

1 Evaluating Four Measures of Water Quality in Clay Pots and Plastic Safe Storage Containers in Kenya

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12

13 Abstract

14 Household water treatment with chlorine can improve microbiological quality and reduce diarrhea.

15 Chlorination is typically assessed using free chlorine residual (FCR), with a lower acceptable limit of 0.2

16 mg/L, however, accurate measurement of FCR is challenging with turbid water. To compare potential

17 measures of adherence to treatment and water quality, we chlorinated recently-collected water in rural

18 Kenyan households and measured total chlorine residual (TCR), FCR, oxidation reduction potential

19 (ORP), and *E. coli* concentration over 72 h in clay and plastic containers. Results showed that 1) ORP

20 served as a useful proxy for chlorination in plastic containers up to 24 hours; 2) most stored water

21 samples disinfected by chlorination remained significantly less contaminated than source water for up

22 to 72 hours, even in the absence of FCR; 3) TCR may be a useful proxy indicator of microbiologic water

23 quality because it confirms previous chlorination and is associated with a lower risk of *E. coli*

24 contamination compared to untreated source water; and 4) chlorination is more effective in plastic than  
25 clay containers presumably because of lower chlorine demand in plastic.

26

## 27 1. Introduction

28 Despite substantial gains in access to improved drinking water sources worldwide since the  
29 Millennium Development Goals were developed and implemented, an estimated 663 million people still  
30 rely on unimproved water sources (UNICEF and WHO 2015). An additional estimated 1.2 billion people  
31 obtain drinking water from improved, but contaminated, water sources. Thus, an estimated 1.8 billion  
32 people lack access to safe water (Onda et al. 2013). Consumption of fecally-contaminated drinking water  
33 is a leading cause of the approximately 502,000 diarrheal deaths worldwide each year (Pruss-Ustun et al.  
34 2014).

35 Chlorination is one of the most widely used, practical, and inexpensive forms of household  
36 water treatment to quickly inactivate most waterborne disease-causing bacteria and viruses (Rosa and  
37 Clasen 2010). In developing countries, liquid (e.g., sodium hypochlorite solutions) and powdered or solid  
38 (e.g., calcium hypochlorite or sodium dichloroisocyanurate) sources of free chlorine are used to disinfect  
39 household drinking water and, in a number of studies, chlorination has been shown to reduce the risk of  
40 diarrheal disease (Arnold and Colford 2007, Clasen et al. 2015).

41 *Escherichia coli* (*E. coli*) is used as an indicator of the microbiologic quality of water (Edberg et al.  
42 2000). However, *E. coli* is difficult to measure in the field and other measureable water characteristics  
43 can be used as indicators of adherence to water chlorination recommendations, serving as proxies for  
44 microbiologic water quality (CDC 2014, OECD and WHO 2003, Crump et al. 2004). Following addition of  
45 chlorine to water, reactions occur that result in free chlorine species and combined chlorine species; the  
46 sum of these two is termed total chlorine. Free chlorine residual (FCR) is the most common measure  
47 used because it indicates the most effective species of chlorine for disinfection. Total chlorine residual

48 (TCR) is less frequently used as a water quality measure because it also detects combined chlorine  
49 species, which are much less effective for disinfection. Oxidation reduction potential (ORP) is another  
50 water chemistry parameter increasingly used in water distribution systems (Hall et al. 2007) and  
51 swimming pools (Kebabjian 1995). ORP is a measure of the tendency of oxidants (e.g., chlorine species)  
52 to be reduced and it therefore provides an indication of the disinfection capacity of the water.

