Rapid Landscape Assessment of Low-Cost Microbiological Drinking Water Tests for Field Use

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Need for novel microbiological water testing approaches

Globally, nearly one billion people lack access to improved water sources and rely on water that is likely contaminated with microbiological and chemical contaminants (World Health Organization [WHO] and the United Nations Children's Fund [UNICEF] 2010). Even with access to an improved water source, there is no guarantee that water is safe to consume. Improved water sources are defined by the WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation by technology rather than by measuring water quality (e.g., levels of microbiological or chemical contamination). Those improved water source technologies include household connections, public standpipes, boreholes, protected dug wells, protected springs, and rainwater collections. One study found that up to 35 percent of improved water sources were not microbiologically safe (WHO/UNICEF JMP 2010).

Individuals in regions that lack safe water, sufficient sanitation facilities, and effective hand-washing resources are more susceptible to suffering from diarrhea-related health complications and death. Diarrheal disease kills an estimated 1.5 million children under age five per year (UNICEF and WHO 2009). Microbiological water testing is an important component for ensuring safe water and reducing negative health impacts associated with diarrheal disease.

The WHO updated guidelines for drinking water quality, which outline a shift to a comprehensive risk-management framework for improved water safety, highlight a growing need for water-testing devices which are appropriate for low-resource settings (WHO 2011). The guidelines provide approaches for both assessing and managing risks that may compromise the safety of drinking water. Those approaches, termed water safety plans (WSPs), encompass all components of the water supply chain—from the catchment of source water to consumer consumption. If implemented correctly, this holistic approach should improve the safety of drinking water. Verification of WSPs (including microbiological testing) is needed to provide a "final check" on the safety of the drinking water supply chain. The recommended frequency of microbiological water testing for verification is based on the type of water supply and the population size that the water supply serves.

Currently available microbiological water tests have a number of limitations within this context. Many of the technologies require access to electricity, refrigeration, and autoclaves that are not readily available in low-resource countries. In addition, specialized laboratory skills are often needed to conduct and interpret water-quality tests. Simple presence/absence (P/A) tests exist, but they do not produce quantitative results, which limits their usefulness.

Project objectives

Recognizing the technology gaps outlined above, the Bill & Melinda Gates Foundation contracted PATH to conduct a landscape assessment of low-cost, low-technology, field-level microbiological water testing technologies. Currently available technologies, technologies that are in development, and new technology ideas that remain to be proven and developed were captured and evaluated. The aim of the work was to identify microbiological drinking water testing technologies with the following specifications:

- Provide quantitative results for thermotolerant coliforms pollution.
- Require no laboratory facility to operate.
- Simple to use and interpret (similar to following a basic cooking recipe).
- Appropriate for use in the field (can be transported by a motorbike or bicycle).
- Low cost.

Methodology

The project was conducted from September through December 2011. Initial efforts focused on evaluating commercially available technologies based on a set of criteria (see Appendix A) provided by the Bill &

Melinda Gates Foundation to better understand the current microbiological water testing market. Primary and secondary research was utilized to identify currently available water testing technologies and capture relevant information. Note that technologies that only use H_2S-producing bacteria as an indicator organism were excluded from the analysis because they do not produce quantitative results and do not use an indicator that is exclusively thermotolerant.

Technologies in development and new ideas that remain to be proven or developed were identified through primary market research conducted primarily via telephone with researchers and industry experts. In addition, representatives from PATH attended and collected information on potential novel water testing approaches at the 2011 University of North Carolina (UNC) Water and Health Conference and the 2011 Water Quality Technology Conference organized by the American Water Works Association. Individuals that are actively developing new technologies or have new technology ideas were identified and invited to participate in the microbiological water testing landscape to share product knowledge. Discussions were focused on evaluating the technologies in development or technology ideas based on a set of criteria (see Appendix A) provided by the Bill & Melinda Gates Foundation. Summary information for technologies in development and new ideas based on the set of criteria provided by the Bill & Melinda Gates Foundation are captured in Appendix B (available upon request).

Primary market research was also conducted with key purchasers or influencers (WHO, UNICEF, and Oxfam) to understand what they would value in a new microbiological water test for use in low-resource countries, gaps in the marketplace, and to gain insight on promising new water testing technologies. In total, PATH talked to 27 people from 16 organizations.

