Sterile drinking water filling of recyclable water bottles for use in Africa

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Contents

Chapter 1

Preface

The present report describe results from a field trip to Daar Es Salam, Tanzania, October 2022. The purpose of the trip was to evaluate water quality of drinking water comming out from Kioo Waters drinking water kiosks. The tour was done in cooperation with Kioo Water represented by Per Nielsen. A deeper description of the outcome from the field trip can be given by contacting Associate Prof. Peter Frigaard, Aalborg University, Denmark. Mail: pf@build.aau.dk. Telf +45 6196 7090.

Chapter 2

Introduction

In Tanzania there is a need for clean drinking water because of an insufficient water supply. The lack of clean drinking water causes a big risk for the population to get sick by waterborne diseases, which in Tanzania constitutes as a large thread because of the lack of access to medication.

Many initiatives have emerged in recent decades to alleviate populations living in areas with a shortage of clean water. This has i.a. led to the fact that in areas like Tanzania you can buy clean bottled water. The bottles is usually made out of PET - a plastic material that also constitutes an environmental burden. There is no proper waste management system in Tanzania that can handle plastic waste and therefore these plastic bottles are disposed of in the environment where it constitutes a contaminant.

To prevent plastic bottles from ending up as a pollution in nature, the solution may be to recycle the bottles. This will also save on greenhouse gases, as less plastic is needed to produce to make new plastic bottles. In addition, there will be an economic benefit for the populations living on clean bottled water, as it is not necessary to buy new bottles, and it will therefore be cheaper to access clean water. The savings that follow by avoiding regular purchases of bottles can possibly lead to more people being able to afford clean water, as the population in areas with a lack of clean water is often also very poor [Friis, 2021].

KIOO Drinking Water Co. (kioowater.com) has therefore worked on the idea of being able to build a station where bottles can be cleaned and refilled with clean drinking water, which will comply with the national requirements specified by the Tanzania Bureau of Standards (TBS). A nozzle has been developed to spray a mixture of ECA water and clean water into a contaminated

bottle which will thereby be cleaned, see figure 2.2. It will be investigated at what concentrations of ECA water and which cleaning time must take place at different sizes of bottles before the standard for drinking water quality is met - the set-up of the cleaning station can be seen in figure 2.1. This is examined by various tests, which are described in more detail in sections 6.

Figure 2.1. Set-up of the cleaning station at the lab at AAU BUILD, used in this report.

Figure 2.2. The sink with two nozzles used for cleaning the cap and the bottle respectively.

Chapter 3

Previous studies

Previous studies have been prepared on KIOO Drinking Water Co.'s previous nozzles. The purpose of the previous studies was to find an ECA-water concentration and a cleaning time that was sufficient to remove the number of viable counts to under 100 at 22 °C and under 50 at 37 °C, furthermore remove the absent of *E. coli* and total coliforms [Tanzania Bureau of Standards (TBS), na].

Friis [2021] has tested several variables and has come to the conclusion that a concentration of 30 ppm ECA water with a cleaning time of 5 sec. at 2.6 bar was sufficient. There was both tested on a 0.45L glass bottle, a 1L PET (polyethylene terephthalate) bottle and a 5L PET bottle. The results are shown on figure 3.1 and the limit values for neither 22 °C nor 37 °C have been exceeded.

