Assessment of Decontamination and Reuse of Disposable Filter Funnels Used in Microbiological Water Quality Tests

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ABSTRACT: To monitor safely managed drinking water services, an increasing number of countries have integrated water quality testing for *Escherichia coli* into nationally-representative household surveys such as the Multiple Indicator Cluster Surveys (MICS). However, plastic waste generated during such water quality testing programs, mostly through the use of pre-sterilized disposable materials, is non-negligible. The objective of this study was to evaluate several re-use protocols for disposable filter funnels used by the MICS water quality test kits. Decontamination and re-use protocols were assessed in centralized laboratory and decentralized field settings and neither yielded positive results. Re-use of 100 mL sterile funnels decontaminated with an alcohol wipe resulted in a higher incidence of false positive results (i.e., positive contamination when processing sterile water), both in the laboratory and field; therefore, a higher proportion of positives tests can be expected if these components are re-used. Further improvements to the decontamination technique and training are needed before material re-use can be reliably adopted. Autoclaving the funnels for re-use is feasible, provided that there is capacity to re-package and distribute funnels in a sterile manner.

KEYWORDS: Drinking water quality, *Escherichia coli*, microbial water testing, plastics waste, multiple indicator cluster surveys (MICS)

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Introduction

In 2015, the United Nations' Sustainable Development Goals (SDGs) were set out to guide world development agendas to the year 2030. Under Goal 6, the mandate was established to "ensure available and sustainable management of water and sanitation for all."[1] Specific to drinking water, Goal 6.1 was established to "achieve universal and equitable access to safe and affordable drinking water for all."[1] Obtaining reliable information on the safety of drinking water supplies is a major undertaking on the national and global scale.[2] The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) jointly gather demographic, social and public health data in collaboration with national governments under the Joint Monitoring Programme (JMP), including information on water supply, sanitation, and hygiene, with the support and use of UNICEF's Multiple Indicator Cluster Surveys (MICS) and its water quality module, alongside other nationwide household surveys.

The MICS water quality testing questionnaire includes verbal questions regarding access and availability of drinking water, and 2 in situ water quality tests carried out by a trained enumerator: 1 from a glass of water in the household (i.e., point-of-use, PoU), and 1 from the source of the glass of water (i.e., point-of-collection, PoC).[3] The water quality tests are processed in or near the household under study and normally uses the membrane filtration technique to enumerate *E. coli*, an indicator of the presence of faecal contamination, in 100 mL of water sample.[4] Such portable water quality test methods require somewhat large quantities of pre-sterilized and pre-packaged disposable materials (depending on the survey size), cost approximately USD $2.40 per test and generate an estimated 10 m^3 of plastics waste for surveys published in 2019 (calculations available in Supplemental Table S2). Aside from the high cost and generation of plastics waste, single-use materials can present logistical difficulties in terms of the transport and distribution before field work, as well as the return transport and disposal. We hypothesized that some single-use materials were sufficiently robust to be cleaned and re-used.

Current guidance on reducing laboratory plastic waste generally assumes access to a centralized laboratory, recycling program infrastructure, and/or high-cost items such as an autoclave,[5,6] thus reducing the potential for plastic waste reduction to be realized in low-resource and portable applications such as the MICS water quality tests. Therefore, the objective of this study was to evaluate different re-use protocols for disposable filter funnels used by the MICS water quality test.

Methods

Overview

MICS water quality tests utilize the membrane filtration technique in order to enumerate presumptive *E. coli*; a 100 mL water sample is passed through a filter of 0.45 µm pore size via vacuum pressure, thus isolating bacteria on the filter. The filter is removed and placed in a single-use petri dish on top of a gel media containing nutrients that are selective for *E. coli* (Compact Dry™ EC plates—Nissui Pharmaceutical Co., Ltd., Japan) and incubated for 24 hours at approximately 37°C. After incubation, each blue colony, measured as a colony forming unit (CFU), is enumerated by visual inspection and is assumed to have originated from a single bacterium.[4] Further details of the water quality sampling and testing are available elsewhere.[4] Two portable field kit designs were used in this study (Figure 1): a standard field kit (SFK; Millipore, USA, see Supplemental Table S1) currently in use by MICS surveys,[4] and a new low-cost field kit (LCFK; glass base from VWR, USA, filter support designed by UNICEF, and not commercially available) that has been trialed during non-MICS surveys in Afghanistan[2] and water quality field research in Malawi.[7,8] An upcoming publication will describe the LCFK in detail, including CAD files to enable others to mass produce this single component.

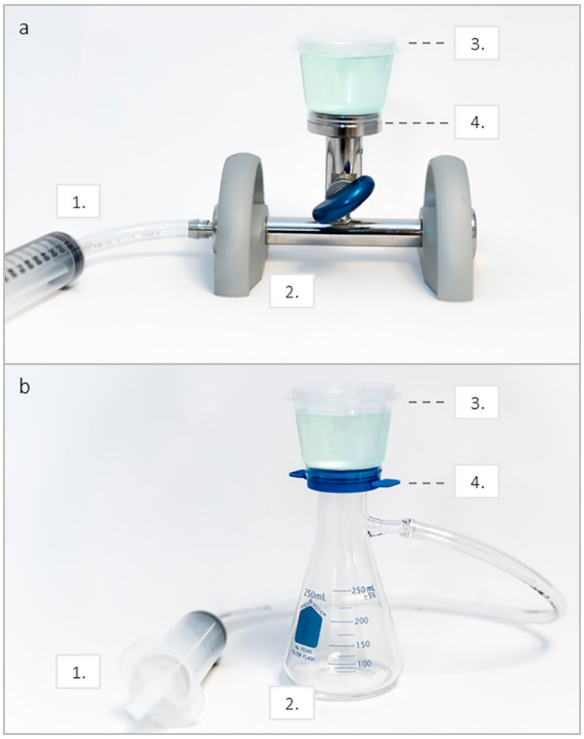

Figure 1. (a) Photo of SFK. (b) Photo of new LCFK. Labels: 1. 100 mL syringe and PVC tube for vacuum application (a 150 mL syringe was used for this work however the standard MICS catalog uses a 100 mL syringe); 2. Filtration base (current or low-cost); 3. Funnel; 4. Filter support (current or low-cost).

