

## **6** Detection of arsenic in urine by a simple, affordable and effective method

**Manzurul Haque Khan, Sk. Akhtar Ahmad, M.H. Salim Ullah Sayed**

Department of Occupational and Environmental Health  
National Institute of Preventive and Social Medicine  
Mohakhali, Dhaka.

### **Abstract**

Wagtech Digital Arsenator is a kit method for quantitative estimation of arsenic in water. This method was modified for estimation of arsenic in urine and compared the data with the standard methods using hydride generator atomic absorption spectrometry and atomic fluorescence spectrometry. Among the four modified methods proposed, method 2 and 5 (addition of potassium iodide, ascorbic acid and stannous chloride) were more sensitive for the estimation of arsenic in urine. The limits of detection and quantification for arsenic were 3.4-3.9 and 11.4-13.0  $\mu\text{g/l}$  of urine respectively.

### **Introduction**

In Bangladesh, chronic arsenic poisoning has become a major public health problem (Ng and Moore, 2005). Chronic arsenic exposure may lead to irreversible damage to several vital organs; moreover arsenic is established as carcinogen (IPCS, 2001; Chen and Ahsan, 2004). Against this backdrop of the potentially fatal toxicity, cessation of exposure to arsenic and providing arsenic safe water are currently the mainstays of management of arsenicosis patient. Recently, to remove the controversies and confusion in diagnosing arsenicosis cases amongst different working groups/organizations/researchers, a uniform arsenicosis case diagnosis and management protocol has been recommended in Bangladesh through an international conference. This national protocol on arsenicosis case detection and diagnosis has been helpful for proper diagnosis of arsenicosis cases throughout the country in the endeavor to identify the actual extent of the problem.

Levels of arsenic or its metabolites in hair, nail and urine are used as biomarkers of arsenic exposure (IPCS, 2001). According to the arsenicosis case diagnosis criterion it is often helpful to establish exposure by demonstrating an excess level of arsenic in drinking water or one of the above-mentioned biological samples. Arsenic in urine is considered as a biomarker of recent past or concurrent exposure. Amongst urine, nail and hair, urine is the most convenient biological material to collect and process for determination of arsenic content.

The problem for determination of arsenic in water, urine, nail and hair in an appropriate laboratory in Bangladesh still persists. The laboratory estimation of arsenic in water and biological samples is available mainly in some institutions of Dhaka but those are expensive and highly technical. The alternative feasible method for arsenic detection in water is the field test kit. In Bangladesh and India, a number of field test kits are being developed and used to detect arsenic in water at the field level (Pande, 2001; Cherukurii and Anjaneyulu, 2005; Steinmaus et al., 2006). Amongst them Wagtech Digital Arsenator gives the quantitative (digital) reading of arsenic content in water. Recently a number of Upazilla Health Complexes in Bangladesh have been enabled to detect arsenic in drinking water using Wagtech Digital Arsenator which is capable of efficiently measuring arsenic in the range of 2 to 100  $\mu\text{g/l}$ . In some cases it becomes necessary to test the arsenic in urine to evaluate the level of exposure particularly amongst arsenicosis patients who are under the mitigation measures (Ahsan et al., 2000). For that reason, a simple and cheap method to detect arsenic in urine is required for use at primary level of health care services.

Through this endeavor an attempt has been made to develop a simple, affordable and effective method to detect arsenic in urine at Upazilla Health Complex as well as at doorsteps of the arsenicosis patients by using Wagtech Digital Arsenator. Development of an easily adoptable urine sample processing and estimation technique using the methodology of arsenic estimation by Wagtech Digital Arsenator would not only aid in diagnosis of arsenicosis cases in rural settings but would also allow us to monitor the cessation of exposure or confirmation of exposure from sources other than drinking or cooking water. Establishment of such facilities at the Upazilla Health Complex level would not only allow us to render the healthcare services to the grass root level but would also save the patients' time and money, and reduce their suffering.

