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Original research and field-related article

Application of two field methods for monitoring microbiological water quality in a polluted tropical environment - A pilot study

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Abstract: (1) Background: Emergencies confront civilian and military healthcare providers with medical and hygienic challenges due to the lack of potable water. This pilot-study aimed to describe the application of two different methods for microbiological monitoring of water in a harsh environment in terms of performance, ease of use, availability, and the possibility of using the results to evaluate water quality. (2) Methods: Samples from raw water, Potable water, and water for consumers were taken from two different camps with the same raw water source. The samples were analyzed by using IDEXX industry-standard methods (Colilert and Enterolert enzymatic test kits) and a combination of membrane filtration and 3M-Petrifilm. (3) Results: The IDEXX method used at the Norwegian Camp are easier to utilize and has a broader range of analyzing kits for drinking water analysis. In addition, IDEXX is better adapted to the requirements of the national legislation. However, the combination of membrane filtration followed by incubation on 3M-Petrifilm ™, as used at the Swedish camp, is a better field alternative compared to traditional bacteriology, as it eliminates the need to produce and store agar plates. (4) Conclusions: This pilot study highlights the need for adapted technical equipment and tools for internal microbiological control of water production in a harsh field environment and may facilitate the use of a relatively simple method for water control and ensure the safety of deployed staff in both civilian and military settings.

Keywords: Austere; Healthcare; Microbiological; Military; Safety; Water.

1. Introduction

Public health emergencies, disasters, and armed conflicts are associated with environmental challenges, of which poor water quality and sanitation might constitute the most critical threats to health [1-3]. Several factors influence the increasing number of water-related emergencies, of which unplanned urbanization, degradation of ecosystem services, and the scarcity of water resources might be the most important [4-8].

Since water supply systems, purification, disinfection, and monitoring processes are inferior in poor or disaster-affected nations [7,9-11], deployed staff on international missions and travelers to wilderness and recreational areas need to know how safe water can be produced and controlled. There are several field methods for water treatment, like heat, ultraviolet light, filtration, and chemical disinfection [8]. However, purification also depends on the microorganisms' type, susceptibility to a specific treatment, host factors, and

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available resources [5,9-10]. Impure water and waterborne diseases are critical risks to the health of both civilian and military service persons and should be mitigated by using suitable water purification and water control methods. [2,4-5,7,9-11].

Previous quantitative and qualitative studies of membrane filtration (MF)/petrifilm™ and IDEXX, a different enzymatic test kit using the MPN (Most Probable Number) principle (e.g., Colilert) — two commonly used methods for microbiological water control — indicate that both methods could be well suited for field application, given the satisfactory conditions. Macy and co-authors reviewed 14 studies comparing Colilert with traditional methods, including MF, regarding coliforms and Escherichia coli (E. coli) and obtained satisfactory consensus, preferably when used in the tropical environment [12]. The same study also compared IDEXX with MF regarding the analysis of coliforms/E. Coli. The results were consistent, but the authors emphasized that accurate results from MF might require higher levels of technical skill and quality control than what might be available in laboratories in developing countries [12]. In a 3-year follow-up study on the natural water, with seasonal variation, repeated analysis of coliforms and E. Coli was made. Based on the results, the authors suggested that Colilert DST (Defined Substrate Technology) gave a more straightforward laboratory protocol, was quicker to process, and was easier to quantify than MF [13].

Another study evaluated total coliforms and E. coli in water samples. IDEXX Colilert 18 and 3M™ Petrifilm™ E. coli/Coliform Count Plates (Petrifilm™ EC plates) were compared against a reference method [14]. 3M Petrifilm performed worse than Colilert 18 for both positive and negative samples (82.7% vs. 70.6%). The authors found that the Petrifilm™ EC plates provide low sensitivity (39.5-52.5%) but high specificity (90.9-78.8%) [15]. The high specificity of 3M Petrifilm was also found in a study using 3M™ Petrifilm Aerobic Count Plates (Petrifilm™ AC plates) for heterotrophic counts and Petrifilm™ EC plates for fecal coliforms and E. coli. By confirming typical distinct colonies on each media type utilizing a golden standard procedure, the authors emphasized the high specificity of Petrifilm™ EC plates for enumeration of fecal coliforms and E. coli in water [16].

In a more recent study, IDEXX Pseudalert/Quanti-Tray was compared to a reference MF-based culture method for the enumeration of Pseudomonas aeruginosa in recreational pool waters [17]. The authors found the IDEXX method is an acceptable alternative to the reference membrane method. It has the advantage of not requiring confirmation testing and providing confirmed counts within 24 to 28 hours of incubation compared to 40 to 48 hours for the reference membrane method [18]. In another study analyzing natural recreational waters, IDEXX Colilert indicated higher values for both coliforms and E. coli compared to a standard method [19]. In contrast, the opposite was true for Enterolert. The authors emphasized that more studies are needed to evaluate the accuracy of the methods if they are to be used for routine monitoring of natural recreational water quality. Once more, the significant advantage of the rapid-test kit, according to the authors, was the short incubation time and high specificity [20].