Decontamination and re-use protocols in this study focussed on filter funnels (item 3, Figure 1) which are disposable and come pre-sterilized in packages of 30. The objective of this study was addressed by assessing re-use protocols in centralized laboratory and decentralized field settings.

Decontamination Techniques for Funnel Re-Use

To assess potential funnel re-use, 2 general decontamination approaches were evaluated: alcohol wipes or autoclaving, selected on the basis described below. Other potential decontamination approaches could include boiling or washing with chlorinated water; however, these methods were not investigated in this study. Laboratory and/or field evaluations were performed depending on the techniques as summarized in Table 1. Based on the methods used, only 1 trial at a time could be processed (i.e., duplicate or triplicate measurements were not possible). However, we undertook multiple trials (minimum 30) for each testing environment (single alcohol wipe, double alcohol wipe, autoclave).

Wipes containing 70% isopropyl alcohol were investigated (150 mm by 170 mm size, PDI Healthcare Inc., United Kingdom) because these products are standard for MICS modules to decontaminate reusable components such as the filter support and forceps during membrane filtration.[4] Other alcohol-based agents include ethyl alcohol (ethanol) and N-propanol (1-propanol or propan-1-ol), however these are not used by MICS so were not evaluated. The PDI alcohol wipes are no longer available from the manufacturer; consequently, current MICS surveys use Medipal Sterile Alcohol wipes (200 mm by 125 mm size, CLH Healthcare, United Kingdom), which similarly contain 70% isopropyl alcohol.

When using the alcohol wipes, care was taken to wipe all water contact surfaces of the funnel at least twice with each wipe, to not touch the inside of the funnel with anything other than the wipe, and, after wiping, the cleaned funnel was placed upside-down on the alcohol wipe. Any further contact time with 70% alcohol wipes has been shown to have a negligible effect on bacterial reduction on plastic surfaces.[9]

Table 1. Summary of decontamination techniques evaluated in this work.

DECONTAMINATION TECHNIQUE	DETAILS	TESTING ENVIRONMENT (NUMBER OF TRIALS)
Single alcohol wipe	Wiping with a single 70% isopropyl alcohol wipe	Laboratory (39) and field (71)
Double alcohol wipe	Consecutive wiping with two 70% isopropyl alcohol wipes; air dried in between wipes	Laboratory (30)
Autoclave	Autoclaving at 121°C and 17 PSI for 15 min	Laboratory (34)

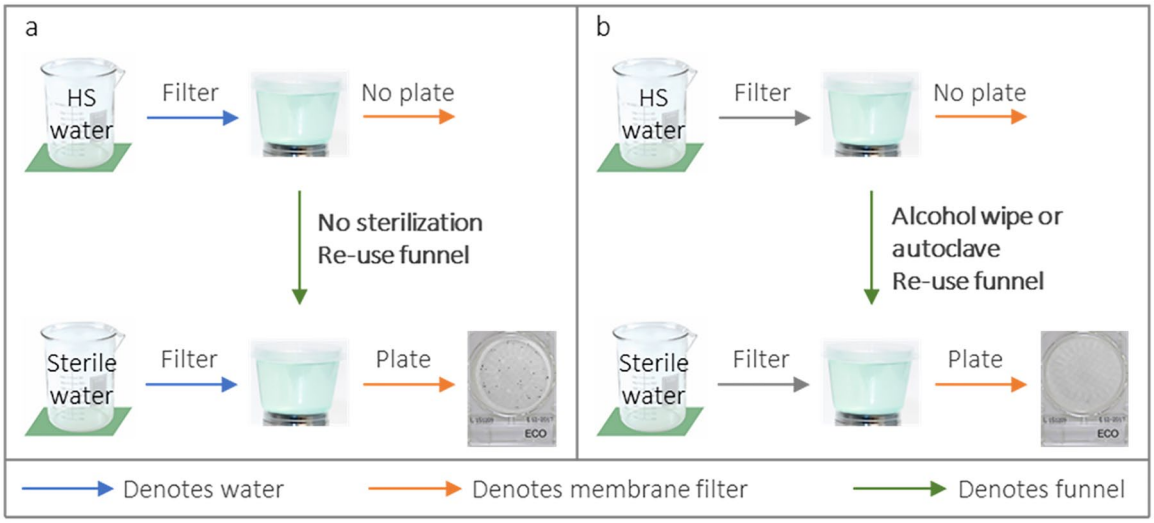

Figure 2. (a) Procedure for establishing a baseline number of *E. coli* remaining in the funnel after funnel re-use with no decontamination. (b) Procedure for assessing decontamination efficacy using an alcohol wipe or autoclave in the laboratory. High spike (HS) water contained approximately 10^5 CFU/100 mL *E. coli*.

For the double alcohol wipe technique, a second wipe was used following the use of a first wipe that was discarded after use. Notably, the double alcohol wipe tests were done as a laboratory verification of possible alternative funnel re-use solutions following the relatively high occurrence of false positive results observed during fieldwork (see "Results" section). To ensure that there was no residual alcohol left over after decontaminating the funnels with the alcohol wipe(s), care was taken that the funnel had fully dried by waiting approximately 1 minute before use. In the lab, to minimize the likelihood of the funnel becoming contaminated during cleaning, gloved hands were sprayed with a 70% alcohol solution (Commercial Alcohols, Canada) prior to each trial. During the fieldwork, gloves were not used during testing, but hands were cleaned immediately prior to each test with a 70% alcohol-based hand sanitizer gel (Purell®, Canada).

The autoclave method for decontamination was also investigated because field water quality testing materials could be repurposed for laboratory use in the host country once MICS activities are concluded. For such scenarios, the funnel could be decontaminated and re-used for other bacteriological assays in a centralized laboratory by, for example, a local water or health authority.

