Heavy Metals Water Testing

*Powered by DNA*

*Fast - Sensitive - Specific - Platform Technology for Metals detection*

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**Advantages**

Platform Technology Allowing Detection of Many Targets
Laboratory Detection Limits in a Portable Instrument
Field Test Results Under One Minute
Research Grade Fluorimeter
Disposable Test Kits

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By
Eric L. Null, Ph.D., Staff Scientist
and
Marty Dugan, VP Marketing & Business Development

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Water Testing Powered by DNA
ANDalyze, Inc.

Introduction
Water pollution is a worldwide problem affecting developing and developed countries alike. Heavy metal contaminants are one prevalent type of water pollutant. They are persistent in the environment once discharged and removal from source waters is necessary to ensure a clean drinking water supply. The problem of heavy metal pollution arises from several sources. Heavy metals such as uranium can naturally exist in ground water. Lead can be present as a result of lead solder in copper piping. Mercury and cadmium can be a result of power plant emissions. Additionally, a variety of industrial processes can produce problematic heavy metal concentrations in discharged water from factories that are harmful to humans and can contaminate agricultural land. Testing for heavy metals at part per billion (ppb) levels is essential to meet international established limits. To meet this need ANDalyze, Inc. has developed heavy metals tests based on catalytic DNA. ANDalyze heavy metals tests are designed to be sensitive, selective, portable, and very easy to use. This paper covers the use, the innovative solution, specifications, applications, and future possibilities of this patented technology.

The Problem with Metals Test Kits: High Detection Limits, Difficult to Use
Limits on heavy metals in waste water and drinking water are often stated in parts per billion (ppb) and are heading in only one direction: down. Current techniques for detection of heavy metals in the low ppb range, such as inductively coupled plasma – mass spectrometry (ICP-MS), are expensive, requiring a six figure investment. These instruments are operated by a trained
scientist and remain stationary once installed. Extensive sample preparation may be required and a single mislabeled sample can result in extensive downtime. These workhorse instruments excel at metals analysis, however a method simpler in implementation is advantageous.

A portable alternative would allow for on-site analysis in real time without expensive sample transportation and preparation. Contaminants could be monitored on a regular basis and high contaminant concentrations can be detected before harm is done. Such metals test kits based on colorimetric technology are currently available, though they come with several caveats. Addition of multiple reagents may be necessary, the test procedure for each metal is different, and the detection limits are usually not single ppb level. The USEPA’s Maximum Contaminant Limits for Lead and Mercury (inorganic) are currently 15 ppb and 2 ppb, respectively.¹ Many colorimetric test kits fail to test below these levels.

The Solution: DNA-based Detection

*ANDalyze has developed heavy metals test kits based on catalytic DNA.*

**Why use DNA?**

Using catalytic DNA technology it is possible to reach single digit ppb level limits of detection and up to million-fold specificity over other metals. Colorimetric methods, though well developed, cannot easily match these capabilities. The selectivity and specificity comes from the ability of DNA strands, obtained through a process known as *in vitro* selection, to bind one metal preferentially over another. Catalytic activity of the DNA strands or rapid binding to metal ions leads to very quick testing times – *e.g.* under one minute for Lead. To allow for portable heavy metals analysis, the selectivity of DNA has been paired with fluorescence detection – a very sensitive technique – in the form of a custom designed portable fluorimeter. The AND1000 fluorimeter is designed to work specifically with ANDalyze sensors. The AND1000 can also be used as a research-grade laboratory instrument due to the incorporation of a photomultiplier tube (PMT) detector. Combining catalytic DNA chemistry with fluorescence-based detection results in detection limits well below the USEPA and other international regulatory limits for dissolved metals in water. Current metals that are detectable include Copper, Lead, Mercury, Zinc, Uranium with several other metals in development.
The combination of catalytic DNA and fluorescence-based detection was developed and patented at the University of Illinois at Urbana-Champaign. Catalytic DNAs (also called DNAzymes or DNA enzymes) are DNA sequences that catalyze the cleavage of another DNA strand (the substrate) in the presence of a specific cofactor. The first step in the development process is obtaining an optimum DNA sequence that catalyzes the substrate cleavage reaction in the presence of a specific metal contaminant. This DNA sequence is obtained through a combinatorial process called *in vitro* selection. Briefly, $10^{14}$-$10^{15}$ DNA sequences are subjected to selection pressure via the repeating steps of incubation with the target, separation of active sequences, and amplification/mutation. Stringency is increased in terms of lower target concentration and shorter reaction times until sufficient catalytic activity is obtained. The next steps of cloning, comparison of DNA sequences, and truncation result in a single DNA sequence with optimum activity. The sequence is modified to add a fluorophore and quencher, two small organic molecules. A fluorophore takes in light of a certain wavelength and emits light at a less energetic wavelength. A quencher dissipates light in the form of heat. Placing a fluorophore and quencher in close proximity via DNA base pairing (complimentary enzyme and substrate DNA strands) quenches the fluorescence process. Separation of the fluorophore and quencher in space due to the metal-specific DNAzyme cleavage of the substrate results in a return of fluorescence signal, which is measured using a fluorimeter.

