Arsenic Exposure Testing

the need for fractionation

Arsenic is a unique toxicant that exists in both toxic and non-toxic forms. This presentation describes how laboratory testing can distinguish between toxic and non-toxic forms involved in an exposure.
This presentation is divided into three sections. First, background information, including common sources of arsenic, the toxicology of arsenic, and laboratory testing used to diagnose arsenic poisoning. The need for incorporating arsenic fractionation in the laboratory evaluation of arsenic poisoning will be justified.

In the second section, arsenic testing options available through ARUP Laboratories as of November 26, 2007 are summarized including test strengths and limitations.

Last, I will provide recommendations for selecting the best test.
Sources of arsenic

- Environment
  - Ground water, soil
  - Air pollution
- Industry
  - Smelters
  - Power plants
  - Treated wood
  - Pottery, glass
  - Pesticides, rodenticides, insecticides, herbicides
- Medicine (arsenic trioxide)
- Food
  - Fish, shellfish
  - Seaweed
  - Food such as rice that are produced where soil and/or water are contaminated with arsenic

Arsenic is found on the Periodic Table of the Elements as Atomic Number 33. Arsenic is part of the Earth’s crust and is concentrated in sea water. As such, it is no surprise that arsenic is common to our environment, including the water we drink, the air we breath, and the food we eat. Arsenic is also a common occupational hazard, particularly in the production of certain sulfide ores. Arsenic is found in pottery and glass manufacturing processes, pesticides, wood preservatives, as a chemotherapeutic agent, and it is naturally occurring in many household products.

Outside of an occupational setting, the most common source of arsenic exposure is through contaminated drinking water or through a diet rich in arsenic-containing food such as seafood. However, arsenic exists in many forms or “species,” each associated with a different risk of toxicity.

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Forms/species of arsenic

- Arsenic exists in many (>30) forms
- The best characterized forms of arsenic (As) that are observed in human urine are
  - Organic arsenic:

  \[
  \begin{align*}
  \text{Arsenobetaine (AsB)} & : \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{As}^+ \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{COOH} \\
  \text{Arsenocholine (AsC)} & : \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{As}^+ \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{OH}
  \end{align*}
  \]

In fact, more than 30 forms of arsenic have been identified. The arsenic that concentrates in seafood and most other dietary sources is generally bound to organic molecules. Through binding to organic molecules, the toxic potential of the arsenic is minimized. The most common forms of organic arsenic observed in human urine in the hours to days following ingestion of seafood include Arsenobetaine and Arsenocholine.
Forms/species of arsenic (cont.)

– Inorganic arsenic (iAs)

<table>
<thead>
<tr>
<th>Arsenic III (As$^{+3}$, arsenite)</th>
<th>Arsenic V (As$^{+5}$, arsenate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>HO - As</td>
<td>HO - As = O</td>
</tr>
<tr>
<td>OH</td>
<td>OH</td>
</tr>
</tbody>
</table>

– Methylated metabolites of inorganic arsenic V:

<table>
<thead>
<tr>
<th>Monomethyl arsenic (MMA)</th>
<th>Dimethyl arsine (DMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>O = As - CH$_3$</td>
<td>O = As = O</td>
</tr>
<tr>
<td>OH</td>
<td>CH$_3$</td>
</tr>
</tbody>
</table>

The arsenic found in the environment, and in most at-risk occupational settings, is inorganic. Of particular interest are the trivalent and pentavalent forms, because they are associated with the highest risk of toxicity. These inorganic species are methylated in vivo to create monomethyl arsenic (MMA) and dimethyl arsine (DMA).

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How arsenic is handled by the human body

• Absorption and bioavailability of arsenic varies with the form/species, the dose, and the route of exposure
• Arsenic circulates bound to globulins and sulfhydryl groups; inorganic forms are incorporated into tissues including bone, hair, and nails
• Metabolism
  – As$^{+5}$ is oxidized to As$^{+3}$
  – As$^{+3}$ is reduced to As$^{+5}$
  – Most inorganic arsenic is methylated, primarily in the liver
    • Monomethyl arsenic (MMA)
    • Dimethyl arsine (DMA), the predominant species found in urine
• Elimination is primarily (>90%) renal
So how is arsenic handled by the body? Absorption and bioavailability of arsenic varies with the arsenic species, the dose of arsenic, and the route of exposure. Once absorbed, arsenic enters the circulation and may bind to globulins and other proteins via sulfhydryl groups.