In the present study, the application of 3M-Petrifilm™ [21], and IDEXX rapid tests [22], used by the Swedish and Norwegian personnel at two military camps in an environmentally harsh area outside Bamako in Mali, is described in terms of performance, ease of use, availability, and the possibility of using the results to evaluate water quality. Bamako represents a tropical area with a large variation in temperature and humidity and with a large amount of airborne particulate pollution including both biological and chemical content. These are environmental factors that might affect the performance of both 3M-Petrifilm™ and IDEXX, as well as the possibility to maintain safe drinking water. In addition, there may be shortcomings in handling fecal contamination from both animals and humans in Bamako, i.e., there might be a risk of fecal contamination of groundwater during the rainy season.

To the best of our knowledge, direct comparisons between membrane filtration combined with 3M-Petrifilm™ and IDEXX's enzyme detection tests have not been made earlier.

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2. Materials and Methods

2.1 Raw water, Potable water, and water for consumers

Utilizing the same raw water, the Swedish (SC) and Norwegian (NC) camps have been equipped with different water purification systems, self-monitoring tools, and methods for microbiological monitoring. SC uses three diverse microbiological barriers to purify water: reverse osmosis, ultraviolet radiation (UV), and finally, chlorination to a chlorine-free level of 0.2-0.5 mg/l. In NC, water is purified using two different disinfection steps: ultrafiltration followed by chlorination to a chlorine-free level of 1.5-1.7 mg/l. Table 1 illustrates each method's purification steps, control measures, and challenges method process [23-28].

Table 1: Overview of the water purification process, control measures, and main challenges with the microbiological methods used.

Camp	SC (Swedish Camp)	NC (Norwegian Camp)
Purification	P 1. Reverse osmosis [24] P 2. UV radiation P 3. Chlorination (level of free chlorine of 0.2-0.5 mg/l)	P 1. Ultrafiltration P 2. Chlorination (level of free chlorine of 1.5-1.7 mg/l).
Monitoring	C 1. Daily: measuring chemical & physical parameters, such as conductivity and chlorine concentration. C 2. Every two weeks, routine control by microbiological control of raw water, potable, and tap water using MF, followed by incubation at 22° C (molds) and 36° C (other tests) on a selective medium [21,25]. C 3. According to the Swedish National Food Agency regulations, yearly, once or twice, raw water, potable water, and tap water samples are sent to an accredited military laboratory for extended microbiological and chemical control [26].	C 1. Daily monitoring and control of chlorine content. C 2. Complement microbiological control every week on raw, potable, and bottled water, using the IDEXX rapid methods for drinking water analysis, followed by incubation [22]. The samples were quantified by the MPN method (Most Probable Number), which is the estimated number of colonies per ml for heterotrophic plate count (HPC) and the estimated number of colonies per 100 ml sample for the other tests. C 3. Yearly, once, or twice, raw water, potable water, and tap water samples are sent to an accredited civilian or military laboratory for extended microbiological and chemical control.
Comments (with ref to paragraphs above)	P 1. BlueBox 4000 RO [27]. C 1. The gradient of conductivity throughout the osmotic water purification process measures the filtration capacity. It thereby serves as an indirect control of the chemical and microbiological quality of the purified water. C 2. 3M-Petrifilm™ is a ready-to-use testing media containing nutrients for the microorganism to be cultured and an indicator that simplifies the reading of the colonies. Available analyzes at SC were quantification of the total number of heterotrophs, coliforms, yeast, and molds as well as Enterobacteriaceae by counting respective colonies (analysis of gram-negative Enterobacteriaceae is a parameter most often used in the study of food but not for drinking water according to the NFA regulations) [21].	P 1. Kärcher WTC 5000 UF [28]. C 1. The Kärcher UF unit, using a membrane pore size of about 0.02 μm, compared to approx. 0.0001 μm for a RO unit is considered to guarantee effective filtration of micro-parasites and bacteria, but not for all viruses. To compensate for the larger pore size, free active chlorine was increased from about 1.0 mg/l to 1.5–1.7 mg/l, i.e., above the recommended value (1.0 mg/l) in the NFA regulations [26]. P1-2 is preceded by softening of the water to reduce the risk of deposits in the pipe network. C 2. IDEXX is a rapid method for drinking water analysis based on enzyme detection techniques. If present, microorganisms produce an enzyme that metabolizes the substrate in the added nutrient indicator. Color change or fluorescence is considered a positive result.

