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BUREAU OF PUBLIC HEALTH LABORATORIES

The Florida Lab Link

"A Biological and Chemical Preparedness Newsletter"

TOTAL ARSENIC IN URINE: IS IT TOXIC?

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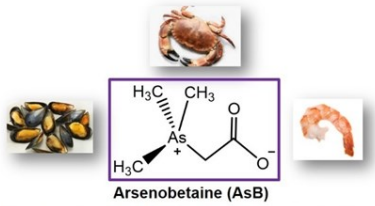
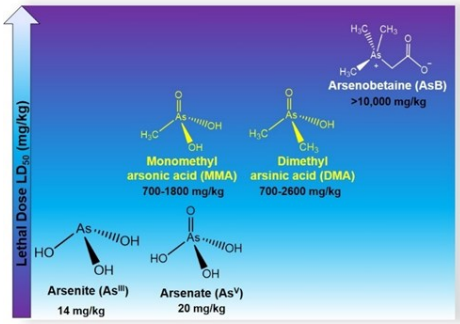
Arsenic is an infamous element that most people consider a toxic poison.[1] Throughout central Florida, arsenic in private well water can exceed the Florida Department of Environmental Protection (EPA) maximum contamination level of 10 µg/L.[2] While drinking water is one source of dietary arsenic, the most common source is through consumption of meat, fish, and poultry which can account for 80% of dietary arsenic intake.[3] The arsenic present in seafood is typically found as the chemical species known as arsenobetaine (AsB), which is generally regarded as 'non-toxic' and rapidly excreted in the urine.[4] The lethal dose in 50% of a population (LD_{50}) of AsB is greater than 10,000 mg/kg.[5] Inorganic arsenic species, arsenite As(III) and arsenate As (V), are much more toxic with LD_{50} of 14 and 20 mg/kg,



Human exposure to AsB occurs predominately through dietary consumption of seafood or shellfish.



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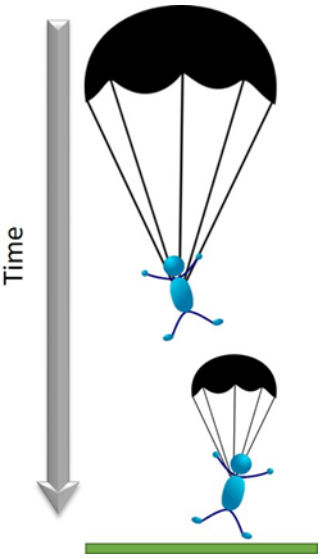
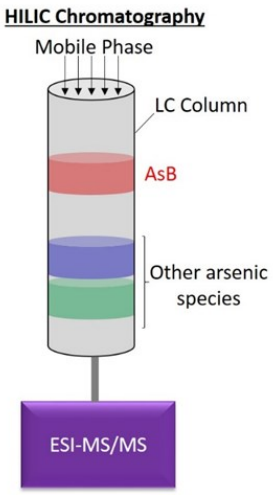
respectively.[6] The human body metabolizes inorganic arsenic species and forms the less toxic species monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA)[7] with LD_{50} of 700-1800 and 700-2600 mg/kg, respectively.[6,8] Consequently, considering the vast differences in toxicity of the arsenic species, quantifying total arsenic in urine does not provide enough information to determine whether the arsenic is toxic.

Traditionally, total arsenic in urine is measured by inductively coupled plasma mass spectrometry (ICP-MS). Liquid chromatography (LC) has been used as a separation technique prior to ICP-MS detection to perform arsenic speciation.[9,10] Molecules of interest interact and adsorb to a solid stationary phase (LC column). Depending on their chemistry, some species adsorb more strongly to the LC column than others, causing the separation, and are then washed off the column using liquid mobile phase(s). Consider a group of skydivers, each with a different size parachute. The skydiver with the smallest parachute will land first, while the skydiver with the largest parachute will land last. Arsenate As(V) interacts with the stationary phase more so like the skydiver with the largest parachute, it exits the column last. AsB is a very polar molecule and does not interact with a non-polar stationary phase so like the skydiver with the smallest parachute, it exits the column first. However, just as it is more difficult to determine the order of landing of several skydivers with small parachutes, it is not ideal for the molecule to speed through the column too quickly because it does not get separated from other molecules.





