

Evaluation of an Arsenic Test Kit for Rapid Well Screening in Bangladesh

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ABSTRACT: Exposure to arsenic in groundwater via drinking remains unabated for millions of villagers in Bangladesh. Since a blanket testing campaign using test kits almost a decade ago, millions of new wells have been installed but not tested; thus affordable testing is needed. The performance of the Arsenic Econo-Quick (EQ) kit was evaluated by blindly testing 123 wells in Bangladesh and comparing with laboratory measurements; 65 wells were tested twice. A subset of the same 123 wells was also tested using the Hach EZ kit in the field and the Digital Arsenator in the laboratory in Bangladesh. The EQ kit correctly determined the status of 110 (89%) and 113 (92%) out of 123 wells relative to the WHO guideline (10 $\mu\text{g}/\text{L}$) and the Bangladesh standard (50 $\mu\text{g}/\text{L}$), respectively. Relative to the WHO guideline, all misclassifications were underestimates for wells containing between >10 and 27 $\mu\text{g}/\text{L}$ As. Relative to the Bangladesh As standard, over- and underestimates were evenly distributed. Given its short reaction time of 10 min relative to the Hach EZ and its lower cost compared to the Arsenator, the EQ kit appears to have several advantages for well testing in Bangladesh and elsewhere.



INTRODUCTION

Concerns about elevated arsenic (As) concentrations in Bangladesh groundwater were first raised in the mid-1990s. As of 2009, an estimated 22 million people were still drinking water that does not meet the Bangladesh Arsenic (As) standard of 50 $\mu\text{g}/\text{L}$ and 5.6 million were exposed to As above 200 $\mu\text{g}/\text{L}$.¹ Exposure to elevated levels of inorganic As is associated with cancers of the skin, bladder, and lung,^{2–4} developmental effects in children,^{5,6} cardiovascular disease,^{7,8} and skin lesions.^{9,10}

The most common action taken by villagers in Bangladesh to reduce As exposure over the past decade has been to switch to a neighboring well that is low in As. This was made possible by a combination of (a) blanket testing of close to 5 million wells with test kits, mostly the Hach EZ, throughout the affected regions between 2000 to 2005 and (b) the spatially heterogeneous distribution of As in groundwater at a spatial

scale of a village.¹¹ In Araihasar upazilla (subdistrict), it has been shown that 90% of the residents lived within 100 m of a low-As well even though close to 50% of the wells were high in As within the same area.¹² The installation of deep tubewells is the second most common form of As mitigation in Bangladesh.^{11–14}

In many regions of Bangladesh, it has been more than six years since the previous nationwide water As testing program was conducted under the Bangladesh Arsenic Mitigation and Water Supply Program (BAMWSP).¹⁵ However, the pace of new well installations has not abated markedly and the proportion of untested wells has therefore been growing.¹⁶ A

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Table 1. Comparison of Arsenic Field Test Methods

	Kits Tested in This Study			Kits Tested by Others	
	ITS Econo Quick	Hach EZ	Wagtech Digital Arsenator	Merck Sensitive	Lyophilized Bioreporter Bacteria
volume (mL)	50	50	50	50	1
reagents	tartaric acid amended with small amounts of iron and nickel sulfate, Zn powder, mercuric bromide strip, potassium peroxymonosulfate (optional)	sulfamic acid crystals, Zn powder, mercuric bromide strip	Sulfamic acid, sodium borohydride, Zn powder, mercuric bromide strip	sulfuric acid, Zn powder, mercuric bromide strip	luminescent whole cell living bacterial biosensor
reaction time (minutes)	10–12 ^a	20–40 ^b	20–40 ^c	N/A	4.5 h (150 samples run in parallel) ^f
cost per test (USD)	0.17–0.6	~0.6	~6.6 ^d	~0.50 ^d	N/A
arsenic readings (µg/LAs)	0, 10, 25, 50, 100, 200, 300, 500, 1000	0, 10, 25, 50, 100, 250, 500	continuous	20, 50, 100, 200, 500	continuous
performance ^e	underestimates (%) overestimates (%)	underestimates (%) overestimates (%)	underestimates (%) overestimates (%)	underestimates (%) overestimates (%)	underestimates (%) overestimates (%)
relative to 10 µg/L	11% ^f 0% ^f	3% ^f , 4% ^g , 5% ^h 2% ^f , 1% ^g , 2% ^h	3% ^f , 0% ⁱ , 10% ^j 7% ^f , 9% ⁱ , 7% ^j	1% ^k 1% ^k	0% ^l 8% ^l
relative to 50 µg/L	4% ^f 4% ^f	2% ^f , 4% ^g , 1% ^h 3% ^f , 1% ^g , 6% ^h	4% ^f , 10% ⁱ , 1% ^j 2% ^f , 6% ⁱ , 4% ^j	1% ^k 3% ^k	4% ^l 4% ^l

