Document: AND013-04-2014

Copper Testing and Site Calibration for Water Testing (High Range) Detection Range: 0.6 – 3.0ppm

April 2014 Edition 2

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2 General Information

Important: Before continuing, please read the entire *ANDalyze 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 safety features of this equipment are not impaired; do not use or install this equipment in a manner other than that specified in this manual. If the equipment is used in a manner not specified by the manufacturer, the safety features of this equipment may be impaired and injury to the operator or damage to the equipment may result.

2.1 Water Testing Guidelines

Note: The below steps are for testing for dissolved, bio-available metal ions in drinking water samples. These steps can also be used for other matrices such as surface, ground, industrial and wastewater which have 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. Large volumes (1L) may be stored up to 12 hours in HDPE containers in a refrigerator or cooler with ice packs if required.

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

Temperature Range

ANDalyze test kits work in the 17 – 35 ˚C (63 – 95 ˚F) temperature range. However, the most accurate and precise results are obtained in the range of 20 - 25 ˚C (68 – 77 ˚F). A change in temperature of several degrees will require an on-site calibration to be performed.

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, please check the pH first to confirm it is within the range prior to mixing with the ANDalyze buffer. The final buffered test solution should be ~pH 7.0 for best results.

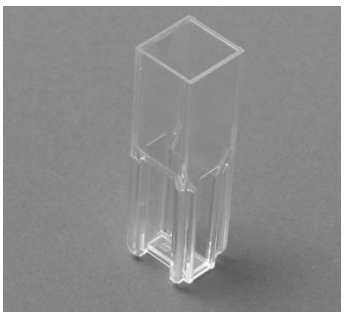
Note: Our tests have shown that environmental samples preserved in acid to a pH < 2 cannot be brought to a pH of 7.0 when mixed with the ANDalyze buffer. These samples have to be first neutralized with NaOH to a pH ~5 before mixing with ANDalyze buffer. Contact ANDalyze customer service for additional application notes on pre-treatment methods.

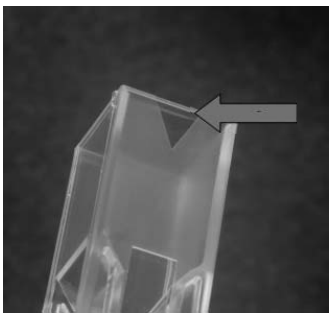
2.2 Sensor Pack: Cuvette and Sensor

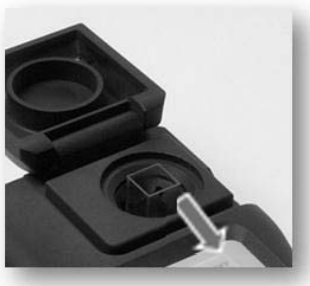
Sensor Pack: Each sensor pack contains a cuvette, a sensor and a desiccant. These are **single use** and must be discarded. The desiccant should be blue in color. If it has turned completely pink in color, the sensor may not perform well.

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.
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

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

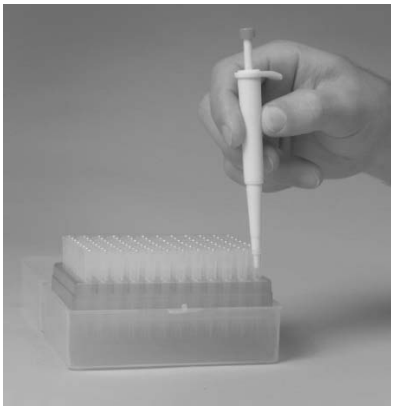
The sample should be injected through the housing into the cuvette at a constant speed of **3 – 5 seconds.** The syringe and housing should be immediately removed and the sample door closed. The **START** button located just below the screen should be pressed immediately to start any measurement**.**

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

2.5 Pipette Use Guidelines

1. **New Pipette 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. Use of tips helps prevent contaminating the pipette.



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

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

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

Important: 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.

Note: The liquid level in the tip should be approximately at the 3rd graduation.

6. Immerse the tip into the liquid present in the sample 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 the pipette.