2.2 Sampling procedures

Three different sampling points were selected at each camp, to be as similar as possible and in line with the definitions of “raw water” “potable water,” and “water for consumers” according to National Food Agency (NFA) regulations [26]. At SC, water samples were taken from the raw water tank, the chlorinated water, and the hygiene container” Ecolog,” respectively. At NC, water samples were taken from the natural water tank, the

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pure water tank in the kitchen (chlorinated), and the hygiene container" Camp Section" (chlorinated), respectively. The six diverse samples were analyzed using methods and available analysis equipment (Table 1). Using an aseptic technique, sampling was performed by adding thiosulphate in chlorinated water sampling bottles according to the ISO standard [23]. A short flush for a few seconds was made prior to sampling at the tapping point. The samples were prepared within one hour from the time of sampling. Free chlorine was measured with the HACHDR300 Pocket colorimeter.

2.3 Microbiological analysis

The laboratories at SC and NC are air-conditioned, i.e., the relative humidity and temperature are kept close to constant, even during the rainy season. All consumables were stored according to the manufacturer's recommendations.

At SC, cultivation was made with 3M Petrifilms™ [21]. The plates contain a water-soluble gelling agent, nutrients for the specific microorganism to be grown, and an indicator that simplifies the reading of the colonies. Quantification is made by counting the colonies manually or by utilizing plate-reading equipment. According to the product information, 3M Petrifilms™ are intended to analyze bottled water. Analysis of other types of water is under the responsibility of the performer [21, 29]. According to the Swedish supplier of 3M-Petrifilm, Triolab (personal communication), 3M Petrifilms™ used in this study, i.e., Aqua Coliform Count Plates (AQCC), Yeast and Mold Count Plates (AQYM), Heterotrophic Count Plates (AQHC) and Enterobacteriaceae Count Plates (AQEB) can be used for non-bottled drinking water.

One hour before sample preparation, 3M Petrifilms™ were moistened with 1 ml of sterile saline. MF was performed according to ISO standard [30] by filtering 100 ml of samples through a 0.45 µm MF-Millipore™ membrane, in which microorganisms with sizes exceeding pore size (bacteria and micro-fungi) stay on the filter. The filter was then placed on a moistened 3M Petrifilm™ and incubated as follows:

- For coliforms (AQCC) and Enterobacteriaceae (AQEB): 36° C for 24 hours.
- For heterotrophs (AQHC): 36° C for 48 hours.
- For micro fungal (AQYM): 22° C for 3-5 days.

Analysis of heterotrophs in raw water was made by filtration of 100, 50, and 10 ml of water, respectively, following the same filter treatment as above. No positive or negative controls were included. After incubation, the number of colonies was counted manually. For coliforms, all red colonies were counted, regardless of whether there were signs of flatulence or not. For Enterobacteriaceae, all red colonies with yellow surrounding zone, red colonies with gas bubbles, and red colonies with yellow zone and gas bubbles were counted. For heterotrophs, all red colonies were counted. For yeast and mold, colonies were differentiated and calculated in accordance with the product manual.

At NC, IDEXX Quanti-tray® was used. IDEXX provides a series of test kits to analyze microorganisms in water based on the detection of specific enzymes in different types of indicator organisms [22]. The tests are delivered as dried powders containing specific enzyme substrates, an indicator, and substances that will favor the growth of the particular bacteria. When bacteria containing the specific enzyme grows, the substrate breaks down, and the indicator is released, resulting in a change in color or fluorescence. Test trays for miniaturized cultivation in liquid medium are provided, containing several communicating cultivation wells so that the reagent mixture does not have to be added to each well. In this study, Enterolert™, Colilert™, and Pseudalert™ test trays were used.

Fast quantification of the result is obtained by utilizing a piece of reading equipment (Quanti-Tray™). Quanti-Tray™ assays were performed by pouring 100 ml of the sample into a 120 ml beaker. An ampoule with a nutrient indicator in powder form for Enterolert-DW, Colilert™, or Pseudalert™ was added. The mixture was poured into the tray for Quanti-Tray™, which was sealed and incubated. The incubation procedure follows below.

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- Coliforms and coli (Colilert™): 35° C for 24 hours.
- Enterococci (Enterolert-DW™): 41° C for 24 hours.
- For P. aeruginosa (Pseudalert™): 38° C for 24 hours.

After incubation, all Ouanti-Tray washers were read in 365 nm UV light concerning fluorescence, according to IDEXX's "51 Quanti-Tray™ MPN (Most Probable Number) Table". In Colilert™, coliforms were read in visible light (yellow) while E. coli fluoresces in UV light. All wells with indicator change, regardless of the degree of cover, were counted. The number was then converted according to IDEXX's "51 Quanti Tray MPN Table" into the corresponding MPN values, as indicated in the table in MPN per 100 ml.