Laboratory Evaluation of Funnel Decontamination Efficacy

A laboratory evaluation of funnel decontamination efficacy was carried out using the SFK and consisted of initially processing 2 test waters (Figure 2), namely: (1) water spiked with *E. coli* to approximately 10^5 CFU/100 mL (high spike, HS); or (2) a sterile, "blank" water. Funnel decontamination efficacy testing consisted of filtering a 100 mL sample of HS test water through the SFK to ensure exposure of the filtration apparatus to *E. coli*. The level of baseline funnel contamination due to such exposure was estimated by processing 100 mL of sterile blank water samples for subsequent *E. coli* enumeration (number of trials, N = 34, Figure 2a). Similarly, the different decontamination techniques (Table 1) were assessed by initially processing 100 mL of HS test water. However, prior to filtering 100 mL of sterile blank water samples, specific decontamination techniques were applied to each funnel (Figure 2b).

After each decontamination technique was applied to the funnel, 2 tests (Figure 3) were conducted to verify whether any decontamination residual (i.e., leftover alcohol after wiping) could potentially compromise subsequent *E. coli* enumeration. Firstly, 100 mL of water containing approximately 1×10^2 CFU/100 mL (enumeration spike, ES), was enumerated in triplicate using new, sterile funnels, in order to provide a baseline enumeration of the ES. Secondly, the ES baseline enumeration was compared to the enumeration of 100 mL of the same batch of ES water, using a different filter funnel subject to 1 of the decontamination techniques.

The above evaluations were carried out in the laboratory by experienced personnel; in addition to these tests, a laboratory evaluation of the double alcohol wipe decontamination method was undertaken with a group of newly trained University of Victoria undergraduate students (N = 16). First, each student plated a standard blank test, using sterile blank water and a new sterile funnel (Figure 4a). Directly following this, each student filtered a 100 mL sample of ES water, cleaned the funnel using 2 alcohol wipes, then processed a 100 mL sample of sterile blank water (Figure 4b). The evaluation protocol carried out by the students (Figure 4b) closely follows that which was carried out by experienced laboratory personnel (Figure 2b), with the exception that ES water was used (as opposed to a HS water) to more closely mimic potential field concentrations.

The HS and ES test water used during the laboratory evaluation of decontamination protocols consisted of different *E.*

coli spike concentrations in a matrix of autoclaved isotonic quarter-strength Ringers solution (Oxoid Ltd., England). *E. coli* was sourced from a commercially-available probiotic Mutaflor® (Pharma-Zentrale GmbH, Germany) which was incubated in Tryptic Soy Broth (TSB, Sigma-Aldrich, Germany), stirred at approximately 500 rotations per minute at 37°C overnight prior to testing (further probiotic *E. coli* details and characterization are available elsewhere[10]).

Mechanical Fatigue Testing

Fatigue testing of funnels was carried out in the laboratory to determine the number of times funnels could be decontaminated and re-used before leakage was observed. Once 100 mL tap water was filtered through the LCFK, the funnels were removed, decontaminated (alcohol wipes or autoclave) and air-dried before repeating the procedure (the number of cycles for each funnel was recorded). This procedure was carried out on 20 funnels for each the single-wipe alcohol decontamination (not conducted for the 2-wipe protocol) and autoclave sterilization methods until leakage between the funnel and the vacuum filter was observed, or until 25 cycles had been carried out; whichever came first.

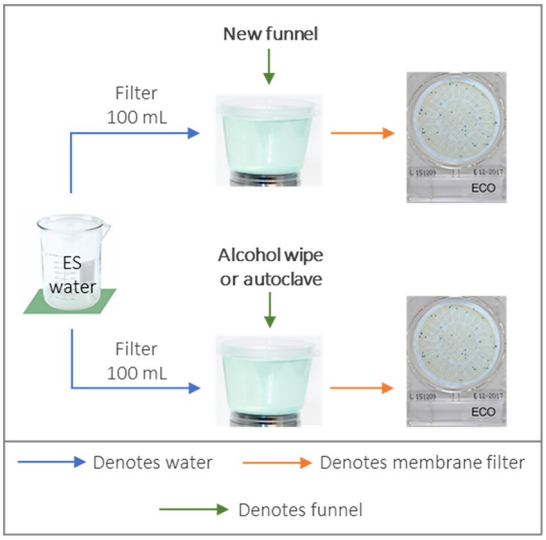

Figure 3. Procedure for the assessment of a residual effect of the alcohol wipe and autoclave decontamination methods. Enumeration spike (ES) water contained approximately 1×10^2 CFU/100 mL *E. coli*.

Field Piloting of Funnel Re-Use

The funnel re-use protocol was piloted during the water quality testing component of a study with a household survey component conducted in April 2019 in Southern Malawi. This study obtained ethical approval from the National Committee on Research in the Social Sciences and Humanities (NCRSH) in Malawi (P.10/18/326) and the Human Research Ethics Board at the University of Victoria (18-1129). The survey consisted of a questionnaire and water quality test and was conducted by 6 enumerators who had undergone 5 days of training on questionnaires and in situ water quality testing, of which approximately 2 days were spent solely on water quality training. The training followed a similar structure to MICS Water Quality Testing Modules (MICS, 2017) with *E. coli* sampling and enumeration of two 100 mL samples: PoU and PoC. In total, 375 randomly selected households were surveyed in informal settlements and rural areas over a 3-week period. In a typical day, each enumerator surveyed 5 households, with a maximum of 20 households surveyed by each enumerator each week.

