High Range (600 – 3000ppb) Copper (Cu) Testing and Site Calibration for Water Testing

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2 General Information

Important: Before continuing, please read the entire AND1000 Fluorimeter User Manual. Pay attention to all danger, warning and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment. Make sure that the protection provided by this equipment is not impaired, do not use or install this equipment in any manner other than that specified in this manual. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the manufacturer may be impaired.

2.1 Water Testing Guidelines

Note: The steps below are for testing drinking water samples. These steps can also be used for other matrices such as surface, ground, industrial and wastewater which has been pre-treated. Contact ANDalyze customer service for additional application notes on pre-treatment methods.

Water Sampling

- For best results use freshly collected sample (unpreserved in acid) for analysis. We recommend that you use the sample within 1 hour (maximum of 2 hours) of collection to minimize any metal loss to the walls of the sample container.

- Once the sample is mixed with ANDalyze sample buffer, test within 15 minutes.

pH Range

The ANDalyze sample buffer that is provided in the sample tubes brings the pH of the test solution to pH 7.0. Generally, the raw sample water can be in the range of pH 5 – pH 8. If you have a sample which is acidic or basic, mix with the ANDalyze buffer and check the pH of this buffered test solution. It should be ~pH 7.0 for best results

Note: Our tests have shown that environmental samples preserved in acid to a pH < 2 cannot usually be brought to a pH of 7.0 when mixed with the ANDalyze buffer. These samples have to be first neutralized with NaOH to a pH ~5 before mixing with ANDalyze buffer. Please contact ANDalyze customer service for instructions related to pre-treatment of highly acidic or highly basic samples.

2.2 Sensor & Cuvette Bag

Sensor Bag: Each sensor bag contains a cuvette, a sensor and a desiccant. These are single use and must be discarded. The desiccant should be blue in color. If it has turned completely pink in color, sensor may not perform well.

2.3 Inserting Cuvette and Sensor

Cuvette: The cuvette has an arrow which should face you when inserted. Insert the cuvette completely so that the fluorimeter lid can close. The meter and cuvette design helps to prevent improper orientation. This is important as the path-length of the emission and detection should not change.

Sensor: The square portion of the sensor can be placed on the cuvette in any orientation with the round sections facing upward. Sensors can only be used once and should be disposed of immediately after use.

2.4 Sample Injection and Measurement

Fluorimeter instrument should be laid flat on a stable surface during a measurement

A buffered solution is prepared in a sample tube as described in the on-site calibration section 3 and test section 4 and this is used for measurements.

A new syringe should be used to withdraw 1 mL water from a sample tube. This syringe can be attached to the top of the housing as shown in the picture.

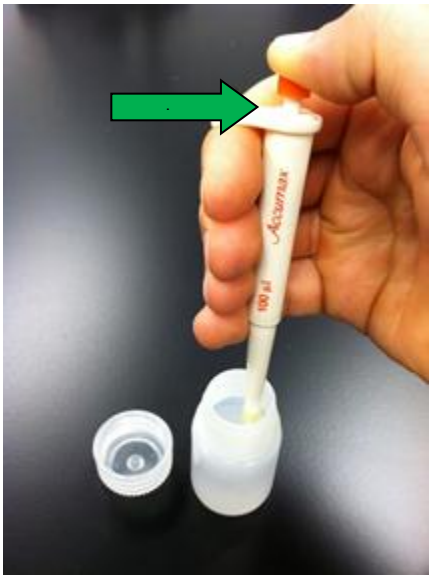
The sample should be injected through the housing into the cuvette at a constant speed of **3 – 5 seconds.** The syringe and housing should be immediately removed and the sample door closed. The START button located just below the screen should be pressed to start any measurement

Important: After each analysis, discard all components used during the analysis including cuvette, sensor housing, sample tube, syringes to avoid cross contamination.

2.5 Pipette Use Guidelines

1. **New Tip –** Attach a new tip by placing the end of the pipette into one of the available tips and pressing down on the pipette body.

Note: Tips are disposable and should never be used more than once.



2. Depress the operating button on the top to the first stop (see photo at left). **DO NOT depress all the way to body.**

3. Immerse the clean tip into the solution to be withdrawn.

4. Release the pressure slowly to withdraw the solution into the tip.

Note: Make sure that the pipette tip continues to be immersed in the solution during release so as to not expose the tip point to air.