53         The World Health Organization (WHO) recommends that FCR in treated water should not fall  
54 below 0.2 mg/L (WHO 2011). For treating water in the home, WHO recommends dosing clear water (<10  
55 Nephelometric Turbidity Units [NTU] turbidity) at 2 mg/L FCR and turbid water (>10 NTU) at 4 mg/L FCR  
56 in order to maintain a FCR of 0.2 mg/L for 24 h after treatment (WHO 2011, Lantagne et al. 2010). Many  
57 studies of household water chlorination rely on a combination of self-reported use of chlorine and FCR  
58 field tests that utilize N,N-diethyl-p-phenylenediamine (DPD) to confirm water treatment. In these  
59 studies, discrepancies between reported and confirmed chlorination have been common (Blanton et al.  
60 2010, DuBois et al. 2006, Gupta et al. 2007, Luby et al. 2008). Potential causes of these discrepancies  
61 include: 1) reliance on water sources with a high content of organic material that rapidly consumes  
62 chlorine (i.e., exerts chlorine demand) (Lantagne 2008); 2) use of clay pots, which are culturally  
63 preferred because they lower water temperature through evaporative cooling, but can exert chlorine  
64 demand (Null and Lantagne 2012, Ogutu et al. 2001); 3) use of wide-mouthed storage containers which  
65 facilitate insertion of hands or other objects that could add organic material and decrease FCR (Wright  
66 et al. 2004); 4) storage of water for periods exceeding 24 hours, a common practice in regions in which  
67 water is scarce or water sources are located far from homes, during which time FCR naturally decays  
68 (Lantagne 2008, Briere et al. 2012, Colindres et al. 2008) and; 5) courtesy, or social desirability, bias, in  
69 which interviewees provide responses to water treatment questions that they believe interviewers  
70 expect, resulting in over-reporting of water treatment (Briere et al. 2012, Luoto et al. 2011).

71 The “real world” problems of turbidity, proper dosing, type of storage container used, time of  
72 storage, and reliance on self-reported water treatment complicate the ability of household water  
73 chlorination program staff to evaluate: 1) whether water has been treated and 2) the effectiveness of  
74 treatment. Simple methods that are feasible for field use are needed to confirm whether, in the absence  
75 of detectable FCR, water was chlorinated and whether this treatment improved water quality. To  
76 address these problems, we conducted a household-based study in western Kenya in which we analyzed  
77 four measures of water quality at five time points in both clay pots, the most commonly used water  
78 storage container (ranging from 62-92% of households) (Blanton et al. 2010, Garrett et al. 2008, O'Reilly  
79 et al. 2008, Parker et al. 2006), and plastic safe storage containers. In particular, we attempted to  
80 determine whether ORP offered advantages over TCR and FCR as confirmatory measures of chlorination,  
81 using *E. coli* concentration as the “gold standard” of disinfection effectiveness.

82

## 83 2. Materials and Methods

### 84 2.1. Study design

85 To assess changes in water quality over time in a real-world setting and to compare four measures  
86 of water quality in two types of water storage containers, we conducted a controlled crossover trial of 2  
87 randomly selected groups of households in western Kenya from August 27-October 19, 2012. In one  
88 group (Group A), water was chlorinated and stored in clay pots typically used for drinking water storage;  
89 in the other group (Group B), water was chlorinated and stored in a plastic safe storage container (Fig.  
90 1). Over the following 72 h, water quality tests were performed for both groups. After a two-week  
91 washout period, the container types were switched between the groups, and the process described  
92 above was performed (Fig. 2).

93

### 94 2.2. Study population

95           We selected a convenience sample of six rural villages in Kisumu County that relied on variety of  
96 community drinking water sources and household water storage. Households with the following  
97 characteristics were eligible to participate: had  $\geq$ one child  $<$ 5 years old; collected and transported  
98 drinking water in 10 liter (L) or 20 L containers (jerry cans or buckets); stored drinking water in a  $\geq$ 15 L  
99 ceramic pots (range 15-30 L) in the home; and were willing to use a plastic safe storage container to  
100 store drinking water for half of the study period and their own ceramic pot for the other half of the  
101 study. Households that did not store drinking water in ceramic pots with  $\geq$ 15 L capacity were excluded  
102 because of the likelihood that stored water would not last for more than one day.

103

### 104 2.3. Enrollment

105           In each of the 6 study villages, we obtained a list of all households with at least one child  $<$ 5  
106 years old from the village chief, or conducted a brief census to obtain the list of households. We then  
107 used a random numbers table to select a sample of households with children  $<$ 5 years old in each of the  
108 six communities. A total of 60 households were initially enrolled in the study. At the time of enrollment,  
109 respondents in households were interviewed about demographic characteristics, and water, sanitation,  
110 and hygiene practices. Electronic questionnaires were verbally administered in Dholuo, the local  
111 language, by trained Kenyan field research assistants.

112

### 113 2.4. Intervention

114           The 60 households were randomly allocated to two groups– Groups A (30 households) and B (30  
115 households) (Fig. 2). Group A households were asked to use their clay pots during the first half of the  
116 study while Group B households were provided a new, 60-L plastic safe storage container with a lid, tap,  
117 and stand.

