

## ARTICLE OPEN



# Novel methods for global water safety monitoring: comparative analysis of low-cost, field-ready *E. coli* assays

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Current microbiological water safety testing methods are not feasible in many settings because of laboratory, cost, and other constraints, particularly in low-income countries where water quality monitoring is most needed to protect public health. We evaluated two promising *E. coli* methods that may have potential in at-scale global water quality monitoring: a modified membrane filtration test followed by incubation on pre-prepared plates with dehydrated culture medium (CompactDry™), and 10 and 100 ml presence–absence tests using the open-source Aquatest medium (AT). We compared results to membrane filtration followed by incubation on MI agar as the standard test. We tested 315 samples in triplicate of drinking water in Bangalore, India, where *E. coli* counts by the standard method ranged from non-detect in 100 ml samples to TNTC (>200). Results suggest high sensitivity and specificity for *E. coli* detection of candidate tests compared with the standard method: sensitivity and specificity of the 100 ml AT test was 97% and 96% when incubated for 24 h at standard temperature and 97% and 97% when incubated 48 h at ambient temperatures (mean: 27 °C). Sensitivity and specificity of the CompactDry™ test was >99 and 97% when incubated for 24 h at standard temperature and >99 and 97% when incubated 48 h at ambient temperatures. Good agreement between these candidate tests compared with the reference method suggests they are suitable for *E. coli* monitoring to indicate water safety.

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## INTRODUCTION

Water quality monitoring has the potential to serve as a critical feedback mechanism to support the development and operation of safe water supplies that promote public health<sup>1</sup>. In many settings, particularly in low- and middle-income countries (LMICs), water quality testing may be limited because the available methods for microbiological testing require dedicated hygienic laboratory space, specialized and expensive equipment, consumables that may be difficult or costly to source locally, and trained personnel. The resource constraints that limit laboratory testing are, in many cases, co-located with resource constraints that limit water safety. There is a pressing need for simple, scalable microbiological water safety tests that can be used in even the most basic settings by non-experts<sup>2–5</sup>.

Simpler, potentially low-cost alternatives to standard membrane filtration assays are now available for detection of *Escherichia coli* and other faecal indicator bacteria (FIB). Some are supported by systematic comparative testing<sup>6–9</sup>. Based on criteria of total cost per test of ≤US\$2 per sample and a lower limit of detection of 1 colony-forming unit (cfu) *E. coli* in 100 ml of drinking-water by culture, we selected two novel assays for evaluation as microbial water safety tests in comparison with EPA Method 1604 (membrane filtration followed by incubation on MI agar)<sup>10</sup>. Our hypothesis was that candidate low-cost assays could yield *E. coli* detection data with high (≥90%) specificity and sensitivity compared with the reference test, both under standard and ambient incubation conditions. Such tests may hold promise for global water quality monitoring such as that required in documenting progress toward Sustainable Development Goal (SDG) 6, to “ensure availability and sustainable management of water and sanitation for all”<sup>11</sup>.