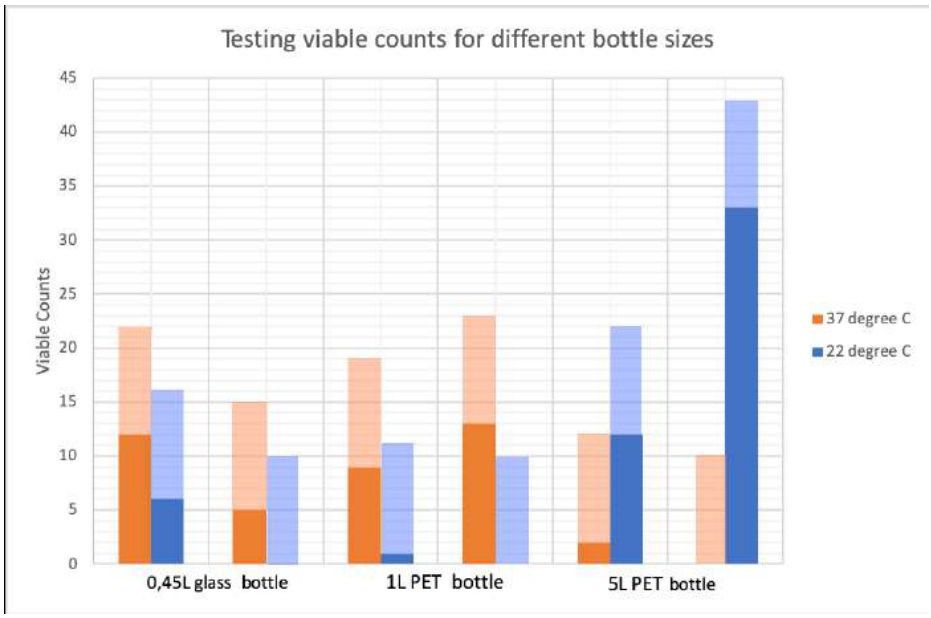

Figure 3.1. Viable counts for different sized bottles, two test from each bottle [Friis, 2021].

Same settings, 30 ppm, 5 sec. and 2.6 bar, have been used to check for coliforms and *E. coli*. Here, a 0.45L glass bottle, a 0.6L PET bottle and a 1L PET bottle have been tested [Friis, 2021]. The result showed no evidence of coliforms or *E. coli*

Chapter 4

Drinking water requirements in Tanzania

In table 4.1, the microbial drinking water requirement for packaged/bottled as well as requirements for potable drinking water can be seen.

Table 4.1. Drinking water requirements set by TBS. "+" means that the criterion is included in the water analyses, while "-" means that there is no testing for the applicable criterion.

	TZS 574 Packaged/bottled drinking water	TZS 789 Drinking (potable) water	Testes
Total viable counts at 22 ° C in 1 mL	Absent	100	+
Total viable counts at 36 ° C in 1 mL	Absent	50	+
Coliform and *E. coli*	Absent	Absent	+
Staphylococcus aureus	Absent	Absent	+
Enterococci	Absent	Absent	+
Salmonella	Absent	Absent	-
Pseudomonas aeruginosa	Absent	Absent	-

Chapter 5

Description of ECA-water

Electro Chemical Activated (ECA) water is made from sodium chloride and water that is treated with electrolysis to form hypochlorus acid [Food Diagnostics, na]. Hypochlorus acid can be used for disinfection, as it act as an oxidizing agents and thereby can destroy enzymes and compounds in microorganisms. Hypochlorus acid is the most effective form of the chlorine disinfectants, but is pH dependant, as hypochlorus will exist as the most dominent form of chlorine around pH 6, but at lower pH chloride will be dominant and at higher pH hypochlorite will be the most dominant. The effect of the disinfection also depends on the concentration of the disinfectant and the reaction time [Karlby et al., 2014].

Chlorine compounds are commonly used to disinfect water to achieve the required quality of drinking water. A benefit of using chlorine is that it has a residual effect. Chlorine can react with other substances such as organic compounds, which will reduce the capacity of disinfection and furthermore the reaction between the chlorine and the organic compounds can result in volatile organic chlorine compounds which can be harmful for humans [Karlby et al., 2014]. In Denmark the quality requirements of the sum of volatile organic chlorine compounds, in drinking water distributed from drinking water plants that uses chlorine compounds as an disinfectant, are 3 μg/L [Miljøministeriet, 2022].

Active chlorine released from hypochlorus acid is approved by the European Chemicals Agency (ECHA) to be used at surfaces in contact with food and water meant for human consumption (product type 4) as well as directly used as disinfectant of drinking water (product type 5) [European Chemicals Agency, na]. ECA water used in this report is the Toucan ECA water from Food Diagnostics which is an approved disinfectant by the Danish Veterinary and Food Administration [Fødevarestyrelsen, na]. The used ECA water has a concentration of 4500 ppm.

Chapter 6

Description of tests for investigating drinking water quality

Based on the investigations and results found in Friis [2021] it has been assessed that there is a basis for the bottles to be cleaned using a concentration of around 5 sec. at 2.6 bar, so that the criteria for viable counts as well as *E. coli* and coliforms are met. However, it is seen that there are variations in the results, why it is necessary to repeat tests. Investigate whether drinking water requirements are met at similar concentrations and cleaning times. It has also been assessed that further studies of the relationship between concentrations and cleaning times are necessary, since one wants to determine the lowest values for concentration and cleaning time respectively that will be able to meet the drinking water requirements. In addition, several different tests will also be included, in order to assess as many criteria as possible, and thus have greater certainty that the requirements are met.