![Figure 1. Schematics of (A) the *in vitro* selection process and (B) catalytic DNA activity in the presence of a metal ion leading to fluorescence signal.](image-url)
Sensor Construction

The core technology developed by ANDalyze is located in the sensor housing. All DNA is chemically synthesized, meaning that no biological organisms are used in the process. Our DNA is not farmed or grown. Active DNA sequences are incorporated into a porous material using a proprietary method and then placed in a plastic housing that allows for liquid flow from a syringe. During testing, the housing is placed over a cuvette and a buffered sample is passed through the housing using a disposable 1 mL syringe. The sample flows through the housing and passes through the porous material containing the DNA. The amount of DNA in each sensor housing is so small that it cannot be seen with the naked eye, though it is easily detected using fluorescence. As soon as the DNA mixes with the liquid sample, an almost instantaneous process, the DNA begins reacting with metal ions in solution. Each metal ion leads to a cleavage event and a release of the substrate strand from the enzyme strand. This release also separates the fluorophore from the quencher, leading to a fluorescence signal that is directly proportional to the metal ion concentration.

AND1000 Fluorimeter

The AND1000 Fluorimeter is a portable self-contained instrument, though it may be connected to a computer via USB if desired to download data. The AND1000 Fluorimeter is used in conjunction with metal-specific kits from ANDalyze, and yet the fluorimeter may be utilized independently as a research-grade instrument. Fluorescence detection was chosen by ANDalyze due to the inherent high sensitivity of the technique and the sensitivity was enhanced further by using a photomultiplier tube (PMT) as a detector, which is uncommon in portable fluorimeters. Excitation is accomplished using a light emitting diode (LED) and an excitation filter. An emission filter at located next to the PMT completes the setup. The AND1000 Fluorimeter is recharged via USB or a wall adapter, is water resistant with an IP54 enclosure rating, and is CE marked.

Advantages of ANDalyze Technology

A great deal of curiosity has resulted from the introduction of catalytic DNA technology to the marketplace. Most people view DNA as a purely biological material and to see DNA used for detection of metals is new and exciting, and rather curious. Why use DNA when ICP-MS and colorimetric techniques are so established? ANDalyze uses DNA because of the host of advantages listed below.
**Advantages of Catalytic DNA Technology:**

- Platform technology – analysis of metals as well as other analytes is possible using the same system with different cartridges and buffers for each target
- Low detection limits – all sensors currently for sale test at or below the USEPA limits for drinking water
- High selectivity - many classic metal analysis reagents react with multiple metals, whereas catalytic DNA has much higher selectivity
- Highly portable
- Reagents are not hazardous
- No contamination issues as kits are disposable
- Low initial investment compared to other solutions

<table>
<thead>
<tr>
<th>Metal</th>
<th>USEPA MCL</th>
<th>ANDalyze LOQ</th>
<th>ANDalyze Detection Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>15 ppb</td>
<td>2 ppb</td>
<td>2-100 ppb</td>
</tr>
<tr>
<td>Copper</td>
<td>1300 ppb</td>
<td>40 ppb (LR)</td>
<td>40-200 ppb (LR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600 ppb (HR)</td>
<td>600-3000 ppb (HR)</td>
</tr>
<tr>
<td>Uranium</td>
<td>30 ppb</td>
<td>2 ppb</td>
<td>2-60 ppb</td>
</tr>
<tr>
<td>Mercury</td>
<td>2 ppb</td>
<td>2 ppb</td>
<td>2-50 ppb</td>
</tr>
</tbody>
</table>

*The Limit of Quantification or LOQ is based on the 10 sigma method, whereas the Limit of Detection or LOD is based on the 3 sigma method. LOQ values are higher than LOD values and are reported instead of LOD. LR= Low Range, HR= High Range


**Applications**

ANDalyze test kits may be utilized in a wide variety of matrices from drinking water to industrial wastewaters and new applications are constantly emerging.