^aTwelve minutes is necessary if the sulfur interference step is used. ^bTwenty minutes is recommended by the arsenic test kit manufacturer; however Van Geen et al., 2005, demonstrated that a 40 minute reaction period reduces inconsistencies in the 50–100 µg/L range. ^cIf the arsenic concentration is below 80 µg/L, then the reaction period is 20 min, otherwise the sample must be diluted and analyzed again resulting in a reaction time of 40 min. ^dAhammadul Kabir. Rapid Review of Locally Available Arsenic Field Testing Kits. DFID. The estimates for the Wagtech Digital Arsenator include equipment costs. ^eComparison of well status relative to the WHO guideline of 10 µg/L and Bangladesh standard for As in drinking water of 50 µg/L assigned by the four kits. The proportion of underestimates and overestimates is calculated relative to the total number of samples tested. ^fPresent Study. ^gVan Geen et al., 2005. ^hSteinmaus et al., 2008. ⁱSankararakeshnan et al., 2006. ^jSafrazadeh-Amini et al., 2011. ^kJakariya et al., 2007. ^lSiegfried et al., 2012.

survey conducted in Araihaazar in 2005 has shown that more recently installed wells were no more likely to be low in As than older wells.¹¹ The Multiple Cluster Indicator Survey in 2006 has shown that the As status of 38% of wells was unknown in Bangladesh,¹⁷ and this has increased to 44% by 2009.¹⁸ In a survey conducted in Singair upazilla, the same area where the present study was conducted, 80% of the surveyed households reported in 2009–2010 that their wells had not been tested previously.¹⁵ There is a renewed and urgent need to redirect households from high- to low-As wells by testing these newly installed and untested wells, which could number in the millions. An affordable and reliable test kit could also help establish a testing capacity locally available to communities throughout the country to monitor periodically low-As water sources, including deep community wells.

The growing proportion of untested wells, and the exposure of villagers in Bangladesh to As resulting from continuing tubewell installations, motivated this evaluation of a field test kit, the Arsenic Econo-Quick (EQ) introduced by Industrial Test Systems Inc. (<http://www.sensafe.com/>). The new kit appeared promising because the prescribed reaction time of 10 min was short and the cost was low (\$0.17/test for a large-quantity order by UNICEF in Bangladesh; \$0.60/test list price in the US). Several studies have been conducted evaluating the effectiveness of As test kits and yielded widely varying results.^{19,20} Ideally, a test kit for As should be light and compact, be easy to use, require a short reaction time, generate minimum quantities of chemical wastes, and be able to accurately measure As concentrations relative to the World Health Organization (WHO) guideline of 10 $\mu\text{g/L}$ as well as the higher Bangladesh standard for As in drinking water.²⁰

METHODS

Recruitment and Sampling. Village workers were recruited by the Christian Commission for Development Bangladesh (CCDB), a local nongovernmental organization, to sample tubewells and deploy the EQ and Hach EZ Arsenic (EZ) test kits. Their educational level ranged from completion of secondary school certificate to higher secondary school certificate (grades 8–13). A total of 123 untested tubewells were randomly selected for testing (twice for a subset of 65 wells) with field kits in villages of Singair and Shibalaya upazilas within the Manikhanj district of Bangladesh. When tubewells were tested in the field more than once, village workers were blinded to the previous results. Each well was tagged with a numbered metal placard for identification. Groundwater from all wells was collected in 20 mL scintillation vials for laboratory analysis. A subset of 60 wells was also tested using the EZ kit for a reaction time that was extended from 20 to 40 minutes, following the demonstration that this modification reduced the likelihood of classifying a well as meeting the Bangladesh standard for As in drinking water of 50 $\mu\text{g/L}$ when it did not.²¹ In addition, a subset of 92 well water samples were tested in the laboratory with the Digital Arsenator (Wagtech).