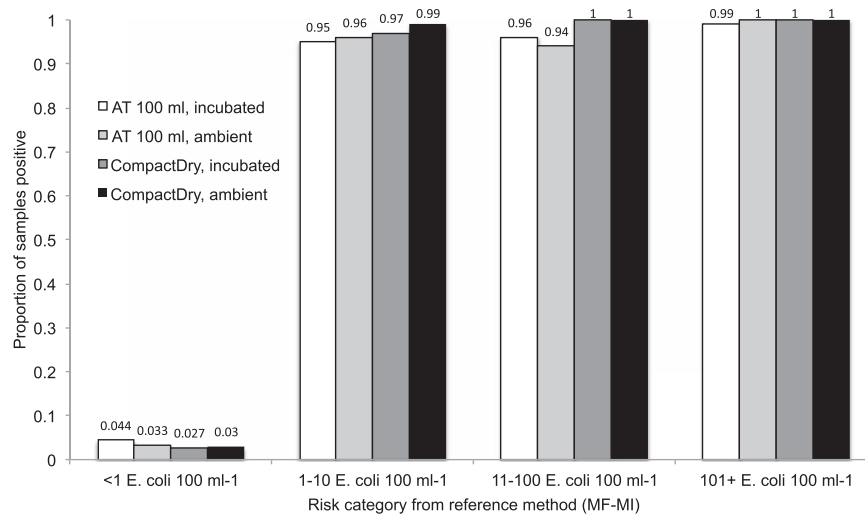
## RESULTS

### Systematic comparison across methods

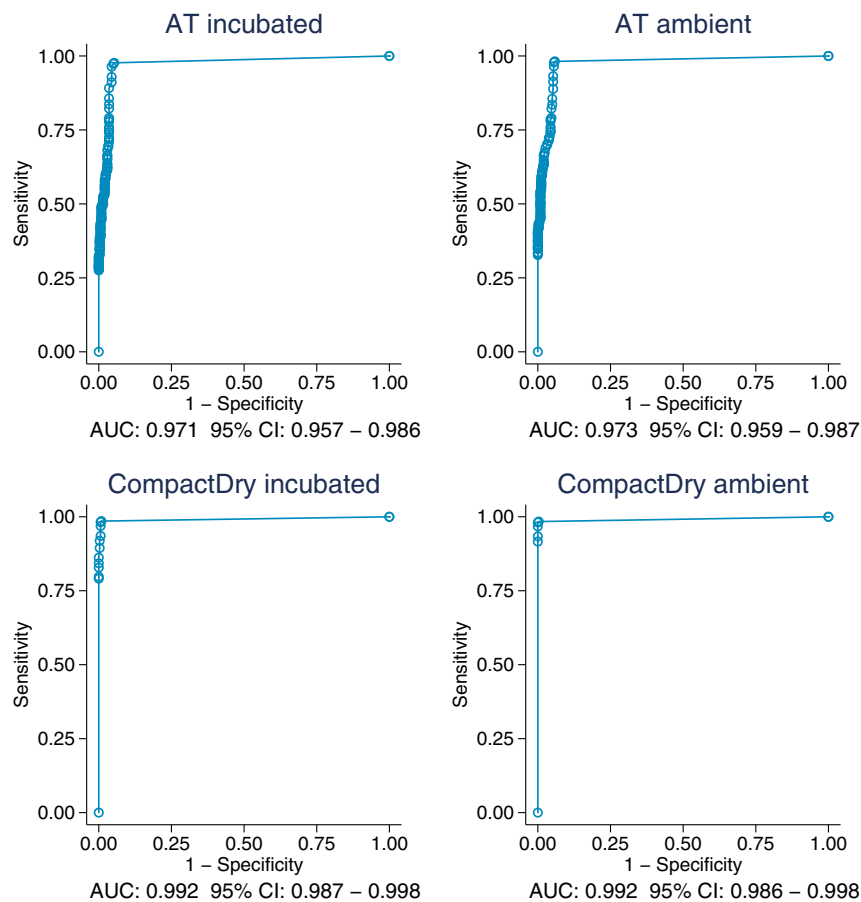
For systematic comparison testing between methods, we collected 315 bulk tap water samples, each assayed in triplicate by all methods ( $n = 945$ ). According to the reference method, the arithmetic mean total coliform count was 70 cfu 100 ml<sup>-1</sup> (standard deviation: 82 cfu 100 ml<sup>-1</sup>) and the arithmetic mean *E. coli* count was 57 cfu 100 ml<sup>-1</sup> (standard deviation 75 cfu 100 ml<sup>-1</sup>). The range of recorded values was <1 cfu 100 ml<sup>-1</sup> to 200 cfu 100 ml<sup>-1</sup> as an upper limit of quantification; therefore, computed means using this upper limit are underestimates of the true mean. Approximately 35% of samples met criteria for “safe” (<1 cfu *E. coli* 100 ml<sup>-1</sup>) and 26% of samples for “high risk” (101+ cfu *E. coli* 100 ml<sup>-1</sup>), according to mean counts from MF-MI.