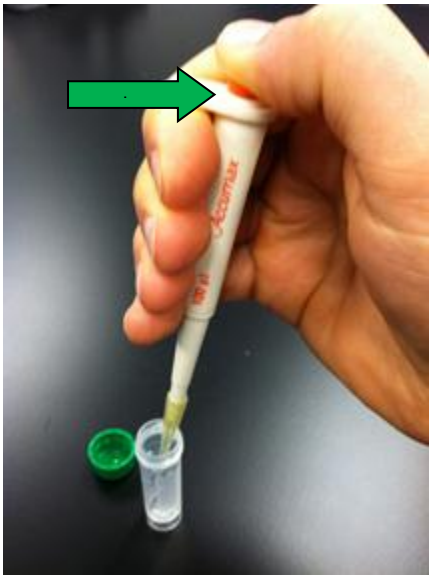
5. Remove the pipette from the solution.

6. Immerse the tip into the liquid present in the tube where the withdrawn solution is to be dispensed.

7. Slowly depress the operating button **ALL THE WAY** to dispense the liquid contained in the pipette tip. (See photo at right)

8. Remove the pipette and discard the used tip.

Note: Dispose of tips immediately after use to prevent possible contamination of pipette.



3 On-Site Calibration

3.1 Required Materials

- AND1000 Fluorimeter
- (2) Sensor Bags with Sensor & Cuvette
- (2) 1mL Syringe
- (2) Sample tubes with buffer
- 0.9 ppm Copper Standard Solution
- 100 µL Pipette and Tips

3.2 Preparing for Site Calibration

When testing water at a new location, the instrument has to be calibrated for accurate readings. Completing the on-site calibration requires two separate solutions and tests. At the end of the process, the fluorimeter is calibrated for testing water at that site and also provides the sample test results for this site. A calibration may only be accurate when originally performed. Do not rely on a calibration to be accurate over long time periods as environmental samples can vary greatly.

Important: On-site calibration must be performed if testing is being done:
- At a new location which has not been previously saved.
- To adjust for significant changes in sampling temperature.
- When beginning to use a new lot of sensors.

1. **Site:** Select the site to be tested or enter a new site as described in section 5 of the AND1000 User Manual.

2. **Collect Test Water** – In a clean cup or container, collect a small volume of water to be tested.

3. Attach a **NEW** tip to the 100µL pipette.

4. Withdraw 100µL of the test water solution using the pipette. (2X)

5. **Mix** - Transfer 100µL into **each** of the sample tubes.

6. **Shake -** Close cap and shake well.

Important: Open tube carefully so that liquid buffer does not fall out.

One tube will be used for analyzing a sample spiked with analyte and the other for analyzing an unspiked sample to calculate an accuracy factor that accounts for recovery of the spike.

3.3 Site Calibration: Sample with Analyte Spike (Step 1 of 2)

7. Attach a **NEW** tip to the 100µL pipette.

8. Withdraw 100µL of the standard solution from the analyte standard solution container using the pipette.

9. **Mix** - Transfer the 100µL into one of the sample tubes filled with sample water.

10. **Shake -** Close cap and shake well. For most accurate results, wait 5 minutes before testing. Incubation is **required** for all environmental water samples.

    Important: Open tube carefully so that liquid buffer does not fall out.

Withdrawing Standard Solution Using Pipette

Transferring Spike Solution to Sample Tube

11. **Pouch –** Open the black bag which contains the sensor (colored plastic housing) and plastic cuvette.

12. **Cuvette –** Place the plastic cuvette in the instrument.

13. **Sensor** - Place colored plastic sensor on the cuvette. (Any orientation). Make sure sensor is seated securely on the cuvette.

14. Enter the Site Calibration screen (as seen in photo to the right) which will appear once a new site has been entered (See section 5 of AND1000 user manual).

Important: Do Not press "Start" until the sample is ready. Follow steps below.

Note: If the unit is left on for more than 2 Minutes without any activity, a screen-saver (black screen) will be activated, press any button to resume operation (Do not press and hold ON/OFF). The instrument automatically turns off if not used for more than 10 minutes.

9. **Test** - With one of the provided syringes, draw 1-ml of water from the spiked sample tube into syringe. Attach the syringe to the housing over the cuvette in the instrument. Maintaining a constant speed (over 3-5 seconds), carefully squeeze the sample water through the housing into the cuvette. Quickly remove sensor housing and syringe and close sample chamber door. Press the **START** button located just below the screen. Remove cuvette when complete.

3.4 Site Calibration: Sample Test (Step 2 of 2)

The Site Calibration screen (as seen in photo to the right) will appear once step 1 of 2 has been completed for a new site.

DO NOT press "Start" until the second sample is ready for injection!