Field Measurements. Although the principle of detection is the same for the three kits that were evaluated, the classic 19th century Gutzeit method, the procedures and reagents used differ (Table 1).²² The first reagent of the EQ kit (part no. 481298), added with a scoop to a 50 mL water sample, is tartaric acid amended with small amounts of iron and nickel sulfate, presumably to accelerate the reaction. A second reagent (potassium peroxymonosulfate) provided with the EQ kit to oxidize hydrogen sulfide that could potentially suppress the

signal was not used. Only hydrogen sulfide levels greater than 10^{-6} M interfere with the measurement and such levels can be ruled out by smell for the majority of groundwater pumped from tubewells in Bangladesh. Skipping this step reduces the total reaction time from 12 to 10 minutes. Unlike the EZ kit, the EQ kit includes a temporary cap for shaking the sample to ensure that the tartaric acid dissolves completely before the next reagent, Zn powder, is added with another scoop. The reference chart provided with the EQ kit displays the yellow to brown range of colors expected for As concentrations of 0, 10, 25, 50, 100, 200, 300, 500, and 1000 $\mu\text{g/L}$. Village workers were instructed not to interpolate their readings between categories but instead to select the As concentration on the chart that matched the color of the test strip most closely. In the few cases that the village workers did interpolate, the reading was converted to the closest reference concentration on the strip and, in the even fewer cases when the reported value was exactly midway between two reference concentrations, the reading was converted to the higher value.

The Hach EZ kit (part no. 2822800; current list price in the US of \$0.60/test) was used for the majority of the tubewells tested under BAMWSP. The EZ kit relies on sulfamic acid crystals to acidify a 50 mL sample. A procedure intended to eliminate interference by hydrogen sulfide, in this case cotton impregnated with Pb acetate, was also eliminated in this study. Village workers were instructed to use a 40 min reaction time and reported the results as 0, 10, 25, 50, 100, 250, or 500 $\mu\text{g/L}$ As. Here too, readings were converted to the nearest reference concentration on the strip when interpolated concentrations were reported.

Laboratory Measurements. Groundwater samples collected in 20 mL scintillation vials were acidified to 1% with high-purity Optima HCl at Lamont-Doherty Earth Observatory at least 48 h before analysis. This has been shown to ensure redissolution of any As that could have adsorbed to precipitated Fe oxides.¹⁴ Water samples were then diluted 1:10 in a solution spiked with ⁷³Ge for internal drift correction and analyzed for As by high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS), which eliminates the isobaric interference of ArCl. Further details are provided elsewhere.^{21,23} The detection limit for As is typically <0.2 $\mu\text{g/L}$, estimated here by multiplying the As concentration corresponding to the blank by a factor of 3. The long-term reproducibility determined from consistency standards included with each run averaged 4% (1-sigma) in the 40–500 $\mu\text{g/L}$ range. This is comparable to the previously reported error estimate for single measurements by HR ICP-MS of 4 $\mu\text{g/L}$ augmented by 2% of the measured concentration.²¹

Although it is designed to be deployed in the field, the Digital Arsenator (Wagtech Part No. WAG-WE10500) was used in the laboratory, as is typically the case in Bangladesh. A subset of 92 well water samples tested with at least one of two other kits were collected in plastic 60 mL bottles. Before analysis, the samples were acidified with 0.3–0.5 mL of 1:1 HCl to ensure redissolution of any precipitated Fe oxides. The Arsenator relies on additions of sulfamic acid and sodium borohydride to a 50 mL sample to generate AsH₃ over a 20 min reaction time, but additional steps in the procedure increase total processing time to approximately 40 min if the water arsenic concentration is found to exceed 100 $\mu\text{g/L}$. If quantification above an As concentration of 100 $\mu\text{g/L}$ is desired using the Arsenator, the sample is diluted and reanalyzed. In addition to its significantly higher purchase price (\$1800 for the reading unit and \$1/test