Inorganic forms may be incorporated into tissues, including bone, hair and nails. As such hair and nails represent good laboratory specimens for evaluating chronic exposure to arsenic. Inorganic forms are also extensively metabolized to help promote elimination in the urine. There are two commonly detected methylated metabolites of inorganic arsenic: monomethyl arsenic (MMA) and di-methyl arsine (DMA). Thus, exposure to inorganic arsenic may be detected based on the appearance of methylated metabolites in urine. In fact, DMA accounts for the vast majority of inorganic arsenic that is recovered in human urine.

Because the vast majority of arsenic is eliminated in the urine, urine is the laboratory specimen of choice for most arsenic exposure testing.

As suggested earlier, it is important to determine which species of arsenic is responsible for an exposure due to the differential potential for toxicity among the species. The relative toxicity of the arsenic species extends over several orders of magnitude. For example, the LD50 in mice, that is, the dose of an arsenic species that is required to kill 50% of mice exposed to that dose, is approximately 1000 times higher for arsenobetaine than for trivalent and pentavalent arsenic.

This type of data demonstrates why organic species are thought to be non-toxic, and the inorganic species are considered toxic.
The signs and symptoms of arsenic poisoning are somewhat non-specific and vary with dose, route of exposure, and duration of exposure. In an acute exposure, gastrointestinal symptoms commonly occur first, although symptoms such as tremor, fever, hypothermia, muscle pain, and multi-organ failure may also occur.

A unique sign of arsenic poisoning that is first observed a few days after an acute exposure to inorganic arsenic are Mees’ lines. Mees’ lines, also known as Aldrich-Mees’ lines are white lines that traverse the width of the finger nails and toe nails. As the nail grows they move towards the end.

However, Mees’ lines are sometimes difficult to detect and are not always present with arsenic poisoning.
Signs and symptoms of chronic arsenic poisoning

<table>
<thead>
<tr>
<th>System</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiopulmonary</td>
<td>Myocarditis, pericarditis, lung cancer</td>
</tr>
<tr>
<td>GI tract</td>
<td>Nausea, vomiting, diarrhea, anorexia</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Peripheral neuropathy, tremor</td>
</tr>
<tr>
<td>Skin</td>
<td>Melanosis, hyperkeratosis</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Jaundice, cirrhosis, cancer</td>
</tr>
<tr>
<td>Renal</td>
<td>Failure, cancer</td>
</tr>
<tr>
<td>Hematologic</td>
<td>Anemia, leukopenia, thrombocytopenia</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Weakness, muscle wasting</td>
</tr>
<tr>
<td>Other</td>
<td>Alopecia, garlic breath</td>
</tr>
</tbody>
</table>

The most common symptom associated with chronic exposure to inorganic arsenic is peripheral neuropathy. However, symptoms of chronic arsenic exposure may appear gradually and may affect essentially all organ symptoms and may appear gradually with chronic low-level exposures to inorganic arsenic.

Diagnosing arsenic poisoning

- **Clinical**
  - History
  - Signs and symptoms
- **Laboratory testing**
  - The best specimen depends on time of exposure relative to time of presentation
    - Acute exposure (within 24 hrs): Blood, Urine
    - Suspected exposure within approximately one week: Urine
    - Chronic exposure (weeks +): Urine, Hair, Nails
Although clinical examination and history are very important, the diagnosis of arsenic poisoning often depends on results of laboratory testing to both confirm and characterize the exposure. The best specimen for the laboratory evaluation of arsenic poisoning is usually urine.

When urine is not available, such as when evaluating exposure in a dialysis patient, or when the exposure to arsenic is known to have occurred within hours of clinical presentation, blood is the best laboratory specimen. Hair or nails are good laboratory specimens when the exposure is thought to have occurred more than three weeks prior to clinical presentation.

### Estimated kinetics of arsenic

<table>
<thead>
<tr>
<th>Species</th>
<th>Serum half-life</th>
<th>Urine Peak</th>
<th>Urine Window of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>As$^{+3}$</td>
<td>2 hrs</td>
<td>10 hrs</td>
<td>1-2 days</td>
</tr>
<tr>
<td>As$^{+5}$</td>
<td>3 hrs</td>
<td>10 hrs</td>
<td>1-2 days</td>
</tr>
<tr>
<td>MMA</td>
<td>40-50 hrs</td>
<td>20 days</td>
<td></td>
</tr>
<tr>
<td>DMA</td>
<td>1-2 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AsB</td>
<td>1-2 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AsC</td>
<td>1-2 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Elimination may be prolonged with acute intoxication.