10. **Test Unspiked Sample** –With one of the provided syringes, draw 1-ml of water from the **second unspiked sample tube** into a new syringe. Attach the syringe to the housing over the cuvette in the instrument. Maintaining a constant speed (over 3-5 seconds), carefully squeeze the sample water through the housing into the cuvette. Quickly remove sensor housing and syringe and close sample chamber door. Press the **START** button located just below the screen. Remove cuvette when complete.

3.5 Applying Results

The results display the site name, a calibration factor associated with that site and the analyte test results for the water used during this process. (The results displayed are for the sample water and not for the sample water containing the spike). To save the factor associated with this site, press "**Apply**".

On-site calibration from this site has been saved. You are now ready to test more samples from this site.

4 Testing a Sample

This section will walk you through the process of testing a water sample for the desired analyte. Please read this entire section before beginning the test as parts of the test will be time sensitive. Be aware and pay attention to all notes.

4.1 Required Materials

- AND1000 Fluorimeter
- (1) Sensor Bag with Sensor & Cuvette
- (1) Syringe
- (1) Buffer Solution Tube
- (1) Sample Cup (Not Provided)
- 100 µL Pipette and Tips

Note: Before using the instrument assure that the fluorimeter device is charged. (See Section 4.1 in the User Manual for Battery Charging Information)

4.2 Testing a Sample

1. **Start-Up** - Initialize the instrument by pressing the ON/OFF button. Instrument will initialize in about 1-2 seconds.

2. **Metal Type** - Ensure that the fluorimeter is on the correct metal screen.

3. **Site** – Confirm that the site being tested is correct.

Do Not press "Start" until the sample is ready!

4. **Pouch -** Open the black bag which contains the sensor (colored plastic housing) and plastic cuvette.

Important: The bag contains a transparent desiccant pouch. This should be blue in color. If desiccant has turned completely pink in color, sensor may not perform well.

4. **Cuvette** - Place the plastic cuvette in the instrument.

5. **Sensor** - Place colored plastic sensor on the cuvette. (Any orientation). Make sure sensor is seated securely on the cuvette.

Note: Unit may go into screen-saver mode after 2 minutes. Press any button to resume (Do not press and hold ON/OFF).

6. **Sample Tube** – Collect water to be tested in a clean cup (not provided).

7. Attach a **NEW** tip to the 100µL pipette.

8. Withdraw 100µL of the test water solution using the pipette.

9. **Mix** - Transfer 100µL into the sample tube.

10. **Shake -** Close cap and shake well.

Important: Open tube carefully so that liquid buffer does not fall out.

11. **Syringe** - With one of the provided syringes, draw 1-ml of water from the sample tube into syringe.

12. Attach the syringe to the housing over the cuvette in the instrument. (Syringe tip will fit into top of sensor)

Note: If the unit is left on for more than 2 Minutes without any activity, a screen-saver (black screen) will be activated, press any button to resume operation (Do not press and hold ON/OFF). The instrument automatically turns off if not used for more than 10 minutes.

13. Maintaining a constant speed (over 3-5 seconds), carefully squeeze the sample water through the housing into the cuvette.

14. Quickly remove sensor housing and syringe and close sample chamber door.

15. Press the **START** button located just below the screen.

16. Remove cuvette when complete.

4.3 Results

The sample will be tested, and results displayed in parts per billion (ppb) or parts per million (ppm) in under a minute.

To save the results, press the **SAVE** button.

Note: A reading of "Below Xppb Limit" indicates the amount of analyte in the sample is below the minimum detectable levels of the instrument. (Min level:0.6ppm Cu (High))

5 Technical Specifications for ANDalyze Sensors

5.1 Detection in Drinking Water

ANDalyze's proprietary Catalytic DNA sensor uses a DNAzyme reaction that fluoresces in the presence of the target contaminant (lead, uranium, copper, etc). The fluorescence of the reaction is measured using the AND 1000 fluorimeter to determine the concentration of the free analyte ion (Pb^{2+}, UO_2^{2+}, Cu^{2+}, etc.) in solution and is reported in parts per billion (ppb) or parts per million (ppm).

Materials Used

- Fluorimeter (Product: AND1000 Fluorimeter)
- Analyte Sensor Kit (Copper100-2)
- Analyte/Metal Standard Solution

Sensor

Performance

Copper dilutions were prepared in test buffer. The Copper100-2 sensor kits and the AND1000 fluorimeter were used to perform the copper test at each dilution (five replicates).
Note: The protocol for using this sensor kit requires mixing 1 part of test solution with 29 parts of buffer, thus test solution is diluted 30 times during the test. The fluorimeter screen displays the results as concentration of copper in the test solution.

Linear Detection Range

0.6 - 3 ppm Copper

Precision

Standard: 1.2 ppm Cu^{2+}
95% Confidence Limits: 0.9 – 1.5 ppm Cu^{2+}

Note: All specifications are subject to change without notice.