For the total number of heterotrophs, SimPlate™ Unit Dose was used. The added reagent solution contains several different enzyme substrates, which produce a fluorescent color under UV light when metabolized by the water-borne bacteria. The analysis was performed by adding an ampoule reagent to 10 ml of water samples and then pouring the mixture into the center of the SimPlate HPC™ (Heterotrophic Plate Count) washer. The tray was covered, and the solution was distributed over the wells. The excess solution was emptied; whereafter, the tray was incubated at 35°C for 48 hours. Reading was made in 365 nm UV light. Wells that indicated fluorescence of some degree were counted and the number was converted to MPN according to the manufacturer's conversion table for" Unit Dose for SimPlate HPC." MPN reading is the estimated number of heterotrophs per ml. Results were re-calculated to MNP per 100 ml to provide a more intuitive picture of demonstrated levels of investigated organisms. MPN was rounded to the nearest integer. No negative or positive checks were carried out.

2.4 Reference values

The results from the samples were compared with each other and against the NFA (National Food Agency)'s regulations on drinking water, as shown below (Table 2) [26].

Table 2. Microbiological reference values for drinking water according to NFA (SLVFS 2001:30). Blank areas mean that there is no limit value for that parameter.

Parameters	Potable drinking water		Drinking water for consumers	
	Appropriate with remark	unfit for human consumption	Appropriate with remark	unfit for human consumption
Cultivable microorganisms at 22° C	10 CFU/ml*		100 CFU/ml	
Intestinal enterococci		Detected in 100 ml		Detected in 100 ml
Coliform bacteria	Detected in 100 ml	10 CFU/100 ml	Detected in 100 ml	10 CFU/100 ml
Escherichia coli		Detected in 100 ml		Detected in 100 ml
Clostridium perfringens			Detected in 100 ml	
Micro fungi			100 CFU/100 ml	
Actinomycete			100 CFU/100 ml	

* 10 CFU/ ml water applies for disinfected water.

3. Results

3.1 Chemical and physical parameters

At SC, the measured water temperatures at the sampling time were 29.0, 28.1, and 38.4 °C (raw water tank, chlorinated water, and hygiene container, respectively). The free, active chlorine concentration was 0.38 and 0.06 mg/l (potable water and hygiene container, respectively). At NC, the measured water temperature was 28.6, 29.0, and 28.0 °C (raw water tank, potable water, and hygiene container, respectively). The free, active chlorine concentration was 1.83 and 1.41 mg/l (pure water tank and hygiene container, respectively).

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3.2 Microbiological parameters

The results from the analysis of six samples taken on January 26th and 27th 2021 are presented in tables 3 and 4 as CFU (Colony Forming Unit) per 100 ml, except for heterotrophs in water taken at NC, that are presented in CFU per ml of water. TNTC (Too-Numerable-To Count) denotes either that the number was too large to count or that the maximum response rate for the test was reached. For raw water, using the MF method (SC), TNTC was reached for all sample volumes (100, 50, and 10 ml) (Figure 1).

Table 3. Result of 3M-Petrifilm™ for six samples taken on January 2021 Samples taken from hygiene containers (HYG) equals "water for consumers."

Camp		**SC**			**NC**	
	Raw water	Potable water	HYG Ecolog	Raw water	Potable water	HYG CampS
Test volume (ml)	100 (50/10)	100	100	100 (50/10)	100	100
Heterotrophs (HPC) (/100 ml), 36° C (48 h)	TNTC	0	0	TNTC	5	20
Coliforms (/100 ml), 36°C (24 h)	7	0	0	7	0	0
Enterobacteriaceae (/100 ml), 36° C (24 h)	4	0	0	6	0	0
Yeast & molds (/100 ml), 22° C (72 h)	35 yeast	0	0	7 yeasts	2 yeasts	1 yeast

Table 4. Result of IDEXX quick test for six samples taken on January 2021. Samples taken from hygiene containers (HYG) equals "water for consumers."For coliforms, both strong and weak positive wells are counted and added.

Camp		**SC**			**NC**	
	Raw water	Potable water	HYG Ecolog	Raw water	Potable water kitchen	HYG CampS
Test volume (ml)	100 (HPC 10 ml)	100	100	100 (HPC 10 ml)	100	100
Heterotrophs (HPC) (/ml), 36°C (48 h)	84 (TNTC)	0	0	84 (TNTC)	0	0
Coliforms (/100 ml), 36°C (24 h)	5+3	0	0	4+2	0	0
Enterococci (/100 ml), 41°C (24 h)	0	0	0	0	0	0
Pseudomonas (/100 ml), 38°C (24 h)	1	0	0	0	0	0