We can change the chemistry of the stationary and mobile phases to change the order and time in which the molecules exit the LC column. Imagine that the skydivers with bigger parachutes have large holes in their parachutes! They are going to reach the ground much more quickly than the skydivers with small, hole-free parachutes. In terms of the chemistry of arsenic speciation, if the stationary phase is polar, AsB will strongly interact and adsorb to it because it is also very polar. This type of chromatography is called hydrophilic liquid interaction chromatography (HILIC). A non-polar organic solvent is required to remove AsB molecules from the column.[11] However, the analysis of non-polar organic solvents is more challenging using Inductively Coupled Plasma (ICP) ionization than other ionization methods.

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Total Arsenic in Urine: Is it toxic? (continued)

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Electrospray ionization (ESI) is compatible with organic solvents, which uses voltage to place a charge on the mobile phase. Gas is applied to nebulize the mobile phase into small droplets. Heat is applied to form smaller and smaller droplets until a charge is placed on the molecules present. In positive polarity, a proton (H) is added to the molecule (M) to form the positively charged $[M+H]^+$ ion. Mass spectrometry (MS) can be used to separate ions based on their specific mass-to-charge ratio (*m/z*) and reject other ions of different *m/z*. Consider making a cup of coffee. The grounds are placed in a filter, and water is added to make coffee. The coffee is the ion of specific *m/z* that you want to detect, and the grounds are the other ions of different *m/z* that are mass filtered/rejected.

The use of tandem mass spectrometry (MS/MS) improves the selectivity of the analyte. The first mass filter is selected for the parent $[M+H]^+$ ion, which then enters another area where the parent ion can be fragmented or broken up into daughter $[M-A]^+$, $[M-B]^+$ and $[M-C]^+$ ions. These daughter ions then can be mass filtered by a second mass filter. Therefore, the parent → daughter ion *m/z* can be measured, which increases the selectivity of the measurement. For example, the molecular weight of AsB is 178, using positive polarity the $[M+H]^+$ is *m/z* 179 which can be fragmented into $[As(CH_3)_3]^+$ at *m/z* 120; $[As(CH_3)_2]^+$ at *m/z* 105; and $[As(CH_2)_2]^+$ at *m/z* 103. Therefore, the *m/z* 179→120 can be used to quantitate AsB, while the 179→105 and 179→103 can be used as confirmation of the presence of AsB. ICP ionization is less selective, only detects arsenic (As^+, *m/z* 75) ion, and must rely heavily on chromatographic retention time (skydivers with different size parachutes) and resolution (ability to separate different peaks from one another) to determine if AsB is present.

The 'gold standard' for mass spectrometry is isotope dilution internal standardization. The molecule of interest can be synthesized using non-radioactive isotopes (deuterium or carbon-13) to create a mass-shift from the native molecule. This isotopically labeled internal standard (ISTD) behaves chemically and chromatographically similar to the native compound. The internal standard can compensate for matrix (e.g. urine) effects, such as ionization suppression which is common using electrospray ionization.[12] The same amount of isotopically labeled internal standard is spiked into the sample of specified amount. The response ratio (analyte to ISTD) is plotted versus nominal concentration for calibration of a quantitative method.

Currently, we are developing a rapid method for the quantitation of arsenobetaine in urine by isotope dilution HILIC-MS/MS. If we detect elevated urinary arsenic by the complementary ICP-MS technique, the HILIC-MS/MS method can then be used to determine the amount of 'non-toxic' arsenobetaine present. In a biomonitoring study