The proportion of samples positive for both CompactDry™ and the AT 100 ml test are shown in Fig. 1, stratified by log<sub>10</sub> *E. coli* counts in the MF-MI reference assay. We plotted receiver-operator characteristic (ROC) curves comparing these two tests to samples taken at the same time and of the same water and assayed by membrane filtration (MF-MI), to provide a measure of replicability of the standard measure of *E. coli* in water (Fig. 2). The ROC curves show the diagnostic performance of the CompactDry and AT tests compared to the MF-MI reference method as the detection limit is varied from ≥1 per 100 mL to the upper limit of detection. We then calculated the Area Under the Curve (AUC), a measure of diagnostic performance based on the ROC curves. For both standard and ambient temperature incubation conditions, the AUC estimates for AT exceed 0.97 and 0.99 for CompactDry™, indicating near-ideal performance compared with the reference method of MF-MI. Tables 1 and 2 provide an overview of test performance characteristics for CompactDry™ and AT assays, respectively. A comparison of results (of individual assays and

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**Fig. 1** Proportion of samples positive with AT and CompactDry candidate test, incubated at 37 °C for 24 h or ambient temperature for 48 h, stratified by risk category of the reference test (MF-MI).



**Fig. 2** *E. coli* ROC curves for candidate tests versus the reference test (MF-MI).

means of assay replicates) with triplicate means of the reference test is provided in Supplementary Tables 1 and 2.

In quantitative estimation, *E. coli* count estimates from the CompactDry™ method showed good agreement with the reference method across a wide range of values, as seen in raw count data plots (Fig. 3), log<sub>10</sub> transformed count data (Fig. 4), and

in Bland-Altman plots (Fig. 5). Spearman's correlation coefficients and Bland-Altman 95% limits of agreement demonstrate that both the ambient temperature and standard incubation conditions yielded consistent agreement between CompactDry™ and MF-MI.

For both novel methods, we noted comparable sensitivity and specificity for sample duplicates incubated at ambient

**Table 1.** Data summary and performance of the CompactDry™ method compared with the reference test.

	CompactDry™, standard temperature incubation, single plate counts <i>n</i> = 945	CompactDry™, ambient temperature incubation, single plate counts <i>n</i> = 945
<i>E. coli</i> count (cfu 100 ml <sup>-1</sup> ) risk category from reference method <sup>a</sup>	Proportion of all samples consistent with reference method range of <i>E. coli</i> count <sup>b</sup>	
<1 ( <i>n</i> = 334)	97%	97%
1–10 ( <i>n</i> = 123)	40%	45%
11–100 ( <i>n</i> = 245)	83%	84%
101+ ( <i>n</i> = 243)	95%	95%
	Test accuracy statistics	
Sensitivity: presence of <i>E. coli</i> (95% CI) <sup>a</sup>	99.5% (98.6–99.9%)	99.5% (98.6–99.9%)
Specificity: presence of <i>E. coli</i> (95% CI) <sup>a</sup>	97.3% (94.9–98.8%)	97.3% (94.9–98.8%)
Positive Predictive Value (PPV): presence of <i>E. coli</i> (95% CI) <sup>a</sup>	98.6% (97.3–99.2%)	98.6% (97.3–99.2%)
Percentage of total samples underestimating risk category	2.3%	2.2%
Percentage of total samples overestimating risk category	12%	11%

<sup>a</sup>Confidence intervals for sensitivity and specificity are “exact” Clopper–Pearson confidence intervals<sup>30</sup>; for PPV, these are standard logit confidence intervals<sup>31</sup>.  
<sup>b</sup><1 cfu 100 ml<sup>-1</sup> (very low risk), 1–10 cfu 100 ml<sup>-1</sup> (low risk), 11–100 cfu 100 ml<sup>-1</sup> (moderate risk), 101+ cfu 100 ml<sup>-1</sup> (high risk).