118

119 2.5. Phase 1

120           Households were contacted in advance and requested to fill their water collection containers (in  
121 most cases, 20-L jerry cans) using water from their usual drinking water source on the morning of the  
122 first home visit and to keep it in the transport containers. During the first home visit, investigators  
123 collected Time 0 (“pre-dose”) water samples by pouring water directly from the transport containers  
124 into test vials and sample bottles.

125           To assess water quality, three water quality and treatment measures were performed using  
126 portable field meters in the home. Water samples collected into 10-mL glass vials were tested for TCR  
127 (mg/L) and FCR (mg/L) (Hach® Pocket Colorimeter™ II, Loveland, CO, USA); water samples collected into  
128 50-mL polypropylene conical tubes were tested for ORP (mV) (Oakton® Waterproof ORPTestr® 10,  
129 Vernon Hills, IL, USA). Additionally, a 100-mL sample was collected in a WhirlPak™ bag containing  
130 sodium thiosulfate, stored on ice, and transported to the laboratory within 4-6 hours of collection for *E.*  
131 *coli* quantification (CFU/100 mL) using membrane filtration (0.45 µM, 47 mm filters) with m-ColiBlue24®  
132 media (Hach®, Loveland, CO, USA). In some cases, because of exceedingly slow filtration rates of water  
133 samples due to high turbidity, we limited the volume of filtrate to 20 or 50 mL of sample and multiplied  
134 positive results by the appropriate proportion factor; samples with no growth were reported as non-  
135 detectable for *E. coli*.

136           In addition, because physicochemical parameters can influence chlorine residuals and other  
137 water quality measures, we also tested samples collected in 50-mL polypropylene conical tubes for the  
138 four following physicochemical parameters: turbidity (NTU) (Hach® 2100Q Portable Turbidimeter,  
139 Loveland, CO, USA), temperature (°C), electrical conductivity (µs/cm), and total dissolved solids (mg/L)  
140 (Oakton® Waterproof Multiparameter PCS Tester 35).

141           In the presence of the head of household, investigators then treated each water transport vessel  
142 with the proper dose of WaterGuard™, a familiar, locally available water treatment product containing

143 1.25% sodium hypochlorite solution. The dose was based on turbidity and the volume of water in the  
144 jerry can; water with turbidity <10 NTU was dosed with a single 3 mL dose of WaterGuard per 20 L and  
145 water with turbidity >10 NTU was dosed with a double dose of 6 mL of WaterGuard per 20 L. Treated  
146 water was then poured into either the household's empty ceramic pot (Group A) or the new plastic safe  
147 storage container (Group B).

148         After 30 min, water samples were collected and tested, as described above. Because the size  
149 and weight of clay storage pots precluded pouring water samples, each head of household was asked to  
150 wash a cup or ladle that was normally used to obtain water so that water samples could be collected;  
151 water samples from the improved plastic storage containers were taken directly from the tap. Heads of  
152 households were asked not to retreat the water or refill the container unless it was completely emptied  
153 out.

154         The household was revisited at 24, 48, and 72 h for a short-follow-up interview about water  
155 addition or treatment since the previous visit, followed by collection and testing of water samples, as  
156 described above. If respondents reported that water or additional disinfectant had been added to the  
157 storage container since the previous visit, they were excluded from the remainder of this phase of the  
158 study.

159

## 160 2.6. Phase 2 (cross-over)

161         The crossover period of the project began after a 2-week washout period. Households were  
162 again contacted in advance and requested to fill their transport containers using water from their usual  
163 drinking water source on the morning of the first home visit and to keep it in the transport containers.  
164 Households in Group A were provided with a plastic safe storage container with a lid, tap, and stand;  
165 Group B households were asked to resume using their ceramic pots for water storage. Water treatment  
166 and testing proceeded in a fashion identical to phase 1. At the conclusion of phase 2, all households

167 were allowed to keep the plastic safe storage containers, stands, and a bottle of WaterGuard for  
168 participation in the study.