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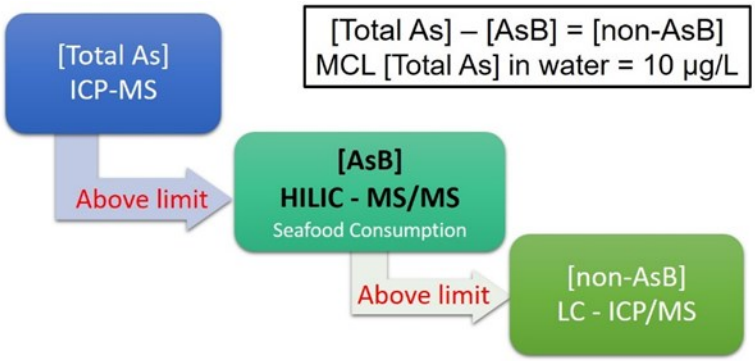
of Hernando county residents in 2012-2013, the Bureau of Public Health Laboratories (BPHL) tested for total arsenic in urine.[13] Even though participants were instructed to avoid seafood consumption, several urine specimens were elevated for total arsenic. Those urine specimens that were determined to be elevated were speciated for arsenic, which was found to be predominately arsenobetaine. By simple subtraction, the non-AsB fraction of total arsenic can be determined: [non-AsB arsenic] = [total arsenic by ICP-MS] – [AsB arsenic by HILIC-MS/MS]. If the non-AsB fraction remains elevated, the more laborious and costly LC-ICP-MS technique(s) should be utilized to determine the arsenic species that are present. The number of urine specimens requiring arsenic speciation by LC-ICP-MS should be a smaller subset as determined from the biomonitoring study. Once the method is validated in our laboratory, we will be able to answer the question: Is the elevated total arsenic due to 'non-toxic' seafood consumption?



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Editor - Betty Wheeler

Mission:
To protect, promote & improve the health of all people in Florida through integrated state, county & community efforts.

Florida HEALTH

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Surgeon General and Secretary

Vision: To be the **Healthiest State** in the Nation

Health Care Provider and Laboratory Notice – June 20, 2017

Detection and Reporting of Emerging Drug Resistant *Candida auris*

The Florida Department of Health (Department) provides the following update to health care providers and laboratories on the detection and reporting of multi-drug resistant *Candida auris*. *C. auris* is an important emerging pathogen that causes invasive infections with high mortality and can be transmitted in health care settings. Initial analysis of *C. auris* infections reported in 2012–2015 from three continents indicates 60% mortality among infected persons. Furthermore, 93% of these isolates were resistant to fluconazole and 41% were resistant to two or more antifungal classes. As of June 12, 2017, CDC has identified 86 cases of *C. auris* infection in eight states. The Department has received a report of one Florida case of *C. auris* infection that was acquired by the patient while receiving health care in another country. There is no evidence of further transmission to date.

Laboratory Identification:

C. auris can be difficult to detect, as conventional biochemical identification has not been reliable. Facilities should suspect *C. auris* if:

- An isolate is identified as *Candida haemulonii*
- An isolate is identified as *Candida* and unable to be further speciated
- An increase in unidentified *Candida* species infections in a patient care unit is identified

Laboratories experiencing an increase in unidentified *Candida* species infections or identification of rare *Candida* species should report the occurrence as described below.

Infection Prevention Measures:

Patients with *C. auris* infection or colonization should be placed in a single patient room on contact precautions. Since *C. auris* can persist on surfaces in health care environments, CDC recommends daily and terminal cleaning and disinfection of rooms of patients with *C. auris* using an Environmental Protection Agency-registered hospital-grade disinfectant effective against *Clostridium difficile* spores.

Reporting:

Any health care provider or laboratorian who suspects or identifies *C. auris* in a patient should notify their county health department epidemiology program or the Bureau of Epidemiology at 850-245-4401 to arrange confirmatory identification of the organism through the state public health laboratory.

Please refer to CDC's recommendations for health care facilities and laboratories for more detailed information, available at: https://www.cdc.gov/fungal/diseases/candidiasis/recommendations.html.