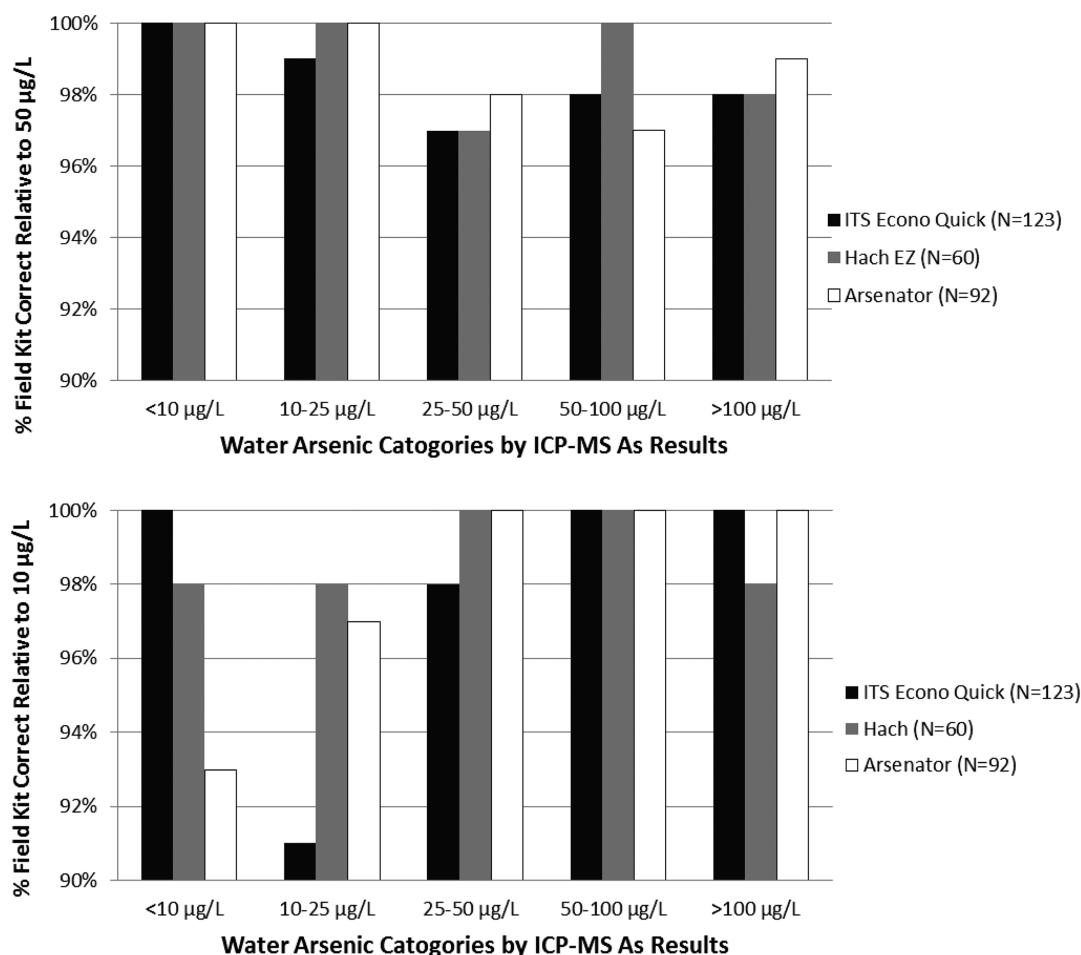


Figure 1. Comparison of ICP-MS and field kit arsenic results relative to 50 µg/L and 10 µg/L.

for reagents), the Arsenator differs from the EQ and EZ kit in that the color of a test strip is measured with a digital reader instead of being estimated visually.