In view of the above, it would be logical to develop an appropriate, simple, affordable, sustainable and effective methodology for estimation of arsenic in urine using the Wagtech Digital Arsenator.

### **Materials and Methods**

In this study Wagtech Digital Arsenator with multipack; water bath with built in temperature controller and timer; graduated flat bottom flasks (Pyrex), stoppers, tubing, pipettes, graduated measuring cylinders, wash bottles, and sample collection bottles were used.

*Chemicals:* Concentrated hydrochloric acid, nitric acid and sulfuric acid were purchased from E. Merck (Germany). Potassium iodide,  $\text{KMNO}_4$ , ascorbic acid and stannous chloride were obtained from Sigma Chemical Co. (USA). Reagent 1 and 2 were from Wagtech Instrument Ltd, UK.

#### *Determination of arsenic:*

##### Method 1 (Direct method)

In this method, it was proposed that urine sample be used instead of water sample with no other modification in the test procedure.

1. Take 25 ml urine in the reaction vessel of the Wagtech Digital Arsenator.
2. Add 25 ml distilled-deionized water.
3. Prepare the bung of the Wagtech Digital Arsenator.
4. Add 2 sachet of reagent 1 (sulphamic acid) to the sample in the reaction vessel and dissolve the acid sufficiently by swirling the reaction vessel.
5. Add 1 borohydride tablet (reagent 2) to the contents of the reaction vessel and insert the bung onto the mouth of the reaction vessel immediately.
6. Wait for 20 minutes.
7. Remove the bung and detach the black slide from the bung.
8. Insert the black slide onto the arsenator and note the reading.
9. Multiply the reading with dilution factor to obtain the arsenic concentration in the urine sample.

##### Method 2

As method 1 did not involve any process for conversion of arsenic V to arsenic III, it was proposed that an iodide-ascorbic acid pre-reduction step be introduced.

1. Take 25 ml urine in a clean 50 ml volumetric flask.
2. Add 1 ml reductant (potassium iodide-ascorbic acid solution).
3. Add distilled-deionized water to make volume up to 50 ml.
4. Transfer contents of volumetric flask to the reaction vessel of the Wagtech Digital Arsenator.
5. Add 2 sachets of reagent 1 (sulphamic acid) to the sample in the reaction vessel and dissolve the acid sufficiently by swirling the reaction vessel.
6. Allow to stand for 30 minutes.
7. Prepare the bung of the Wagtech Digital Arsenator.

8. Add 1 borohydride tablet (reagent 2) to the contents of the reaction vessel and insert the bung onto the mouth of the reaction vessel immediately.
9. Wait for 20 minutes.
10. Remove the bung and detach the black slide from the bung.
11. Insert the black slide onto the arsenator and note the reading.
12. Multiply the reading with dilution factor to obtain the arsenic concentration in the urine sample.

#### Method 3

Method 2 involved a pre-reduction step introduced for conversion of arsenic V to arsenic III, it was proposed that an iodide-ascorbic acid would be used as reductant in acidic pH.

1. Take 25 ml urine, in a clean 50 ml volumetric flask.
2. Add 1 ml reductant (potassium iodide-ascorbic acid solution).
3. Add 15 ml concentrated hydrochloric acid.
4. Add distilled-deionized water to make volume up to 50 ml.
5. Transfer contents of volumetric flask to the reaction vessel of the Wagtech Digital Arsenator.
6. Allow to stand for 30 minutes.
7. Prepare the bung of the Wagtech Digital Arsenator.
8. Add 1 borohydride tablet (reagent 2) to the contents of the reaction vessel and insert the bung onto the mouth of the reaction vessel immediately.
9. Wait for 20 minutes.
10. Remove the bung and detach the black slide from the bung.
11. Insert the black slide onto the arsenator and note the reading.
12. Multiply the reading with dilution factor to obtain the arsenic concentration in the urine sample.