ANDalyze test kits were originally developed for testing drinking water and do not require sample preparation. Drinking water may include water from a utility or municipality, bottled water, and treated spring and well water. In short, any water intended for human consumption.

The utility of ANDalyze test kits has been expanded to include waters such as:

- Environmental fresh waters (rivers, lakes, streams, and ground water)
- Seawater
- Industrial process wastewater
- Municipal final effluent
A simple filtration step is required for these waters due to their turbidity. In rare cases pH adjustment and iron interference protocols may be necessary, and an ANDalyze Iron Interference accessory kit may be purchased from ANDalyze if such treatment is necessary.

Seawaters and wastewaters may be tested after filtration and dilution steps. These waters are often turbid, and a simple filtration step is required. Seawater does not usually require pH adjustment, though certain wastewaters may require pH adjustment before proceeding with testing steps. ANDalyze test kits include a buffer material that adjusts the pH to the optimum range for the sensors. Some wastewaters may exceed the buffering capacity. Be sure to follow the product guidelines for each sensor to determine whether the sample pH is within range. A simple ten-fold dilution step follows and may be accomplished using standard laboratory equipment or the ANDalyze Dilution accessory kit.

ANDalyze has developed an Environmental Water Testing Solution Note that guides the user through testing environmental waters and research is ongoing with additional matrices. ANDalyze test kits may be able to detect heavy metals in matrices not listed here. Please contact ANDalyze with details of any matrix you would like to test.

**Colorimetric Test Kit Limitations**

The most common portable test kits for detection of heavy metals rely on colorimetric techniques, some of which date back over 100 years. These common methods have undergone numerous improvements over the years. However, the limits of detection are seldom at the single ppb level and are more likely to be found above 50 ppb. In order to test below ever-decreasing USEPA MCLs, alternative techniques are necessary.

In addition, the colorimetric test steps and reagents vary for each metal and hazardous reagents such as concentrated acids and cyanide may be required. Finally, design of reagents that will react specifically with a single metal is very difficult and therefore false positives are a concern. These difficulties are in contrast to the platform technology developed by ANDalyze which utilizes the same general test steps for all metals, while testing below the USEPA MCLs.

**Cost Advantages of the AND1000 Portable Fluorimeter**

Laboratory methods such as ICP-MS, commonly used for analysis of metals in water, involve a tremendous initial investment of hundreds of thousands of dollars and must be operated by highly trained chemical analysts. The drive towards portable instrumentation is beneficial from a financial standpoint as well as a technical standpoint. On-site testing provides quick results, allowing issues to be corrected in a timelier manner and minimizing water sample chain of custody concerns. Though portable instruments are generally cheaper than laboratory instrumentation, the cost varies greatly with technique and instrument capabilities. Lower detection limits and higher capabilities usually mean greater expenditure and increased operator experience requirements. The AND1000 Portable Fluorimeter is designed to provide a user-friendly experience with low detection limits and advanced capabilities, while costing much less than competitive portable instruments. Another portable technology with low
detection limits, anodic stripping voltammetry or ASV, is commercially available although the initial investment is ten times higher than the DNAzyme/fluorimetric based solution and ASV instruments require regular maintenance of electrodes.

**Advanced Water Testing Applications**

ANDalyze is currently exploring a wide range of possibilities for future product lines. Our basic technology is applicable to a wide range of targets, not simply metals, and a variety of detection schemes. A few possibilities are discussed below and we would be glad to elaborate on any or all of these applications.