For heterotrophs in raw water at SC and NC, we noticed a maximum response rate (TNTC) on SimPlate™ HPC™, i.e., 84 positive wells (Figure 2 a), corresponding to a table value of $\geq$ 73.8 CFU/ml. If higher bacterial counts are to be quantified, the sample must be diluted, which was not done in this study. Raw water sample analysis from SC and NC with Enterolert™ and Colilert™ produced a "null" and "positive" result, respectively, resulting in a positive signal (yellow color) in six wells from the NC water sample. According to the MPN table, six positive wells correspond to 6.4 CFU/100 ml for coliform bacteria. The result for raw water at SC was eight wells with yellow color (Figure 2 b). According to the MPN table, eight positive wells correspond to 8.7 CFU/100 ml. None of the wells (SC + NC) fluoresced, indicating the samples were negative for E. coli. Sample

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analysis with Pseudalert™ gave a positive well from the raw water sample from SC (Figure 2 c), equivalent = 1.0 CFU / 100 ml. Other samples were negative. The total number of heterotrophs at 36°C and Pseudomonas are NFA parameters in evaluating bottled water. Although no sampling of bottled water was performed in the present study, heterotrophs were measured at both camps as they are seen as relevant indicators also for tap water and drinking water. In addition, Pseudomonas were measured at NC. It should be noted that the yearly reference sample of raw water at SC was taken on February 2nd. The result for Pseudomonas (CFU = 1) and Enterococci (CFU = 0) was identical with that measured with IDEXX five days earlier.

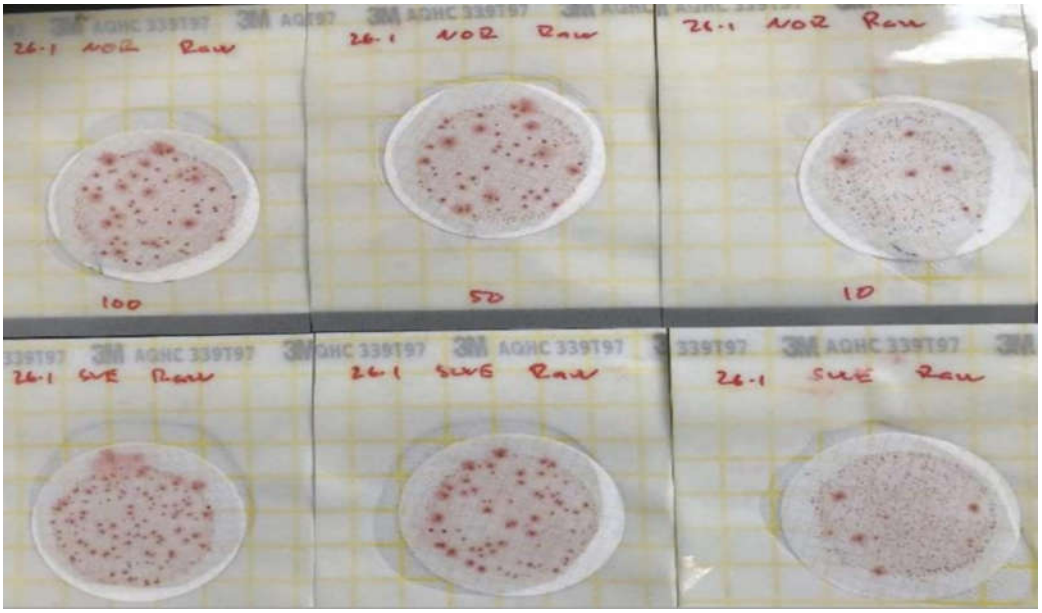

Figure 1. 3M-Petrifilm™ total number of heterotrophs in raw water at SC (lower part of the picture) + and NC (upper part of the picture) for 100, 50, and 10 ml, respectively.

Figure 2: A: SimPlate™ HPC raw water SC, **B** Colilert™ raw water SC, **C**. Pseudalert™ raw water SC.

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3.3 Temporal variation of coliforms in raw water at NC and SC

Routine samples of raw water from SC and NC are taken from the raw water tank at each camp, respectively, both water tanks being located close to the joint raw water source. In figure 3, available results from the analysis of coliforms in raw water samples taken during autumn 2021 at NC (brown line) and SC (blue line) are shown. Routine sampling at the SC and NC are made independently from each other; however, on one occasion, in week 42, sampling was made on the same date (CFU = 2 at SC, MPN = 0 at NC). As can be seen, both methods detect higher amounts of coliforms during weeks 31-34. In the sample taken during week 34 at NC, 22 of the 84 wells were fluorescent, indicating the presence of E. coli. Although data are too limited for statistical evaluation, there is a tendency towards higher counts of coliforms with the IDEXX method.