#### Method 4

This proposed method aimed for estimation of total arsenic estimation (inorganic + organic) excluding arsenobetaine. In this method, sulphamic acid had been replaced by concentrated hydrochloric acid. And it includes a digestion and a pre-reduction step.

1. A 25 ml aliquot of the urine sample was taken and kept in a 100 ml conical flask (with stopper).
2. 15 ml concentrated hydrochloric acid was added and the whole was agitated for 2-3 minutes for intimate mixing.
3. The mixture was heated in a water bath at 60-70° C for an hour.
4. To the mixture 5 ml of 5 mM KMnO<sub>4</sub> solution [for total arsenic estimation (inorganic + organic)] was added.
5. The mixture was again heated in a water bath at 60-70° C for an hour.
6. Then the contents were cooled down to room temperature and 4 ml of 100% aqueous potassium iodide was added.
7. Add deionized water to make volume up to 50 ml.
8. The contents were mixed thoroughly and allowed to stand for 20 minutes for complete reduction.
9. Prepare the bung of the Wagtech Digital Arsenator.
10. Add 1 borohydride tablet (reagent 2) to the contents of the reaction vessel and insert the bung onto the mouth of the reaction vessel immediately.
11. Wait for 20 minutes.

12. Remove the bung and detach the black slide from the bung.
13. Insert the black slide onto the arsenator and note the reading.
14. Multiply the reading with dilution factor to obtain the arsenic concentration in the urine sample.

#### Method 5

Method 5 involved a pre-reduction step introduced for conversion of arsenic V to arsenic III, it was proposed that stannous chloride and potassium iodide would be used as reductant. The method was adopted from the methodology for arsenic estimation in water by NIPSOM kit with modifications.

1. Take 25 ml urine in a clean 50 ml volumetric flask.
2. Add 1.0 mg stannous chloride.
3. Add 1.0 mg potassium iodide.
4. Add distilled-deionized water to make volume up to 50 ml and dissolve the content sufficiently by swirling the reaction vessel.
5. Add 2 sachets of reagent 1 (sulphamic acid) to the sample in the reaction vessel and dissolve the acid sufficiently by swirling the reaction vessel.
6. Transfer contents of volumetric flask to the reaction vessel of the Wagtech Digital Arsenator.
7. Allow to stand for 30 minutes.
8. Prepare the bung of the Wagtech Digital Arsenator.
9. Add 1.5 g zinc powder to the contents of the reaction vessel and insert the bung onto the mouth of the reaction vessel immediately.
10. Wait for 20 minutes.
11. Remove the bung and detach the black slide from the bung.
12. Insert the black slide onto the arsenator and note the reading.
13. Multiply the reading with dilution factor to obtain the arsenic concentration in the urine sample.

#### *Test for compatibility of chemicals used and interference of urine matrix*

##### Distilled-deionized water (Test 1)

A blank sample using distilled-deionized water instead of urine sample was prepared and in accordance to the procedure described above and the arsenic content of the blank was obtained.

##### Urine (Test 2)

A urine sample from a non-smoker individual who had not ingested any sea-food in the preceding 2 weeks and had no history of arsenic exposure through drinking water. It was prepared in accordance to the procedure described above and the arsenic content of the blank was thus obtained.

#### *Dilution factor*

Dilution factor =  $50 \div$  volume of urine sample used

## Results and Discussion

For the purpose of method development initially arsenic estimations for distilled-deionized water (Test 1) or urine (Test 2) were undertaken to see if any of the chemicals used produced interference in the estimation of arsenic. It was expected that for the methods described above, using distilled-deionized water as sample no hydride of arsenic would be produced and therefore the estimated arsenic level would be 0  $\mu\text{g/l}$ .

