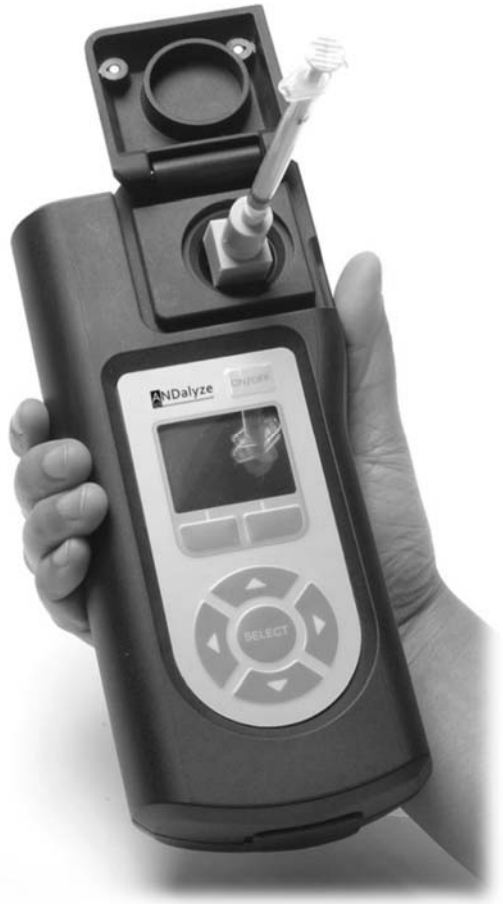
Document: AND010-07-2018

Mercury Testing for Water Testing Detection Range: Positive/Negative at 2ppb (Up to 50ppb)

July 2018 Edition 1



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1 Testing a Sample

Important: Prior to operating this equipment, we highly recommend you read this entire manual in addition to the *ANDalyze Fluorimeter User Manual*. Pay attention to all danger, warning and caution statements. 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.

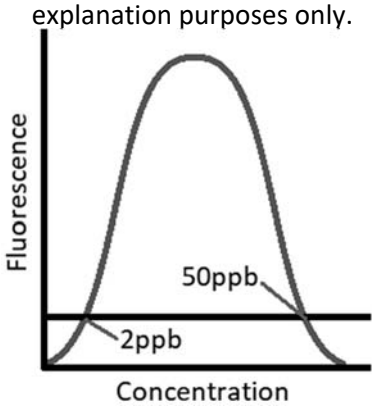
1.1 Qualitative Mercury Sensor

The mercury sensor is a qualitative sensor that shows a Positive/Negative result. A "Positive" test result indicates that mercury is present at levels greater than 2 ppb. A "Negative" result indicates the water contains less than 2ppb mercury.

This test does <u>not</u> require on-site calibration. The mercury sensor has a signal dependence to mercury which is sigmoidal instead of linear. Therefore, a 4-point calibration would be required for accurate quantitative results which pose a challenge during on-site use.

In addition, the fluorescence signal is hyperbolic which allows for two different concentrations to produce an equivalent fluorescence (see image at right). Therefore, there are chances for false positives and negatives at mercury concentrations above 50ppb in the sample. To address these potential false results the kit contains materials that will help users to confirm both positive and negative results. This process may require multiple tests per sample and thus sets of testing materials (sensor, buffer vial, syringe, etc.)

* Not actual curve. For explanation purposes only.



1.2 Sample Collection and Conditioning

<u>Water Sampling</u>

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. This is particularly important for testing trace lead levels. 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.

<u>Temperature Range</u>

ANDalyze test kits typically work with water samples at 17 – 35 ˚C (63 – 95 ˚F) but for most accurate results, use sample at 20 - 25 ˚C (68 – 77 ˚F). Cold samples can be easily warmed by holding the sample vial in your hand for a short period. The sample quickly assumes the temperature of the sensor kit components so if the kit is at room temperature (~23C/73F), the sample will also likely be at that temperature during testing.

<u>pH Range</u>

The ANDalyze sample buffer that is provided in the sample tubes brings the pH of the test solution to pH 7.4. 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 7.4 for best results.

