and must be re-started from point 1. The Aquaprobe's sleeve should also be removed in order to reduce the Probe's thermal mass.

Procedure, Point 1, 0.5ppm calibration:

1. Remove the Probe Sleeve. Remove the storage cap from the pH electrode, wash the Probe in distilled water, dry the probe thoroughly then gently lower the Probe into the warm **0.5ppm** solution.
2. Switch on the Aquameter and leave until the temperature and Fluoride readings are completely stable. A minimum of five minutes is recommended.
3. Ensure the temperature of the solution is between 20ºC and 40ºC (68ºF - 104ºF).
4. To start the calibration, press the MENU key then select Calibration>Full Cal>AUX ELETRODES>FLU to enter the Fluoride calibration screen.
5. Move the cursor downwards to point 1, which is the 0.5ppm calibration.
6. Press the OK button on the Aquameter to begin the calibration process.
7. A calibration report value will be displayed after the calibration is complete, it is useful to record these values for troubleshooting purposes.

Procedure, Point 2, 5ppm calibration:

1. Remove the probe from the 0.5ppm solution and wash thoroughly in deionised water. Dry the probe then gently lower it into to the warm **5ppm** solution.
2. Leave until the temperature and Fluoride readings are completely stable. A minimum of five minutes is recommended.
3. **Ensure the temperature of the solution is within 1 degree of the previous 0.5ppm calibration point.** If the solution is not, calibration will fail.
4. To start the calibration, press the MENU key then select Calibration>Full Cal>AUX ELETRODES>FLU to enter the Fluoride calibration screen.
5. Move the cursor downwards to point 2, which is the 5ppm calibration.
6. Press the OK button on the Aquameter to begin the calibration process.
7. A calibration report value will be displayed after the calibration is complete, it is useful to record these values for troubleshooting purposes.

Procedure, Point 3, 0.5ppm COLD calibration:

1. Remove the probe from the 5ppm solution and wash thoroughly in deionised water. Dry the probe then gently lower it into to the **cool 0.5ppm** solution.
2. Leave until the temperature and Fluoride readings are completely stable. A minimum of five minutes is recommended.
3. **Ensure the temperature of the solution is at least 10ºC cooler than the previous 5ppm calibration point.** If the solution is too warm, calibration will fail.
4. To start the calibration, press the MENU key then select Calibration>Full Cal>AUX ELETRODES>FLU to enter the Fluoride calibration screen.

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5. Move the cursor downwards to point 3, which is the **COLD** 0.5ppm calibration.
6. Press the OK button on the Aquameter to begin the calibration process.
7. A calibration report value will be displayed after the calibration is complete, it is useful to record these values for troubleshooting purposes.

CONTROL

Check the calibration report values are within the acceptable ranges.

The calibration report value for the point 1 calibration can vary with sensor age and type, this is as a guide only. The important indicator for electrode health is the acceptable range for point 2 calibration. Once the value is out of this range the electrode needs to be replaced.

Fluoride
The example value for the point 1 calibration is ~ -360mV
The acceptable range for the point 2 calibration is +/- 30-80mV to point 1
The acceptable range for the point 3 calibration is between point 1 and 2

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30/07/25	0	Creation	CP
01/08/25	1	Review and fine adjustment	GP

Notes