**Table 1:** Results of arsenic estimation in deionized-distilled water and urine samples by different methods

| <i>Method</i> | <i>Sample</i>  | <i>Observation</i>   | <i>Comment</i>                         |
|---------------|----------------|----------------------|--|
| 1             | Test 1 (Water) | 0 $\mu\text{g/l}$    | No reaction on test paper              |
| 1             | Test 2 (Urine) | 0 $\mu\text{g/l}$    | No reaction on test paper              |
| 2             | Test 1 (Water) | 0 $\mu\text{g/l}$    | No reaction on test paper              |
| 2             | Test 2 (Urine) | 0 $\mu\text{g/l}$    | No reaction on test paper              |
| 3             | Test 1 (Water) | >100 $\mu\text{g/l}$ | Silvery black coloration of test paper |
| 3             | Test 2 (Urine) | >100 $\mu\text{g/l}$ | Silvery black coloration of test paper |
| 4             | Test 1 (Water) | >100 $\mu\text{g/l}$ | Silvery black coloration of test paper |
| 4             | Test 2 (Urine) | >100 $\mu\text{g/l}$ | Silvery black coloration of test paper |
| 5             | Test 1 (Water) | 0 $\mu\text{g/l}$    | No reaction on test paper              |
| 5             | Test 2 (Urine) | 0 $\mu\text{g/l}$    | No reaction on test paper              |

When “Test 1” and “Test 2” were used as expected no color change of test paper was detected for methods 1, 2 and 5 and the digital arsenator reading for both samples in the mentioned methods were 0  $\mu\text{g/l}$ . Whereas in methods 3 and 4 silvery black coloration of test paper were observed and dilution of >100  $\mu\text{g/l}$  digital arsenator reading was obtained (Table 1). Even when 1:10 diluted hydrochloric acid was used in place of concentrated hydrochloric acid similar silvery black coloration of test paper was observed. Moreover, atomic fluorescence spectrometry (AFS) results for “Test 1” and “Test 2 (urine)” revealed that there was no detectable arsenic in samples.

It was apparent from the above observations that methods 3 and 4 in which hydrochloric acid and reagent 2 (borohydride tablet from Wagtech) possibly should not be used together. The initial experiments indicated that arsenic

estimation in urine might be possible through method 1, 2 and 5. Thereafter a urine sample was spiked with known amounts of arsenic (10, 20, 30, 40 and 50 µg/l) and arsenic estimation was carried out by method 1, 2 and 5.

The rates of arsenic recovery at different concentration levels by method 1, 2 and 5 are shown in Table 2.

**Table 2:** Arsenic recovery rates by methods 1, 2 and 5 in spiked urine samples

| <i>Arsenic in spiked urine samples (µg/l)</i> | <i>Amount of arsenic (µg/l)</i> |                 |                 |
|---|---------------------------------|-----------------|-----------------|
|   | <i>Method 1</i>                 | <i>Method 2</i> | <i>Method 5</i> |
| 0   | 0                               | 0               | 0               |
| 10  | 7<br>70%                        | 8<br>80%        | 7<br>70%        |
| 20  | 13<br>65%                       | 16<br>80%       | 16<br>80%       |
| 30  | 21<br>70%                       | 28<br>90%       | 26<br>86.7%     |
| 40  | 28<br>70%                       | 37<br>90.5%     | 36<br>90%       |
| 50  | 35<br>70%                       | 46<br>92%       | 47<br>94%       |

Subsequently arsenic estimation in 20 urine samples was attempted by methods 1, 2 and 5. Each sample was estimated three times by each method (1, 2 and 5). Initially 25 ml of the sample was used and the volume of sample was made up to 50 ml with distilled-deionized water thus having a 2-fold dilution of the test sample. Whenever the test result exceeded 100 µg/l five-fold dilution was used (5 ml urine sample was used in place of 25 ml).

In all the methods some variation of results were observed. In most cases the variation could have arisen because of variable lag time between initiation of hydride generating reactions and insertion of the assembled bung on to the mouth of the reaction vessel. This could be the logical explanation to the variation of results of a sample by a particular method on repeated runs.

Substantial variations between results obtained in methods 1 and 2; and methods 1 and 5 were observed even when the same sample tested. And in some samples the differences were statistically significant (Table 3: samples 1, 3, 5, 6, 9, 10, 11 and 12). Test results between method 2 and method 5 had very little differences and the differences were not statistically significant ( $p > 0.05$ ).