**Table 2.** Data summary and performance of the AT method compared with the reference test.

<i>E. coli</i> count (cfu 100 ml <sup>-1</sup> ) risk category from reference method <sup>a</sup>	AT, standard temperature incubation <i>n</i> = 945		AT, ambient temperature incubation <i>n</i> = 945					
	100 ml	10 ml	100 ml	10 ml				
<1 ( <i>n</i> = 334)	+	–	+	–	+	–	+	–
	14	320	1	333	11	323	0	334
1–10 ( <i>n</i> = 123)	117	6	0	123	118	5	0	123
11–100 ( <i>n</i> = 245)	235	10	46	199	230	15	43	202
101+ ( <i>n</i> = 243)	241	2	205	38	243	0	209	34
	Test accuracy statistics							
Sensitivity: presence of <i>E. coli</i> (95% CI) <sup>a,b</sup>	97.1% (95.4–98.2%)		51.4% (46.9–56.0%)		96.7% (95.0–98.0%)		51.6% (47.1–56.2%)	
Specificity: presence of <i>E. coli</i> (95% CI) <sup>a,b</sup>	95.8% (93.1–97.7%)		99.8% (98.8–100%)		96.7% (94.2–98.3%)		100% (99.2–100%)	
Positive predictive value (PPV): presence of <i>E. coli</i> (95% CI) <sup>a,b</sup>	97.7% (96.2–98.6%)		99.6% (97.3–99.9%)		98.2% (96.8–99.0%)		100%	
Percentage of total samples underestimating risk category <sup>a,b</sup>	1.9%		25%		2.1%		25%	
Percentage of total samples overestimating risk category <sup>a,b</sup>	1.5%		0.11%		1.2%		0%	

<sup>a</sup>Using the following thresholds: <1 cfu 100 ml<sup>-1</sup> for 100 ml test and 11 cfu 100 ml<sup>-1</sup> for 10 ml test.

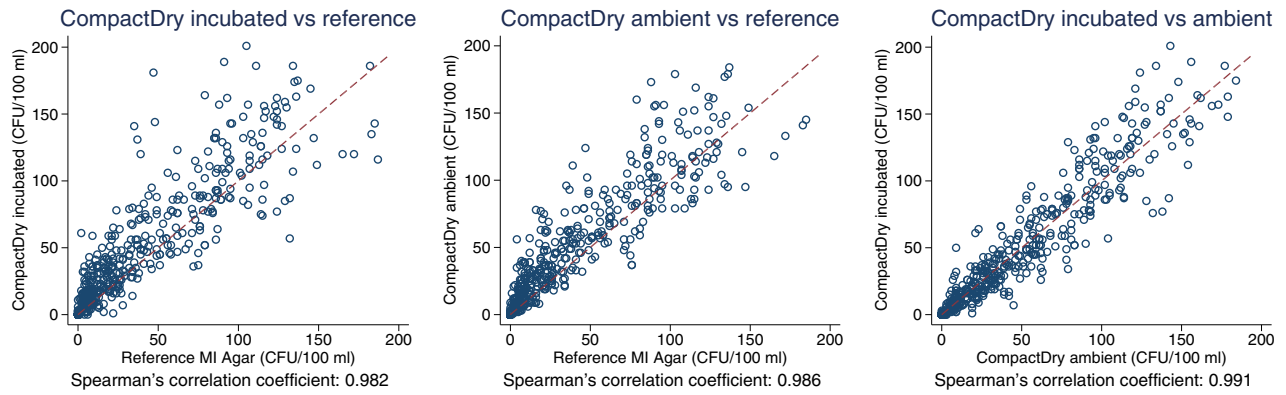
<sup>b</sup>Confidence intervals for sensitivity and specificity are “exact” Clopper–Pearson confidence intervals<sup>30</sup>; for PPV, these are standard logit confidence intervals<sup>31</sup>.

temperature in the testing space, generally (Tables 1 and 2), suggesting these ambient temperature conditions would yield equivalent results to standard incubation for these methods.