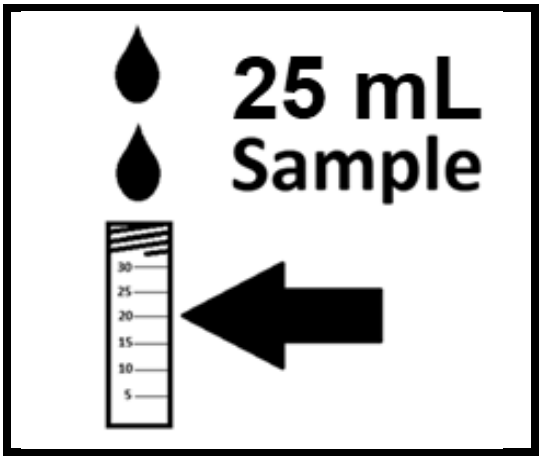
<u>Conditioning</u>

To improve testing accuracy, we recommend adding 2 drops of hydrogen peroxide from provided dropper bottle to every 25 mL of sample water (except for tap water sample). Do not test for other analytes using this sample.

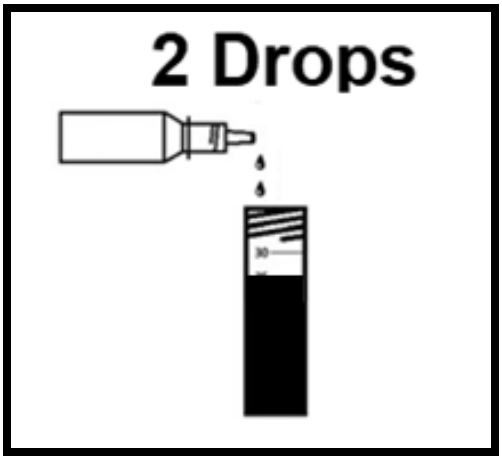
Required Materials

- (1) 50mL Vial with Cap
- 10 mL Hydrogen Peroxide in Dropper Bottle

Note: Between tests, rinse vials with new sample water to clean.



1. Fill 50mL vial to **25mL mark** with fresh sample water.



2. Add 2 drops of Hydrogen Peroxide to 25mL sample OR 4 drops to 50mL sample (for False Negative Test)

3. Screw on lid and shake to mix. **Wait 3-5min** for best results.

1.3 Procedure Considerations

Important: 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. Please see Section 3 for additional notes on *Sample Pretreatment* methods.

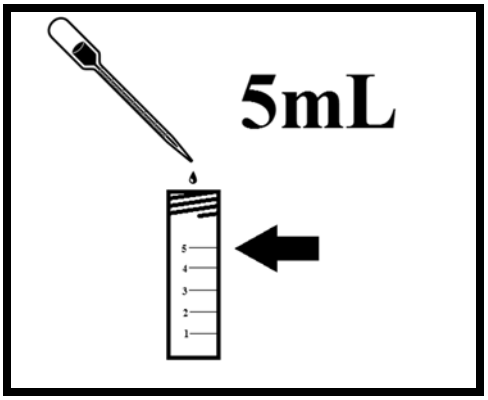
- Consistency is important for improved accuracy. Repeat your steps as closely as possible (examples: 3-5sec injection of precisely 1mL, close lid, and press start immediately)
- All components in a sensor kit (cuvette, sensor housing, 1mL syringe, buffer vial, pipette tip) are meant to be used only once and are disposable. No special treatment required as all materials are non-hazardous.
- Kits are color coded and labeled for each metal. For example, Mercury testing will use a gray sensor housing, buffer caps, and fluorimeter display.
- Test with fluorimeter on a flat surface (i.e. Inner surface of provided fluorimeter case)
- Open the 5mL vials carefully so that the buffer liquid buffer does not spill out. When adding the sample water, you can slowly pour the water into the vials up to the 5mL mark and use one of the provided disposable plastic pipettes to transfer water accurately. Do not exceed the 5mL mark for accurate results.
- If the unit is left on for more than a few minutes without any activity, a screen-saver (black screen) will be activated (user settable); press any button to resume operation (Do not press and hold ON/OFF).
- 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.