Several tests are therefore necessary, which is why different sizes of bottles will be examined at different concentrations and cleaning times. Each combination will be replicated to make sure that the results for each test has a more sure statistics basis.

It will be taken as starting point that the tests is happening at a pressure on 2.6 bar while the concentration of ECA-water will vary respectively at 5, 15 and 30 ppm and the cleaning time will vary between 3, 5 and 10 seconds. In addition to test for viable counts (22 °C and 36 °C) as well as coliforms and *E. coli*, there will also be examined for staphylococci and enterococci.

The bottles used (1000mL, 1500mL, 5000mL and 10000mL) are shown in figure 6.1.

Figure 6.1. Bottle sizes used for testing from left to right; 10000mL, 5000mL, 1500mL and 1000mL.

Chapter 7

Procedure

7.1 Contamination of the bottles

In the following the procedure for contamination of the water bottle will be described.

1. Mix a bottle with sewage water and tap water so the dilution factor will be equal to 30.
2. Mix and wait 30 minutes for contamination.
3. After contamination pour out the content.
4. Clean the bottle for the given time and concentration.
5. Fill the bottle with tapped water and let the retention time be 30 minutes.
6. Take water samples for the different tests. The procedure of the different tests are described in the following.

7.2 Test for viable counts acc. ISO - 6222

For the viable counts test, the Yeast extract agar will be used.

1. Take 1 ml of the test sample into a 90 mm diameter Petri dish.
2. Add 15-20 ml of molten agar medium, and mix by gentle rotation. The time between addition of the test sample and addition between molten agar may not exceed 15 min.
3. Let the medium settle, and afterwards invert the plate.
4. Incubate at least one plate for each temperature (22 $\pm$ 2°C for 68 $\pm$ 4 hrs and 36 $\pm$ 2°C for 44 $\pm$ 4 hrs).

5. Examine plates for colonies directly after incubation. Results is expressed as colony-forming units per mililitre (cfu/ml).

[EUROPEAN COMMITTEE FOR STANDARDIZATION, 2000a]

7.3 Test for *E. coli* acc. ISO - 9308-2

For this test the Colilert is used.

1. Add a single snap pack of Colilert to 100 ml of test sample.
2. Shake gently to ensure mixing.
3. Pour the sample into a Quanti-Tray and seal with a Quanti-Tray Sealer.
4. Incubate the Quanti-Tray at 36 °C for 18 to 22 h.
5. Examine the Quanti-Tray after incubation. Wells with yellow colorization indicates presence of coliform bacteria, see figure 7.1.
6. Examine the tray under UV light in a UV viewing cabinet. Wells that exibit a degree of fluorescence shall be regarded as positive for the presence of *E. coli*, see figure 7.1.
7. Results are expressed as cfu/100 ml, and is found using a 97-wells Quanti-Trey/2000 MPN sheet.

[EUROPEAN COMMITTEE FOR STANDARDIZATION, 2014]

(a) *(b)* *(c)*

Figure 7.1. (a) Colilert test material, (b) Quanty-Trey/2000 indicating coliform bacteria in the yellow wells, (c) Quanty-Trey/2000 indicating *E. coli* in the fluorescent wells.

7.4 Test for Enterococci acc. ISO - 7899-2

For this test the Slanetz and Bartley medium and Bile-Aesculin-azide agar is used.

1. Pour Slanetz and Bartley medium into a 55 mm diameter Petri dish and allow medium to set.
2. Take 150 ml of sample from the 1 L bottle, 300 ml from the 1,5 L bottle, 600 ml from the 5 L bottle or 600 ml from the 10 L bottle.
3. Filtrate the water sample through a 0,22 μm membrane filter
4. Place the filter onto the Slanetz and Bartley medium in the Petri dish, and invert the plates.
5. Incubate the plates at 36 °C for 44 ± 4 h.
6. Examine the plates after incubation. Typical colonies show a red, maroon or pink color.
7. If typical colonies are present the filter must be transferred to a plate with Bile-aesculin agar, without inverting the filter.
8. Re-incubate at 44 °C for 2 h.

