Water Analysis in the

New technologies coupled with human factors are key to reaching safety goals.

Deborah L. Illman

Worldwide, 1.1 billion people lack access to safe drinking water, and 2.4 billion are without basic sanitation, according to estimates by the United Nations (UN).

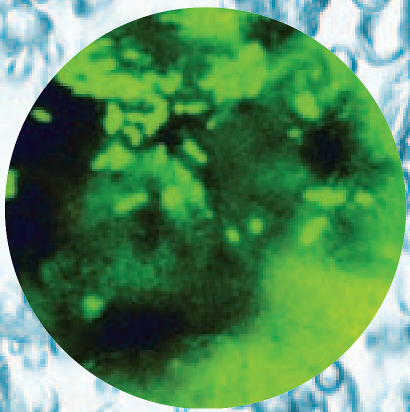
Those are just two of the startling statistics highlighted in *Water: A Shared Responsibility*, a UN report issued this year (*1*; Figure 1 on p 5269). The UN General Assembly has declared an "International Decade for Action", with the goal of halving the figures listed above by the year 2015.

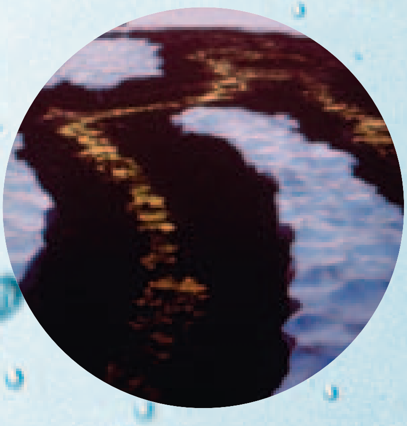
Every day, ~6000 children around the world die from waterborne illnesses. Arsenic in well water compromises the health of millions. Threats from naturally occurring geological sources add to pressures stemming from population growth, industrial development, mining, and agriculture.

All around the globe—developed countries included—we are facing a critical shortage of potable water, explains Mark A. Shannon, director of the WaterCAMPWS, a National Science Foundation Science and Technology Center headquartered at the University of Illinois, Urbana–Champaign. An increasing population means less water is available per person. For human use, we can capture at most one-third of the water that is deposited on our continents, and the surface water is almost fully utilized already. Methods to reuse water are critically needed. Regions are starting to draw more from aquifers—a situation that will be unsustainable unless these supplies are replenished. "The coupling between water, scarcity, and aquifers is a huge and growing problem," he emphasizes. Essential to meeting international goals for safe drinking water are the analytical tools that may help characterize the extent of water-quality problems (box on p 5271) and support water-treatment efforts. Water contaminants such as arsenic, fluoride, and organic pollutants have yet to be fully characterized in many regions of the world. Developing countries may lack the laboratory infrastructure needed to characterize these problems. Furthermore, field-testing methods have been limited in availability, problematic to develop and implement, and, especially in the case of arsenic, plagued with performance problems.

BRANDX PICTURES

Developing World





Recent efforts to improve test kits and to develop innovative new approaches for simple, inexpensive, field-portable devices are an important part of the solution—but not the whole answer. Human factors, such as international partnerships, ultimately help or hinder the efforts to develop new analytical tools to address the world's water needs.

Enemy number one

The primary water-quality problem in the developing world is waterborne disease (Figure 2). The conventional tests for the problem measure indicator bacteria—coliforms and *E. coli*—and require a laboratory facility and trained personnel. One alternative to laboratory methods is Colilert, a simple test kit sold by IDEXX Laboratories, Inc., that uses dry chemicals and test tubes and produces a result within 24 h. It is approved by the U.S. Environmental Protection Agency (EPA) and widely used around the world. But this approach requires incubation, and an incubator might not be available or affordable in the field. Some resourceful souls have found a way to incubate the samples with body heat, according to a newsletter by Robert Metcalf, who is a microbiology professor at California State University, Sacramento, and who has been teaching people in western Kenya how to test and solar-pasteurize water (*2*).

WHO/P. VIROT

Community water.

In many parts of the world, community water sources, like this one near Alem Kitmama (Ethiopia), are the norm.