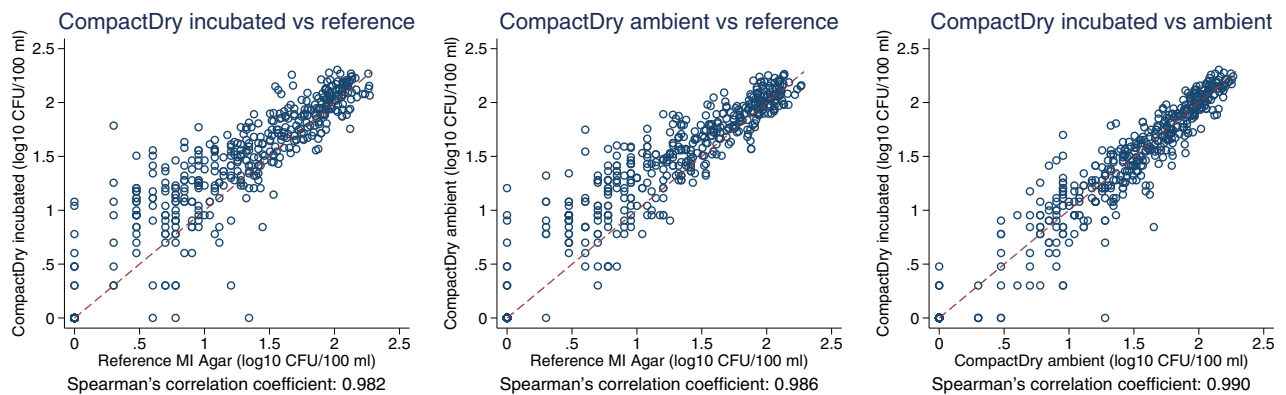
For 10 ml AT tests, poor sensitivity at both standard and ambient incubation temperatures (51.4 and 51.6%, respectively) suggests that 10 ml tests are not likely to be reliable for detection of *E. coli* counts at  $\geq 11$  cfu 100 ml<sup>-1</sup> (the theoretical detection limit for a 10 ml test) when compared to a reference method using 100 ml samples. Among AT 10 ml tests, almost half (49%) of samples were negative when the reference method yielded counts of  $\geq 11$  cfu 100 ml<sup>-1</sup> (Table 2).

## DISCUSSION

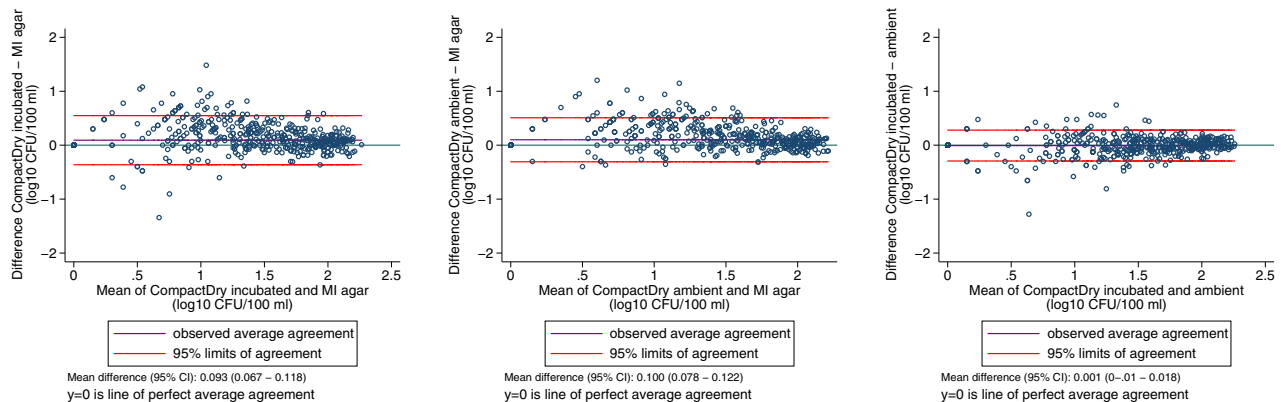
The SDGs aim to track global progress in drinking-water safety. SDG global targets include measurement of *E. coli* in drinking-water sources, a reasonably reliable and widely used indicator of microbiological water safety<sup>12–14</sup>. Current methods for measuring microbial water safety at scale are not feasible, given the specialized training, resources, and facilities required for *E. coli* assays used in water safety monitoring and regulation in economically rich countries<sup>2</sup>. New, globally scalable approaches are needed that can provide equivalent water safety information at lower cost where these resources are lacking.



