Assessment of disinfecting contaminated water bottles for reuse within potable water requirements in Tanzania.

Line Møller Friis
Master thesis
Water and Environmental Engineering
Aalborg University

AALBORG UNIVERSITY
STUDENT REPORT

Department of Built Environment
Division of Water and Environment
Thomas Manns Vej 23
DK - 9220 Aalborg Ø
www.en.build.aau.dk/

Title:
Assessment of disinfecting contaminated water bottles for reuse within potable water requirements in Tanzania.

Project:

Master Thesis

Project Period:

1. September 2020 - 1. january 2021

Author:

Line Møller Friis

Supervisor:

Peter Frigaard

Cooperation partner:

Per Nielsen (KIOO Drinking Water Co.)

Pages of rapport: 75
Appendix pages: 25
Handed in: 31. December 2020

Synopsis:

Tanzania is a developing country, where the majority of the population are suffering from poverty and experiencing the severe consequences of climate change, resulting in great risks of waterborne diseases and related deaths due to inaccessibility to clean water. Both national and international initiatives have focused on increasing access to clean water, however contaminated bottles and containers for transporting the water remain a risk. This thesis has investigated the possibility of a cleaning unit that allows for the criteria of potable water, following the standards in Tanzania, to be withheld for water refilled in a cleaned bottle. The unit disinfects the bottles using ECA water, a sodium chloride based solution that inactivates the bacteria by oxidation, and does not require a rinse of the bottle after use. Cleaning time, concentration and water pressure were adjusted until a steady results of viable counts within the criteria for potable water could be met, where as little water as possible was wasted for cleaning. Alongside, the nozzle underwent physical modifications. It was found that a concentration of 30 ppm for 5 seconds of cleaning time with a water pressure of 2,6 bar will allow for the criteria to be met, and decrease the amount of viable counts of up to 99% from initial state of water in an uncleaned bottle. Furthermore an absence of *Coliforms* and *E. coli* is found, and insignificant levels of ATP is found on cap and mouth piece of bottle, and surface area of cleaning unit, indicating limited risks of transmitting diseases between users.

AALBORG UNIVERSITET
STUDENTERRAPPORT

Institut for Byggeri, By og Miljø
Sektion for Vand og Miljø
Thomas Manns Vej 23
DK - 9220 Aalborg Ø
www.build.aau.dk/

Titel:
Vurdering af disinfektere forurenede vand flasker til genbrug under kravene for drikke vand i Tanzania.

Projekt:

Kandidatspeciale

Projekt periode:

1. September 2020 - 1. Januar 2021

Forfatter:

Line Møller Friis

Vejleder:

Peter Frigaard

Samarbejdspartner:

Per Nielsen (KIOO Drinking Water Co.)

Sidetal projekt: 75
Sidetal bilag: 25
Afleveret: 31. December 2020

Resume:

Tanzania er et udviklingsland, hvori fattigdom og konsekvenserne af klima forandringer præger størstedelen af befolkning, hvilket resulterer i store risikoer for vandbårne sygdomme og dertilhørende dødsfald som følge af utilgængeligheden af rent vand. Både nationale og internationale intiativer har fokuseret på at øge tilgængeligheden af rent vand, hvilket angiver en risiko i forurende beholdere, som benyttes til at transportere vandet i. Dette studie har undersøgt muligheden for at lave en renseenhed, der kan rense en flaske, således at det genopfyldte vand i flasken kan overhold krave for sikkert drikkevand, angivet i Tanzanias standarder. ECA vand er et disinfektionsmiddel baseret på natriumklorid, som bruger oxidations potentiale til at deaktivere bakterier, hvor efterskylning af flaske er unødvendigt. Ved at justere på parametrene rensetid, koncentration of vandtryk, benyttes kimtal til at vurdere hvorvidt kravene for drikkevand kan opnåes. Sideløbende er dysen til rengøring blevet modificeret. Ved en koncentration på 30 ppm og en rensetid på 5 sekunder ved et vandttryk på 2,6 bar er det muligt for kimtal at overholde kravene til drikkevand. Hertil vil kimtallet reduceres med up til 99% sammenlignet med vandkvaliteten i en flaske, der ikke var rengjort. Der er testet for *Coliforms* og *E. coli*, hvor et komplet fravær var fundet, hvilket opfylder kravene til drikkevand. Ubetydelige mængder af ATP var fundet på overflade arealer af dysen, gevindet i proppen og på flaske hovedet, hvilket indikere begrænsede smitterisiko imellem brugere.