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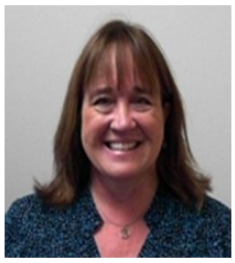
Meet the staff



Lylah Seaton, Biosafety Outreach Officer
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Please welcome our newest team member. Lylah Seaton is one of two Biosafety Outreach Officers for the Florida Department of Health Bureau of Public Health Laboratories. She is coming to us from the Bureau of Public Health Laboratories in Tampa where she was the Lead Arbovirology Scientist responsible for overseeing the serological testing of arboviral diseases such as Dengue, West Nile, and Zika. Originally from Jacksonville, she graduated from Jacksonville University with a degree in Marine Sciences and received her Medical Technology degree from the University of North Carolina Chapel Hill. She then received her Master's in Public Health and Graduate Certificate in Infection Control from the University of South Florida. She is ready to come to your facility and provide you the training and tools to create and foster a culture of safety in your laboratory. Ms. Seaton can be contacted by phone at (904) 791-1569 or by email at Lylah.Seaton@flhealth.gov.

Biosafety risk assessment and lab biosafety training

The Bureau of Public Health Laboratories biosafety outreach officers (BOOs) are currently offering a course in biosafety risk assessment and laboratory biosafety to clinical laboratory institutions. The training consists of two sessions that are approximately one hour each and offered on-site at no charge to the facility. The first session discusses biosafety risk assessment and the second session focuses on biosafety in the clinical laboratory.

Biosafety risk assessment is a systematic process of evaluating the potential risks involved in a laboratory procedure and determining the measures needed to manage any gaps or risks identified. The BOOs have created standard operating procedures and resource documents to assist clinical hospital laboratories in biosafety risk assessment and laboratory biosafety. This session will train clinical laboratory personnel how to use these documents to perform risk assessments in their laboratory.

The second session is for anyone who works in the laboratory or is responsible for a safe working environment. Topics include general laboratory biosafety, the use of biological safety cabinets (BSCs), choosing correct personal protective equipment, proper use and removal of gloves, and spill cleanup. This training awards Florida clinical laboratory and nursing continuing education credits.

For more information or to schedule training, contact Ed Kopp at (813)233-2260 (Edgar.Kopp@flhealth.gov) or Lylah Seaton at (904)791-1569 (lylah.seaton@flhealth.gov).

Chemical Threat (CT) Preparedness Training

The CT laboratory coordinators continue to reach out to the health and medical community by offering training for CT preparedness at hospitals and county health departments (CHDs). This training covers chemical terrorism awareness and the collection of clinical specimens after a chemical terrorism event. Hospital and CHD staff play an important role in the response to a chemical exposure event when clinical specimens are collected for analysis. For your convenience and to increase participation, this training can be presented at your facility. Each course lasts approximately one hour with one 15-minute break between courses. Florida clinical laboratory and nursing continuing education credits will be offered. Training manuals, "hands on" exercise materials, and CT preparedness kits will be provided. This training is recommended for physicians, nurses, epidemiologists, emergency department personnel, phlebotomists, hospital and health department laboratory personnel, and others who may collect clinical specimens. Contact the CT laboratory coordinators in your region for more information (see the Bureau of Public Health Laboratories Directory for contact information).

Laboratory Response Network (LRN) Training—Biological Defense

The Bureau of Public Health Laboratories is currently offering an LRN sentinel laboratory training course at no cost to you at your facility. This training follows the American Society for Microbiology (ASM) Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases. Scheduling the training at your facility is a relatively easy process. Determine when you would like to have the training and how many people will be attending. A time will be set up that is convenient for all. The training materials are provided, as well as the biodefense reference manuals for your laboratory.

The training syllabus includes: 1) an overview of the LRN; 2) the ASM protocols for ruling out potential bioterrorism agents and how to refer a sample to the state LRN Public Health Reference Laboratory when a bioterrorism agent cannot be ruled out; 3) the role of the sentinel laboratory in responding to pandemic influenza; 4) a brief introduction to packaging and shipping of infectious substances; 5) an introduction to the CDC Select Agent Program; and 6) the College of American Pathologists Laboratory Preparedness Exercise (CAP LPX).

This class awards Florida clinical laboratory continuing education credits based on five hours of instruction. Please contact Betty Wheeler at (904) 791-1568 (Betty.Wheeler@FLhealth.gov) to schedule a class for your facility.

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