3 On-Site Calibration

3.1 Required Materials

- ANDalyze Fluorimeter
- (2) Sensor Packs with Sensor & Cuvette
- (2) 1mL Syringe
- (2) Sample tubes (with buffer)
- Copper Standard Solution (0.9 ppm)
- 100 µL Fixed Volume Pipette and Tips(1) Clean sample collection cup (Not Provided)

3.2 Preparing for On-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 test results for the site. A calibration may only be accurate when originally performed, as changes in the sample matrix, temperature, and sensor lot will affect the results. Do not rely on a calibration to be accurate over days as environmental samples can vary greatly. If in doubt, perform On-site Calibration

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 ANDalyze Fluorimeter User Manual
2. **Collect Sample Water** – In a clean cup or container, collect a small volume of water to be tested.
3. Remove the lid from **two sample tubes** containing the liquid buffer

   Important: Open tube carefully so that liquid buffer does not fall out.
4. Attach a **new** tip to the 100µL fixed volume pipette.
5. Withdraw 100µL from the **Sample Water** container and transfer into one of the sample tubes filled with buffer.
6. Repeat step 5 to transfer 100uL sample water into the **second** sample tube filled with buffer.

   Note: One tube will be used for analyzing a sample spiked with Copper and the other for analyzing an unspiked sample.

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

1. Attach a **new** tip to the 100µL fixed volume pipette.

2. Withdraw 100µL of the Copper Standard Solution from the bottle using the pipette.

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

4. **Shake -** Close cap and shake well. For most accurate results, wait 5 minutes before testing. Metal standard spiked for on-site calibration is **required** to incubate for all environmental water samples.

Withdrawing Standard Solution Using Pipette

Transferring Spike Solution to Sample Tube

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

5. **Pouch –** Open the sensor pack which contains the sensor (colored plastic housing) and plastic cuvette.

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

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

8. 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 ANDalyze Fluorimeter 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 a few 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 5-15 minutes (user settable).

9. **Test** - With one of the provided 1 mL syringes, draw 1 mL buffered sample water from the spiked sample tube into the syringe.

10. 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. Immediately press the **START** button located just below the screen. Remove the cuvette when complete.

3.4 On-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!

1. Open a **NEW** sensor pack and place the plastic cuvette in the Fluorimeter and the colored plastic sensor on the cuvette. Make sure sensor is seated securely on the cuvette.

2. With one of the provided 1 mL syringes, draw 1 mL buffered sample water from the **second unspiked sample tube** into a new syringe.

3. 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. Quickly remove sensor housing and syringe and close sample chamber door. Immediately press the **START** button located just below the screen . Remove the cuvette when complete.

4. All waste produced during the test can be disposed of in normal public receptacles. Materials and liquids used are not considered harmful to people or the environment and do not require special disposal. The plastic waste is made from recyclable plastics and can be disposed of per local guidelines.

3.5 Applying Results

The results display the site name, a calibration factor associated with that site, and the Copper test results for the water sample used during this process.

Note: The results displayed are for the sample water and not for the sample water containing the spike. **The results should be treated as an actual test and can be seen in the results menu screen.**

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

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

Note: Before using the ANDalyze fluorimeter assure that it is charged. (See Section 4.1 in the *ANDalyze Fluorimeter User Manual* for Battery Charging Information)

4.2 Testing a Sample

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

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

2. **Sensor Type** - Ensure that the fluorimeter is on the correct sensor screen.

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

Unless the Instructional Screens are turned ON, Do Not press START until the sample is ready!

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

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

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

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

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

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

9. Withdraw 100µL of the **Sample Water** container using the pipette.

10. Transfer the 100µL sample water into the provided sample tube containing liquid buffer.

11. **Shake -** Close cap and shake well by shaking.

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

12. **Syringe** - With one of the provided syringes, draw 1 mL of buffered sample water from the sample tube into syringe.

13. 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 a few 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 5-15 minutes (user settable).

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

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

16. Immediately press the **START** button located just below the screen.

17. 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 2ppb Limit” or “Above 100ppb Limit” indicates the amount of Lead in the sample is outside the recommended detectable levels of the instrument.

4.4 Waste Disposal

All waste produced during the test can be disposed of in normal public receptacles. Materials and liquids used are not considered harmful to people or the environment and do not require special disposal. The plastic waste is made from recyclable plastics and can be disposed of per local guidelines.