**Organics and Biological Organisms**

DNA sequences used by ANDalyze do not have to be catalytic in nature. DNA sequences may also be obtained by *in vitro* selection that simply bind to a target rather than undergo a catalytic reaction upon target binding. These nucleic acid sequences are called “aptamers,” a term derived from the Latin word *aptus* meaning “fit.” Aptamers can be selected to bind a wide range of targets from insecticides to proteins to whole cells. With a short development cycle almost any target, chemical or biological, can be analyzed using the ANDalyze platform system. Please inquire if you have a target in mind.

**Dipstick Technology**

Are you interested in qualitative or semi-quantitative sensors for metals or other targets? By conjugating DNA sequences to gold nanoparticles instead of fluorophores the ANDalyze detection scheme can be expanded to lateral flow or “dipstick” tests. Appearance of a red band indicates target presence and the intensity of the band may be compared to a chart to determine approximate concentration. These inexpensive tests are very user friendly.

**In-line Applications**

In-line monitoring systems are becoming more and more important in drinking water, industrial processing water and wastewater analysis. To ensure the highest levels of safety, monthly sampling just isn’t enough. Are you interested in sampling daily, hourly, or even higher frequencies? Are you interested in a set it and forget it system that works with minimal intervention? Please contact ANDalyze with suggestions or comments related to in-line monitoring.

**Summary**

This new water testing technology for heavy metals creates not only a clear operational advantage for testing and analyzing water, but it also sets a new path for the potential of field testing technologies by creating a platform instrument on which a long list of inorganic, organic and microbiological contaminants can be detected and analyzed at field sites. It also will enable a broader list of users to test water. Trained chemists and water resource engineers won’t be the only ones testing water. General technicians, service personnel and students will undoubtedly uncover a much clearer understanding of the locations, sources and levels of
metals contamination around the world. This innovation will bring a new understanding of the levels of contamination in communities, industry and regions. It is a new tool to help better manage our global environmental resources and industrial water technology.

Contact

For more information on the technology behind Water Testing Powered by DNA please contact ANDalyze at:

Offices

ANDalyze, Inc.
800 Boylston Street
16th Floor
Boston, Massachusetts 02199
USA

Laboratory

ANDalyze, Inc.
2109 S Oak Street, Suite 102
Champaign, IL 61820 USA

Contact us by Email: info@andalyze.com

Contact us by Telephone: +1 888 388 0818 or +1 217 328 0045

References

Appendix

Specifications for the ANDalyze Lead100 Sensor

Performance

Lead dilutions containing 0, 5, 10, 15, 25, 50, 75, 100, 150, 200 ppb Pb\(^{2+}\) were prepared in DI water. Five replicates were used for each test at each dilution.

Limit of Detection (LOD)

\[
\begin{align*}
1 \text{ ppb Pb}^{2+} \\
\text{Based on 3 sigma method}
\end{align*}
\]

Limit of Quantification (LOQ)

\[
\begin{align*}
2 \text{ ppb Pb}^+ \\
\text{Based on 10 sigma method}
\end{align*}
\]

Linear Detection Range

\[2 - 100 \text{ ppb Pb}^{2+}\]

Precision

\[
\begin{align*}
\text{Standard: } 15 \text{ ppb Pb}^{2+} \\
95\% \text{ Confidence Limits: } 13 - 17 \text{ ppb Pb}^{2+}
\end{align*}
\]

Coefficient of Variation (CV):

\[0–200 \text{ ppb Pb}^{2+} \pm 15\%\]

Note: All specifications are subject to change without notice.

Interference

Interference tests were done with a 30ppb Pb\(^{2+}\) solution plus the potential interfering ion. The interference tolerance levels represent the concentration above which the lead 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.