Project limitations

Limitations are inherent in the scope and timeline of the project. The information gathered for this report is not comprehensive and was dependent upon the willingness of developers, manufacturers, and potential purchasers to engage. The interviews were conducted on a nonconfidential basis and thus sensitive information may have been withheld. While the research did not reveal new technology developments at large manufacturing companies, due to the proprietary nature of their development work, it is likely that these types of companies would keep information about new developments confidential prior to launch of a new technology. In addition, much of the technology information reflects information provided by the developer and may be prone to bias. PATH aimed to control for this by talking with multiple sources and utilizing secondary research. It was not within the scope of this project to conduct business and technical due diligence. The findings from this report should be augmented with these additional evaluations before significant investments are considered.

Currently available water testing technologies

Twelve manufacturers were identified that currently make 35 distinct microbiological water testing technologies. The current manufacturers were identified through web-based searches and other secondary data sources.[1] Additionally, the current list of manufacturers was vetted with select industry experts to ensure that key players were represented. These manufacturers are based in the United States, Canada, or Europe. Their technologies test for one or more of the following indicator organisms: total coliforms, thermotolerant coliforms and *Escherichia coli (E. coli)*.[2] In addition, the technologies utilize either P/A, most probable number (MPN), or a direct count of colonies methods for detecting and/or quantifying

[1] Including market research conducted under the Aquatest Research Program.
[2] Exception: Charm Sciences' Fast Phage product tests for viruses that infect *E. coli* (somatic coliphage).

microbiological contamination. All currently available technologies also required 18-hour or longer incubation times.[3] The price per test excluding capital equipment was found to be between US$4 and $7.[4]

Ten of the currently available technologies were confirmed to be approved by the Environmental Protection Agency (EPA) for drinking water, and six of those ten are manufactured by Idexx suggesting significant EPA regulatory hurdles.[5] Some of the manufacturers mentioned that they do not seek EPA approval because it is not needed in the markets in which they operate. Additionally, many membrane filtration manufacturers mentioned that EPA approval is not needed for their technologies because the membrane filtration method is approved.

Only two manufacturers were identified that currently market technologies that meet the desired specifications outlined by the Bill & Melinda Gates Foundation for this landscape assessment. Charm Sciences' ColiGel technology utilizes a bag that contains a gelling agent and selective medium to detect total coliforms and *E. coli*. The water sample can be directly added to the bag and incubated at 35°C for 28 hours. After the incubation period, colonies that grow in the gel can be counted to determine the level of contamination. An ultraviolet (UV) light is needed to detect *E. coli* contamination. Charm Sciences has another technology (PathoGel) that is identical to ColiGel except it also contains a H_2S P/A indicator. An assumption for this exercise is that portable incubators are available for testing in the field.

Bluewater Biosciences' Coliplate technology also met the desired specifications. For this test, the water sample can be poured directly into a 96-well microplate to detect total coliforms and *E. coli.* The sample is then incubated at 35°C for 24 hours. The wells that change color are counted to determine the MPN for total coliform and the wells that fluoresce under UV light are counted to determine the MPN for *E. coli*.

While the technologies outlined above meet the desired specifications, there are still limitations. As mentioned previously, both tests require incubation. In addition, neither technology is EPA approved for drinking water. Discussions with Charm Sciences revealed that the upper limit of quantified results for *E. coli* may be lower than membrane filtration methods. The key limitation for Bluewater Biosciences' Coliplate technology is that it does not test 100 mL of water, which is the required sample size to meet WHO guidelines. These guidelines state that *E. coli* and thermotolerant coliforms "must not be detectable in any 100 mL sample" (WHO 2008).

Technologies in development and technology ideas

Ten different potential new microbiological water testing technologies have been identified during this project. All of these technologies are intended for field use and could test for thermotolerant coliforms in water. Many of these technologies include novel components or approaches that could be adapted for other functions, such as the ability to detect a range of indicator organisms—thus providing platforms for further innovation or development. In addition, the research revealed that diverse technology approaches (e.g., molecular amplification methods, antibody detection, novel devices for chemistry reactions, and spectrum technologies) are being pursued. These efforts are at many different stages of product development. For the purposes of the landscape assessment, the stages have been simplified. Each technology was classified into one of the following development stages: idea, proof of concept, prototype, validation, or adaption of a currently available technology.

New technology ideas included in the landscape assessment were considered to be novel approaches for microbiological water treatment that have not yet been proven or developed. If funding were made available, the respondents to the market research would be interested in participating in future

[3] Exception: Charm Sciences' Fast Phage product has a six-hour incubation time.
[4] This excludes membrane filtration kits. Consumables for these kits are often lower but capital costs are high.
[5] No EPA regulation information was identified for many technologies.

development activities. Organizations with technologies classified as being in the proof-of-concept stage have developed a rough prototype that is under investigation for feasibility. No technologies in the fully functional prototype development stage were identified. The technologies that were classified in the validation stage had been tested in field and/or laboratory studies. These technologies may still need additional design revisions and validation studies. In addition, two research groups were working to adapt a currently available technology to provide additional applications.