Furthermore it appears that in cases where the requirement of use of increasingly diluted sample, increasing variation of results of a sample would most likely occur. Therefore, it appears that method 2 and method 5 probably could be used for estimation of arsenic in urine as a screening tool for arsenic in urine subject to validation against a laboratory technique.

**Table 3:** Comparison of results of arsenic estimation in urine samples by methods (1, 2 and 5), HG-AAS and AFS

| Sample number | Amount of arsenic in urine ( $\mu\text{g/l}$ ) by various methods |               |                 |        |        |       |
|---------------|---|---------------|-----------------|--------|--------|-------|
|               | Method 1  | Method 2      | Method 5        | HG-AAS | HG-AAS | AFS   |
| 1             | 57 $\pm$ 1.4  | 79 $\pm$ 1.4  | 80 $\pm$ 2.8    | 68.0   | 87.0   | 90.0  |
| 2             | 9 $\pm$ 1.4   | 12 $\pm$ 0    | 13 $\pm$ 1.4    | 14.6   | 13.2   | 16.8  |
| 3             | 55 $\pm$ 1.4  | 69 $\pm$ 1.4  | 67 $\pm$ 1.4    | 69.2   | 70.3   | 73.9  |
| 4             | 19 $\pm$ 1.4  | 22 $\pm$ 2.8  | 23 $\pm$ 1.4    | 36.3   | 9.5    | 33.2  |
| 5             | 184 $\pm$ 5.6   | 285 $\pm$ 0   | 287 $\pm$ 3.5   | 303.3  | 310.0  | 315.8 |
| 6             | 195 $\pm$ 4.2   | 282 $\pm$ 3.5 | 282.5 $\pm$ 3.5 | 337.0  | 330.1  | 306.4 |
| 7             | 48 $\pm$ 2.8  | 59 $\pm$ 1.4  | 61 $\pm$ 1.4    | 33.7   | 71.3   | 72.6  |
| 8             | 48 $\pm$ 2.8  | 60 $\pm$ 2.8  | 60 $\pm$ 1.4    | 29.0   | ND     | 81.2  |
| 9             | 169 $\pm$ 1.4   | 232 $\pm$ 3.5 | 230 $\pm$ 7.0   | 230.0  | 228.1  | 248.0 |
| 10            | 55 $\pm$ 1.4  | 73 $\pm$ 4.2  | 72 $\pm$ 2.8    | 86.3   | 96.6   | 98.4  |
| 11            | 68 $\pm$ 2.8  | 120 $\pm$ 2.8 | 122 $\pm$ 2.8   | 161.0  | 138.0  | 146.0 |
| 12            | 73 $\pm$ 4.2  | 119 $\pm$ 1.4 | 118 $\pm$ 2.8   | 173.0  | ND     | 156.3 |
| 13            | 11 $\pm$ 1.4  | 19 $\pm$ 1.4  | 18 $\pm$ 2.8    | 24.3   | 26     | 21    |
| 14            | 5 $\pm$ 1.4   | 10 $\pm$ 2.8  | 9 $\pm$ 1.4     | 16.4   | ND     | 10.3  |
| 15            | 0   | 0             | 0               | 6.2    | ND     | ND    |
| 16            | 0   | 0             | 0               | ND     | ND     | 2.01  |
| 17            | 11 $\pm$ 1.4  | 16 $\pm$ 2.8  | 15 $\pm$ 1.4    | 5.6    | 4.2    | 7.2   |
| 18            | 0   | 0             | 0               | ND     | ND     | 0.9   |
| 19            | 10 $\pm$ 2.8  | 23 $\pm$ 1.4  | 20 $\pm$ 0.0    | 30.0   | ND     | 25.4  |
| 20            | 0   | 0             | 0               | ND     | ND     | ND    |

Data are mean of consistent pair  $\pm$  SD

Table 3 also shows the results of the arsenic estimation for the urine samples obtained from different laboratories using hydride generation atomic absorption spectrometry (HG-AAS) and AFS. Variation of results was observed between the laboratories. Such variations could have arisen out of the technique employed, conditions of sample storage, time lapse between sample collection and testing, or level of expertise. It is difficult to comment on performance of individual laboratory.