1.4 Testing

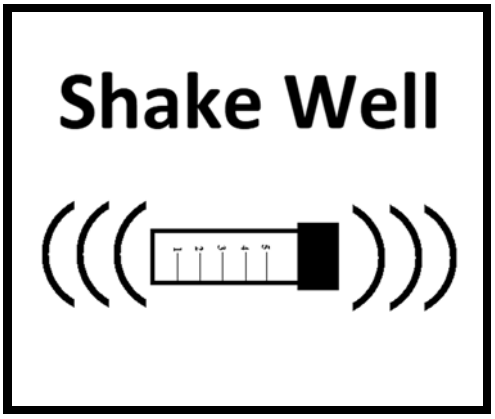
Required Materials

- AND1100 Fluorimeter
- (1) Sensor Pack
- (1) 1mL Syringe
- (1) 5mL Buffer Vial
- (1) Transfer Pipette
- "Conditioned" Sample

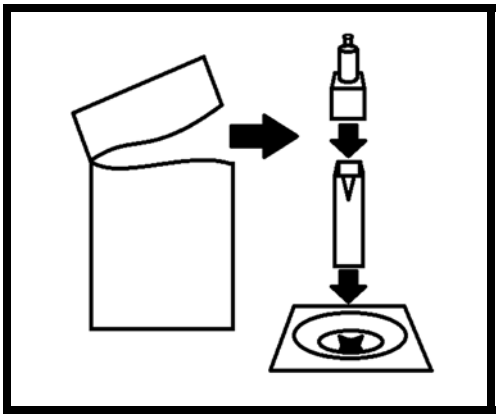
1. Press **ON/OFF** to initialize AND1100. Confirm correct Sensor and Site setting.



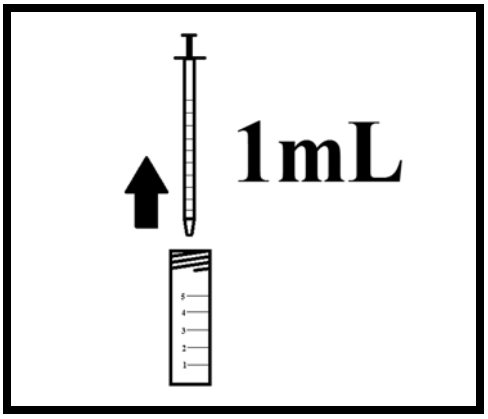
2. Fill vial containing buffer to the 5mL mark with sample water using transfer pipette.



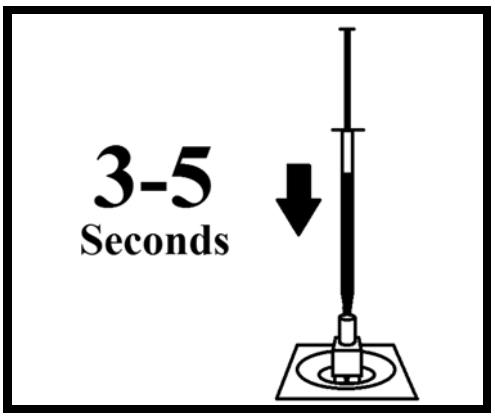
3. Screw on lid and shake to mix.



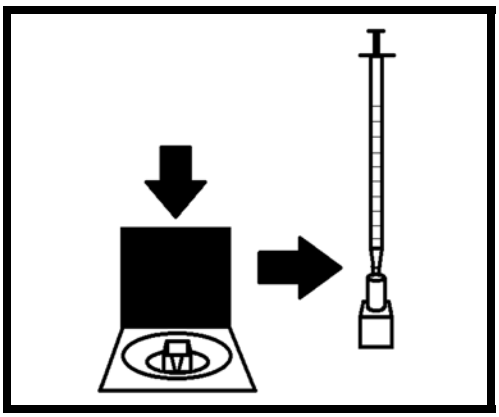
4. Open sensor pack, placing cuvette into AND1100 test chamber (triangle mark forward) and gray sensor housing onto cuvette.



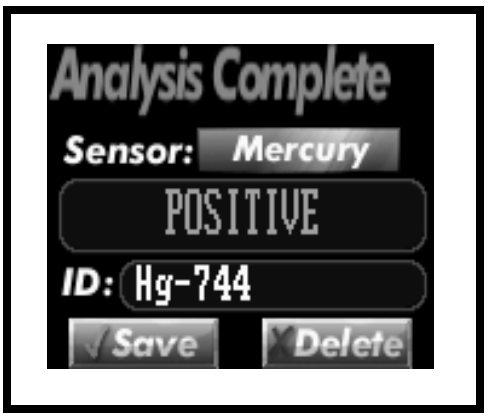
5. With a new syringe draw 1mL of sample from vial.