Discussions with industry experts and researchers revealed that with new water testing technologies and ideas, they are attempting to improve on one or more of the identified technology gaps described on page 1. These intended improvements include the following: time to result, suitability for the field, ease of use, and reduced cost. All of these technologies and ideas could potentially test for thermotolerant coliforms. For technologies that are in earlier stages of development, it is important to note that the technical challenges are less well understood, and the risk of not meeting target technology specifications is much higher.

As a reference point, improvements of new water testing technologies and ideas are evaluated against currently available membrane filtration field kits (see table below). Using these kits, it takes approximately 24 hours to obtain test results for *E. coli* and total coliform enumeration. As a result, any new water testing technology or idea that can provide test result in 18 hours or less is considered an improvement. Membrane filtration field kits are designed for field use but require components including incubators, battery chargers, and UV lamps. These components contribute to the weight of the kits which are often 10 kg (22 lbs) or more. In addition, to run the tests, the users must regularly purchase consumables such as membrane filtration pads, culture media, and petri dishes. Often these consumables are packaged in a replacement kit. Consumables needed, capital equipment, transportability and electricity requirements were all considered when comparing suitability for field use.

Membrane filtration kits also require some laboratory training to perform the tests and are subject to intertechnician variability. Any new water testing technology or idea that only requires skills similar to those required for basic kitchen processes is considered an improvement on ease of use. For these tests only, gross measurements are required and interpretation should be relatively simple (e.g., assess a color change). Lastly, an improvement in cost is defined to be any reduction over the cost of currently available tests (approximately US$5 per test). The price per test for membrane filtration kits will depend on the number of tests performed as the capital costs of the durable components are amortized over the number of uses. The $5 per test estimate assumes that the capital equipment for a technology is used for approximately 1000 tests.[6]

[6] Assumes the price for capital equipment is approximately $4000 (based on current product landscape findings) and the price per test for consumables is $1.

Technologies in development and technology ideas: Gap-filling potential

(Table is organized in declining order of technology readiness)

Name	Technology Approach	Stage of Development	Time to Result	Suitability for Field	Ease of Use	Lower Cost
Membrane Filtration Field Kits* (Reference Point)	**Quantitative direct count**	**Available technology**	**24 hours**	**Multiple components are needed and they are heavy**	**Requires some laboratory training**	**$5 per test estimate**
Idexx Colilert 18 + ATP[α] meter	Measure ATP luminescence using Idexx 18 reagents	Existing product adaptation	X 3 to 4 hours	X	X	
Idexx Colilert 10-mL tubes + 3M Petrifilm	Using two available tests to get a MPN result	Existing product adaptation		X		
Aquatest*	MPN in a self-contained device	Validation[ǂ]		X	(X)[#]	
Compartmentalized Bag Technology*	MPN in a bag	Validation		X	X	X
Photonic Biosystem Coliform Analyzer*	Measuring UV light growth using an instrument	Validation	X 8 hours (less for highly contaminated water)	X		
DelAgua Quicktest*	Antibody coupled to beads with instrumented photographic detection	Proof of concept	X 20 to 40 minutes	X	X	
Plastic Gel Bag or Absorbent Pad Bag*	Colony count in a bag	Proof of concept		X	X	X
Water Canary	UV spectrum analysis	Proof of concept	X seconds	X	X	Limited information available
Antibody on Paper	Antibody on paper that measures light intensity using a cell phone	Idea	X 30 seconds to a few minutes	X	X	Limited information available
Molecular approaches (PCR/LAMP)‡	Molecular	Idea	X 1 to 4 hours			Limited information available

Definitions:

Time to result: faster than current technologies on the market (18 hours).
Suitability for field: improvements on number of consumables, capital equipment, transportability, reliance on electricity, etc.
Ease of use: requires basic cooking type skills and easy to interpret.
Reduced cost: costs targeted to be lower cost than the currently available technologies ($5 estimate).

[α] Adenosine Triphosphate (ATP).
* Technology can test 100 mL.
ǂ Aquatest is currently in pilot production and has created low-volume production capacity.
Aquatest is thought to be generally easy to use, but interpretation may require more sophisticated skills than tests with fewer chambers. Aquatest has achieved proof of concept for a cell phone-based automatic reader/interpreter.
‡ Polymerase Chain Reaction (PCR) or Loop-Mediated Isothermal Amplification (LAMP).