The need for rapid, simple tests for fecal contamination of water is not confined to developing countries. In coastal areas of the U.S., for example, monitoring beaches for the indicator species *E. coli* and *Enterococcus* by laboratory analysis can take more than a day. During that time, conditions can change, and swimmers can be put at risk. Research coordinated by the Southern California Coastal Water Research Project (SCCWRP) has focused on new rapid tests for these indicator species. A recent evaluation compared quantitative PCR (QPCR), a genetic method that quantifies a DNA target by means of a fluorogenic probe; transcription-mediated amplification (TMA), a genetic method that uses a fluorogenic probe to target RNA rather than DNA; dual-wavelength fluorimetry, which utilizes the same fluorogenic substrates as the IDEXX method but is coupled with an advanced optical detection system to produce results after an incubation period of just a few hours; and an immunological dipstick method. Results indicated that the QPCR and TMA methods appear particularly promising, and additional testing is ongoing, says Stephen Weisberg, executive director of SCCWRP. But even so, these are not necessarily techniques that would be feasible to deploy in developing countries.

Ironically, the effort to avoid pathogens in surface waters in Bangladesh by switching to well water has resulted in a calamity of tragic proportions by exposing residents to arsenic in the groundwater. This situation was exacerbated by the inability of early test kits to accurately determine which wells were safe and which were not.

Progress on arsenic testing

Arsenic finds its way into groundwater by the dissolution of minerals from rocks and from industrial sources. Toxic As(III) and As(V) are formed when buried vegetation decays and causes the reducing conditions that lead to arsenic contamination. These conditions are found worldwide in alluvial sediments, such as those in Bangladesh and West Bengal (India), where as many as 35–50 million people are exposed to unhealthful levels of arsenic. Chronic exposure can lead to vascular diseases; skin lesions; and in the long term, cancers of the bladder, liver, kidney, and skin.

The arsenic problem arose with the decision many years ago to stop using surface sources of drinking water and to begin digging millions of tube wells, so called because of their design (*3*). Although this shift has been accompanied by a decrease in infant mortality from microbial diseases, it has exposed millions to arsenic. Because of the time it takes for cancers from arsenic to show up, the true toll on human health has yet to be fully tallied.

In the late 1990s, international organizations decided to use field kits to test tube wells in Bangladesh and West Bengal. Wells that had arsenic levels >50 µg/L, the guideline value in Bangladesh, were painted red for unsafe; the wells that had values below the threshold were painted green. Those kits were based on the mercuric bromide stain method, or Gutzeit method. In this test, inorganic arsenic is reduced to arsine gas, which reacts with mercuric bromide on a test strip; this strip, which can be compared to a reference color scale, turns from white to yellow or brown if arsenic is present. Some 1.3 million samples from tube wells were analyzed.

But work by Dipankar Chakraborti of Jadavpur University in Calcutta (India) and colleagues called into question the effectiveness and reliability of the early arsenic field kits when their laboratory results showed that a large percentage of wells were colored red or green incorrectly (*4*). For the range of 50.1–100 µg/L, for example, ~47% of the wells were colored green (i.e., safe) on the basis of results from one kit, even though they were determined by laboratory methods to contain arsenic at >50 µg/L. This high false-negative rate has serious implications for public health.

These events have stirred debate over the comparative benefits of field kits versus laboratory testing. Some have argued that the lab approach is preferable because it provides better accuracy and can achieve low cost per sample with high throughput. Others contend that the sheer volume of samples to test, long turnaround time, and transport logistics, combined with a lack of facilities and skilled personnel in developing countries, make the laboratory approach unrealistic and the field kits the only feasible solution (*3*).