Preface

This thesis is written in collaboration with Soacha Aps with Per Egede Nielsen as interlocutor, whom are the co-owners of KIOO Drinking water Co., a Danish owned Tanzanian company producing bottled drinking water. The company briefed with an idea of making sterile refilling of water meeting drinking water criteria for reusable water bottles in Africa through water refilling station, and prevent the buying of new PET bottles with water by allowing a hygienic, fast and cheap replenishment of used water bottles. The products for the project will be physically produced by Lsm:steel spånteknik with a baseline in a disinfection product produced by food diagnostics. Lastly, Huse Design will design the station with robustness and simple functionality in focus. The projects innovation process has received funding from Access2innovation, a danish platform with aim to create sustainable business in East Africa.

The thesis focuses on validating the concept by finding an optimal solution for disinfecting and replenishment of bottles, where the water quality in the refilled bottles will meet the Tanzanian standards for potable drinking water, once the bottle has been cleaned. The thesis is written in period 1^{st} September 2020 to 1^{st} January 2021.

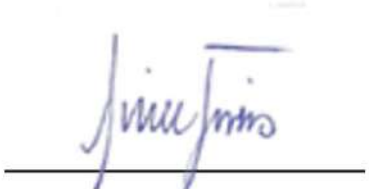
A thank you goes to Peter Frigaard as a supervisor on this thesis for guidance and discussion of ideas, to Jytte Denker for assistance with experimental procedures, and to Per Nielsen for assistance with physical development of the nozzle and discussion of ideas.

Reading guide

The thesis consist of a main report and attached appendices. The appendixes are referred to throughout the report and can be found after the bibliography.

References are made with the Harvard reference method and references are put into brackets with author or organisation followed by year i.e. (The UN, 2020). References are placed after the sentences where used. All references are placed in alphabetic order in the bibliography, found at the end of the report.

Figures and tables are numbered by the chapter number followed by the number of the order presenting them e.g. figure 3.3 is the third figure in chapter three.



Line Friis

Table of contents

Introduction 1

The United Nations have set 17 sustainable development goals for the world to ensure a better future (The United Nations, 2020). Good health and well being is defined through education, access to food, clean water and sanity, and housing reasonable for location. Climate actions are necessary to secure the access for victuals and reduce global inequalities through innovative communities where reasonable consumption and production allows for natural life on land and sea. The consequences of the present climate change include more extreme weather events as droughts and heavy rainfall (NASA, n.d.).

For developing countries, where access to clean water is limited due to restricted sewerage, droughts alone or followed by heavy rain events are catastrophic and causes starvation, homelessness and health risks. Globally, initiatives have been made throughout the past decades to better the access to clean water, however 29% of the world population continue to lack access to clean water (World Health Organisation, June 2019). The lack of access to clean water forces people to use contaminated water from surface water or pumped directly from the groundwater in drought seasons. Great health risks due to transmission of waterborne diseases, that could be prevented, are a common threat due to poor sanitation in the community, where sewage water is disposed directly into rivers or onto the ground, here-through reaching the groundwater or contaminating surface water (Makoye, n.d.). For rural areas the distances to water sources can also be a concerning matter, where the possibly contaminated water will be transported in non-sanitary containers, in addition to the potential dangers of travelling long distances. In cities with access to tap water, poor sanitation, outdated sewerage, and lack of cleaning results in the water not reaching the criteria for clean water, concluding in bottled water being the primary source for clean water suitable for consumption (Kjellén, n.d.). Bottled water is often sold in thermoplastic bottles, PET, that can cause an environmental threat, as it is non-biodegradable. In addition, the costs of bottled water surpasses the costs of tapped or surface water, which conflicts with the economical troubles of developing countries, where poverty among the civilisation is common. Innovation in terms of reusing plastic bottles and containers is therefore a necessity, both environmentally and economically, to ensure access to clean water (The united republic of Tanzania, ministry of Water, October 2015).

Warm weather improves the conditions for many waterborne diseases, increasing the health risks followed by consuming contaminated water. Many African countries struggle with lack of water and face further struggles as droughts become more common. In addition, Africa consists of many developing countries, where access to medicine that could help against health risks associated with contaminated water is limited. Tanzania is an African country where inequality within the country is large, and more than two thirds live below the line of poverty (Ministry of Foreign affairs of Denmark, 2020). Furthermore, 43% of the population lack access to safe water and only 30% have access to improved sanitation (water.org, 2020).