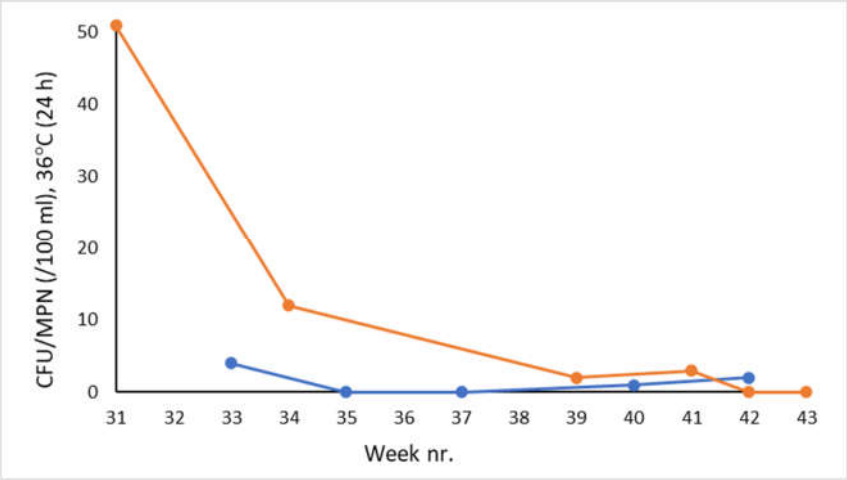

Figure 3: Temporal variation in coliforms per 100 ml raw water during week 31-43 at NC, using IDEXX (brown line) and SC, using 3M (blue line).

In figure 4, all available results on coliforms in raw water during 2021 from NC (brown stacks, 10 samples) and SC (blue stacks, 9 samples) are presented, including the double analysis taken for comparing IDEXX with 3M on identical water samples (January 26th and 27th). In addition, the result from the yearly taken reference analysis at SC (grey stack) on February 2nd is included (CFU = 2). For practical purposes, a maximum value of 15 has been set at the y-axis. The actual value on January 24th was 35 CFU, which was the highest value of coliforms measured in raw water at SC during 2021. The actual MPN value on August 3rd was 51 at NC. Taken over the entire year, there is a tendency toward higher counts of coliforms with the IDEXX method. However, due to the low number of samples and large temporal variation of coliforms in raw water, data are not suitable for statistical evaluation (mean and STDEV = 6,3 and 9,9 for 3M and 9,1 and 15,1 for IDEXX).

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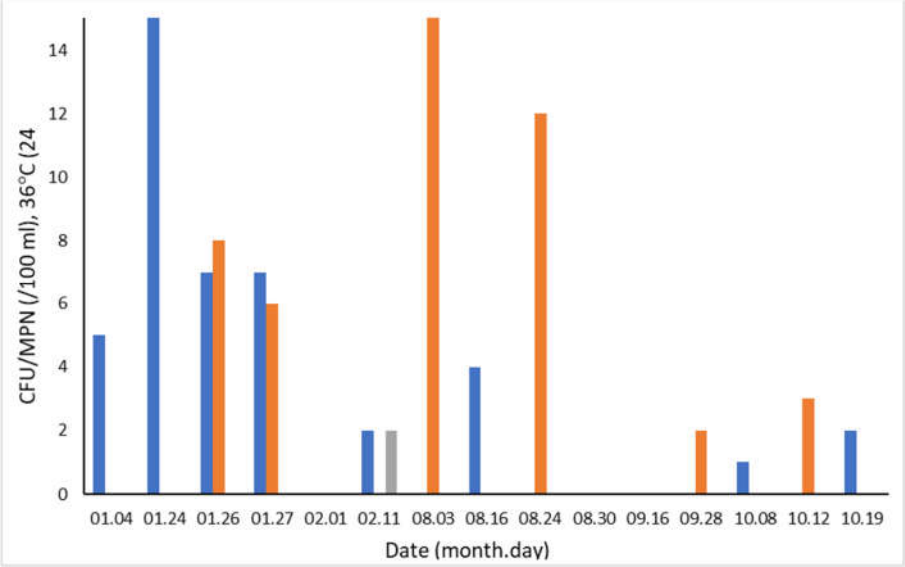

Figure 4: Temporal variation of coliforms per 100 ml raw water at NC (brown stacks) and SC (blue stacks). Reference analysis (grey stack) was made according to technical standard DIN EN ISO 9308-1:2017-09. Dates with no visible stack equals CFU/MPN = 0.

3.4 Temporal variation of other microorganisms in raw water at SC and NC

From samples taken during the rainy season (week 31 35 in figure 5), the number of Enterobacteriaceae (blue stack) and yeast/molds (green stack) in raw water at SC varied between 0 and 8 CFU (5 samples), and 5-40 CFU (5 samples), respectively. Compared to the four samples taken during January and February 2021, the number of Enterobacteriaceae and yeast/molds was not considered to be elevated during the rainy season. With a few exceptions, the number of heterotrophs in the samples taken during 2021 was TNTC.