169

## 170 2.7. Data analysis

171 Interview data were entered into personal digital assistants (PDAs) and uploaded into an Access  
172 (Microsoft, Redmond, WA, USA) database. Chemical and microbial data were collected on hardcopy  
173 forms, entered into an Excel database, and analyzed with SAS® 9.3 software (Cary, NC, USA). TCR, FCR,  
174 turbidity, and *E. coli* concentration had skewed distributions and were categorized according to the  
175 following metrics. For descriptive purposes, *E. coli* was categorized according to WHO risk thresholds as  
176 non-detectable or 1-10, 11-100, or >100 CFU/100 mL (WHO 1997). Since WHO guidelines and public  
177 health interventions are aimed at complete removal of *E. coli*, we further categorized data into a  
178 dichotomous presence/absence for modeling. FCR was categorized as <0.2 or ≥0.2 mg/L, as this is the  
179 minimum recommended concentration by the WHO Guidelines for Drinking Water Quality for  
180 infrastructure treated water (WHO 2011). TCR was similarly categorized as <0.2 or ≥0.2 mg/L based on  
181 previous research that utilized this strategy to assess chlorine treatment efficacy and storage time in  
182 ceramic pots (Null and Lantagne 2012). Water samples were categorized as turbid when turbidity  
183 measures were ≥10 NTU, in reference to chlorine dosing recommendation for turbid water. The primary  
184 outcomes of interest were TCR, FCR, ORP, and *E. coli*.

185 To investigate water quality differences in clay pots and plastic safe storage containers across  
186 the five time intervals, two-way within-subjects random effects models were constructed; logistic  
187 regression models for the outcomes TCR, FCR, and *E. coli* and linear regression for ORP. Interaction  
188 terms for storage container and time interval were significant for all four primary outcomes (TCR, FCR,  
189 ORP, and *E. coli*). For results stratified by water storage container type, we present estimates from  
190 separate repeated measures models for binary outcomes using generalized estimating equations (GEE)



191 and an autoregressive correlation structure. Odds Ratios (OR) and 95% Confidence Intervals (CI)  
192 computed from robust standard error estimates are reported. ORP mean differences are computed  
193 from random effects linear regression models and Tukey adjusted p-values are reported. All models  
194 adjusted for turbidity.

195

## 196 2.8. Ethical considerations

197 The study protocol was approved by the Ethical Review Committee of the Kenya Medical  
198 Research Institute (protocol 2324) and the Institutional Review Board of the Centers for Disease Control  
199 and Prevention (protocol 6313). Written informed consent was obtained from all participants. Data  
200 were maintained in an encrypted file in a password-protected computer. Personal identifiers were  
201 destroyed after all data were collected.

202

## 203 3. Results

### 204 3.1. Demographic characteristics and baseline water, sanitation, and hygiene practices

205 A total of 60 respondents were enrolled in the study. Five households were excluded from the  
206 study because respondents weren't available for one or more of the intervention phases; ultimately 25  
207 respondents remained in Group A and 30 respondents comprised Group B. The median age across  
208 participating respondents was 27 (range 17-55) and all were women. Fewer than half (n=23) had less  
209 than a complete primary school education and only one, in Group B, had electricity. The majority (85%)  
210 of study households used improved water sources and 60% of respondents reported that they treated  
211 water stored in their homes. Of 32 households that reported treating their water, 24 (75%) reported  
212 using WaterGuard; 2 (6%) reporting using other chlorine-based products, 5 (16%) reported boiling, and  
213 12 (38%) reported using a cloth to filter water. Fewer than half (47%) of households had an improved

214 sanitation facility. Soap was present in 93% of households and 56% of respondents were able to  
215 demonstrate proper handwashing technique.

216

### 217 3.2. Water testing: clay pots

218 Water sources used for dosing experiments in clay pots included rain (40%), surface water  
219 (24%), springs (18%), piped networks (16%), and ground water (2%). The median turbidity of water  
220 samples in clay pots at Time 0 was 37 NTU (range 0-300 NTU) (Table 1). Turbidity measures did not vary  
221 widely over the 72 h testing period. Median TCR and FCR values at Time 0 were 0.1 mg/L; over three-  
222 fourths of samples were <0.2 mg/L for both TCR and FCR. The median ORP was 393 mV (range 196-597  
223 mV). At Time 0, 83% of water samples were contaminated with *E. coli*. Water had a median pH 6.8, 25°C  
224 temperature, electrical conductivity of 106  $\mu\text{s}/\text{cm}$ , and 78 mg/L total dissolved solids; these median  
225 measures did not vary widely over the 72 h testing period.