Facing the consequences of climate change, longer drought seasons will worsen the possibility for agriculture, that holds 25% BPD and 80% of the workload in Tanzania, and hereby escalate poverty within the country (Makoye, March 2017*a*). In addition, a drastic yearly increase of nearly 3% in the population within the past century, increasing the demand for water, causing more dense neighbourhoods, and resulting in larger amounts of sewage pollution (Ministry of Foreign affairs of Denmark, 2020). Despite several both national and international initiatives to improve sewerage systems in cities and make water more accessible in urban and rural areas, a decrease in percentage of the population with access to water and sanitation occurs, as a result of the population growth (Shore, 2020).

Problem Analysis 2

United Republic of Tanzania is located in East Africa, a former German followed by British colony before becoming independent in 1964. Tanzania consists of a variety in landscapes from mountain region in the north, with Africa's largest mountain; mount Kilimanjaro, and densely forested, bordering the Indian Ocean to the east with areas of mangrove swamps, whereas the central and southern part, being part of the eastern African plateau, consist of arable and grass land, respectively. The western part of Tanzania is part of the African great lakes region, consisting of both endorheic basins and rivers mounding into the Indian Ocean, resulting in a total of 6,5% (61,336 km^2) of the lands area to be water (figure 2.1) (Britannica,, N.d*b*).

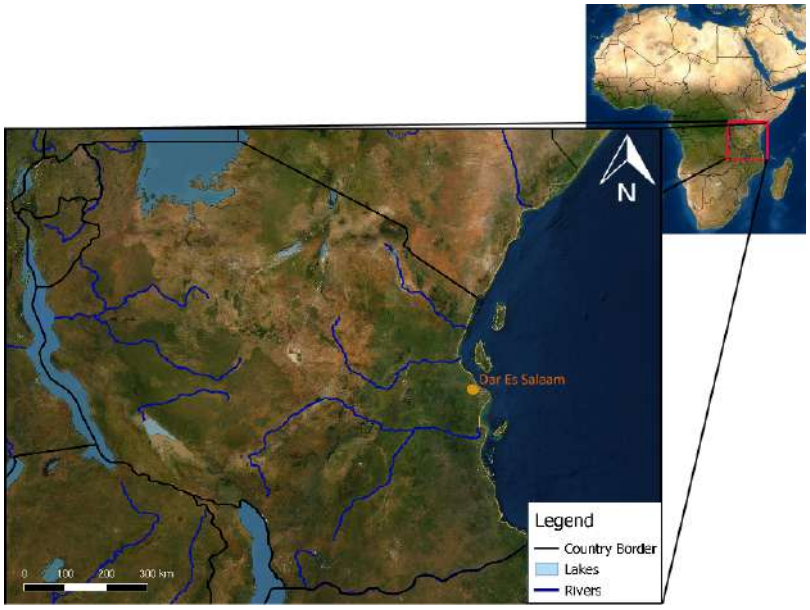


Figure 2.1: *Location of Tanzania, where 6,5% of land area is water.*

The landscapes of Tanzania are reflected in the climate classification in the country, ranging from tropical climate with dry winters in the grass land, hot summers in arid areas in the centre of Tanzania with minimal precipitation, and temperate dry winters in the remaining area (figure 2.2). Dry winters indicate wet summers, influenced by Monsoon, where at least 70% of the annual precipitation is received in the warmest six months (Arnfield, November 2020).

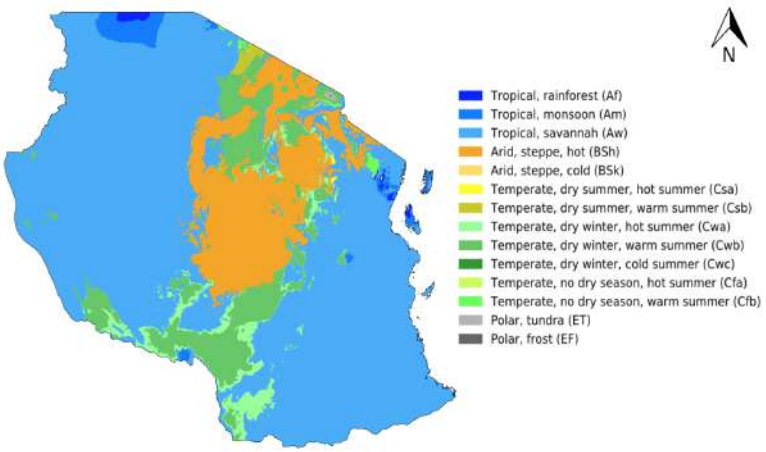


Figure 2.2: *Climate Classification of Tanzania (Arnfield, November 2020).*

The climate classifications are intensifying along with the consequences of climate change, causing higher temperatures, more flooding and longer and more severe droughts, threatening the livelihoods for millions of Tanzanians (USAID, January 2012). Severe droughts will increase the demand for water both for farming and household purposes. In contrast, flooding will cause an increase in contamination of available water, where higher temperatures improves the conditions for many waterborne diseases (Makoye, February 2012).