9. Examine the plates after incubation. Typical colonies show a tan to black color, and shall be counted as enterococci.
10. Results are expressed as cfu/ml.

[EUROPEAN COMMITTEE FOR STANDARDIZATION, 2000b]

Figure 7.2. The filter is removed with sterile forceps near a bunsen burner.

Figure 7.3. Set up of the membrane filtration apparatus, as the filter is placed.

7.5 Test for Staphylococci acc. ISO - 6888-1

For this test the Baird Parker agar is used.

1. Pour 15-20 ml of complete Baird Parker agar to a Petri dish, and allow medium to set.
2. Take 0,1 ml of the test sample and pour into the Baird Parker agar plate.
3. Carefully but quickly spread the added sample over the surface of the agar with a spatula.
4. Allow plates to dry for 15 min. at laboratory temperature.
5. Invert the plates.
6. Incubate the plates at 36 ± 2 °C for 24 ± 2 h.

7. After incubation examine the plates for colonies showing black or grey color, and mark the colonies at the bottom of the plate.
8. Re-incubate the plates at 36 ± 2 °C for 24 ± 2 h.
9. Examine the plates for new colonies, and mark the colonies.
10. Results are expressed as cfu/ml.
11. If any colonies are present a confirmation is undertaken by a tube test.

[EUROPEAN COMMITTEE FOR STANDARDIZATION, 2021]

Chapter 8

Test of cleaning time and concentration

In the following the results of the tests performed at the laboratory of AAU BUILD in Aalborg, Denmark, will be shown. Each test was replicated 4 times to reduce uncertainty, and to ensure that the tests are representative. If 1 out of the 4 replicates failed the requirements, the performance of the test is concluded to have failed. If 1 out of the 4 replicates failed the requirements, it was investigated whether the test failed because of an error. If it was suspected that there was an error at the specific test, the test was excluded from the results, and the test was concluded to have passed the water quality requirements.

8.1 Test for 3 seconds cleaning time at 5ppm

Table 8.1. Test for 3 seconds cleaning time at 5ppm

3 seconds of cleaning with a concentration at 5ppm.						
Bottle type	Coliform	*E. coli*	Viable Counts 22 °C	Viable Counts 36 °C	Staphylococci	Enterococci
1000 mL	Failed	Failed	Passed	Passed	Passed	Passed
1500 mL	Failed	Failed	Passed	Passed	Passed	Passed
5000 mL	Failed	Failed	Passed	Passed	Passed	Passed
10000 mL	Failed	Failed	Passed	Passed	Passed	Passed

8.2 Test for 5 seconds cleaning time at 5ppm

Table 8.2. Test for 5 seconds cleaning time at 5ppm

5 seconds of cleaning with a concentration at 5ppm.						
Bottle type	Coliform	*E. coli*	Viable Counts 22 °C	Viable Counts 36 °C	Staphylococci	Enterococci
1000 mL	Passed	Passed	Passed	Failed	Failed	Passed
1500 mL	Passed	Passed	Passed	Failed	Passed	Passed
5000 mL	Passed	Passed	Passed	Failed	Failed	Passed
10000 mL	Passed	Passed	Passed	Failed	Passed	Passed

8.3 Test for 5 seconds cleaning time at 15ppm

Table 8.3. Test for 5 seconds cleaning time at 15ppm

5 seconds of cleaning with a concentration at 15ppm.						
Bottle type	Coliform	*E. coli*	Viable Counts 22 °C	Viable Counts 36 °C	Staphylococci	Enterococci
1000 mL	Passed	Passed	Passed	Failed	Failed	Passed
1500 mL	Passed	Passed	Passed	Failed	Passed	Passed
5000 mL	Failed	Passed	Passed	Failed	Failed	Passed
10000 mL	Passed	Passed	Passed	Failed	Failed	Passed

8.4 Test for 10 seconds cleaning time at 30ppm

Table 8.4. Test for 10 seconds cleaning time at 30ppm

10 seconds of cleaning with a concentration at 30ppm.						
Bottle type	Coliform	*E. coli*	Viable Counts 22 °C	Viable Counts 36 °C	Staphylococci	Enterococci
1000 mL	Failed	Passed	Passed	Passed	Passed	Passed
1500 mL	Failed	Failed	Passed	Passed	Failed	Passed
5000 mL	Passed	Passed	Passed	Passed	Passed	Passed
10000 mL	Failed	Passed	Passed	Passed	Failed	Passed