The number of Enterococci (red stack) and Pseudomonas (yellow stack) in raw water at NC reached a peak during week 31-34, with MPN = 13-51 and 24-200 respectively. During week 39-43, four samples were analyzed with respect to Enterococci and Pseudomonas, all with MPN = 0, except for week 42 (MPN= 1 för Enterococci). With a few exceptions, the number of heterotrophs in the samples taken during 2021 was TNTC.

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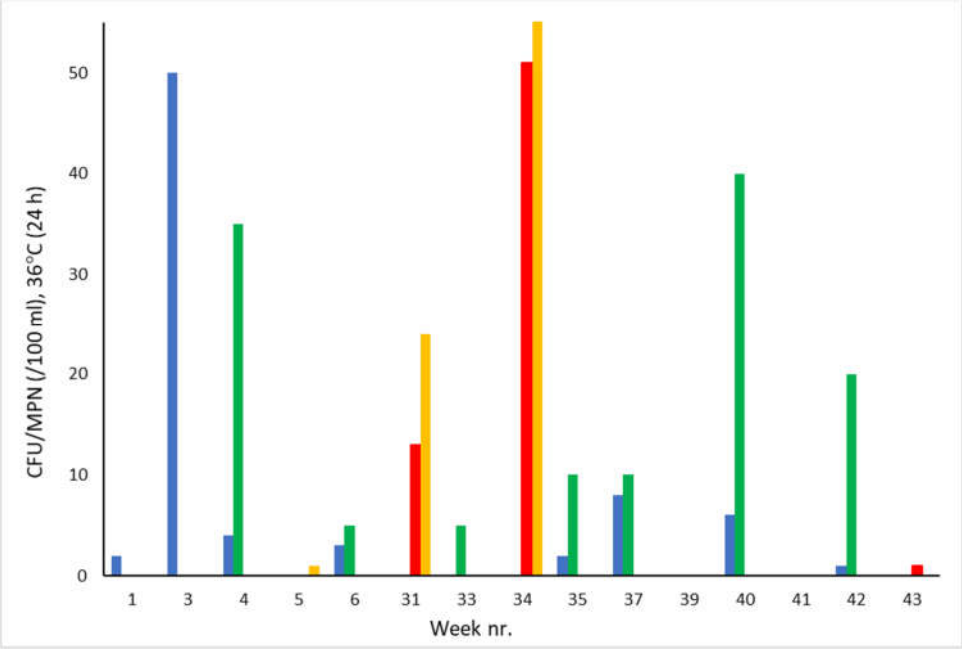

Figure 5: Temporal variation of Enterobacteriaceae (blue stack), yeast/molds (green stack) in raw water at SC and of Enterococci (red stack) and Pseudomonas (yellow stack) in raw water at NC during 2021. Weeks with no visible stack equals CFU/MPN = 0. For practical purposes, a maximum value of 55 has been set at the y-axis. The actual count for Pseudomonas in raw water at NC during week 34 was MPN = 200, i.e., the highest value measured in raw water at NC during 2021.

4. Discussion

4.1 Methods for microbiological monitoring

The possibility to compare the consistency of the results between the two methods is limited to those parameters that could be analyzed with both methods, i.e., heterotrophs at 35 and 36 °C, respectively and coliforms. Based on the six samples (12 analyses), the agreement regarding coliforms was good both qualitatively and quantitatively. In the study conducted by Hörman et al. [15], the authors concluded that Coliert 18 overestimated the total number of coliform bacteria, while Petrifilm EC underestimated the number when compared with the reference method [14]. One possible reason for the higher E. coli counts detected with Colilert, according to the authors, is its ability to induce recovery of injured and stressed coliforms and E. coli in the sample, while the methods including MF are known to reduce the recovery of the target organisms. In the present pilot study, based on 19 raw water samples taken in 2021, there is a tendency of higher counts of coliforms with the IDEXX method, although it cannot be statistically evaluated

The sensitivity with MF/3M-Petrifilm was found to be high in terms of low heterotrophic counts, while high bacterial counts were easier to quantify with MF/3M-Petrifilm since it is easy to control (reduce) the amount of water filtered. For both 3M-Petrifilm and IDEXX, the number of heterotrophs in raw water was too high to quantify [21-22]. In Hörman et al., the authors found that the Petrifilm EC plates have low sensitivity due to small sampling volume, i.e., 1 ml. [15]. However, they did not filter the sample but instead inoculated 1 ml of the sample directly on the 3M film. With the reservation of being a small pilot study, our present study indicates that the combination of MF and 3M-Petrifilm results in sensitivity for coliforms that are in line with the Colilert analysis.