Waterborne diseases and lack of water continues to be a problem within the country, as large amounts of surface water are regularly becoming contaminated. Lack of proper sewerage systems results in waste flowing freely in streets or being dumped in surface waters. Surface water is the main source for watering for agriculture, cleaning and consumption purposes. Despite precaution such as boiling water, the issues with bad sanitation causes reoccurring troubles with contaminated water and following diseases. In addition, the general access to medicine to overcome waterborne diseases is by far limited and expensive. The inequality in the country both economically and related to access to medical care, causes citizens in rural areas to be at further risks to waterborne diseases. This consists of 66% of the population in the country (Britannica,, N.d*b*). Furthermore, these citizen face additional troubles living far away from water access, in terms of transporting water to homes and dangers involved in making daily travels (Prüss-Ustün et al., 2016).

2.1 Waterborne diseases

In Tanzania, 13% of all registered deaths are related to water, sanitation and hygiene (Prüss-Ustün et al., 2016). Contaminated water can contain a variety of microorganisms, such as bacteria, parasites, virus or chemicals from factory waste or fertilisers. Although chemicals can cause serious intoxication, the diseases caused by pathogenic microorganisms are the main cause for high death rates related to waterborne diseases in Tanzania. Lack of sewerage systems combined with an increase in flooding events, as well as bad sanitation, cause human and animal excreta to end in surface water, or infiltrate to groundwater, before being used for cleaning or drinking, and ultimately being consumed by humans (figure 2.3) (Prüss-Üstün et al., 2008).

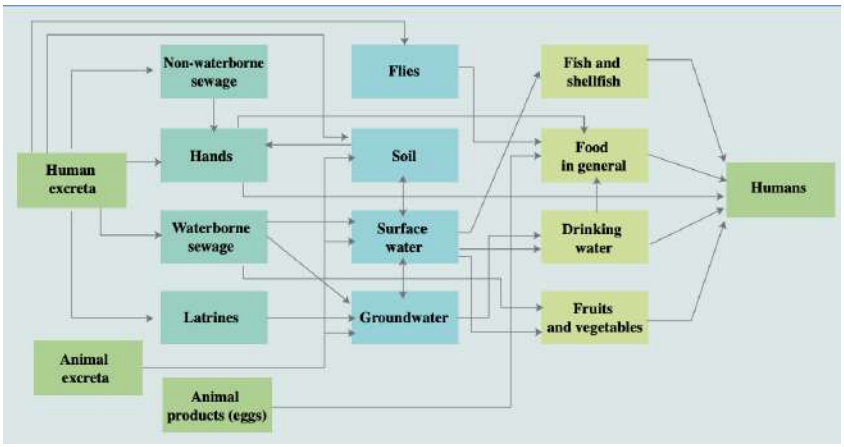


Figure 2.3: *The disease burden related to water, sanitation and hygiene (Prüss-Üstün et al., 2008).*

The most common diseases related to water, sanitation and hygiene are *tuberculosis*, diarrhoeal diseases, *hepatitis*, *gastroenteritis*, *malaria*, and the consequence of malnutrition as a reaction from diarrhoeal disease (Outwater et al., 2013). Diarrhoeal disease is a symptom of an infection, that can be hosted by either bacterial, viral or parasitic microorganisms. Typically, microorganisms causing diarrhoeal diseases are spread by faeces-contaminated water, however tuberculosis and malaria are airborne and carried by mosquitoes, respectively, and thereby related to poor hygiene. Dehydration and lack of necessary salts are the main cause of diarrhoea associated deaths, however bacterial infections have become an increasing cause. Additionally, malnutrition caused by diarrhoea weakens the immune system and will worsen the course of illness. The most typical diarrhoea diseases are *E. Coli* infection, *hepatitis A*, *Rotavirus* and *gastroenteritis*. The three last aforementioned infections are caused by a virus, whereas *E. Coli* infection is caused by a bacteria (World Health Organisation, May 2017).