The information provided reflects information gathered during market research as well as the opinion of the authors. Every effort was made to ensure accuracy of the information. Complete business and technical due diligence were outside the scope of this project.

All of the microbiological water testing technologies and ideas listed above have the potential to obtain a quantitative result for thermotolerant coliforms. However, tradeoffs were identified between ease of use and the levels of sensitivity, specificity, and precision. For example, the Water Canary device was identified as having the most potential for ease of use. This development effort is targeted at creating a push-button technology that automatically sends global positioning system-tagged data to a centralized location within seconds. However, this technology may be developed to detect only broad microbiological contamination and not a specific indicator organism. In addition, the current prototype tests approximately 10 mL so it does not meet the WHO-100 mL recommendation for water testing. The developers acknowledge that the technology will likely lack the sensitivity, specificity, and precision of currently available technologies. The idea for this device is to provide a warning of poor water quality, similar to a canary in a coal mine; follow-up microbiological testing may be needed for some samples to confirm results.

Similar tradeoffs were observed for the two MPN technologies. For example, Aquatest has 11 chambers (one large and ten small) while the Compartmentalized Bag Technology (CBT) has only five chambers. The higher number of chambers makes Aquatest more precise than the CBT. In addition, Aquatest has a higher range (1 to 210 colonies in 100 mL) than the CBT (1 to 100 colonies in 100 mL). Both technologies are capable of testing 100 mL of water.

The tradeoff for the higher precision of Aquatest is a higher level of sophistication required for interpretation of the results. Early adopter studies currently being conducted by the Aquatest program will provide valuable feedback on ease of use for the device. The CBT has been used by trained, though not highly educated, Demographic Health Survey (DHS) workers in a study in Peru. In this study, three 100 mL water samples were collected from the same water source. One test was performed by a DHS worker in the field using the CBT, the second sample was sent to a reference laboratory that also used the CBT, and the third sample was sent to a reference lab for membrane filtration. No significant differences in microbiological contamination were observed among the three methods. In addition, when users were asked to rank the technology on ease of use on a scale of one (easy) to ten (challenging), the average score was a three.

Conclusions

The microbiological water testing landscape assessment revealed that significant interest exists among technology developers in creating technologies that address the gaps in the market. Requests for participation in the primary research for the landscape assessment were met with a high level of interest and engagement. Technology developers overall are willing to be further engaged with the Bill & Melinda Gates Foundation as needed. Interestingly, potential improvements in cost were only identified for two bag technologies being developed at UNC. Many of the technologies are attempting to reduce the requirement for equipment and consumables, making them more suitable for field use. In addition, a high proportion of development efforts are focused on reducing the time to result from the 18 hours which is the lowest of the commercially available products (total coliforms and *E. coli*). Lastly, significant tradeoffs in ease of use and technology sensitivity, specificity, and precision were observed.

Many of the landscape analysis participants mentioned that any one technology will not suit the needs of all end-users. Technologies that are intended for use in the community to elicit behavior change may be different than technologies intended for use by individuals performing microbiological water surveillance. More specifically, many respondents mentioned the importance of creating technologies with very simple interpretation for community use. In addition, a technology used for disaster relief may benefit from different product specifications. In these circumstances, the ability of a technology to automatically send data to a centralized location may be valuable.

Recommendations

The following areas may benefit from additional resources:

Technology due diligence: Independent third-party testing or other evaluation may help further differentiate technologies. This is particularly important for technologies that are in earlier stages of development with little data to support stated target specifications.

Charm Sciences' gel bag: Charm Sciences' gel bag was the only currently available technology identified that met all of the desired specifications and also met the WHO guidelines for testing 100 mL. Further testing and evaluation of this technology could lead to immediate access to a currently viable solution.

Association between microbiological water testing and health: Some respondents mentioned a need to show an association between specific microbiological water testing technologies and health outcomes. WHO water safety plans are still evolving, and a new quantitative risk-assessment framework was recently published by the WHO Network for Household Water and Safe Storage (WHO 2011). Expert respondents indicated these efforts are likely to advance slowly towards acceptance of a specific relationship of water contamination levels to health risk. Providing practical guidelines for manufacturers on how microbiological contamination relates to health could help guide future development efforts. More specifically, this information could help inform difficult tradeoffs between technology sensitivity, specificity, and precision, and ease of use.