6. Attach syringe to top of sensor housing. Over 3-5 seconds, squeeze sample into cuvette.



7. QUICKLY remove sensor housing and syringe, close AND1100 lid, and press Start.



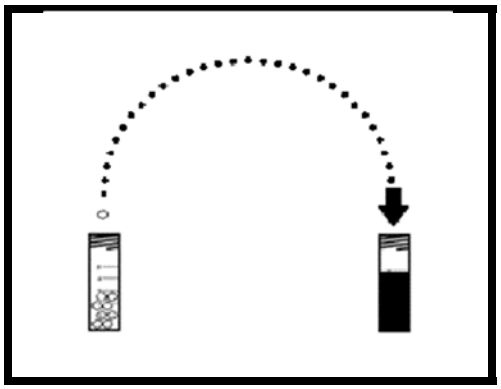
8. When prompted, remove cuvette to view and save results. Discard all materials used.

1.5 Test for False Positive

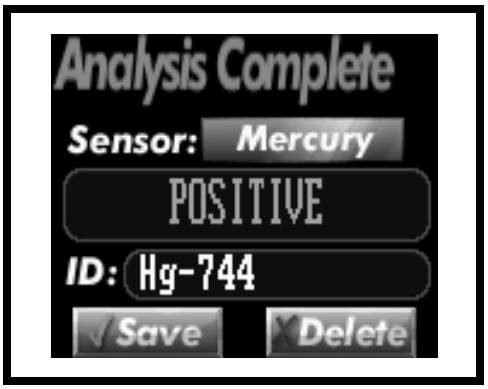
If your test result for mercury is positive, the validity can be tested by adding a mercury chelator to bind to the mercury ions making them undetectable by the sensor.

Required Materials

- AND1100 Fluorimeter
- (1) Sensor Pack
- (1) 1mL Syringe
- (1) 5mL Buffer Vial
- (1) Transfer Pipette
- "Conditioned" Sample
- (1) Chelator Pellet



1. Add one chelator pellet to a **NEW** buffer vial. **The pellet will NOT dissolve.**



2. Follow the FULL Testing procedure in Section 1.4 using buffer vial with pellet.

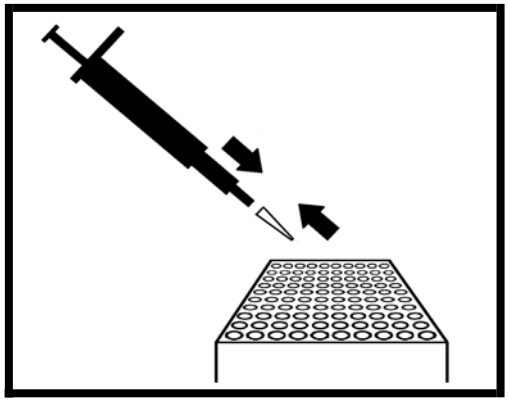
If the result of this sample containing the mercury chelator is **NEGATIVE**, the original positive result is valid. If the result is **POSITIVE**, an interference in the sample matrix may have led to a false positive.

1.6 Test for False Negative

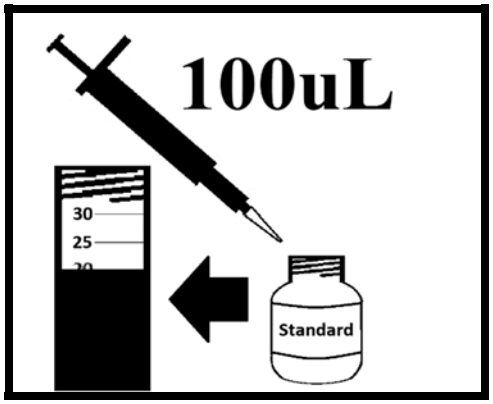
If your test result for mercury is negative, the validity can be tested by testing against a solution with a known level of mercury added.

Required Materials

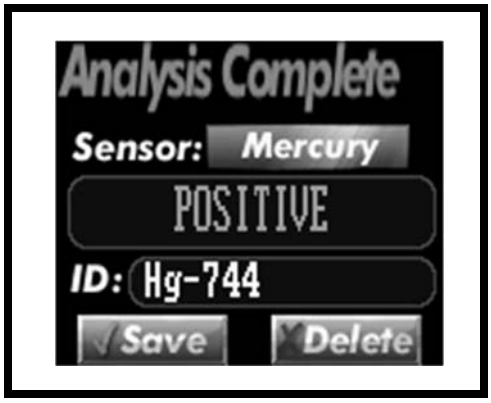
- AND1100 Fluorimeter
- (1) Sensor Pack
- (1) 1mL Syringe
- (1) 5mL Buffer Vial
- (1) Transfer Pipette
- 100 µL Pipette
- Pipette Tip
- Mercury Standard Bottle
- **50mL** "Conditioned" Sample