8.5 Test for 10 seconds cleaning time at max ppm (74ppm)

Table 8.5. Test for 10 seconds cleaning time at 74ppm

10 seconds of cleaning with a concentration at 74ppm.						
Bottle type	Coliform	*E. coli*	Viable Counts 22 °C	Viable Counts 36 *degree*C	Staphylococci	Enterococci
1000 mL	Failed	Failed	Passed	Passed	Passed	Passed
1500 mL	Failed	Passed	Passed	Passed	Passed	Passed
5000 mL	Passed	Passed	Passed	Passed	Passed	Passed
10000 mL	Passed	Passed	Passed	Passed	Failed	Passed

8.6 Discussion and conclusion

The results showed no trend on which bacteria the cleaning worked best on. Even at the highest concentration allowed on the used pump we still see some bacteria in some of the tests. This could be that the concentration is not high enough. However, it is noticeable that the higher the concentration is, the higher is the overall pass rate.

It should also be mentioned that the contamination method used is a dilution from sewage water, which probably is more extreme than the real world as it contains way more bacteria. This could also explain why even at the highest ECA-water concentration used some of the flask still fail.

The fact that there is no real trend there can not be drawn any conclusion on which cleaning time and concentration there should be used.

Chapter 9

Testing the effect of ECA

In the following chapter the results of the effect of ECA-water will be shown. The difference in the cleaning methods will be; one bottle with no cleaning referred as ”w/o cleaning”, one bottle with only cleaning with water referred as ”water” and the last one cleaning with ECA concentration with max ppm (74ppm) referred as ”ECA”. The tests do not include Enterococci cause the previous tests showed no indication of Enterococci.

9.1 Viable Counts

9.1.1 Viable Counts 22 °C

As seen on figure 9.1, the threshold is exceeding the 100 CFU and therefore not qualified to drink. There is still some CFU when cleaned with water, but within the threshold. When the bottle is cleaned with ECA there is no CFU at all.

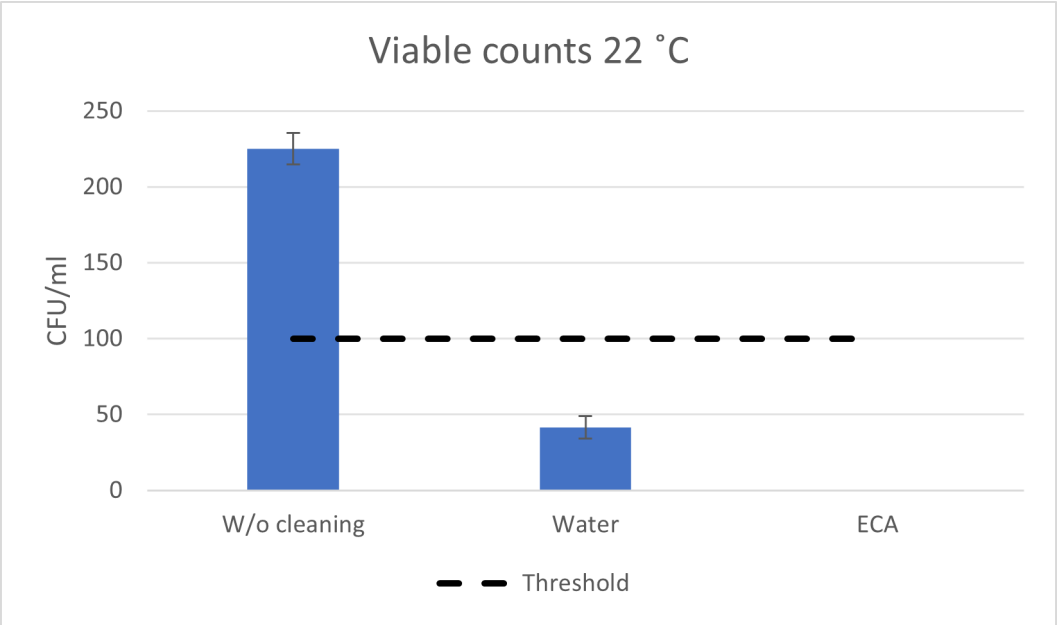

Figure 9.1. CFU for the different cleaning methods at 22 °C

9.1.2 Viable Counts 36 ˚C

As seen on figure 9.2, the viable counts is exceeding the threshold of 50 colony forming units (CFU) when no cleaning is performed. When cleaned with water the CFU is just below the threshold, with one of the samples exceeding the threshold. When the bottle is cleaned with ECA-water, there is a few or none observations of CFU.