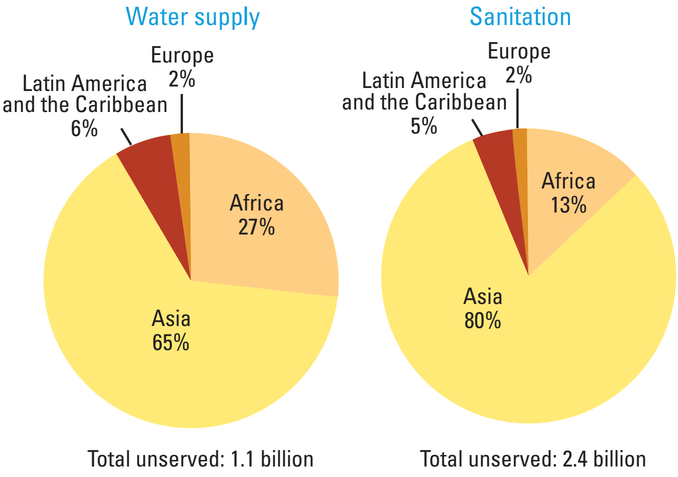


FIGURE 1. Distribution of unserved populations.

1.1 billion people worldwide are without safe drinking water, and 2.4 billion are without basic sanitation. In Latin America and the Caribbean, 66% of the households have access to water that is piped into their homes, and 66% have access to sanitation that is linked to a sewage system. In Asia, the figures are 49% and 18%, respectively, and in Africa, 24% and 13%, respectively. (Adapted from Ref. 11.)

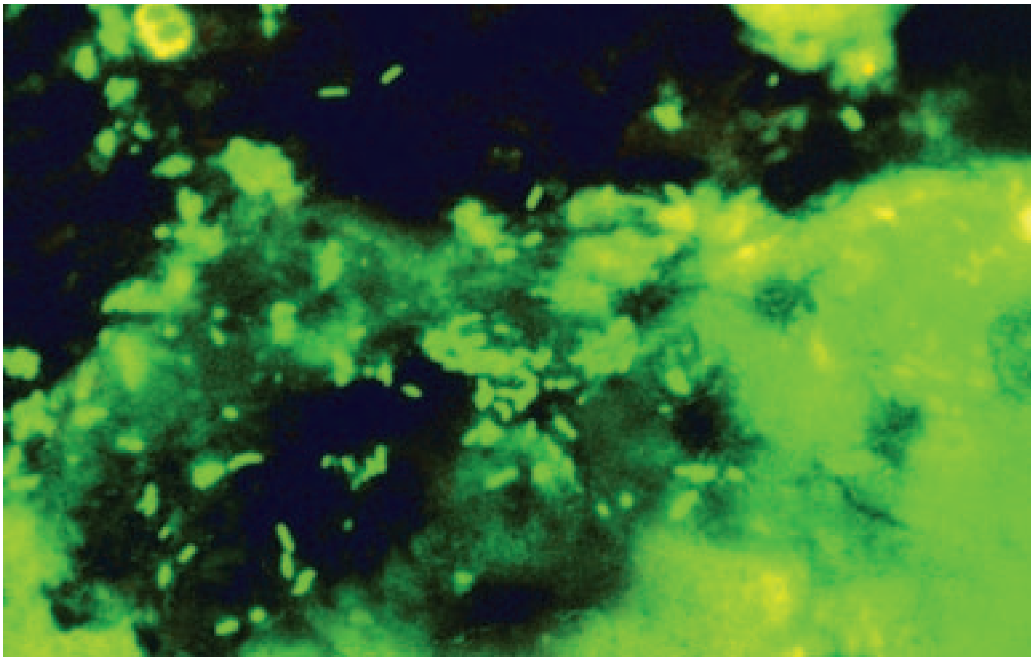
Compounding the issue are several other factors. High spatial variability in arsenic levels—that is, variability among wells that are near each other—means that each site has to be screened individually. And it's not clear to what degree the quality of well water may change over time; repeated testing may be needed. But testing millions of wells once is already a daunting task.

An additional factor supporting the use of field kits arises from the recent development of an inexpensive well-sampling device that permits testing of water quality as a well is being constructed (*5*). This step is advantageous because arsenic concentrations may vary significantly with depth. Use of the device in conjunction with an accurate arsenic field kit could provide real-time feedback that may be used to identify the depth at which the water meets drinking-water standards.

The scope of the analytical challenge grew when the first study of severe arsenic contamination in northern Vietnam was published in 2001 by Michael Berg and colleagues at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) working with scientists at the Centre of Environmental Chemistry at the Hanoi University of Science (Vietnam) (*6*). The researchers estimated that >10 million people in Vietnam had been exposed to unhealthful arsenic levels.