New applications: Respondents hypothesized about trends for new water test applications. Mentioned uses included monitoring and evaluation of water and sanitation interventions, DHS, enhanced JMP data collection or tools for safe water entrepreneurs such as water kiosks. However, these new applications do not represent significant sales, and respondents' opinions varied as to the timing and scale of these new applications.

Market barriers: Idexx is the market leader for microbiological water testing technologies. One respondent with regulatory expertise said that Idexx's cost of production is less than its competitors because of the high volume of technologies that they manufacture. It is difficult to invest in infrastructure to scale-up production unless high volumes can be justified. In addition, the shelf life for many microbiological water testing technologies is one to two years. Therefore, technologies must get purchased and used rapidly after they are manufactured.

Many of the technology developers said that finding partners is challenging. To convince partners to invest, they need to be able to produce high volumes to reduce costs and sell the technologies quickly. This is especially challenging when working in low-resource markets because demand is less well understood and distribution systems are more complex. Several developers said that to form partnerships they desired additional commercialization assistance including identifying market segments, forecasting demand, and evaluating distribution networks.

Interesting late-stage technologies: The microbiological technology landscape revealed a number of interesting early- and late-stage technologies in development. As mentioned previously, it was difficult to differentiate technologies that are in early-stage development due to the lack of available data. Three technologies were identified that have undergone some level of validation studies. Descriptions for each of these late-stage technologies are listed below to showcase unique features that could address gaps in the marketplace.

- **Aquatest:** Development of the Aquatest system was funded by the European Union and the Bill & Melinda Gates Foundation. This test was designed for field use where laboratory testing may be costly, inefficient, or impossible. A single self-contained device is used for sample collection, mixing,

incubation, results, and finally, disinfection. The system also includes an incubator that uses boiling water as its heat source and a specially designed UV reader. The device is currently in low-volume pilot production and over 10,000 devices have been given to early adopters for use and evaluation in over 17 countries. Many outputs of the Aquatest project such as market studies, national regulatory profiles, and methods documents will be made widely available. Aquatest is currently seeking commercialization partners for manufacturing scale-up and distribution.

- **Compartmentalized Bag Technology:** The CBT technology is unique in that it is relatively simple to use and has a low environmental footprint. In addition, the technology could achieve lower costs than currently available microbiological water testing technologies. However, it may be difficult to measure accurate volumes in bag technologies. A technology with a rigid structure will have more accurate measurements and MPN results. Feedback from discussions with respondents indicated that EPA approval for bag MPN technologies may be challenging because of the flexible nature of the bag. It may be possible to address this limitation by adapting the bag to a reusable rigid carrier frame or including a measurement step in the method that utilizes a rigid disposable or disinfectable collection vessel.
- **Photonic Biosystems:** Photonic Biosystems predicts that its Coliform Analyzer will be available in 2012, making it the most advanced in terms of development of all the technologies in the landscape assessment. The technology has made improvements over the standard membrane filtration equipment and has reduced the time to result to eight hours with a novel optical sensor instrument. The company mentioned the instrument can detect "high levels of contamination" in between 30 and 60 minutes. It is important to note that the instrument to detect contamination is currently estimated to cost $5,000. However, it is also possible to perform the test without the instrument and count colonies using the Photonic Biosystem membrane filtration components. These membrane filtration components are claimed to be easier to use and less prone to contamination than traditional membrane filtration. The company is interested in pursuing low-resource markets because of the potential advantages that their technology has over currently available field membrane filtration kits.

  Photonic Systems has received substantial military funding for development, currently has a conditional military purchase contract, and is actively seeking commercial partners. This dynamic development is worth tracking as it has the potential to change the water-test landscape significantly.

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Appendix A: Assessment criteria

For all technologies, the following information was captured:

1. Contact information for manufacturer and/or developing laboratory.
2. Description of technology approach.
3. Technology Readiness Level.
4. Indicator organism(s) targeted (e.g., *Escherichia coli*, total coliforms, and others).
5. Required operator skill.
6. Time to result.
7. Sensitivity per 100 mL of water.
8. Supplies required for preparation and field operation.
9. Weight and size.
10. Number of samples that can be processed/incubated in a single run.
11. Capital costs (target costs for new technologies).
12. Recurrent costs (target costs for new technologies).
13. Shelf life of reagents.
14. Advantages and limitations of the underlying approach.

For technologies currently still in the idea or the development stage, information captured also included:

1. Time to market.
2. Anticipated manufacturing arrangements.
3. Intellectual property considerations.

An excel database was generated to capture the information for each of the criteria listed above.