226 Thirty minutes after chlorination (Time 0.5 hr), median TCR and FCR levels increased to 1.2 and  
227 0.9 mg/L, respectively (Table 1). Median ORP increased to 541 mV (range 392-757 mV), with 93% of  
228 samples increasing by >10% of the time 0 value. *E. coli* were non-detectable in 83% of samples. By 24 h,  
229 FCR was <0.2 mg/L in 61% of samples and TCR was <0.2 mg/L in 31% of samples. Approximately 40% of  
230 samples had ORP values 10% of the time 0 value. *E. coli* were non-detectable in 74% of samples. At 48 h,  
231 51% of TCR and 67% of FCR values were <0.2 mg/L and the median ORP measurement decreased to  
232 slightly lower than the time 0 value. The percentage of samples with non-detectable *E. coli* decreased to  
233 48%. By 72 h, median TCR was 0.2 mg/L and FCR was 0.1 mg/L; 35% of samples had no detectable *E. coli*.

234 Compared to Time 0 values and adjusted for turbidity, water treated with the recommended  
235 amount of chlorine and stored in clay pots was significantly less likely to contain *E. coli* for up to 48 h  
236 (Table 2). Although FCR levels were significantly more likely to be >0.2 mg/L at 30 min than at Time 0, by  
237 24 h FCR was significantly more likely to have fallen below the threshold of 0.2 mg/L. However, TCR

238 levels were less likely to have fallen below the 0.2 mg/L threshold over the entire 72 h time period than  
239 at baseline. At 30 min and 24 h post treatment, ORP was significantly higher than at Time 0. By 48 h,  
240 ORP values were not significantly different than at Time 0.

241

### 242 3.3. Water testing: plastic safe storage containers

243 Source water used for dosing experiments in plastic safe storage containers include rain (44%),  
244 surface water (22%), springs (18%), piped networks (13%), and ground water (4%). The median turbidity  
245 of water samples in plastic containers at time 0 was 28 NTU (range 0-300 NTU) (Table 3). Turbidity  
246 measures did not vary widely over the 72 h testing period. At Time 0, median TCR and FCR were 0.1  
247 mg/L and <0.1 mg/L, respectively, with over three-fourths of samples <0.2 mg/L for both TCR and FCR.  
248 The median ORP was 387 mV (range 252-556 mV). At Time 0, 87% of water samples were contaminated  
249 with *E. coli*. Water had a median pH 7.2, 24°C temperature, electrical conductivity of 104 µs/cm, and 69  
250 mg/L total dissolved solids; these median measures did not vary widely over the 72 h testing period.

251 Thirty minutes after chlorination, median TCR and FCR levels increased to 1.3 and 0.8 mg/L,  
252 respectively (Table 3). Median ORP increased to 541 mV (range 392-747 mV), with 89% of samples  
253 increasing by >10% of the Time 0 value. *E. coli* were non-detectable in 91% of samples. By 24 h, median  
254 FCR decreased to 0.3 mg/L, median TCR was 0.7 mg/L, and 15% of water samples had fallen to within  
255 10% of the time 0 ORP value. *E. coli* remained non-detectable in 85% of samples. At 48 h, 17% of TCR  
256 and 33% of FCR values were <0.2 mg/L and the median ORP measurement remained higher than the  
257 Time 0 value. *E. coli* were non-detectable in 90% of samples. By 72 h, median TCR was 0.6 mg/L and FCR  
258 was 0.3 mg/L, the median ORP value was higher than at Time 0, and 80% of samples had no detectable  
259 *E. coli*.

260 When compared with Time 0 values and adjusted for turbidity, water treated with the  
261 recommended amount of chlorine and stored in a plastic safe storage containers was significantly less

262 likely to contain *E. coli* across all time points, indicating a protective effect for up to 72 h (Table 4). Both  
263 FCR and TCR levels were significantly less likely to be <0.2 mg/L than the Time 0 values over the entire  
264 72 h time period. Up through 24 h, mean ORP was significantly higher than Time 0 ORP values, however,  
265 by 48 h ORP values were not significantly different than Time 0 values.