This discussion of best specimen is validated by considering the elimination kinetics of each arsenic species. Thus, the opportunity to detect inorganic arsenic in blood is limited to a few hours after exposure in persons with normal renal function, due to a half-life in the blood of roughly 2 hours. In contrast, detection of inorganic arsenic, particularly as represented by the methylated metabolites, is possible for approximately 3 weeks with urine. Organic forms of arsenic are generally eliminated within 48 hours after exposure. This is the basis for many laboratories requesting patients refrain from seafood or other common dietary sources of organic for 72 hours prior to specimen collection.
Choosing the best arsenic test

- Total arsenic
  - Determines if arsenic exposure has recently occurred
  - Adequate to rule-out clinically significant arsenic exposure if arsenic concentration falls below the upper limit of the reference interval
  - Inconclusive if arsenic concentration exceeds the reference interval or biological exposure index (BEI)
  - Best for hair and or nail specimens as incorporation represents inorganic arsenic only
  - May be subject to a common analytical interference from argon chloride

- Total inorganic arsenic
  - Useful for diagnosis of arsenic poisoning and occupational monitoring
  - Eliminates need to refrain from dietary sources prior to specimen collection
  - Fails to determine the which arsenic species(s) is present
  - May be subject to a common analytical interference from argon chloride

- Fractionation or speciation of arsenic
  - Useful for diagnosis of arsenic poisoning and occupational monitoring
  - Eliminates need to refrain from dietary sources prior to specimen collection
  - Determines the proportion and concentration of clinically significant species
  - Useful for evaluating arsenic metabolism, elimination, and decontamination
  - Resolves a common analytical interference from argon chloride

Once the specimen is collected, there are at least three types of arsenic testing available. Most analytical methods are designed to measure total arsenic concentrations. As long as the total concentration of arsenic detected is within normal limits, this test choice is adequate. This test is also adequate for hair and nail testing because only the inorganic forms of arsenic are incorporated into tissues. However, if the total concentration of arsenic in urine is elevated, it is impossible to know whether the elevated concentration is due to non-toxic organic species, toxic inorganic species, or some combination.

To determine which species is responsible for an elevated arsenic concentration, the analytical method used to detect and quantify the arsenic must be designed for measuring inorganic arsenic. Selectively measuring the inorganic arsenic will eliminate the need for dietary restriction prior to specimen collection.

A step above a total inorganic arsenic test is one that can independently identify and quantify clinically relevant species. Such a test will detect fractions or individual species of arsenic present in the specimen, and are therefore referred to as fractionation or speciation tests.

This category of tests will provide the most interpretive information regarding an arsenic exposure, and is particularly useful for determining the proportion of arsenic species involved, as well for evaluating patterns of arsenic metabolism, elimination, and decontamination.
Interpretation of random urine arsenic concentrations

• Reference intervals based on total arsenic concentrations:
  0.7 - 19 µg/L
  10 - 30 µg/L
  2.3 - 31 µg/L
  1.6 - 40 µg/L
  5 - 50 µg/L
  2.3 - 100 µg/L
  0.5 - 197 µg/L

• Reference intervals based on the sum of (inorganic arsenic) + (methylated metabolites)
  Non-occupational population: < 15 µg/L
  Occupationally exposed persons: < 35 µg/L (= end of work week BEI)

NOTE: threshold concentrations associated with signs and symptoms of toxicity range from 25 - 200 µg/L of (inorganic arsenic) + (methylated metabolites)

Once a result has been obtained, how should it be interpreted? Is the result elevated or not? Toxic or not? Defining an elevated and/or toxic concentration of arsenic is dependent on the reference interval used. There are many published reference intervals that vary based on what population was included, whether dietary restrictions that minimize the exposure to organic arsenic were imposed, and what analytical technology was employed.

A widely accepted reference interval is based on the Biological Exposure Index defined by the American Conference of Governmental Industrial Hygienists for occupationally exposed persons. The index, < 35 µg/L, is based on the summed concentrations of inorganic and methylated metabolites of arsenic measured in a random urine specimen collected at the end of a work-week.