RESULTS AND DISCUSSION

ICP-MS Data. Concentrations of As measured in groundwater from 123 tubewells by HR ICP-MS ranged from 0.2 to 452 µg/L, with a mean of 60 µg/L. The set of samples was roughly evenly split between 51 (41%) tubewells containing 0.2–10 µg/L As (and meeting the WHO guideline for drinking water), 38 (31%) tubewells with 10–50 µg/L As that do not meet the WHO guideline but still meet the Bangladesh standard, and 34 (28%) tubewells with >50 µg/L As. In this analysis, ICP-MS data are used as the reference to compare the performance of the field kits.

Performance of the EQ Kit. Readings in the field using the EQ kit were identical for 47 out of 65 wells that were analyzed twice. For only one out of the 18 remaining duplicates did the readings differ by more than one category (<10 µg/L, 10–25 µg/L, 25–50 µg/L, 50–100 µg/L, >100 µg/L). Relative to the WHO guideline of 10 µg/L, the EQ kit correctly determined the status of 110 (89%) out of 123 wells (Table 1). All 13 misclassifications relative to the WHO guideline were underestimates for wells containing between 10 and 27 µg/L range As, the only category (10–25 µg/L) for which the EQ kit performed less well than the EZ kit and the Arsenator (Figure 1). The EQ kit correctly determined the status of 113 (92%) out of 123 wells relative to the Bangladesh standard of 50 µg/L.

One of the misclassifications was an underestimate for a well containing 193 µg/L according to duplicate EQ kit measurements as well as EZ and Arsenator determinations. This suggests that the sample bottle collected from this well for ICP-MS analysis was most likely mislabeled in the field. Excluding this well, four of the EQ misclassifications were overestimates for wells containing 25–50 µg/L As and the other five were underestimates with three samples in the 50–100 µg/L range and two in the >100 µg/L range. Relative to either 10 µg/L or 50 µg/L, the EQ kit correctly identified the status of 91–100% of well within each of the five categories of As concentrations as measured by ICP-MS (Figure 1).

Performance of the EZ Kit. Relative to 10 µg/L and 50 µg/L, the EZ kit underestimated the As content of only 2 wells (3%) and 1 well (2%) respectively out of a total of 60 that were tested (Table 1). The number of wells for which the As content was overestimated using the EZ kit relative to either threshold was 1 (2%) and 2 (3%), respectively (Table 1). Relative to either 10 µg/L or 50 µg/L, the EZ kit correctly identified 97% to 100% of well waters for all five categories of As concentrations as measured by ICP-MS (Figure 1).

Comparison with Laboratory Measurements. A different way to evaluate the performance of the EQ and EZ kits is to compare concentrations inferred from the visual readings across the entire range of ICP-MS measurements. Although such a comparison is informative, it is less relevant to public health and policy than the binary classification described above. For this comparison, the boundary between each range of As