To undertake sample enumeration, the MICS protocol[4] was followed, with 2 exceptions: first, the LCFK was used (Figure 1b) to process all samples; second, the funnels were decontaminated and re-used instead of 1 new funnel per test as per the

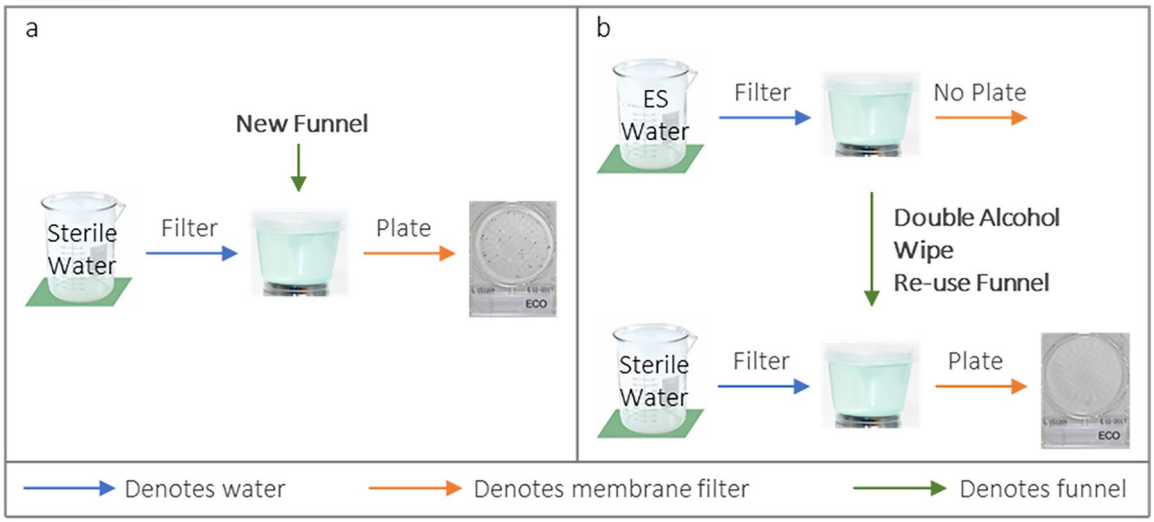

Figure 4. Procedure for the assessment of decontamination effectiveness with a group of newly trained students. (a) Procedure for establishing a baseline number of *E. coli* remaining in the funnel after funnel re-use with no decontamination. (b) Procedure for assessing decontamination efficacy using an alcohol wipe or autoclave in the laboratory. Enumeration spike (ES) water contained approximately 1×10^2 CFU/100 mL *E. coli*.

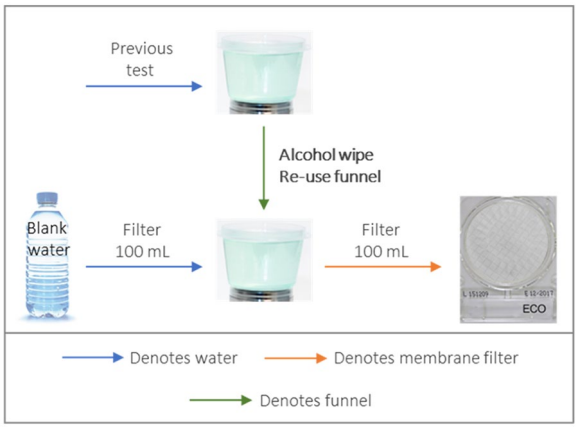

Figure 5. Procedure for assessing decontamination effectiveness in the field.

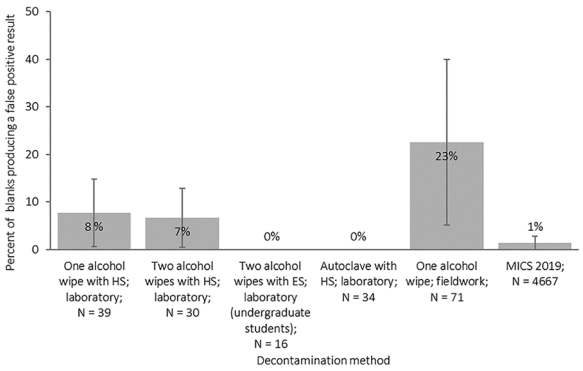

Figure 6. Percent of blank tests (using sterile or mineral water for the laboratory and field tests, respectively) returning a count of >1 CFU/100 mL; Error bars represent the Bernoulli variance; "N," denotes the number of blank samples processed; MICS proportion calculated using a weighted average by number of blank tests conducted in each survey (see Supplemental Table S2).

MICS protocol. Before use, funnels were cleaned with 1 alcohol wipe (the same wipe as used to clean forceps and funnel support contact area), with care taken to not touch the inside of the funnel during cleaning. Following testing, the funnels were dried with a towel and stored in the enumerators' backpack with the rest of the kit components. Each enumerator was provided with 2 new funnels at the outset of each week (1 for main use and 1 as a backup), with each funnel being used for a maximum of 24 water quality tests.

The effectiveness of funnel decontamination (with a single alcohol wipe) was evaluated in the field by processing a 100 mL sample of a freshly-opened bottled of water "field blank" (Figure 5). During the field program, each enumerator conducted 1 field blank test per day, or approximately 4 per week per enumerator, for a total of 71 blank tests. Field tests were conducted following the third household survey of each day. Bottled water used for field blanks was tested beforehand by the supervisors to ensure no *E. coli* was detected.

Statistical Methods

Relevant geometric mean colony counts and upper/lower 95% Confidence Intervals (CIs) were computed for all blank (laboratory and field) test data, using a value of 0.5 CFU/100 mL, half of the minimum detectable *E. coli* (1 CFU/100 mL), for non detects. Colony counts greater than 1×10^2 CFU/100 mL were recorded as 1.01×10^2 CFU/100 mL in accordance with MICS protocols.[4]

The proportion of positive blank tests were calculated for previous MICS surveys and for laboratory and fieldwork. For field data, the proportion of positive blank tests was computed as a function of the enumerator who conducted the test, the day of the week and the week number (first, second, or third weeks). Statistical comparisons were drawn using a binomial regression. Model outputs were used as the input into a 1-way analysis of variance (ANOVA). Significant ANOVA results were further analyzed using multiple comparisons with the post hoc Bonferroni correction, to reduce the likelihood of a Type I error.