**Fig. 3** Scatter plot of *E. coli* count data from **a** CompactDry™ versus the reference test, incubated; **b** CompactDry™ versus the reference test, ambient; and **c** CompactDry™ incubated versus ambient.



**Fig. 4** Scatter plot of  $\log_{10}$  transformed *E. coli* count data from **a** CompactDry™ versus the reference test, incubated; **b** CompactDry™ versus the reference test, ambient; and **c** CompactDry™ incubated versus ambient.



**Fig. 5** Bland-Altman plots showing  $\log_{10}$ -transformed microbial count data for **a** CompactDry™ versus the reference test, incubated; **b** CompactDry™ versus the reference test, ambient; and **c** CompactDry™ incubated versus ambient.

Based on criteria of cost, specificity of culturable *E. coli* as a target, and ease of use, we identified two novel water quality tests that represent potential for scale in international monitoring programs. These two tests join a suite of available methods that may be suitable to measure progress toward SDG 6.1, which calls for “universal and equitable access to safe and affordable drinking water for all” by 2030. We hypothesized that these methods could yield sensitive and specific estimates of *E. coli* in drinking water samples compared with an internationally accepted standard method for *E. coli* enumeration. Overall, our results from the ROC analysis on 945 data points from drinking water sources in

Bangalore, India, suggest that both candidate tests, at ambient temperature and standard-temperature incubation, yield comparable estimates for *E. coli* presence in 100 ml samples. The CompactDry™ method shows good quantitative agreement with MF-MI across risk categories, and both methods provide highly sensitive and specific information on the presence or absence of *E. coli* in 100 ml sample volumes. Because CompactDry™ requires the use of sterile membrane filtration or another concentration step for any assay volume greater than 1 ml, this method will probably find greatest use in applications where quantitative *E. coli* counts are the desired endpoint. A presence-absence 100 ml

AT assay is likely to be the most efficient and field-ready<sup>15</sup> option when quantal (presence-absence) data will suffice.

Our results also demonstrate the importance of using an appropriate volume for adequate sensitivity in quantal tests, as 10 ml was not sufficient for many of the samples tested in this study to yield information consistent with the reference method. Even though a 10 ml test should have a lower limit of detection of 10 cfu 100 ml<sup>-1</sup>, the performance of 10 ml quantal AT tests was poor in reliably detecting  $\geq 11$  cfu 100 ml<sup>-1</sup> *E. coli* (medium or high risk) in drinking water samples. This finding has potential implications for other methods that have been proposed for use in field surveillance, including a similar novel 10 ml *E. coli* method<sup>16</sup> and a 30 ml H<sub>2</sub>S test<sup>17</sup> proposed as lower-cost alternatives to more standard methods. Though these methods may be important tools for rapid surveys and to identify high-risk sources, any test using less than 100 ml assay volume will not be able to yield reliable estimates of drinking water safety at scale where the normative goal remains absence of culturable *E. coli* in 100 ml samples.