In general, IDEXX test kits are relatively easy to use compared to MF and cultivation on 3M-Petrifilm. In addition, IDEXX provides a broader range of analysis kits for drinking water analyses that has a better agreement with the analyses prescribed in NFA regulations [26]. 3M-Petrifilm presently lacks film for enterococci, pseudomonas, or film specific

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for E. coli [12-13,15-16]. Sample preparation with IDEXX does not require laboratory experience in the same way as MF. On the other hand, sample preparation with IDEXX requires an UV lamp and equipment for sealing the trays, in addition to sampling trays

An advantage of MF/3M-Petrifilm™ is its similarity with traditional bacteriology with outgrown colonies that can be used for further cultivation and analysis. In comparison with traditional bacteriology, the combination of MF followed by incubation on 3M-Petrifilm is feasible and cost-effective since it eliminates the need to produce and store diverse types of agar plates. However, the possibility to isolate single colonies from the 3M Petri films for further typing is of minor importance in an international field environment, due to limited access to laboratories with sufficient analytical methodology. One advantage with 3M-Petrifilm™ though is its ability to analyze micro-fungi.

4.2 Interpretation of water quality

One of the main challenges in Mali concerns the dramatic differences between rain and dry season. Torrential rain on dry, cracked impermeable soil inevitably poses a high risk of penetration of surface water and fecal contamination. Based on the available samples analyzed in 2021, the raw water did have a notably variation in coliforms, with peaks connected to the rainy season. The highest number of coliforms measured during 2021 at SC, i.e., 35 CFU on January 24th, did occur in close connection with flooding of the waste water tank at NC. This indicate that waste water possibly can penetrate in to the swedish piping system. At NC, E. coli. as well as pseudomonas and enterococci were detected in raw water during the rainy season, indicating that surface water and fecal contaminants can be present in the raw water during this period.

The relatively high raw water temperatures, from around 28 °C up to 38 °C, imply a potentially favorable environment for microbial growth if nutrient sources are present [4]. Intermediate storage of water in rubber tanks, as applied in both camps, causes an additional risk since rubber of certain qualities can act as substrates for microorganisms, in particular micro-fungi and actinomycetes. Based on our results, there is no indication of "uninhibited" growth of micro-fungi, even though yeast appears to occur.

There is a large difference in the number of heterotrophs in purified water compared to untreated water, indicating that the purification systems are efficient at both camps. Furthermore, analysis of purified water indicated that the water is safe according to the NFA (National Food Agency)'s regulations on drinking water, although free active chlorine at NC can be above the recommended limits of the NFA regulations at [26].

4.3 Limitations

This pilot study contains several limitations. Firstly, the supply chain is uncertain since all consumables have to be ordered and transported from within the EU. This limits the possibility to expand sampling procedures, for example with dilution series, in which sterile packaged water, etc., is needed. Secondly, the lack of time associated with the limitation of the mission facilitated only one sampling occasion, using the experimental material available at SC and NC. In addition, a quantitative comparison of the quality of drinking water between the two camps would have required a higher number of samples.

Elucidating the efficiency of the methods needs further sampling, including multiple analyses from each sample. However, such analyses are difficult to perform under the prevailing harsh field conditions. Furthermore, there were limited possibilities to follow up on the results by sending the samples to an accredited laboratory under the general conditions and due to limited options for refrigeration and cold transportation.

5. Conclusions

In this pilot study, we describe the application of two different methods for microbiological monitoring of water in a harsh environment during field operations, where laboratory resources are scarce, and supply chains are limited in terms of consumables for the methods. The study aims at giving a brief overview of the technical performance, the

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ease of use, and the availability of the methods. In addition, the study provides an example of how the result can be used to interpret temporal trends in the water quality.

Compared to MF and cultivation on 3M-Petrifilm™, IDEXX analysis kits are easier to utilize. In addition, IDEXX has the advantage of a broader range of analyzing kits for drinking water analysis and a better agreement with the examinations prescribed in the NFA regulations. Additionally, 3M-Petrifilm™ presently lacks film for enterococci, pseudomonas, or film specific for E. coli. Therefore, although IDEXX is more costly than MF, it might be a better option for some laboratories in developing countries because of the ease of use in the laboratory or if certain species, such as enterococci, pseudomonas, or E. coli, are to be analyzed. However, the combination of MF followed by incubation on 3M-Petrifilm ™ is a field alternative compared to traditional bacteriology, as it eliminates the need to produce and store agar plates. To obtain a statistically significant evaluation of the techniques used in the present study, repeated sampling must be done.

The rainy season in Bamako is intense with the heaviest rainfall in August. The changes in water quality are expected as the risk of intrusion of surface water and fecal pollution thereby becomes significantly higher. This is a probable explanation for the elevated levels of the indicators for human intervention and fecal contamination detected in this study.

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Author Contributions: F.C. and K.K.F. conceptualized the study. F.C., K.K.F., and H.L. conducted the research and collected data. F.T., F.C, and A.K.M. analyzed the data and wrote the first draft. All authors contributed to editing and expanding the manuscript and approved the final version.

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