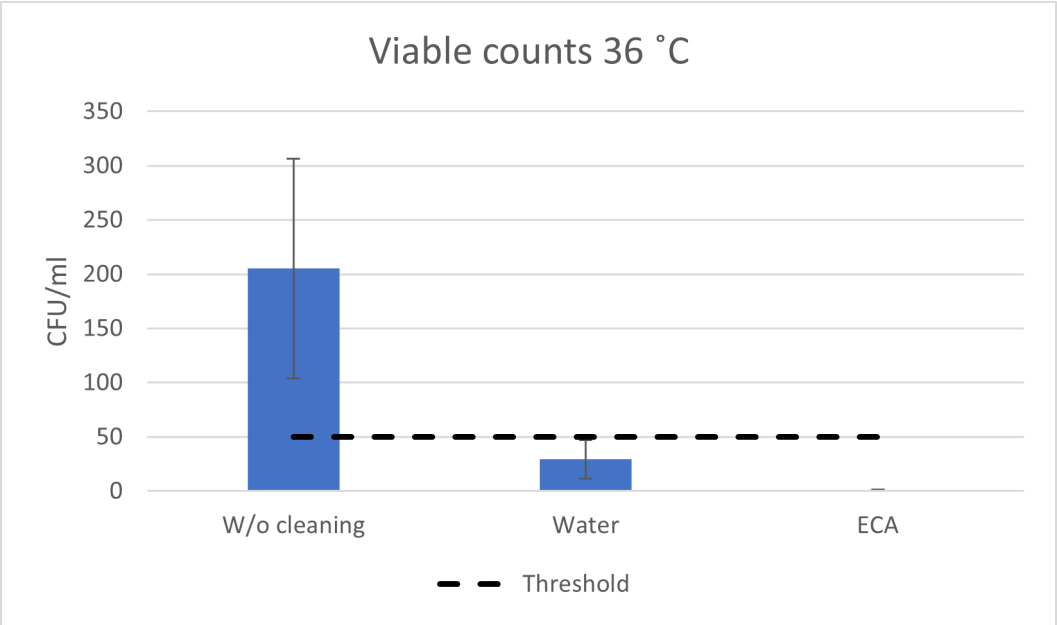

Figure 9.2. CFU for the different cleaning methods at 36 °C

9.2 *E. coli*

Figure 9.3 shows the Most Probable Number (MPN)/100 ml for *E. coli* for the different cleaning methods. It shows that the highest MPN is found in the bottle that did not receive any cleaning. Then there is a significant reduction in the MPN when we clean the bottle with just water. And almost everything is gone when we clean the bottle with ECA-water.