For each decontamination technique, an ANOVA test was used to test for differences in ES counts when new funnels were used, as compared to decontaminating and re-using the funnels. A separate ANOVA test was undertaken for the 1 alcohol wipe and autoclaving decontamination methods; ES data were analyzed based on batch (i.e., blocking in 1-way ANOVA), so that comparisons were only drawn on counts within each batch of ES. A Kruskal-Wallis rank sum test (the non-parametric version of an ANOVA) was undertaken for the 2 alcohol wipe decontamination method as the dataset violated the normality assumption. All datasets used for an ANOVA were tested to verify the normality assumption using a Shapiro-Wilk's test.

Results from all statistical tests used in the analysis were considered significant at the $\alpha \leqslant 0.05$ significance level. In the case of statistically significant ($P \leqslant .05$) results, the data were examined further to determine if the result would have an impact on the categorization of results into such risk categories. All statistical tests were performed with R statistical software, in RStudio, version 3.6.3. Water quality data were, in some cases, compared to the a priori waterborne risk categories defined by Lloyd et al[11]: "very low risk" (<1 CFU/100 mL); "low risk" (1-10 CFU/100 mL); "moderate risk" (11-1×10^2 CFU/100 mL); "high risk" (1.01×10^2-1×10^3 CFU/100 mL).

Results

Funnel Decontamination Efficacy and Field Effectiveness

The proportion of blank tests that returned a positive ($\geqslant 1 \times$ CFU/100 mL) result during laboratory and fieldwork are displayed in Figure 6. The proportion of positive blank tests in the laboratory and fieldwork using 1 alcohol wipe to decontaminate the funnels was found to be significantly higher than the proportion reported in MICS surveys in 2019[12–19] ($P = .02$ and $P < .01$, respectively; Table 2). When funnels were decontaminated using 2 alcohol wipes (either with HS or ES water

Table 2. Summary of *P*-values for Binomial *t*-test analysis of proportion of positive blank test, using all funnel decontamination methods in laboratory and fieldwork.

	MICS	ONE ALCOHOL WIPE; LABORATORY	TWO ALCOHOL WIPES; LABORATORY	TWO ALCOHOL WIPES WITH ES WATER; LABORATORY	AUTOCLAVE; LABORATORY	ONE ALCOHOL WIPE; FIELD
MICS	N/A	0.02[a]	0.07	0.80	0.63	<0.01[a]
One alcohol wipe; laboratory		N/A	0.74	0.28	0.07	<0.01[a]
Two alcohol wipes; laboratory			N/A	0.33	0.10	0.02[a]
Two alcohol wipes with ES water; laboratory				N/A	N/A[b]	0.02[a]
Autoclave; laboratory					N/A	<0.01[a]
One alcohol wipe; fieldwork						N/A

[a]Significant result.
[b]Not possible to compute; both rates of positive blanks 0%.

preceding the blank) or the autoclave in the laboratory, the proportion of positive blank tests did not differ significantly from that reported in MICS surveys from 2019[12–19] (*P* = .07, .80, and .63, respectively, Table 2). The proportion f positive blank tests in the laboratory did not differ when using 1 or 2 alcohol wipes (either with HS or ES water preceding the blank; *P* = .74 and .28, respectively; Table 2). For more information on false positive rates reported in MICS surveys conducted in 2019 (N = 4667), see Supplemental Table S2.

The summary of counts resulting from blank tests when the funnels were re-used and decontaminated with the alcohol wipe(s) in the laboratory and field are shown in Figure 7. The geometric means for the laboratory blank test data were all <1 CFU/100 mL (upper and lower 95% CI both <1 CFU/100 mL) using 1 or 2 alcohol wipes, or the autoclave, to decontaminate the funnel. In the laboratory, when no decontamination took place between processing the HS water and processing and plating the blank water (Figure 2a), a baseline contamination average of 42 CFU/100 mL (95% CI 37-47 CFU/100 mL) were enumerated in the blank water.

All field blank tests produced a geometric mean of <1 CFU/100 mL (95% CI <1-2 CFU/100 mL), and exclusively positive field blank tests produced a geometric mean of 3 CFU/100 mL (95% CI <1-7 CFU/100 mL). There was no significant difference between the frequency of positive field blank test results with respect to the day of the week it occurred, week number, or the enumerator (excluding supervisors) who conducted the blank test (*P* = .13, .09, .80, respectively), indicating that a positive blank result was equally likely to happen to all enumerators during fieldwork, regardless of the day and week. None of the field blank tests conducted by the experienced counters, either during training or fieldwork, produced positive results.

In laboratory testing for the presence of a decontamination residual, there was no statistical difference between the enumeration of ES water when new funnels were used, compared to decontaminating and reusing the funnels using any of the decontamination methods examined. An ANOVA test comparing differences in ES counts when new funnels were used, as compared to decontaminating and re-using the funnels, yielded *P*-values of .99 and .07 for 1 alcohol wipe and autoclaving decontamination methods, respectively. For the 2 alcohol wipe decontamination method, a Kruskal-Wallis Wilcoxon rank sum test for differences in ES counts when new funnels were used, as compared to decontaminating and re-using the funnels, yielded a *P*-value of .30.

Counts from positive field blank tests were compared to their respective preceding water quality samples (Figure 8), and the correlation between the 2 was not found to be significant (*P* = .50). Colony counts greater than 1×10^2 CFU/100 mL were recorded as 1.01×10^2 CFU/100 mL; counts higher than this did not have an impact on the correlation analysis.

Mechanical Fatigue Testing

No funnels leaked or showed signs of wear at any time during testing, regardless of the decontamination type (either an alcohol wipe or autoclave), up to the maximum of 25 uses (after which trails ceased). During field implementation of funnel re-use with the new LCFK, no leakage was reported by the enumerators or by the experienced counters. The funnels, which are intended for single use, are robust enough for a minimum of 25 uses with decontamination by either alcohol wipe or autoclave, confirming our hypothesis.