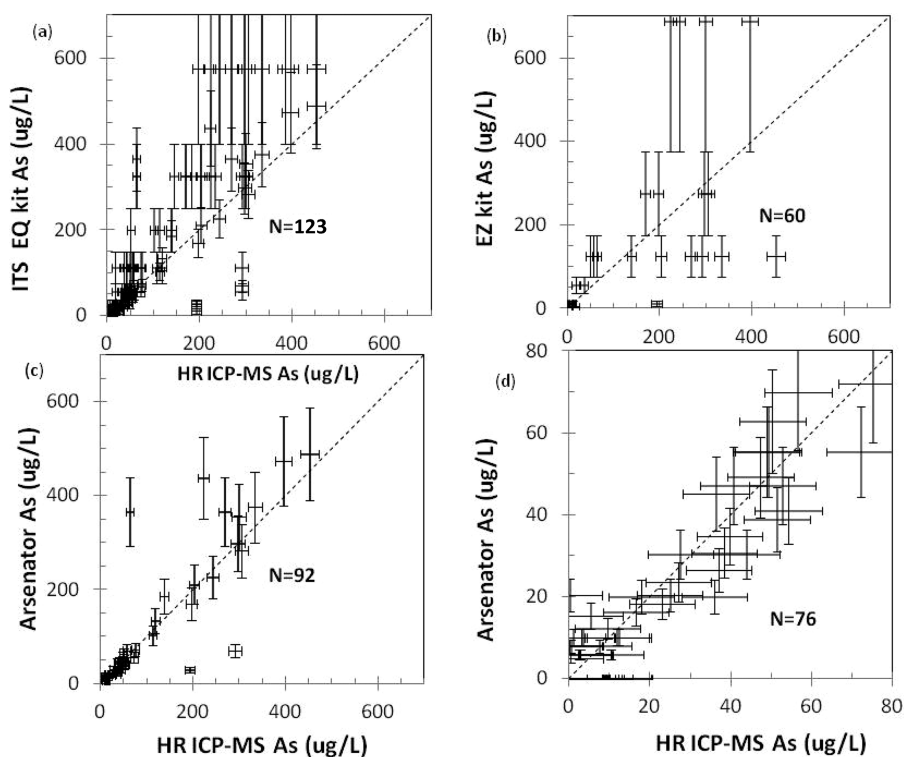


Figure 2. Comparison of As concentrations in water samples measured by inductively coupled plasma mass spectrometry (ICP-MS) compared with the outcome of field and laboratory testing with three different kits. One tubewell with an ITS EQ reading of $1000 \mu\text{g/L}$ and an actual concentration of $395 \mu\text{g/L}$ is excluded from (a) for clarity. The data in (d) are an expanded version of the same data shown in (c). Horizontal bars indicate the estimated 2-sigma errors for HR ICP-MS measurements (22). Vertical error bars in (a) and (b) indicate the full range of As concentrations ranges for the ITS EQ and the EZ kit listed in the text, respectively. Vertical errors bars in (c) and (d) correspond to an estimated error of $\pm 10\%$ of the reported Arsenator readings. The one-to-one relationship indicating a perfect match is shown as a dotted line.

concentrations was set midway between each of the readings illustrated on the two kit's reference charts (Figure 2). The rationale is that the actual As concentration is as likely to be slightly below or as slightly above the reported reading. In the case of the EQ kit, the resulting 8 categories are 0–5, 5–17.5, 17.5–37.5, 37.5–75, 75–150, 150–250, 250–350, and $\geq 500 \mu\text{g/L}$. Even when considering these relatively wide ranges and 2-sigma error estimates for the ICP-MS measurements, the EQ kit consistently overestimates the As content of well water above $150 \mu\text{g/L}$ by about a factor two (Figure 2a). For the EZ kit, the first 4 categories are the same as for the EQ kit and the next 3 are 75–175, 175–375, and $\geq 500 \mu\text{g/L}$. Unlike the EQ kit, discrepancies between EZ kit readings and ICP-MS measurements are not systematically distributed relative to the line corresponding to an exact match (Figure 2b).

Performance of the Arsenator. Correspondence between the Arsenator and ICP-MS measurements (Figure 2c) is improved relative to either of the kits used in the field, including for As concentrations in the 0–80 $\mu\text{g/L}$ range (Figure 2d). The 4 clear outliers, two of which stand out based on EQ and EZ kit readings as well, likely indicate mislabeling in the field, and possibly exchanged labels. The Arsenator was comparable with the EZ and EQ kits with respect to classifying the status of wells relative to the 10 and 50 $\mu\text{g/L}$ thresholds but performs less well (93%) for well water with $< 10 \mu\text{g/L}$ [As] as determined by ICP-MS (Figure 1).

Previous Studies. In Jakariya et al. 2007, an evaluation of the Merck sensitive kit (Table 1) in comparison to laboratory measurements was conducted for 12,532 tubewells in Matlab, Bangladesh.²⁴ The proportion of underestimates and over-