1. Place a new pipette tip onto 100uL fixed pipette.



2. Using pipette, withdraw 100uL of Mercury Standard and dispense into a 50mL sample vial containing **50mL of "conditioned" sample.**



3. Follow the FULL Testing procedure in Section 1.4 using the sample containing the mercury "spike".

If the result of this sample containing the "spike" is **POSITIVE**, the original negative result is valid. If the result of this spiked sample is **NEGATIVE**, the sample matrix leads to false negatives due to interference.

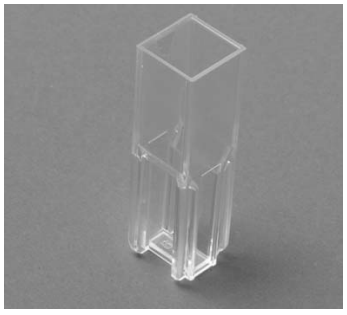
2 General Information

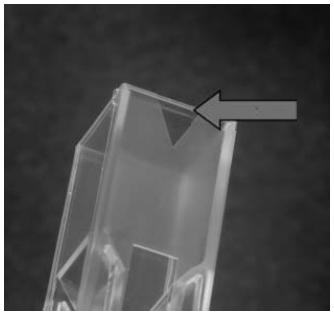
2.1 Sensor Pack: Cuvette and Sensor

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.

2.2 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 help 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.





2.3 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.

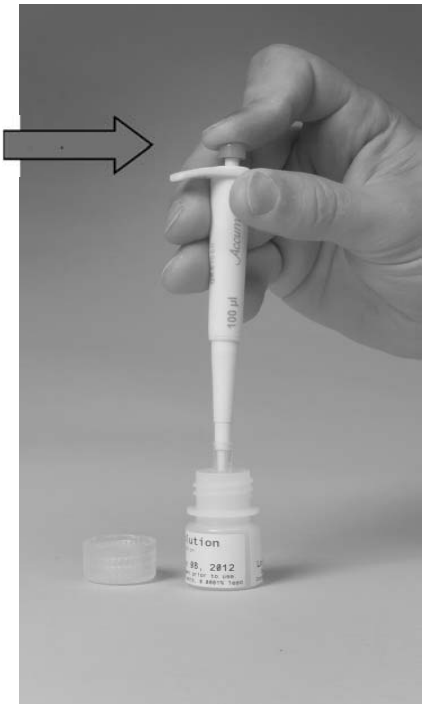
The sample should be injected through the housing into the cuvette at a constant speed over **3 – 5 seconds.** The syringe and housing should be immediately removed and the sample door closed. The **START** button located just below the screen should be pressed immediately to start any measurement**.**

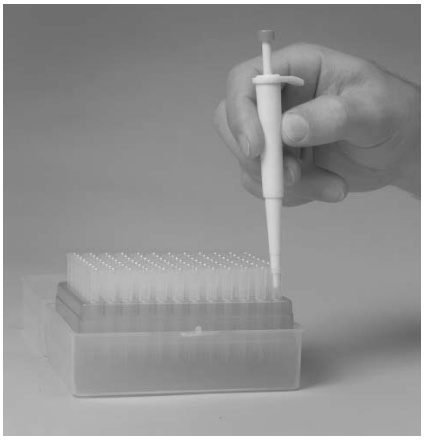
Important: After each analysis, discard all components used during the analysis including cuvette, sensor housing, sample tube, and syringes to avoid cross contamination.

2.4 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 Sample Pretreatment

ANDalyze test kits are designed for use "out of the box" with drinking water; however, they can be used for analysis of other water matrices with some minor protocol modifications. This section contains instructions for various pretreatments that may be required for samples obtained from other sample sources such as those indicated in the chart below and which pretreatments are recommended and/or required them:

		Filtration	Dilution	pH Adjustment	Acid Digestion	Other*
	ANDalyze Kit Part Number:	**AND900**	**AND901**	**AND903**	**N/A**	**N/A**
Drinking	**Municipal**					
	Municipality					
	Plumbing/Pipes	**Recommended**				
	Ground					
	Wells	**Required**	**Recommended**			
Natural	Mining	**Required**	**Recommended**	**Recommended**		**Recommended**
	Hazmat sites	**Required**	**Recommended**			**Recommended**
	Surface (River/Pond/Lake)					
	Environmental	**Required**				
	Irrigation	**Required**				
	Salt Water					
	Brackish	**Required**	**Required**			**Recommended**
	Seawater	**Required**	**Required**			**Recommended**
Waste	**Industrial**					
	Inlet Boiling/Cooling Tower	**Required**	**Required**	**Recommended**	**Recommended**	**Recommended**
	Process Wastes	**Required**	**Required**	**Recommended**	**Recommended**	**Recommended**
	Municipal					
	Final Effluent	**Required**	**Required**	**Recommended**	**Recommended**	**Recommended**

*** May require additional testing to identify potential other interference**

Each matrix type may require one or more of the following pre-treatment kits, which are available through ANDalyze. However, individual components have been listed also, which may be purchased through a scientific supply company as well.

ANDalyze Dilution Kit

- 50 mL Self-standing sample tube
- 5 mL Fixed Volume Pipette & Tips
- Reagent grade deionized or distilled water

ANDalyze pH Adjustment Kit

- Sodium Hydroxide Neutralization Solution, 1% (w/v) sodium hydroxide in a dropper bottle
- Nitric Acid Neutralization Solution, 1.5% (w/v) nitric acid in a dropper bottle
- pH paper

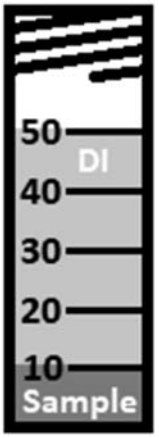
ANDalyze Filtration Kit

- 0.45 µm Nylon filter, 25 mm diameter (Nalgene)
- 20 mL Syringe
- 50 mL Self-standing sample tube

3.1 Dilution

Dilution involves taking a small amount of sample water then adding distilled or deionized (DI) water (aliquot), which contain no interferences, to make a new solution with a larger volume but a lower concentration than the original sample. Dilution is needed for accurate readings when the target metal ion is present at a concentration higher than the linear detection range (i.e. 100ppb for lead). If the concentration is unknown, the device will generally indicate an "Above Limit" on the results page.

With any dilution, the displayed result (concentration) after the test, must be multiplied by a factor to account for the dilution. This is the number usually followed by an "X" when describing a dilution (i.e. 4X dilution contains 1 part sample and 3 parts DI water). This factor can be calculated by dividing the Total Volume by the Sample Volume (i.e. 50mL total volume divided by 5mL sample = 10, "Negative" result indicates the concentration is below 20ppb in original solution).



Use of 50mL Sample Vials (Included with Kit)

- Using the graduations on the 50mL sample water vial, users can make a 2X to 10X dilution by adding sample to the 10mL mark and then filling to 20mL (2X), 30mL (3X), 40mL (4x), or 50mL (5x) mark with DI water. Higher dilutions can be obtained by adding sample to the 5mL mark and then filing to the 30mL (6X), 35mL (7X), 40mL (8X), 45mL (9x), or 50mL (10x) mark with DI water.
- For even higher dilution, the 100uL pipette used for Site Calibration (and new pipette tip) can be used. By adding 100uL of sample to the 50mL vial and then adding DI water to a specific mark, users can achieve 50X through 500X dilutions.

Using ANDalyze Dilution Kit (Purchased Separately)

By withdrawing 5mL of sample with the 5mL fixed volume pipette, adding it to the 50mL self-standing tube then filling with DI water to the 50mL mark, a 10X dilution can be created.

Important: After dilution, all solutions should be shaken well.

Note: Use of glassware is not recommended for samples with metal due to adsorption to the walls.

3.2 pH Adjustment

ANDalyze sensors perform best when the sample pH is between 5 and 8. Samples with a pH greater than 8 or below 5 will not test reliably so it is required to adjust the pH of the sample to be within this range before site calibration or testing. Samples above pH 10 should not be tested even with pH adjustment.

Note: Tests have shown that environmental samples preserved in acid to a pH < 2 cannot be brought to a pH of 7.4 when mixed with the ANDalyze buffer. These samples must be first neutralized with NaOH to a pH ~5 before mixing with ANDalyze buffer.

Procedure

1. **Check the sample pH** using pH paper.
2. **Adjust the sample pH**
   - **If the sample is below pH 5** addition of a dilute sodium hydroxide solution is necessary. To a 25 or 50mL volume of sample, add the Sodium Hydroxide Neutralization Solution dropwise with stirring or with shaking between addition of each drop. Do not adjust beyond pH 5.