Discussion

General

Considering the laboratory evaluation, we found no additional benefit (in terms of proportion of false positive blank results) to wiping the funnels down a second time. Autoclaving funnels for re-use was only undertaken in the laboratory; such a method

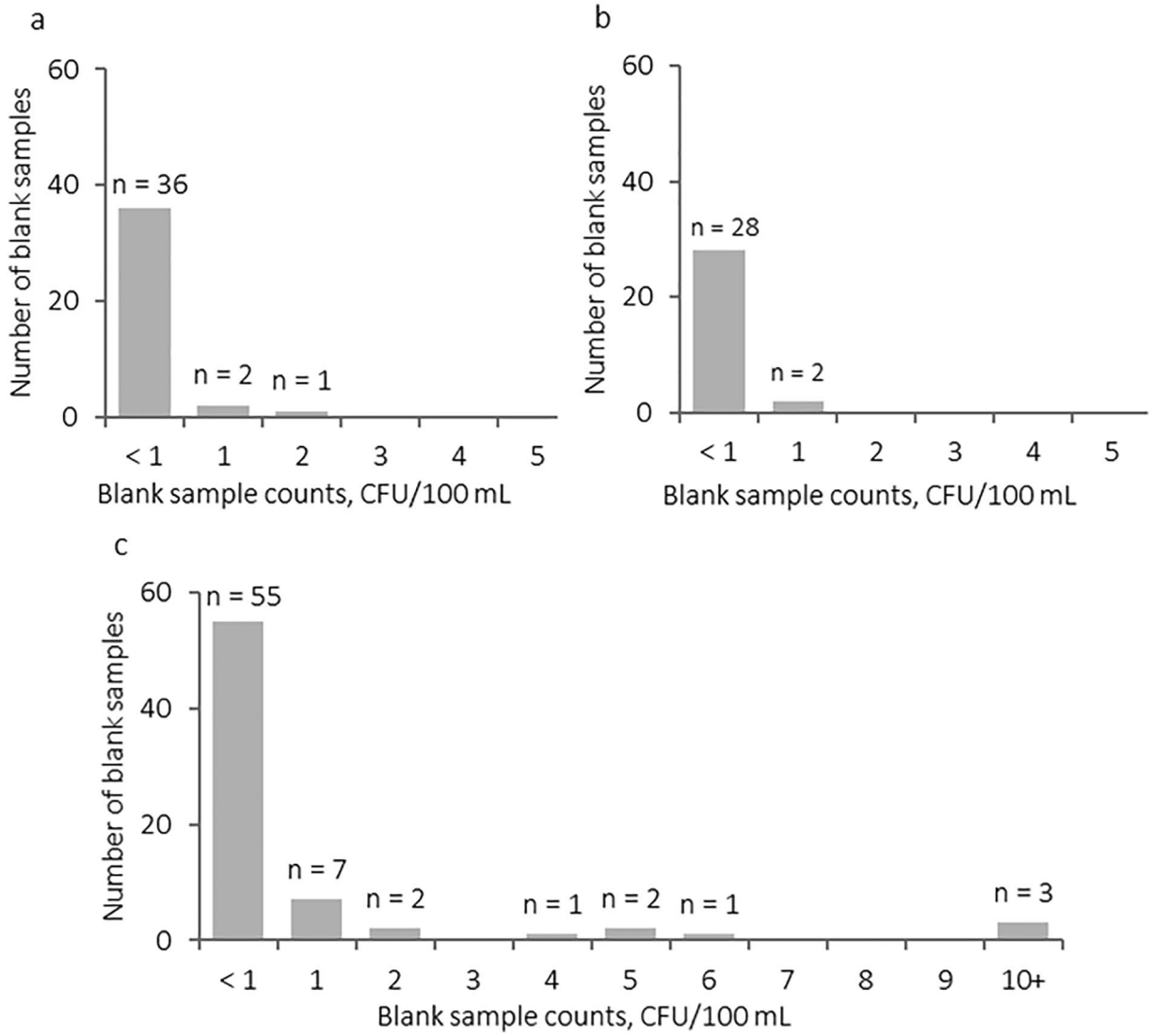

Figure 7. (a) Distribution of counts resulting from blank tests in the laboratory using 1 alcohol wipe to decontaminate funnel (N = 39). (b) Distribution of counts resulting from blank tests in the laboratory using 2 alcohol wipes to decontaminate funnel (N = 30). (c) Distribution of counts resulting from blank tests in the fieldwork using 1 wipe to decontaminate funnel (N = 71).

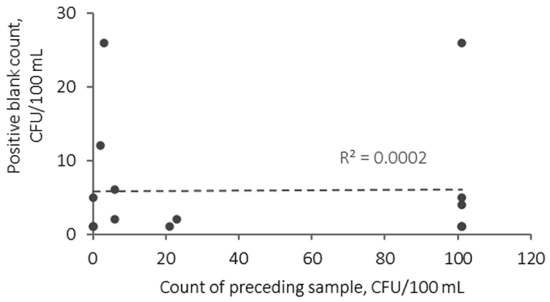

Figure 8. Correlation between positive field blank test counts and respective preceding samples. A value of 1.01×10^1 CFU/100 mL was used for counts $>1.01 \times 10^1$ CFU/100 mL.

could present logistical difficulties for fieldwork, such as the need to carry used funnels for the remainder of the day or week until they are returned to a central location for decontamination (as opposed to disposing them), and the need for funnels to be re-packaged in sterile wrapping for re-use. However, if the funnels are being re-used in a centralized laboratory or if there is capacity to re-package and redistribute funnels in a sterile manner, autoclaving funnels is a viable alternative to disposing of the funnels after a single use. Low-cost alternatives to autoclaves can be used; for example, the use of pressure cookers to sterilize reagents in low-resource areas.[20]

There were noticeable differences between laboratory- and field-based assessments, in terms of both the frequency and magnitude of positive blank test results (Figures 6 and 7; Table 2), although protocols in the laboratory and field were, in principle, the same and all surfaces (including gloves or hands as appropriate) were cleaned with 70% alcohol (liquid solution or hand sanitizer gel in the laboratory and field, respectively) prior to testing. The laboratory and field contexts were characterized by several main differences: where tests were performed (i.e., a benchtop as opposed to a plastic board on the ground); the person conducting the tests (i.e., experienced laboratory personnel vs newly trained enumerators); and the composition of

blank water and water preceding each blank (discussed below, "Correlation of Positive Field Blank Tests with Preceding and Succeeding Water Quality Data").