A bacterial biosensor with a lower detection limit of ~4 µg/L, developed by Jan Roelof van der Meer at the University of Lausanne (Switzerland) and colleagues, potentially offered a simple, field-portable, and inexpensive approach that might be applied in Vietnam. Genetically engineered bacteria in the sensors produce a reporter protein that, in the presence of arsenic, can be detected by bioluminescence or colorimetry.

Berg and van der Meer worked with Pham Thi Kim Trang of the Hanoi University of Science and colleagues to conduct a large-scale environmental test of the biosensor on 194 samples



CDC

FIGURE 2. Waterborne disease.

Fluorescence staining reveals *E. coli* in the intestine of an 8-month-old child with chronic diarrhea. In vulnerable people, primarily children <5 years old and the elderly, *E. coli* can cause hemolytic uremic syndrome, which destroys red blood cells and leads to kidney failure. The various types of infectious diarrheas caused 1.96 million deaths worldwide in 2001. Of those, 1.3 million were children <5 years old.

from the Red River and Mekong River Delta regions (*7*). High iron concentrations in these samples required the development of a pretreatment step to remove that interference. Of 38 samples tested in the range of 10–100 µg/L, only 5 samples, or 13%, were found to be false negatives; in other words, these samples were found by the laboratory method to have concentrations >100 µg/L (142–176 µg/L). Two samples were false positives. This performance far surpassed that of the earlier chemical test kits, and the work was selected by *Environmental Science & Technology* as its 2005 environmental technology paper of the year.


CDC/MARK L. ROSENBERG

Sign of trouble.

Even in the developed world, sewage leaks contaminate drinking water and force authorities to close swimming areas. In this 1976 photograph, fluorescent dye reveals sewage spilling into the drinking-water supply at Crater Lake National Park, Ore.

Similarly, Craig M. Steinmaus, Christine M. George, and Allan H. Smith at the University of California, Berkeley, and David A. Kalman, chair of environmental health at the University of Washington, have evaluated the performance of two new arsenic field-testing kits, the Hach EZ and the Quick Arsenic kits (*8*). In this study, both kits were able to identify all water samples that had arsenic concentrations >15 µg/L as being above the WHO guideline value (10 µg/L). That is, no false negatives occurred for samples that had >15 µg/L of arsenic.

With a cutoff of 10 µg/L, the false-positive and false-negative rates for the Quick Arsenic kit were 14.8% and 2.7%, respectively, and those for the Hach EZ kit were 7.4% and 4.6%, respectively. “This compares very well to the 8% false negatives and no false positives that the bioreporter assay measured for 112 samples in the <10-µg/L range in Vietnam,” says van der Meer.

The estimated cost per sample with the chemical field kits in the study by Steinmaus and colleagues was relatively low, ~$1–2/sample. But the researchers believe the most important advantage is convenience. “Traditional laboratory analyses involve collecting and shipping samples to specialized laboratories that may be hundreds or thousands of miles from the water source,” they wrote. “With field test kits, measurements can be completed on-site, and results can be provided to the water consumers almost immediately after sample collection.” The ability to communicate findings and provide timely advice about drinking-water safety is critically important to community-based intervention programs in remote areas, they note.

Compared with chemical test kits, “the advantage of the biosensor is that it’s much faster and cheaper and generates less waste,” says Berg. “You can have a throughput of 200 samples per day.” The cost, he says, is ~2¢/sample. The biosensor has a shelf life of 2 months if stored under refrigeration. (When it is stored at room temperature, the sensitivity decreases.) And there is a risk of cross-sensitivity to other analytes. Field-testing methods in general “cannot compete with laboratory instruments,” says Berg. “The biosensor is most appropriate for larger throughput of samples—rapid screening, ‘yes or no, there is a problem.’”

The important thing, emphasizes van der Meer, is to have a reliable test for arsenic in water samples. “When we started our work on the biosensor, there was a real gap in the sense that most chemical test kits were not sensitive enough and they generated a lot of waste,” he says. “Local people were mimicking the same sort of test . . . with the same problems.” Work is ongoing in his lab to simplify the method by incorporating a colorimetric paper strip, among other improvements. He is also using the biosensor to detect arsenic in rice, which can take up the toxin from contaminated irrigation water. Interestingly, bacteria in the biosensor detect only the inorganic forms As(III) and As(V).