     Note: pH change from 4 to 5 is rapid, requiring a half drop or less. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to increase pH from 3 to 4, or many more may be required.

   - **If the sample is above pH 8** addition of a dilute nitric acid (1.5 %) solution is necessary. For highly basic water samples, acidification may be insufficient as metal ions may have already precipitated out of solution.

     Note: pH change from 9 to 8 is rapid, requiring a half drop or less depending on matrix. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to decrease pH from 10 to 7, or many more may be required.

3.3 Filtration

Before performing a Site Calibration or testing any water sample that may contain high turbidity or particulates, it must be filtered to remove suspended solids.

Procedure

1. Draw ~20 mL of sample into a 20mL syringe.
2. Securely attach the syringe filter to the syringe.
3. Slowly push the sample through the filter into the self-standing vial. The sample should be clear and the filter may no longer be white.

   Note: If the sample is collected off-site and transported to a laboratory for testing, the EPA recommends that the user ensures that the sample is stirred (e.g. place a stir-bar in the bottom of a 1L HDPE Nalgene bottle filled with sample on a stir plate) while filling the syringe to ensure homogeneity. For most applications ANDalyze recommends simply shaking well.

   Note: If a sample contains a great deal of suspended solids the syringe filter may clog after elution of 10-20mL of sample. In this case, discard the clogged filter and use a fresh filter to continue filtering the sample.

4 Technical Specifications

4.1 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 ANDalyze 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
- Mercury 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

Mercury dilutions containing 0 - 150 ppb Hg^{2+} were prepared in DI water. Five replicates were used for each test at each dilution.

Mercury Detection Range

2 – 50 ppb mercury

(Above 50 ppb mercury, there can be false negatives. If it is known that sample may contain very high levels of mercury, please dilute your sample in DI water).

Note: All specifications are subject to change without notice.

4.2 Interference

Interference tests were done with a 0 or 1 ppb mercury in DI water plus the potential interfering ion. The interference tolerance levels represent the concentration above which will there is a false positive for a 0ppb mercury sample, or false negative where mercury is present. Data represents an average of at least three replicates.

Interfering ion	Interference level	Above tolerance level will cause:
Calcium, Ca^{2+}	80 ppm	False negative
Manganese, Mn^{2+}	100 ppm	False negative
Ammonium, NH_4^+	100 ppm	False negative
Copper, Cu^{2+}	0.5 ppm	False negative
Iron, Fe^{3+}	15 ppb	False negative
Aluminum, Al^{3+}	20 ppb	False negative
Magnesium, Mg^{2+}	30 ppm	False negative
Chloride, Cl^-	200 ppm	False negative
Sulfate, SO_4^{2-}	1500 ppm	False negative
Dihydrogen phosphate, $H_2PO_4^-$	2000 ppm	False negative
Bicarbonate, HCO_3^-	800 ppm	False negative
Zinc, Zn^{2+}	200 ppm	False positive
Cobalt, Co^{2+}	140 ppm	False positive
Cadmium, Cd^{2+}	60 ppm	False positive
Nitrate, NO_3^-	>10000 ppm	No interference
Sodium, Na^+	>3700 ppm	No interference

5 Consumables and Replacement Items

- Fluorimeter (Part Number: AND002)
  - Kit Includes: AND1100 Fluorimeter
    USB to MINI-B Cable
    100µL Fixed Volume Pipette and Tips
    pH Test Strips
    User Manual

- Sensor Kit (Part Number: AND014)
  - Kit Includes: (25) Sensor Packs with Sensor & Cuvette
    (25) Sample Tubes (with buffer)
    (25) 1 mL Syringes
    (15) Disposable Transfer Pipettes
    (3) 50mL Sample Vials
    1 dropper bottle of Hydrogen Peroxide
    3 mL Mercury Standard Solution (1.0 ppm Hg^{2+})
    Tube containing Mercury Chelator pellets
    Instruction Manual
    Material Safety Data Sheets (MSDS)

6 Contact Information

Contact us by Email:

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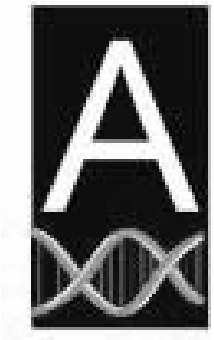

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