Our results are consistent with and complementary to one recent study comparing the use of AT for *E. coli* monitoring in environmental waters in Switzerland and Uganda<sup>18</sup>. This study compared quantitative results from AT with other, more standard tests: IDEXX Colilert-18®, m-TEC, and m-ColiBlue24®. In the previous study, AT showed high sensitivity ( $\geq 97\%$ ) for *E. coli* detection compared with reference methods, with an estimated 6% of AT samples being false positives. In our study, sensitivity of 100 ml AT tests was 97.1% among samples at standard incubation temperature and 96.7% under ambient incubation conditions, with 14 (4.2%) and 11 (3.3%) false positives, respectively. A finding that is potentially divergent between the studies, however, relates to performance at ambient temperatures. In Bangalore, where the range of ambient incubation temperatures was 25–30 °C (mean: 27 °C), we observed good agreement between test results at the two temperatures with ambient-temperature plates read at 48 h, concluding that ambient temperature incubation consistent with these conditions would yield comparable *E. coli* count data. Genter et al.<sup>18</sup> reported reduced performance at lower than standard incubation temperatures, though the condition tested was 24 °C with plates counted at 42 h post-inoculation. A comparison of results between these studies suggests that 25 °C may be a minimum recommended threshold for ambient temperature incubation using AT media. If possible, incubation at 37 °C for 24 h is likely to yield optimal results.

## METHODS

### Sample collection and processing

We collected drinking water samples from 14 locations in Peenya, approximately 11 km from the city centre of Bangalore, India. Peenya has a resident population of ~800,000 people and a population density of ~1300 people per square km. We collected water samples from taps at businesses and public taps across an area of 15 km<sup>2</sup>; all sources were from the mains water supply serving residential, commercial, and industrial areas. We collected samples across 37 non-consecutive sampling days in 2017–2018, typically visiting 10 or fewer tap sources per day. At each sample point, we collected three samples of water totalling ~2000 ml from the tap into sterile Whirl-Pak bags containing sodium thiosulfate. Following collection, samples were placed on ice until microbiological testing by all methods within 5 h of collection. Each sample was tested in triplicate by (1) the standard reference method (MF-MI), (2) the AT presence-absence test (100 and 10 ml volumes), (3) and a modified CompactDry™ test. On each sample day, we ran negative controls of each method using sterile dilution water.

As a standard method and basis for comparison, we measured *E. coli* in samples by filtering undiluted and diluted samples through 47-mm diameter, 0.45 µm pore size cellulose ester filters in sterile magnetic membrane filter funnels; membranes were incubated on MI agar for 24 h at 35 °C (this method is abbreviated throughout as “MF-MI”). MI agar detects *E. coli* by cleavage of a chromogenic β-galactoside substrate to detect total coliforms (TC) and a fluorogenic β-glucuronide substrate to detect *E. coli*, producing distinctive color TC colonies and blue fluorescing

*E. coli* colonies under long-wave UV light at 366 nm<sup>19–21</sup>. Our methods conform to EPA Method 1604<sup>10</sup>, widely used globally as a standard method for detection of *E. coli* in water. *E. coli* and TC count data were reported as colony forming units (cfu) per unit volume of water. As the sources tested were drinking water sources, we assayed only 100 ml volumes, assigning an upper limit of quantification of 200 cfu per plate, deeming colonies “too numerous to count” beyond this number (TNTC).

The AT presence-absence *E. coli* test was developed and piloted as part of a behavior-change randomized controlled trial (RCT) in rural India<sup>1</sup>. The semi-quantitative test uses the open-source Aquatest (AT) broth medium<sup>22</sup> with a resorufin methyl ester chromogen<sup>23</sup> (Biosynth AG, Switzerland) and subsequent incubation. Briefly, water samples are measured to 10 and 100 ml volumes using single-use volumetric cylinders containing pre-measured AT medium. Following incubation for 24 h at 37 °C or 48 h at ambient temperature (mean: 27 °C, range 25–30 °C), a color change from yellow-beige to pink-red indicates the presence of *E. coli*, and the combination of the two containers is used to determine the test result. Results can be interpreted as <1 *E. coli* per 100 ml (both containers negative, “safe”); 1–10 *E. coli* per 100 ml (large container positive, small container negative, “unsafe–low to medium risk”); or  $\geq 10$  *E. coli* per 100 ml (small container positive, “unsafe–medium to high risk”).