estimates during this survey were low relative to the WHO guideline (1% and 3%, respectively) and Bangladesh arsenic standard (1% and 3%). Two previous evaluations were conducted using Hach EZ test kit and had comparable findings to our present study, the first evaluation was conducted in Bangladesh,²¹ and the second was conducted in the United States in Fallon, Nevada.²⁵ The proportion of underestimates and overestimates relative to the WHO guideline or the Bangladesh standard were all below 6% in both studies (Table 1). The Wagtech digital arsenator has been used in two previous studies in Bangladesh.^{26,27} The proportions reported by Sankararakrishnan et al. were slightly higher for underestimates at 10% (compared to 4% found in the present study) and 6% for overestimates (compared to 2%).²⁶ In contrast, Safarzadeh-Amiri et al reported a higher proportion of underestimates relative to 10 $\mu\text{g/L}$ than found in the present study (10% vs 3%) and a lower proportion of underestimates relative to 50 $\mu\text{g/L}$ (1% vs 4%).²⁷ Using a novel form of lyophilized bioreporter bacteria, Siegfried et al. presented field kit results that were comparable in terms of performance to the three kits evaluated in the present study.²⁸ However, deploying this kit requires considerably more training and the kit seems cumbersome to use in the field.

Practical Implications. Past debates over the usefulness of field kits for testing the As content of tubewell water in Bangladesh and other affected countries have been fraught in part with the notion that it is important to be able to distinguish concentrations around the Bangladesh standard of 50 $\mu\text{g/L}$. There is essentially no known threshold below which As exposure has no deleterious health effects and, without

evidence to the contrary, the impact should be assumed to be proportional to dose. Nevertheless, correct classification relative to either the 10 $\mu\text{g}/\text{L}$ or the 50 $\mu\text{g}/\text{L}$ threshold has implications because test results have been shown to be a key factor determining whether a household switches to a different well.^{29,30} Our results show that all methods evaluated here, the EQ, EZ kits, and the Digital Arsenator, are quite similar with respect to underestimating or overestimating As concentrations relative the Bangladesh standard. The comparison also shows that the Arsenator can provide a relatively inexpensive form of quality control for field kit measurements.

Relying on the EQ does increase the chance relative to the EZ kit and Arsenator that a well whose As content is marginally above the WHO guideline will be considered safe because of its singular tendency to underestimate the 10–27 $\mu\text{g}/\text{L}$ range as <10 $\mu\text{g}/\text{L}$. Further, the EQ slightly increases the chance relative to the EZ kit and the Arsenator that a well whose As content is below the Bangladesh standard for As will be considered unsafe. This could be a serious shortcoming only in those villages where the proportion of unsafe wells is particularly high, as it would reduce the opportunity for switching among private wells. Although overestimation of As concentrations above 150 $\mu\text{g}/\text{L}$ by the EQ kit has no implications for classifying wells relative to the WHO guideline or the Bangladesh standard, recalibration of the reference color strip is recommended. The EQ kit has the advantages of a shorter reaction time and a lower cost relative to the EZ kit and the Arsenator.

Significance. The Bangladesh Arsenic Mitigation Water Supply Project sponsored by the World Bank, UNICEF, and other organizations between 2000 and 2005 was the largest of its kind to test for As in well water in any country. A significant proportion of these tubewells were probably incorrectly classified as safe relative to the Bangladesh standard of 50 $\mu\text{g}/\text{L}$ because the manufacturer's recommended reaction time of 20 min was used.²¹ Considering that a 2009 national survey conducted by UNICEF and the Bangladesh Bureau of Statistics has found that nearly half of the wells in the country were untested,¹⁸ there is an urgent need for expanding the availability of well testing at the village level.

The Ministry of Local Government and Rural Development Cooperatives of Bangladesh, in collaboration with UNICEF and several other developmental agencies, recently piloted a pay-for-use (fee-based) well-testing program for As through the local government in 8 upazillas of Bangladesh. In an evaluation of the program conducted in Meherpur Sadar upazilla, it was found that a majority of households were switching to drinking water sources identified by the pay-for-use testing to be safe with respect to As.^{31,32} The advantage of pay-for-use testing is that it provides a financial incentive for the tester to seek out untested wells. An expansion of this testing program at the national scale is being planned. Another massive blanket testing campaign that is free of charge would likely again reduce As exposure, but would probably also delay the viability of commercial or subsidized testing for several years. The pros and cons of testing-for-a-fee versus free blanket testing need to be carefully weighed.

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Notes

The authors declare no competing financial interest.

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