Coefficient of Variation (CV):

0.6–3 ppm Cu^{2+} ±20%

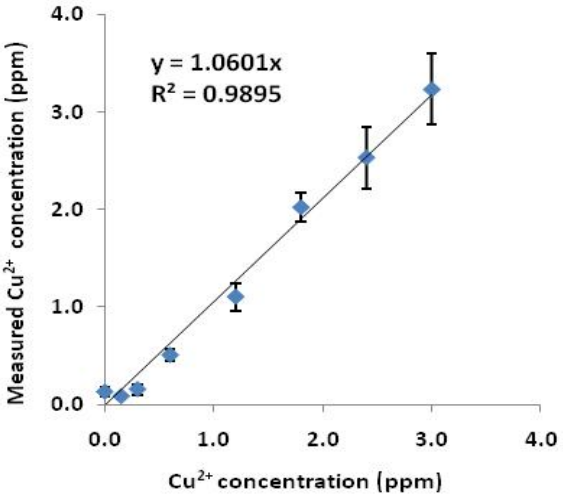
5.2 Interference

Interference tests were done with a 1.5 ppm copper solution plus the potential interfering ion. The interference tolerance levels represent the concentration above which the copper concentration is changed to ±10%. Data represents an average of at least three replicates. For each interference test, an on-site calibration with the particular water matrix (containing the interfering ion) was performed. Note that the tolerance to interfering ions may seem extremely high. This is due to the fact that the sample matrix gets diluted 30 times during the test, thus diluting the interferences.

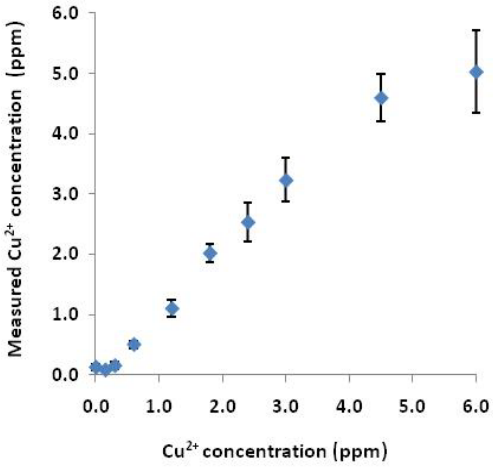
Interfering ion	Interference level
Calcium, Ca^{2+}	15000 ppm
Magnesium, Mg^{2+}	6000 ppm
Zinc, Zn^{2+}	150 ppm
Aluminum, Al^{3+}	9 ppm
Copper, Cu^{2+}	30 ppm
Iron, Fe^{3+}	30 ppm
Iron, Fe^{2+}	1.5 ppm
Cadmium, Cd^{2+}	150 ppm
Mercury, Hg^{2+}	9 ppm
Manganese, Mn^{2+}	300 ppm
Lead, Pb^{2+}	3 ppm
Ammonium, NH_4^+	7500 ppm
Carbonate, CO_3^{2-}	15000 ppm
Phosphate, PO_4^{3-}	1500 ppm
Chloride, Cl^-	30000 ppm
Nitrate, NO_3^-	9000 ppm
Sulfate, SO_4^{2-}	15000 ppm

5.3 Product Accuracy Ranges

The graphs depict the average measured Copper concentration as displayed on AND1000 fluorimeter (y-axis) vs. the known concentration of the Copper standards (x-axis). Error bars depict the standard deviation from at least five measurements. Graph 1 shows the linear detection range is 0.6 – 3 ppm copper. For higher concentration of copper (tested up to 6 ppm copper), the accuracy decreases (Graph 2). Note that a different sensor pack (Product name: Copper100-1) is available for detecting copper in the range of 20-200 ppb copper.



Graph 1: Average Copper Conc. vs. Known Conc.



Graph 2: Accuracy vs. Linear Detection Range

6 Consumables and Replacement Items

- Fluorimeter (Product: AND1000 fluorimeter)
  - Includes Fluorimeter and USB to MINI-B Cable. Capable of measuring multiple metals.

- Sensor Kit (Products: Copper100-2)
  - Equipment for (30) Tests and/or Calibrations

    Kit Includes: (30) Analyte Sensors
    (30) Cuvettes
    (30) Test Buffers
    (35) Syringes
    (15) Disposable Transfer Pipettes
    15 mL Copper Standard Solution – 0.9 ppm Cu^{2+}
    100µL Automatic Pipette with Tips
    Instruction Manual
    Material Safety Data Sheets (MSDS)

7 Customer Service Contact Information

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