2.2 Tanzania's current sewage system

In additional to further challenges aggravated by climate changes, drastic growth in population pressures the existing sewage network in cities. Focusing on the cities, only a minority is connected to sewerage network, and places without, a septic tank or latrine is used. As emptying of these are expensive, the waste most often ends up in streams or storm drains. Despite the well-knowings of the citizen that this pollutes, the alternative is an unrealistic priority economically (Makoye, September 2017*b*). Eventually, the water will end up in either the Indian Ocean, a nearby lake or river and hereby polluting. This solution in unsustainable in polluting surface waters that is used for agricultural and consumption purposes and in an economical aspect by polluting the Indian Ocean, that is a main income tourist-wise. The sewage network uses superficial wastewater treatment as stabilisation ponds as the main method, and only few of the larger cities have more advanced treatment, however most are unable to meet national standards for discharge (Government Portal Content Committee, October 2015). Some areas are not connected to a treatment plant or there is a lack of capacity, and instead the sewage water is led into the Indian Ocean (figure 2.4) (Chiara, November 2018)).

Figure 2.4: *Sewage water is led into the Indian Ocean instead of receiving treatment due to lack of capacity (Chiara, November 2018).*

Despite many both national and international initiatives to better the water system, the sewerage network has undergone very few improvements since the beginning of the 2000's. Most importantly, the seriousness of sanitation has become common knowledge among a higher share of the population (Shore, 2020). In addition, the sewage authorities are ambitious with easing sewage disposal in cities using pipelines to connect different suburban areas to disposal at wastewater treatment plants with the aim of preventing sewage spilling in the streets. Following an increase in population, especially found as an increase in the density within urban areas and cities, space for renewal of sewage piping is limited and inequality within the country is reflected in which areas that are prioritied for the renewal. These are typical wealthier neighbourhoods where emptying of septic tanks are affordable. The result herefore is that suburban areas, that suffer from flooding and depend on streams for water access, are downgraded and the pollution continues (figure 2.5) (Makoye, September 2017*c*).

Figure 2.5: *Streams filled with contaminated water (EEPCO, December 2009).*

2.3 Distribution and access to water

In co-relation with the lack of sewerage network, a water supply network is equally problematic. Access to water in rural areas consists of either surface water, wells, or private financed water-towers, from where small towns can collect their water. In water tanks, the water is stored for long periods of time before being distributed to the users, which also involves a risk.

In cities, with Dar Es Salaam as an example, the distribution of drawn water consists of 30% from shallow wells, 17% from surface water sources, and only 8% make use of public taps. The remaining 45% buy water from vendors, which for many is a costly option (figure 2.6). The Tanzanian government generally advises against consumption of tap and surface water, as the quality is questionable. This is due to outdated and leaky pipelines, as well as water that has undergone little to none treatment, where growth conditions for microorganisms are great. The main disinfectant method an average citizen can use is to boil water, which kills off many microorganisms, however not to full completion. Furthermore the water supply from public taps is with limits dependent on demand (Kjellén, n.d.).