Using 35 µg/L as the upper limit of normal is also reasonable considering that this concentration is near the lower limit of concentrations thought to be associated with signs and symptoms of toxicity. The precise threshold for toxicity related to inorganic arsenic exposure is, however, very dependent on the individual patient and whether the exposure is acute or chronic.

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Conclusion

Total arsenic concentrations in urine that exceed 35 µg/L must be fractionated to determine if arsenic exposure is clinically significant.

Because it is impossible to determine if a total urine arsenic concentration greater than 35 µg/L is due to inorganic or organic species of arsenic, it is reasonable to conclude that specimens in which the total urine arsenic concentrations exceeds 35 µg/L should be fractionated or speciated to definitively evaluate the toxic potential of the exposure.
Arsenic testing (urine) at ARUP Laboratories

*effective November 26, 2007*

Tests at ARUP in which arsenic fractionation is automatically performed when total urine arsenic > 35 µg/L

<table>
<thead>
<tr>
<th>Test code #</th>
<th>Test name</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0025000</td>
<td>Arsenic with Reflex to Fractionation</td>
<td></td>
</tr>
<tr>
<td>0099475</td>
<td>Heavy Metals Panel 3</td>
<td>Includes arsenic, mercury, lead</td>
</tr>
<tr>
<td>0020572</td>
<td>Heavy Metals Panel 4</td>
<td>Includes arsenic, mercury, lead, cadmium</td>
</tr>
<tr>
<td>0025055</td>
<td>Heavy Metals Panel 6</td>
<td>Includes arsenic, mercury, lead, cadmium, copper, zinc</td>
</tr>
</tbody>
</table>

NOTE: Arsenic Fractionation (Test code # 0020734) is also available without determination of the total arsenic concentrations

ARUP Laboratories offers several urine-based tests designed to detect and quantify the total concentration of arsenic. With these tests, the specimen is automatically fractionated to determine which species is present, whenever the total arsenic concentration exceeds 35 µg/L. This testing process minimizes the time required
or determining the clinical significance of an elevated concentration, as well as the need to collect a second specimen.

Whenever the total arsenic concentration exceeds 35 µg/L, the total concentration is reported first, and the fractionation results are reported later. Fractionation is performed using HPLC to separate the species, and ICP-MS to quantify the species.

To simplify reporting, the fractionation results are provided based on three fractions: total organic, total inorganic, total methylated metabolites, all in terms of µg/L. Arsenic fractionation is also available separately, without the determination of total arsenic.

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**Strengths of ARUP’s arsenic fractionation test**

- Separates and quantifies three clinically significant arsenic fractions in urine by HPLC-ICP/MS:
  - Inorganic (most toxic)
  - Methylated metabolites of arsenic (moderately toxic)
  - Organic (not considered toxic)
- Eliminates need for dietary restrictions (e.g. no fish or seafood) prior to specimen collection
- Resolves common analytical interference in ICP/MS due to the presence of argon chloride
- Useful tool for studying patterns of arsenic metabolism and renal elimination, and monitoring decontamination

The benefits of arsenic fractionation over total arsenic determinations include better interpretive value of the results, eliminating the need to require dietary restrictions prior to specimen collection for arsenic testing, and the ability to study the patterns of arsenic observed over time, particularly following an exposure to inorganic arsenic.

An additional strength of this HPLC-ICP-MS methodology is that it eliminates argon-chloride that is sometimes generated by conventional ICP-MS and can lead to falsely elevated arsenic concentrations.

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Limitations of ARUP’s arsenic fractionation test

- Not all species of arsenic can be identified; the sum of the fractions may not equal the total arsenic concentration determined separately.
- Although possible that exposures to inorganic arsenic as far as 3 weeks prior to specimen collection will be detected via the methylated metabolites, the test is best applied to detecting arsenic exposures that occurred less than 1 week previous to specimen collection.
- Low-level exposures (i.e., < 10 µg/L of any single arsenic species) are not reliably detected.
- The source of arsenic exposure cannot be determined.
- Specimens preserved with acid are unacceptable due to pH-related effects on chromatography and potential inter-conversion of species.

As with any test, there are also limitations. For the arsenic fractionation test offered at ARUP Laboratories, it is important to realize that all species of arsenic may not be detected. As such, it is possible that the sum of those species detected and quantified will not equal the total arsenic concentration determined separately. In addition, the test may not reliably detect low-level exposures or exposures that occurred more than one week previous. It is important that urine specimens submitted for arsenic testing not be acidified because acid-based preservatives will adversely affect the performance of the analytical method.