“My initial feeling was that the biosensor would have commercial potential,” says van der Meer. “At one point, we tried very hard to find companies in Europe that might be interested. But the problem is that most companies hesitate, because they say although there is a need, it’s really a nonmarket—these kits should not cost anything at all.” A patent taken out by Eawag on the technology ultimately was sold to the UFZ Centre for Environmental Research in Leipzig (Germany).

Another problem for manufacture in Europe is that the biosensor uses a genetically modified organism. “For laboratory use, it’s not a problem since it is a closed test system. But for production purposes, this falls into a different class of regulations,” says van der Meer. “So instead, we work with local laboratories and show that the test works. From there, they can manage to find ways to develop it; for example, in Vietnam there is a central government lab, and they teach smaller local labs.”

One of the problems with developing a test kit of any kind, says van der Meer, is to make it work in the field. “In Bangladesh, there was no central lab, the basics were lacking, and people had no idea what to do. They would say, ‘You have these nice micropipettes—we don’t have those. So, how shall we pipette 10 µL plus 100 µL?’” The implementation issues are country-specific, he says, in terms of infrastructure, skill level of the local personnel, and sociopolitical factors.

Even when laboratories are well equipped, explains Walter Giger, former head of the chemical pollutants department at

Selected water-quality problems in developing countries (*12*).

Problem	Occurrence
Pathogens	Most significant risk to water quality
Metals (e.g., arsenic)	Associated with certain geological conditions or with agricultural or industrial use
Pesticides	Localized areas of agricultural use; runoff; aerial transport
Algal toxins	An array of neuro-, hepato-, and cytotoxins are produced by a range of cyanobacteria; typically found in water with elevated nutrient levels
Nitrates	Widespread; natural and agricultural sources
Fluoride	Localized areas, depending on geology
Organic compounds	Common sources are industry and transport; includes aromatic and aliphatic hydrocarbons, halogenated compounds, and persistent organic pollutants

Eawag, a continuous struggle is quality assurance and quality control. How precise are the measurements? What about interlaboratory comparisons? "These considerations need to be incorporated from the very beginning," he emphasizes.

ELISAs for pesticides and toxins

In South America, the use of the ELISA as a simple and affordable way to monitor pesticides and cyanobacterial toxins in water may offer some valuable insights on international cooperation in support of water analysis.

A pilot study to measure several pesticides in groundwater in an agricultural region of Uruguay involved a collaboration between researchers at the University of California, Davis (UC Davis) and university and government scientists in Montevideo (*9*). The work was supported by a grant from the Fogarty International Center of the U.S. National Institutes of Health (NIH).

Agriculture and livestock are critical to the Uruguayan economy, and much of the agriculture is done on small family farms in a zone near Montevideo. Use of agricultural chemicals is "essentially unregulated" there, say the researchers, and data were lacking on whether pesticides might be contaminating the drinking water. But they note that the "traditional instrumental technology for pesticide residue analysis is too expensive and too labor-intense to meet the region's needs. Major data gaps on pesticides in the environment exist because of the lack of ability to analyze large sample loads rapidly and cost-effectively."

The pilot study provided a way to match UC Davis's capabilities in the ELISA method with this need, and it afforded an opportunity for science to provide input to public policy in Uruguay on how to optimally invest very limited resources for environmental monitoring, explains coauthor Jerold A. Last, a professor in the department of pulmonary and critical care medicine at UC Davis. He notes that his colleague Bruce Hammock has had a long-term interest in ELISAs for environmental monitoring; Hammock had on hand the first ELISAs that were applied in the study.

A critical factor was education. "We brought the senior scientists from Uruguay up here to learn the assays, and they then went back with materials and started training technicians in the government agencies and students. They started preparing their own reagents and antibodies," says Last.

The 2-year study examined concentrations of 2 triazine herbicides and the carbamate insecticide carbaryl and its major metabolite 1-naphthol. Pesticides were detected at low concentrations or were below detection limits, and in all cases, they were well below the maximum contaminant levels established by EPA. "To our knowledge, this is the first study of its type in Uruguay," wrote the researchers, "and perhaps the first systematic approach to monitoring for organic pesticides in groundwater water sources in the temperate region of South America."