It should be noted that positive blank test results do not necessarily result from re-using funnels. Positive blank tests can result from a number of factors including, but not limited to, contaminated blank water, contaminated forceps or filtration head contact area, accidental contact of membrane filter with non-sterile surfaces or improper opening/closing of petri dishes. The funnel decontamination and re-use in this study was the only methodological difference to the standard MICS technique and there are 2 plausible ways in which funnels could become contaminated during re-use: cross-contamination from the previous sample, with residual *E. coli* not sufficiently decontaminated by the alcohol wipe, or by hand contact with the inside of the funnel inside during cleaning. Although hand cleanliness has never been evaluated in the context of field-based membrane filtration techniques, during the MICS survey the hand does not reach inside of the funnel.[4] It should be noted that some MICS surveys have reported higher-than-average (1%) positive blank tests; notably, surveys conducted in Cote d'Ivoire and The Gambia reported percentages of positive blank tests of 8.2% and 6.2%, respectively,[2] and such an investigation was not indicated by these surveys to discern the contamination source. The comparison of false positive rates in this study was made against the pooled results from MICS surveys (see Supplemental Table S2) and we did not examine heterogeneity in false positive rates between surveys or survey teams.

Occasionally, in the field, when funnels were decontaminated and re-used with an alcohol wipe, there were pale yellow or colorless colonies present on the blank test results, even if *E. coli* colonies were absent. Therefore, it is unlikely the alcohol wipes achieved full "sterility"—full reduction of all bacteria to numbers below detection minimum—but rather, in cases where <1 CFU/100 mL was reported, the alcohol wipe reduced the *E. coli* to numbers below the detection minimum (1 CFU/100 mL). This may also be the case during MICS surveys, however as per the MICS methods,[4] only blue colonies (presumptive for *E. coli*) are counted and recorded by enumerators, so any such occurrences have not been reported by the surveys.

Log_{10} Reduction Achieved by Alcohol Wipe in the Laboratory

In the laboratory work, a "baseline" was established to estimate the number of *E. coli* remaining on the funnels after processing the HS water (Figure 2a). The results for the established baseline (42 CFU/100 mL; 95% CI 37-47 CFU/100 mL) indicated that, using either 1 or 2 alcohol wipes, a reduction of 1.6 log_{10} CFU/100 mL was achieved. It should be noted that this reduction value is limited by the relatively low numbers of *E. coli* present on the funnel following the filtration of HS water. It is not possible to make such a comparison for the field data because no such baseline was established to estimate the contamination remaining on the funnels if no decontamination were to take place. The alcohol wipes used in this study are intended for use in a healthcare setting[9,21] and a study examining bacterial reduction by a similar disposable wipe on hospital computer keyboards indicated that bacterial reduction of up to 99.99% (4 log_{10}) is possible.[21]

Correlation of Positive Field Blank Tests with Preceding and Succeeding Water Quality Data

Although it is important to minimize positive blank tests whenever possible, it is not clear how best to address them when they do occur, either for specific field teams or for an overall survey such as those performed for MICS. In this study, we tried to assess whether there was any evidence to suggest that the positive blank tests observed in the field were correlated with the preceding blank tests (Figure 8), for which we find no evidence. In light of such lack of evidence, it seems plausible that the field positive blank results did not originate from the preceding water, but perhaps from setting-related environmental contamination or accidental contact of the funnel during the course of cleaning. Similar such correlation between positive blank tests and the preceding water quality test is not available from MICS survey findings reports published in 2019[12–19] (summarized in Supplemental Table S2).

Such a correlation analysis was not conducted for laboratory results, however the HS water preceding blank tests in the laboratory consistently had counts on the order of 10^5 CFU/100 mL, several orders of magnitude higher than that seen in the field. It therefore seems plausible that in the laboratory, the positive blank test results may have originated from a residual contamination of the preceding HS water, which was spiked to a concentration not seen in the field.

Effect of Funnel Re-use on Categorical Risk Assessments

Typically, the *E. coli* enumeration data gathered in MICS surveys are used to make risk assessments based on a priori waterborne risk categories defined by Lloyd et al.[11] For water having a count of 1 or 2 CFU/100 mL lower than the cut-off for the next highest risk category (eg, 9 CFU/100 mL or 1×10^2 CFU/100 mL), or for water in the very low risk category (<1 CFU/100mL), a contaminated funnel could raise the risk assessment. Based on this, 2 hypothetical projections were conducted by subtracting from the non-blank counts obtained during the fieldwork (i.e., PoC and PoU samples), to ascertain the possible blank-adjusted risk categories of the water quality data.

First, based on the result that a positive blank result was equally likely to happen to all enumerators during all points in the field, we subtracted 1 CFU/100 mL, the geometric mean of all field blank test data, from the count of all non-blank water quality data. The adjusted assumed risk category assessments[11] are presented in Table 3; under this analysis, 38 of 563 (6.7%)

Table 3. Projected shift in risk assessment data of non-blank samples, according to an estimated shift of –1 CFU/100 mL to every non-blank sample. Risk categories defined according to Lloyd et al.[11]

ASSUMED BLANK-ADJUSTED RISK CATEGORY	RISK CATEGORY ACCORDING TO WATER QUALITY DATA COLLECTED				
	VERY LOW	LOW	MODERATE	HIGH	TOTAL
Very low	184	33	0	0	217
Low	0	93	5	0	98
Moderate	0	0	139	0	139
High	0	0	0	109	109
Total	184	126	144	109	563

Table 4. Projected shift in risk assessment data of non-blank samples, according to an estimated shift of –3 CFU/100 mL to 23% randomly selected non-blank samples. Risk categories defined according to Lloyd et al.[11]