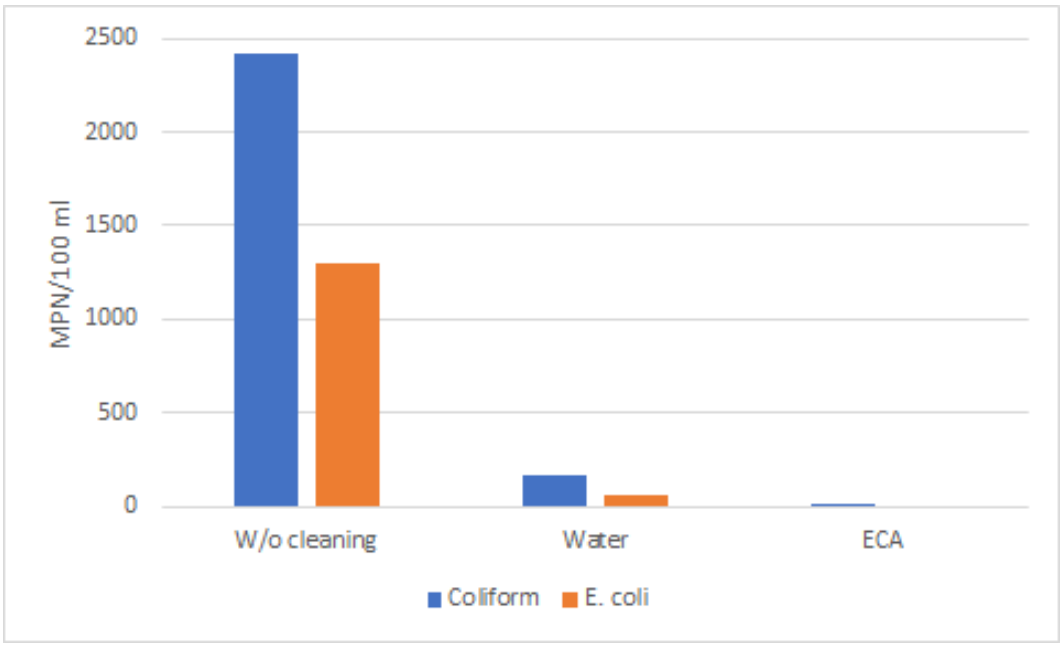

Figure 9.3. MPN of coliform and *E. coli* for the 3 different cleaning methods.

On figure 9.4 the test results are shown, where yellow wells indicate coliform organisms.

(a) *(b)* *(c)*

Figure 9.4. (a) Shows the coliforms (yellow wells) from no cleaning, (b) shows the coliforms only with cleaning with water, (c) shows the coliforms from cleaning with ECA water.

9.3 Staphylococci

As shown on figure 9.5, the CFU, is highest in the bottle where there was no cleaning. A reduction in CFU is seen when the bottle is cleaned with only water, and then no CFU is seen when the bottle is cleaned with ECA-water.

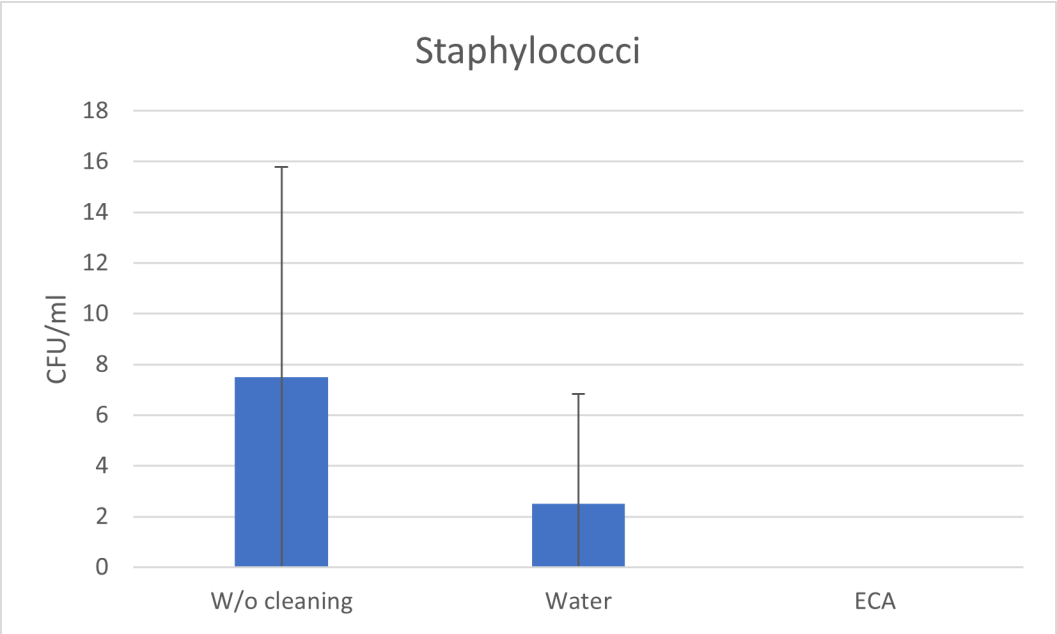

Figure 9.5. Caption

9.4 Discussion and conclusion

As we can see on the tests there is a clear trend that the bottle do not meet the requirement for any of the tested parameters, and in all cases it's way above the allowed requirements as shown in table 4.1 on page 6. If thee bottle is cleared with only water, then the requirements is not always met eg. in *E. coli* and staphylococci. And therefore is not sufficient enough to clean the bottle with water only. Last we see with the addition of ECA to the water which is used to clean with, we see that the bottle meet almost every requirement is met, except we see coliform bacteria. Thise values are extremely high, and the bottle is contaminated with raw waste water, which might not be the case out in the public, and there for the conditions might not be that extreme. The conclusion of the test is if you add ECA water to your cleaning water then there will bee a large reduction in the bacteria, so therefore the addition of ECA water is recommended.