Comparison of results obtained by the different laboratory methods and by methods 2 and 5. Inter-laboratory variability had made the situation difficult in term of drawing highly valid conclusion in favor of the arsenator-based methods (methods 2 and 5). The most striking consistent observation was that the arsenator-based methods (methods 2 and 5) in almost all the instances had provided a lower estimate of the arsenic level in urine sample than the methods by HG-AAS or AFS. This difference could be because in the laboratory based methods the arsine was always generated from the sample in a closed system and therefore there was almost no scope of loss of generated arsine before quantification. On the other hand, variable loss of arsine occurred during the time that lapsed between dropping the reagent 2 (borohydride tablet from Wagtech) into the reaction vessel and insertion of the fully assembled bung into the opening of the reaction vessel. Longer the time lapse higher the loss as was evident from repeated testing of a sample.

The lowest detection limits by method 2 and 5 were 3.9 and 3.4  $\mu\text{g/l}$  respectively. The limits of quantifications were 13.0 and 11.4  $\mu\text{g/l}$  respectively.

## **Conclusion**

Based on the above findings it can be concluded that it is possible to estimate arsenic in urine using digital arsenator with modified methodology (methods 2 and 5). Though the estimates of arsenic content obtained through use of digital arsenator would be lower than that obtained by established laboratory techniques (AAS or AFS), the Wagtech Digital Arsenator with modified methodology could be used at Upazilla level as an initial screening tool for establishing evidence of arsenic exposure.

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## WAGTECH DIGITAL ARSENIATOR

Wagtech Digital Arsenator is a system manufactured by Wagtech International Ltd. Berkshire, UK designed for quantitative estimation of arsenic in water (manufacturer reference: Wag-WT0900, Wagtech Digital Arsenator). The Digital Arsenator is a fully portable arsenic detection system featuring a portable battery-operated digital photometer capable of giving accurate digital readings of the discoloration of the filters as a result of the reaction of the two reagents with the arsenic in water. It gives a direct readout of arsenic in water specifically within the critical range of 2-100 µg/l.

The Digital Arsenator includes the following: two reagents [one in tablet form (reagent 2) and the other in pre-measured individually packed sachets (reagent 1)], a digital photometer, a reaction vessel, a dilution graduated tube, five filter holders for the collection and detection of arsenic, five filter holders (scrubber) for the elimination and removal of any arsine gas, 10 hydrogen sulphide filters, a color comparison chart, waste disposal bags and a spare battery for the photometer. As an accessory for the Digital Arsenator, a multi-pack generator is

available with enough reaction vessels and filter holders to enable five tests to be run simultaneously (manufacturer ref. no. Wag-WT0920, Arsenic Generator Multi-pack).

The system is based on hydride generation technique. The reagent 1 is sulphamic acid (a solid form of sulfuric acid) while the reagent 2 is sodium borohydride tablet. Reagent 1 and reagent 2 when sequentially added to a sample of water containing arsenic a hydride of arsenic, arsine ( $\text{AsH}_3$ ) is formed and it comes off from the solution in gaseous form. The hydride of arsenic on generation is then allowed to pass through an arsenic collection filter (a mercury bromide treated filter) and react with mercury bromide in it. A color change in the arsenic collection filter develops depending on the amount of hydride of arsenic available ( $\text{AsH}_3 + \text{HgBr}_2 > \text{H}_2\text{As} - \text{HgBr} + \text{HBr}$ ). The product has a light yellowish color, further reaction with arsenic leads to brownish products. This developed color is read off as  $\mu\text{g/l}$  (ppb) by the digital photometer of the arsenator.