We request that urine specimens be collected and transported without any added preservatives.
Recommendations

Indications

- Unexplained clinical symptoms consistent with arsenic poisoning
- Routine exposure screening (i.e., occupational) with interest in arsenic only
- Arsenic exposure known due to accident, intentional ingestion, water contamination, etc.

Order Test 0025000:
**Arsenic with Reflex to Fractionation**

NOTE: Total arsenic concentrations = 2,000 µg/L are usually clinically significant and may not require fractionation to make decisions regarding patient care; clients may cancel automatic fractionation if arsenic concentrations are = 2,000 µg/L

The most common reasons for ordering an arsenic test are based on clinical symptoms consistent with arsenic poisoning that are otherwise unexplained, routine exposure monitoring, and known exposures to arsenic. In these cases, the best test to order is the Arsenic with Reflex to Fractionation test in urine. This test will determine a total arsenic concentration first. If the total arsenic concentration exceeds 35 µg/L, the
result will be reported and the specimen will be reflexed to fractionation, the results of which will be reported shortly thereafter.

If the total concentration of arsenic exceeds 2,000 µg/L, the Client will be contacted to determine whether the specimen should be fractionated or not.

As it is unlikely that a concentration of 2,000 µg/L could be achieved through exposure to dietary organic species of arsenic alone, a total concentration this high will most likely be clinically significant and the added value of fractionation may be minimal.

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**Indications (cont.)**

- Confirmed arsenic exposure based on previous laboratory testing
  - Total arsenic concentration in urine determined previously to be >35 µg/L
  - Interest in specific arsenic fraction(s) regardless of concentration

**Order Test 0020734:**

*Arsenic Fractionation*

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When arsenic exposure is known based on previous laboratory testing, or when there exists interest in specific arsenic fractions regardless of the total concentration, the fractionation test may be ordered directly and in doing so, avoid determination of total urine arsenic concentrations.
Indications (cont.)

• Unexplained clinical symptoms consistent with heavy metals poisoning
• Routine exposure screening (i.e., occupational) relative to arsenic and other metals

Order appropriate Heavy Metals Panel:
0099475 arsenic, mercury, lead
0020572 arsenic, mercury, lead, cadmium
0025055 arsenic, mercury, lead, cadmium, copper, zinc

When the suspected toxicant is not known or when exposure to other clinically significant toxic elements is possible, it may be preferred to order a Heavy Metals Panel. With any of the three Heavy Metals Panels available, the specimen is automatically fractionated when the total urine arsenic concentration exceeds 35 µg/L.
Additional recommendations

- Additional biological testing
  - Blood
    - When urine is not available
    - Recent (within hours) arsenic exposure
  - Hair, nails, or other tissues (not performed by ARUP)
    - Chronic and/or historical (> 3 wks from presentation) arsenic exposure
    - Forensic purposes
    - Death investigations

- Environmental testing (not performed by ARUP)
  - Useful to investigate the source(s) of exposure: water, soil, food, unknown powders, air pollution, etc.

Other testing related to arsenic exposure may be warranted based on the situation. For example, detection and quantification in blood is appropriate when the exposure is very recent, or when urine is not available.

Collection of hair or nails may be most appropriate for evaluating historical exposure to arsenic and to support forensic or death investigations.

Hair and nail testing is not currently available through ARUP Laboratories. To evaluate the source of an arsenic exposure, environmental testing may be useful and is available through specialty environmental laboratories.

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Summary and Conclusions

- Urine is the best specimen for detecting arsenic exposures that occurred within the 3 weeks prior to clinical presentation
- Arsenic fractionation is the best way to determine whether an exposure is related to toxic inorganic or non-toxic organic forms
- ARUP Laboratories offers several testing options wherein urine specimens with total arsenic concentrations greater than 35 µg/L are automatically fractionated

In summary, the best specimen for detection of arsenic exposure in most cases is urine. When results in urine are elevated, it is necessary to identify which arsenic species (toxic or non-toxic) is responsible for the exposure to provide a clinical interpretation and develop a management plan for the patient.

Currently the best way to identify which arsenic species are responsible for an exposure is through fractionation, such as the test offered through ARUP Laboratories for specimens that produce a total urine arsenic concentration greater than 35 µg/L. Thank-you for your attention.

SLIDE: 20 References

- 2007 TLVs and BEIs, a publication of the ACGIH.