ASSUMED BLANK-ADJUSTED RISK CATEGORY	RISK CATEGORY ACCORDING TO WATER QUALITY DATA COLLECTED				
	VERY LOW	LOW	MODERATE	HIGH	TOTAL
Very low	184	20	0	0	204
Low	0	106	3	0	109
Moderate	0	0	141	0	141
High	0	0	0	109	109
Total	184	126	144	109	563

water quality samples underwent a shift in risk category, with 33 of 126 (26%) low risk (1-10 CFU/100 mL) shifted to very low risk (<1 CFU/100 mL). Water quality data which are projected to have given a result of <1 CFU/100 mL (very low risk category) are the most impacted by occurrences of positive blank results. Under this analysis, all water quality data collected with a count of 1 CFU/100 mL (low risk) is assumed to have returned an adjusted count of <1 CFU/100 mL (very low risk). Although some of our collected water quality data may have returned their respective counts without any supposition of contamination (i.e., not all water quality data collected with 1 CFU/100 mL were contaminated), this projection demonstrates the impact of contamination on samples in the very low or low risk categories.

The second hypothetical projection was based on the result that 23% of the field blank tests returned a positive result. In this projection we subtracted 3 CFU/100 mL, the geometric mean of the positive field blank tests, from a randomly selected 23% of field non-blank water quality data. The resulting blank-adjusted risk category assessments are presented in Table 4; under this analysis, 23 of 563 (4.1%) water quality samples underwent a shift in risk category, with 20 of 126 (16%) low risk (1-10 CFU/100 mL) shifted to very low risk (<1 CFU/100 mL). Under this second analysis, low risk samples are not as highly impacted as the first analysis, under which all samples having 1 CFU/100 mL were shifted into very low risk.

A paired Wilcoxon rank-sum test was used to assess differences between the risk categories[11] of collected water quality data and each projection, revealing statistically significant differences between the collected water quality data and both the first and second projections depicted in Tables 3 and 4 (both comparisons $P < .01$).

Future Implementation of Funnel Re-Use

There was no indication that any residual alcohol remaining on funnels following decontamination by alcohol wipe or autoclave, and the funnels did not leak during any testing either the laboratory or field up to a maximum 25 uses. Therefore, the main barrier to implementing the practice of re-using funnels in MICS surveys may be the observed incidences of positive blank test results.

If further implemented, the proportion of positive blank test results would have to be reduced via intensive training and/or refresher training during fieldwork. Close supervision would need to be implemented to evaluate practices that may lead to contamination of the funnel, membrane filter, or sample. Such a reduction of false positive blank tests may be possible as none of the field blank tests conducted by skilled counters either during training or fieldwork produced positive results. In addition, we find promising the result of 0% false positive results by undergraduate trainees in the laboratory when a double wipe was used and with the previous sample having approximately 1×10^2 CFU/100 mL concentration (Figure 4). However, the sample size for this finding was small (N = 16) and this testing

would need to be repeated. Alternate chemicals for surface decontamination, such as 70% ethyl alcohol (ethanol)[22] and chlorine-based, phenol-based and quaternary ammonium-based wipes[21] have been found to be comparable to 70% isopropyl alcohol (used in this study) in the medical context. Based on these findings, it would appear to be unlikely that alternative commercially available disinfectant wipes would greatly improve results in this study.

We note that other field-based membrane filtration techniques such as the DelAgua kit utilize re-useable funnels made of stainless steel.[23] In the field, these funnels are decontaminated by sealing the top and bottom of the funnel and burning methanol within the sealed space. When methanol is burned in the low-oxygen space, formaldehyde gas is created, which acts as a disinfectant, although is not as simple as using alcohol wipe(s). The DelAgua kit has been recommended for portable water quality testing[24] and is commonly used to undertake field-based water quality monitoring.[20,25-28]

The generation of plastics waste by water quality testing conducted to monitor progress toward SDG 6.1 is somewhat at odds with SDG goal 12.6, established to "substantially reduce waste generation through prevention, reduction, recycling, and reuse."[29] Plastics waste generated by scientific research is non-negligible; the University of Exeter bioscience department estimated their plastic waste consumption to be approximately 247 tonnes in 2014[30]—generated in 1 year by 1 department laboratory in 1 university. There have been calls to reduce consumption of single-use plastic items in laboratory work,[30] for example by re-using items such as pipettes[5] or recycling nitrile gloves[6] if possible.

To generate the MICS surveys published in 2019[12–19] (English language only), 31 354 household water quality questionnaires were conducted, for a total of 65 506 water quality tests, including blanks (see Supplemental Table S2). If funnel decontamination and re-use had been implemented for all MICS surveys published in 2019, an estimated 10 m^3 or 525 kg of plastic waste could have been saved and a cost savings of approximately USD $1000 would have been realized, simply for funnels, excluding all other disposable supplies required for MICS surveys such as petri dishes (see Supplemental Table S2). Although the results of this study were not positive, reducing the cost, logistical barriers and plastics waste via decontamination, and re-use of water quality test kit components is a valuable idea and is worth pursuing further.

Conclusion

The objective of this study was to evaluate different re-use protocols for the funnels used in water quality testing. The proportion of positive blank tests during both laboratory and fieldwork when alcohol wipes were used to decontaminate funnels for re-use was significantly higher than those achieved in the MICS programs published in 2019 (7% and 23% for laboratory and field respectively, compared to 1% for MICS). Therefore, although it is worthwhile to study the idea further, we recommend that funnels not be decontaminated via alcohol wipe for re-use in MICS surveys unless blank test results can be improved. Given the reductions in cost and waste, as well as the logistical difficulties presented in the transport, distribution, and disposal of the funnels as they are currently used, we recommended that further work be conducted to either find a way to re-use the current funnels, or switch to using a funnel designed for re-use. Such work would support a meaningful reduction in plastic waste by MICS surveys and would simultaneously support SDGs 6.1 (drinking water for all) and 12.5 (reduce waste generation). Autoclaving the funnels for re-use is feasible, provided that there is capacity to re-package and redistribute funnels in a sterile manner.

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Supplemental material

Supplemental material for this article is available online.

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