Chapter 10

Examination of the local water in Tanzania

An examination of the local water was performed in Tanzania. The purpose was to get a more realistic result from local water, local bottles and local people as contamination rather than sewage water as used in prior tests.

The sampling was done on the local ”kiosk” which represent the local conditions and how the pump, tank, pipe and pouring will occur. The procedure changed a little bit, since the bottles did not had to be contaminated, as they already was from used local bottles. The cleaning time was 10 seconds. The concentration of the ECA-water used in cleaning was unknown as the dosage only had a knob with a min and max settings witch control the pulse speed and is not linked to the water flow as the pump used in Denmark. The other difference was that the refill water was not from local distribution but was packed water poured in a storage tank. The test methods remained the same, although the replicates was halved. The kiosk at the factory site is from now on referred as ”at the factory”, and the kiosk at the public is from no on referred as ”in the public”. There is no difference between the kiosks, only the location.

Figure 10.1. Set up of the work space in Tanzania.

Figure 10.2. Bottles of different treatment used for examination.

10.1 Results

Table 10.1. Different bottle tests in Tanzania

Bottle type	Coliform	*E. coli*	Viable Counts 22 °C	Viable Counts 36 °C	Staphylococci	Enterococci
New Bottle	-	-	Failed	Failed	Failed	Passed
Used bottle without cleaning (at the factory)	Failed	Passed	Failed	Failed	Failed	Passed
Used bottle with water cleaning (at the factory)	Passed	Passed	Failed	Failed	Failed	Passed
Used bottle with ECA cleaning 1 (at the factory)	Failed	Passed	Failed	Failed	Failed	Passed
Used bottle with ECA cleaning 2 (at the factory)	-	-	Failed	Failed	Passed	Passed
Used bottle with ECA cleaning 3 (at the factory)	Passed	Passed	Failed	Failed	Failed	Passed
Used bottle with ECA cleaning 4 (at the factory)	Passed	Passed	Failed	Failed	Failed	Passed
Used bottle filled with raw water, with ECA cleaning	Passed	Passed	Failed	Failed	Failed	Passed
Raw water	Passed	Passed	Failed	Failed	Passed	Passed
Used bottle without cleaning 1 (in the public)	Passed	Passed	Failed	Failed	Passed	Passed
Used bottle without cleaning 2 (in the public)	-	-	Failed	Failed	Passed	Passed
Used bottle with ECA cleaning 1 (in the public)	Passed	Passed	Failed	Failed	Passed	Passed
Used bottle with ECA cleaning 2 (in the public)	-	-	Passed	Passed	Failed	Passed

10.2 Discussion and conclusion

The tests done at the factory for viable counts for both temperatures showed extreme growth and the petri dish was overgrown made it impossible to count the actual number but was over 100. This was caused by the fact that the storage container was filled with water weeks before and was left in a temperature around 30 °C. This made the container the perfect place for bacteria growth. This was not the case for the water at the public kiosk, since that was filled with fresh water when the test was made. This is most likely why every test failed the viable counts. Even

tho the first test in the public failed in viable counts and perhaps the 2 other without cleaning, could be due to that the container had a little left over water which also has been there for weeks, and that has probably been filled in the first 3 bottles, and then the clean water in the last.

The pump used to dose the ECA-water and water was not the same as used in Denmark. The one used in Denmark was flow based, and you can control exactly how many ml/L the pump should dose. The one in Tanzania just had a constant pump rate, which resulted in a super high ECA-water concentration at the start of a flow in situations when the was started. In order to decrease the amount of ECA-water been used, a pump like the one used during testing in Denmark should be used.

One conclusion from the tests made in Tanzania is that in situations where the water needs to be stored in a container for longer periods, there must be some sort of disinfection/inhibition for bacteria growth. That could be some sort of cooling or an approved disinfectant for food. Furthermore, another pump can be looked into, to better control the dose of ECA-water, and also reduce the risk of the water to taste of chlorine.

Though, the overall conclusion from the tests in Tanzania is the the cleaning system installed in the Kioo Water drinking water kiosks is able to disinfect plastic bottles, so they can be reused.

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