266

### 267 3.4. Comparison of clay pots and plastic safe storage containers

268 Using two-way random effects models and adjusting for turbidity, we assessed differences in  
269 water storage containers and time points for TCR, FCR, and *E. coli*. Water container type was a  
270 statistically significant effect modifier for time interval, thus we present results stratified by either water  
271 container or time interval. There were statistically significant differences between clay pots and plastic  
272 safe storage containers for TCR, FCR, and *E. coli* at 48 and 72 h. Despite no differences in water quality  
273 measures between storage containers at pre-treatment, 30 mins and 24 h, the odds of having a positive  
274 *E. coli* result were greater in clay pots compared to plastic containers at 48 ( $p=0.0002$ ) and 72 h  
275 ( $p=0.0004$ ). The odds of having TCR <0.2 mg/L were significantly greater in clay pots than plastic  
276 containers at 24 ( $p=0.0199$ ), 48 ( $p=0.0023$ ), and 72 h ( $p=0.0061$ ); likewise, the odds of having FCR <0.2  
277 mg/L were significantly greater in clay pots than plastic containers at 24 ( $p=0.0370$ ), 48 ( $p=0.0014$ ), and  
278 72 h ( $p=0.0245$ ) (Table 5).

279 If TCR or FCR was  $\geq 0.2$  mg/L in stored water, regardless of container type or time, there was a  
280 decreased likelihood that *E. coli* was present. This association was stronger for TCR  $\geq 0.2$  in plastic  
281 containers (OR 0.08, 95% confidence interval [CI] 0.04-0.16) than in clay pots (OR 0.44, 95% CI 0.27-  
282 0.75); likewise, this association was stronger for FCR  $\geq 0.2$  in plastic containers (OR 0.25, 95% CI 0.14-  
283 0.44) than in clay pots (OR 0.43, 95% CI 0.26-0.69).

284

## 285 4. Discussion

286 To our knowledge, this is the first study in which a controlled chlorination experiment at the  
287 household level tested four water quality measures, including ORP, for a period of up to 72 hours. This  
288 evaluation yielded several key findings. First, ORP served as a reasonable proxy for chlorination in plastic  
289 containers up to 24 hours, but was not a good proxy after 24 hours as ORP decreased to near pre-  
290 treatment levels. ORP was also not a good proxy in clay pots because the level was not significantly  
291 different at 24 hours than pre-treatment. The ease of ORP measurement using a probe and without a  
292 need for reagents offers the advantage of convenience, while the main disadvantage is the initial  
293 investment in the ORP meter. Second, chlorinating various types of source waters at recommended  
294 doses resulted in a statistically significant increase in the percentage of stored water samples that had  
295 no detectable *E. coli* for up to 72 hours, even as FCR fell below the recommended minimum  
296 concentration of 0.2 mg/L and ORP decreased to pre-treatment levels. Third, as expected, TCR persisted  
297 above 0.2 mg/L over a longer period than FCR. There was a statistically significant association between  
298 TCR values  $\geq 0.2$  mg/L and non-detectable *E. coli* in stored water, which presents the possibility of TCR  
299 serving as a useful proxy measure of water quality.

300 This evaluation also demonstrated that chlorination at the recommended dose was more  
301 effective at eliminating detectable *E. coli* for up to 72 hours in plastic safe storage containers than in  
302 traditional clay pots, even when adjusting for source water turbidity. This finding most likely occurred  
303 because FCR was significantly more likely to persist at higher concentrations over time in plastic versus  
304 clay containers. These findings are expected, consistent with other studies (Ogutu et al. 2001, Quick et  
305 al. 1996), and plausible because clay pots often have organic materials on the surface that exert chlorine  
306 demand and facilitate biofilm growth (Murphy et al. 2009). In addition, clay pots have wide mouths,  
307 which permit the insertion of hands or other objects potentially increasing chlorine demand and the risk  
308 of recontamination. In this study, by testing water stored in clay pots that had been in use in households  
309 rather than new clay pots, chlorine demand in the pots may have been greater and likely to decrease

310 FCR levels at a faster rate than in new pots, thereby possibly biasing results toward the null. However, at  
311 least one study has shown no difference in chlorine behavior between new and used clay pots (Ogotu et  
312 al. 2001). In addition, the evaluation of water storage in used clay pots more accurately represents  
313 actual household circumstances. One caveat to this finding is that we used new plastic safe storage  
314 containers that initially would have been free of biofilm, so their performance might decline over longer  
315 periods of use as biofilm formed on the inner surface (UNICEF and WHO 2015, Arnold and Colford 2007,  
316 Jagals et al. 2003). Further study is needed to evaluate this possibility.