The work is being applied in the rice industry. "The major rice-growing province drains into a major pristine wetland," says Last. "The Uruguayan researchers recently have developed an agreement with one of the agricultural agencies to start looking at potential runoff." Food safety is a growing focus, given the

WHO/P. VIROT

Essential routine.

A girl carries water away from a community source near Alem Kitmama (Ethiopia).

need to certify products for export as free of pesticide residues. “Again, the ELISA techniques are ideal for this.”

WHO/P. VIROT

Promoting sanitation.

A woman stands in a hygienic kitchen in Delhi. Local volunteers spread the word about disinfection of drinking water and good hygiene practices.

Another key factor appears to be a country’s ability to generate its own test kits and materials. Last notes that the university has transferred the technology to a private-sector organization that is manufacturing ELISA kits for use in-country.

A further extension is the use of ELISA techniques to detect cyanobacterial toxins. Sewage and agricultural runoff to the Rio de la Plata, an estuary on which Montevideo sits, can provide the kind of elevated nutrient conditions ideal for incubating cyanobacteria, which produce an array of toxins. Recreational water use there is a major economic activity, and closing the beaches has tremendous financial consequences. “When scums of cyanobacteria are visible, [the authorities will] close the beaches, but otherwise they tend to be reluctant to do so,” says Last. But wind and current make visual detection less than reliable. “Being able to assay for the toxins gives you real-time output rather than something like, ‘Hey, it looks green today.’”

Last says that the single polyclonal antibody used in this ELISA method has cross-reactivity to a family of toxins, a factor that simplifies the assay. And although the single result “may not be directly linear with toxicity, it is certainly an adequate indicator to say, ‘We have a problem here,’” he says. This technique has been used for two summer seasons at four major sites on the river. Researchers measure the bacterial mass and correlate it with the ELISA results. In making the decision to close a beach, “no one is willing yet to depend totally on the assay, but so far the correlations have been excellent,” says Last (*10*).

Future directions

Recent events have brought into sharper focus the need for better analytical technologies for the developing world. But it is not clear to what extent that goal will drive method development in the U.S. and Europe, where technologies for homeland security and rapid laboratory methods to meet environmental or food-safety goals get the most attention. It remains to be seen what dividends, if any, these efforts will pay for developing countries.

However, one example that may show promise for simple, low-cost field tests is the catalytic DNA sensor developed by Yi Lu of the WaterCAMPWS and colleagues.

These sensors use DNAzymes—segments of DNA with enzymatic activities—that can bind analytes of interest. By coupling the DNAzymes with fluorophores or gold nanoparticles, the researchers can use them as sensitive and selective fluorescent or colorimetric sensors for a variety of analytes. A fluorescent sensor for Pb^{2+} was found to have a detection limit of 0.2 ppb, which is below the 15-ppb level for drinking water, say the researchers.

Interestingly, the impetus for simplifying the catalytic DNA sensor as a colorimetric paper test strip arose not to serve the needs of the developing world but to engage secondary-school students. Now, developing a simple, low-cost kit that could be used in the field is “exactly where we are going,” says Lu.

This sensor has become the focus of a start-up company, Dzyme Tech, funded by EPA, NIH, and an Illinois venture-capital firm. The lead sensor would offer better selectivity than conventional kits, which have high rates of false positives, says Lu.

Time will tell whether this particular approach will be successfully commercialized and added to the toolkit of methods to help reach UN drinking-water goals. Those targets may give analytical chemists a chance to tap not only the very best of emerging technologies but even some old-fashioned, seat-of-the-pants know-how as well.

“Chemists from the 1930s, for example, knew a lot about color reactions, about how to do wet-chemical manipulations,” says Kalman. “The more I encounter it, the more appreciative I am of what analytical chemists and qualitative inorganic chemists did back in the 1950s and earlier.” This expertise might be re-harvested and combined with new technologies or substrates, he says, to make it usable for paper strips and test kits. And “some of the old technology may require almost rediscovery.”

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