The CompactDry™ *E. coli* test (Nissui Pharmaceutical, Japan) used membrane filtration as in Method 1604<sup>10</sup>, except 99 ml of sample water was filtered through the filter and this was placed on a pre-sterilized, single-use plate with dehydrated culture medium. The method allows for straightforward identification of *E. coli* and total coliforms (TC) via chromogenic media<sup>24–26</sup>. Plates were rehydrated with 1 ml of sample water (for 100 ml sample volume), allowing for computation of cfu per 100 ml. When testing 10 ml samples, 9 ml of sample was filtered and the filter placed on the plate rehydrated with 1 ml of sample water. As in the reference method, the use of a filtration column is necessary, requiring the use of a suction pump, sidearm flask or manifold, and sterile filtration column for each sample. By using inexpensive, single-use, pre-sterilized plates, however, the method does not require the preparation and sterilization of microbiological media or the re-sterilization of plates. Results were recorded after 24 h incubation at 35 ± 2 °C and after 48 h at ambient temperature (mean: 27 °C, range 25–30 °C). As for MF-MI, *E. coli* and TC count data were reported as colony forming units (cfu) per 100 ml sample. Values above 200 cfu were recorded as TNTC and assigned a figure of 200 cfu when used in computing means.

### Statistical analysis

We performed each method in triplicate in each bulk tap water sample. Our primary analysis assesses comparability of the methods across all individual replicates in parallel. We also compared data from each candidate test with the arithmetic means of three replicates of the reference test as a “true” value for that sample. We calculated sensitivity, specificity, and positive predictive values for detection of *E. coli* ( $\geq 1$  *E. coli* per 100 ml) in both candidate tests and for both temperatures compared with the reference method.

After entering all data in Excel, we used Stata 16 (StataCorp, College Station, TX, USA) for primary data analysis, with further visualization and calculations in R. Descriptive statistics were used to characterize the water quality testing results from standard-temperature- and ambient-temperature-incubated samples using both continuous and categorical data, in unmodified and log<sub>10</sub> form. Of particular interest were the correlations between estimates within *a priori* risk strata for *E. coli*: <1 cfu 100 ml<sup>-1</sup> (very low risk), 1–10 cfu 100 ml<sup>-1</sup> (low risk), 11–100 cfu 100 ml<sup>-1</sup> (moderate risk), 101+ cfu 100 ml<sup>-1</sup> (high risk). These categories are commonly used to indicate levels of waterborne disease risk; existing evidence suggests an association between *E. coli* counts and diarrheal disease though the correlation may be non-monotonic, weak, and not necessarily always present<sup>12–14,27</sup>.

We used Bland-Altman and scatter plots to visualize results across tests. In data analysis of log<sub>10</sub>-transformed counts, we replaced non-detects with an integer value of “1” as the lower detection limit in assays. In Bland-Altman plots, we used linear regression to calculate mean differences accounting for replicate samples. To further compare results of candidate tests with the reference method, we used receiver operating characteristic (ROC) curves<sup>28</sup>. The ROC curves describe how the sensitivity and specificity of CompactDry and AT tests vary as the threshold used to define a positive reference test result increases from  $\geq 1$  per 100 ml to the upper limit of detection. Accuracy of a novel assay compared with the standard is measured by the area under the ROC curve (AUC), combining both sensitivity and specificity to evaluate

the overall performance of the candidate tests compared with the reference method. We calculated confidence intervals for AUC estimates accounting for clustering of replicates for each water sample<sup>29</sup>.

### Reporting summary

Further information on research design is available in the Nature Research Reporting Summary.

### DATA AVAILABILITY

All data generated during this study are available at Open Science Framework: <https://osf.io/k637w/>

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### AUTHOR CONTRIBUTIONS

JB and RB conceived and designed the study. AB processed all water samples. JB and RB analyzed the data and JB, AB, and RB wrote the manuscript.

### COMPETING INTERESTS

The authors declare no competing interests.

### ADDITIONAL INFORMATION

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