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

- ANDalyze Fluorimeter
- Copper (high) Sensor Kit
- Analyte/Metal Standard Solution

Sensor

Associated US Patents

8,815,156B2, 6,706,474, 7,192,708, 6,890,719, 7,332,283

Performance

Copper dilutions were prepared in test buffer. The Copper (High) sensor kits and the ANDalyze 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.

Precision

Standard: 1.2 ppm Cu^{2+}

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

Coefficient of Variation (CV):

0.6–3 ppm Cu^{2+} ±20% or 0.3ppm, whichever is greater

Note: This data is for tests in DI water. Environmental and other matrix variations will be higher.

Linear Detection Range

0.6 - 3 ppm Copper

Note: All specifications are subject to change without notice.

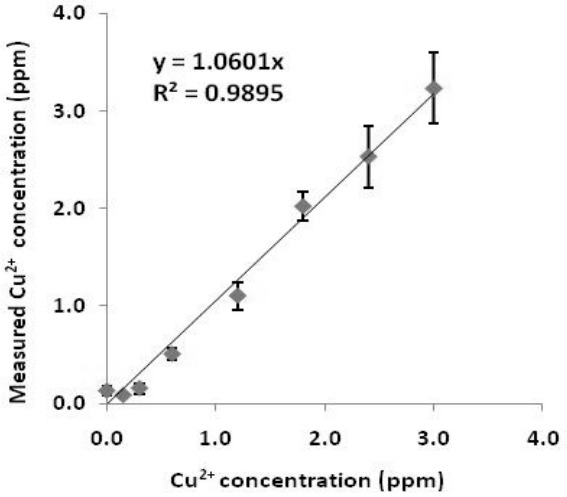
5.2 Interference

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.

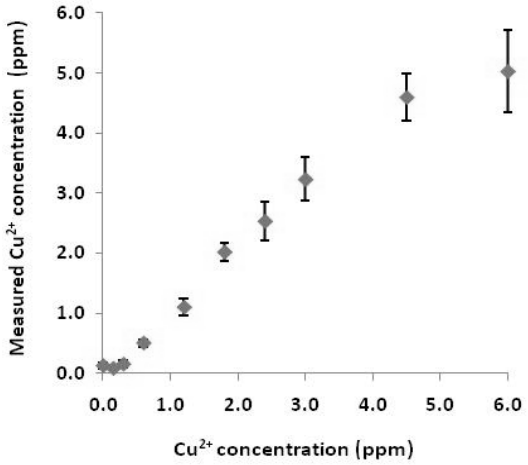
Interfering ion	Interference level
Calcium, Ca^{2+}	15000 ppm
Magnesium, Mg^{2+}	6000 ppm
Zinc, Zn^{2+}	150 ppm
Aluminum, Al^{3+}	9 ppm
Copper, Cu^{2+}	30 ppm
Iron, Fe^{3+}	30 ppm
Iron, Fe^{2+}	1.5 ppm
Cadmium, Cd^{2+}	150 ppm
Mercury, Hg^{2+}	9 ppm
Manganese, Mn^{2+}	300 ppm
Lead, Pb^{2+}	3 ppm
Ammonium, 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

5.3Product Accuracy Ranges

The graphs depict the average measured Copper concentration as displayed on ANDalyze 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 (Part Number: AND012) is available for detecting copper in the range of 40-200 ppb copper.



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



Graph 2: Accuracy vs. Linear Detection Range

6 Consumables and Replacement Items

- Fluorimeter (Part Number: AND002)
  - Capable of measuring multiple metals.
  - Includes: Fluorimeter
    USB to MINI-B Cable
    100µL Fixed Volume Pipette and Tips
    pH Test Strips

- Sensor Kit (Part Number: AND013)
  - Equipment for (25) Tests and/or Calibrations
  - Kit Includes: (25) Sensor Packs with Sensor & Cuvette
    (25) Sample Tubes (with buffer)
    (25) 1 mL Syringes
    5 mL Copper Standard Solution (0.9 ppm Cu^{2+})
    Instruction Manuals
    Material Safety Data Sheets (MSDS)

7 Customer Service Contact Information

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