317       ORP proved to be a poor proxy of drinking water disinfection after 24 hours because, although  
318 ORP is increasingly used to monitor disinfection capacity of water in distribution systems and swimming  
319 pools, a higher concentration of chlorine is often used in those systems (i.e., FCR 1-3 mg/L) than in  
320 stored drinking water, resulting in higher post-treatment OPR values. When treating water for human  
321 consumption, palatability is an important consideration, and chlorine concentrations that would results  
322 in elevated ORP for greater than 24 hours, such as those used in treatment facilities or swimming pools,  
323 would be unpalatable in drinking water stored in household containers. For ORP to meet its potential as  
324 a field measurement of effective household water treatment over periods <24 hours guidelines would  
325 need to be developed for interpretation of measures.

326       The practical importance of the above findings can be appreciated when considering other  
327 studies of chlorination in which reported rates of chlorine use were high but measured FCR in water  
328 samples were low (Colindres et al. 2007, Lantagne and Clasen 2012, Harshfield et al. 2012, Mong et al.  
329 2001). In those studies, it was not possible to determine whether the high reported rates were a result  
330 of social desirability or courtesy bias in which water treatment was not actually performed, or a result of  
331 a poor indicator (i.e., FCR) for turbid water treated with hypochlorite, for water treated with  
332 hypochlorite >24 hours before testing, or both. Findings of this study suggest that, because TCR persists  
333 longer than FCR in stored water, it may serve as a better proxy measure for adherence to recommended

334 treatment with sodium hypochlorite. Additionally, the statistically significant association between TCR  
335  $\geq 0.2$  mg/L and non-detectable *E. coli* in stored water suggests that TCR may also serve as a rough,  
336 though imperfect, proxy measure for water quality. While not completely free of *E. coli* contamination,  
337 water remained improved up to 72 hours as compared with its pre-dose quality. Recent research found  
338 a positive association between the risk of child diarrhea and increasing *E. coli* concentration in drinking  
339 water; the dose-response relationship observed suggested that even modest improvements in water  
340 quality can provide a health benefit (Luby et al. 2015). However, TCR would be a less reliable proxy  
341 measure of water quality in populations that use clay pots for water storage, particularly if the water  
342 were stored over a period of several days before being replenished. Populations that prefer clay pots  
343 because of evaporative cooling of stored water would likely be difficult to motivate to switch to plastic  
344 water storage containers. In this case, chlorination promotion campaigns would need to take into  
345 account the properties of clay pots, particularly those with wide mouths that permit the introduction of  
346 hands or other objects, and recommend daily treatment of stored water.

347         This study had several important limitations. First, we cannot be certain that households did not  
348 chlorinate water before our first visit or between visits over the 72 hour study period, even though we  
349 requested that they not do so. If water had been chlorinated between visits, or non-chlorinated water  
350 had been added to containers, then our data would not provide an accurate representation of the  
351 behavior of chlorine, ORP, or *E. coli* over time. The steady decrease of TCR and FCR that we observed  
352 over time during both study periods suggest that the population adhered to our request. Second, TCR  
353 and FCR were detected in some source waters (primarily surface, rain, and spring water); this finding  
354 might be related to false positive results related to DPD interference from chemicals present in water  
355 and warrants further research. Third, during both study periods, there was attrition in the number of  
356 households at each visit as participants used up the water that had been placed in their containers  
357 before Time 0 (pre-dosing), which decreased the precision of our findings. Fourth, because clay pots are

358 cumbersome and heavy, we were not able to directly sample stored water but instead relied on the use  
359 of a ladle or cup. While we observed household members washing these collection vessels before  
360 sampling, we cannot be certain of the effect they had on water quality. Finally, this study was conducted  
361 in a limited geographical area and is not representative of the larger Kenyan population, or other  
362 populations. Although the findings were consistent with known behavior of residual chlorine in stored  
363 water and *E. coli* exposed to chlorine, further study in other populations would help determine how  
364 broadly applicable our findings are.

365

## 366 5. Conclusions

- 367 • ORP may be a useful proxy to confirm chlorination for periods up to 24 hours in plastic  
368 containers, but further study is needed to verify its utility.
- 369 • Most stored water samples disinfected by chlorination remained significantly less contaminated  
370 than source water for up to 72 hours, even in the absence of FCR.
- 371 • TCR may be a useful proxy indicator of microbiologic water quality because it confirms previous  
372 chlorination and is associated with a lower risk of *E. coli* contamination compared to untreated  
373 source water.
- 374 • Chlorination is more effective in plastic than clay containers presumably because of lower  
375 chlorine demand in plastic.

376

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388

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