<table>
<thead>
<tr>
<th>Interfering Ion</th>
<th>Interference level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium, Ca(^{2+})</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Magnesium, Mg(^{2+})</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Zinc, Zn(^{2+})</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Aluminum, Al(^{3+})</td>
<td>0.1 ppm</td>
</tr>
<tr>
<td>Copper, Cu(^{2+})</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>Iron, Fe(^{3+})</td>
<td>0.04 ppm</td>
</tr>
<tr>
<td>Cadmium, Cd(^{2+})</td>
<td>15 ppm</td>
</tr>
<tr>
<td>Mercury, Hg(^{2+})</td>
<td>0.03 ppm</td>
</tr>
<tr>
<td>Manganese, Mn(^{2+})</td>
<td>150 ppm</td>
</tr>
<tr>
<td>Ammonium, NH(_4)(^{+})</td>
<td>2000 ppm</td>
</tr>
<tr>
<td>Carbonate, CO(_3)(^{2-})</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Phosphate, PO(_4)(^{3-})</td>
<td>50 ppm</td>
</tr>
<tr>
<td>Chloride, Cl(^-)</td>
<td>2000 ppm</td>
</tr>
<tr>
<td>Sulfate, SO(_4)(^{2-})</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Nitrate, NO(_3)(^-)</td>
<td>5000 ppm</td>
</tr>
</tbody>
</table>

Product Accuracy Ranges

The graphs below depict the average measured Pb\(^{2+}\) concentration as displayed on the AND1000 fluorimeter (y-axis) vs. the known concentration of the lead standards (x-axis). Error bars depict the standard deviation from five measurements. Figure 1 shows the linear range of 0 - 100 ppb Pb\(^{2+}\). For higher concentrations of Pb\(^{2+}\) (tested up to 200 ppb Pb\(^{2+}\)), the accuracy decreases (Graph 2). Samples containing higher than 100 ppb Pb\(^{2+}\) can be diluted 1:1 and re-analyzed.
Graph 1: Average Pb²⁺ Conc. vs. Known Conc.

Graph 2: Accuracy vs. Linear Detection Range
Glossary

Platform Technology
A hardware and/or software solution that allows future expansion of the product line and increased capabilities without the need to purchase additional equipment.

Deoxyribonucleic Acid (DNA)
The most common definition of DNA is a nucleic acid that carries genetic instructions in living organisms. However, DNA can also be synthesized in a laboratory. Through a process known as in vitro selection, pieces of DNA can be found that bind to metals, small molecules, or cells in a highly specific manner. These short sequences can then be turned into sensors.

Fluorescence/Fluorophore/Fluorimeter
Fluorescence is a process in which a substance or molecule absorbs one wavelength of light and emits a less energetic wavelength of light, e.g. absorbing blue light (more energetic) and emitting green light (less energetic). A fluorophore is a molecule that fluoresces and in the case of ANDalyze technology, is covalently attached to DNA. A fluorimeter is an instrument that measures fluorescence by shining a more energetic wavelength of light on a sample and measuring the less energetic light emitted by the sample. Fluorescence is extremely beneficial in analytical procedures due to its high sensitivity.

In Vitro Selection
A combinatorial chemistry technique performed in a laboratory to obtain short DNA or RNA strands of non-biological origin that bind to a target in a highly specific manner. The process uses repeating cycles of selection, amplification, and mutation. The selection step eliminates inactive DNA strands, amplification increases the population of active DNA strands, and mutation introduces changes in the DNA strands that may increase affinity for the target.

Parts per billion (ppb)
A dimensionless quantity (no units, e.g. grams) used to denote abundance or concentration of a substance. One ppb denotes one part per 1,000,000,000 parts. An equivalent expression in water analysis is µg/L or micrograms per liter. A related expression is ppm, or parts-per-million, which is equivalent to one part in 1,000,000 parts or 1 milligram per liter of water.

Colorimetric
Colorimetric tests rely on generation of color, often by complex formation between an organic ligand and a metal ion. The color may be measured using a colorimeter, color comparator, or other device. Some colorimetric methods have been available for over 100 years, though despite decades of improvements many detection limits are not at the single digit ppb level.

Maximum Contaminant Levels (MCLs)
Part of the National Primary Drinking Water Regulations (NPDWRs) which are legally enforceable standards set by the United States Environmental Protection Agency (USEPA). All ANDalyze test kits test below these limits.
Inductively Coupled Plasma – Mass Spectrometry ICP-MS
A hyphenated or hybrid analytical instrument used for elemental analysis, including analysis of metals in water. The sample is ionized using inductively coupled plasma at 10,000 degrees and components are separated and detected by mass using a mass spectrometer. Metal speciation studies may require additional equipment, e.g. determination of trivalent chromium vs. hexavalent chromium. A well-respected technique with low detection limits. Requires a six-figure upfront investment and a trained analyst to operate.