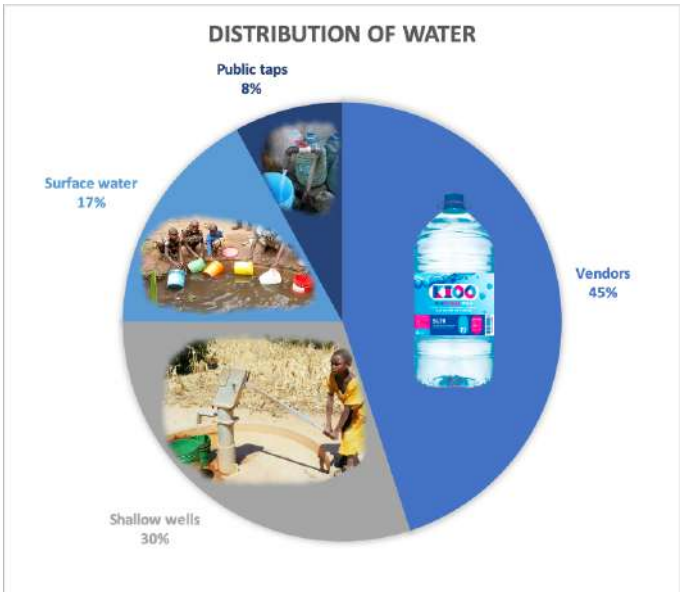


Figure 2.6: *The distribution of water source in Dar Es Salaam (Kjellén, n.d.).*

The troubles involved with using wells consists of the uncertainties involving pollution. Disregard infiltration of contaminated water, wells are at high risks of contamination at extreme rain events, the safenes of using water from wells will be met with uncertainty, as surface water from rain mixed with waste may enter the well. In addition to the risks involving infiltrated contaminated water, groundwater can also be chemically polluted, either naturally or from factory dumping. Naturally, the hydrogeochemical characteristics of large parts of northern Tanzania contains alarmingly high levels of fluoride for drinking purposes and is therefore unsuitable (British Geological Survey, 2000). Furthermore, natural water and soil bacteria is found in water taken from wells and boreholes.

Lastly, when using public taps, surface water or any other distribution option, the water will have to be transported back to houses, where containers are mainly used. Filling a container with contaminated water, results in contaminating the bottle, and hereby contaminating the following refills in the container (figure 2.7). The safest option for getting clean water is thereby to buy fabric bottled water, that has undergone further treatment and where a clean container is used. This does not correspond with the economical possibilities for many citizens, whom will instead choose the option of reusing large containers and refill them with water of questionable quality, nor with the environmental issues related to increase the amount of PET that will be disposed.

Figure 2.7: *Refilling containers with contaminated surface water (Eric Sturdza Investments, April 2020).*

The Tanzanian government is aware of the problems involving quality of water in general, and the lack of access to clean water. Therefore the Tanzanian Bureau of Standards, TZS, has made a standard catalogue with specifications, from where a certification can be achieved, for both packaged/bottled drinking water and drinking (potable) water (figure 2.8), which will promote water that has been cleaned (Tanzania Bureau of Standards, n.d.). As the requirements for potable water is to be met at the water work, local treatment for community piped water is generally advisable in Tanzania. Initiatives with origin in Dar Es Salaam, has focused on making the water treatment more effective by increasing water production by up to 50% within the city and increasing the access to piped water by limiting the storage time in tanks where bacteria growth can take place. The result showed an increase of 12% in the household taps free from *E. Coli* in a timespan of two years, however only reaching 64% of the household taps being free from the pathogen (MCC, May 2019).

TZS 574: 2016 (2nd Ed) -EAS 153: 2014	2016	**Packaged/ bottled drinking water - Specification** Prescribes the quality requirements for packaged/bottled drinking water. It does not include the requirements for natural mineral water **ICS: 13.060.20**
TZS 789: 2019 (3rd Ed) EAS 12: 2018	2019	**Drinking (potable) water - Specification** Prescribes the quality requirements and methods of sampling and test for drinking (potable) water intended for human consumption and distributed through community piped water systems servi **ICS: 67.060.20**

Figure 2.8: *Specification of what the standards requirements can be used for once met; potable water or bottled water (Tanzania Bureau of Standards, n.d.).*

In comparison, in Denmark, the requirements for bottled water are less strict than those for tap water, and furthermore has separate requirements for water at the waterworks and in the pipes (Aalborg Kommune, 2014). Denmark is the only place worldwide that can use the natural filtration that groundwater goes through as a cleaning method, meaning that chloride is not added to the water for cleaning purposes. In many countries the smell of chloride is equivalent to clean and safe water from the tap (Abildgaard, December 2017).

2.3.1 Drinking water requirements in Tanzania

The requirements for bottled drinking water is more strict than those for potable water. To achieve a certification for packaged/bottled drinking or potable water, the requirements state an absence of the following:

- *E. Coli*
- *Coliforms*
- *Salmonella*
- *Enterococcus*
- *Staphylococcus Aureus*
- *Pseudomonas Aeruginosa*
- *Viable counts*

For potable water viable counts of microorganisms are allowed. The requirements, following those of packaged water, to be met are as stated:

Parameter	Potable water
Total viable counts at 22°C in 1 mL	100
Total viable counts at 37°C in 1 mL	50

The test method are based on International Organization for Standardization by testing the microbial characteristics as colony-forming unit per 100 ml. The requirement is listed as absence, which involves results of less than one and not detected in order to pass (Tanzania Bureau of Standards, n.d.).

Coliforms and *Salmonella* are bacteria part of the *enterobacteriaceae* family of gram negative bacteria. *Salmonella* is of animal origin and is transferred to humans by eating raw meat or animal biproducts, or through contamination by manure. The bacteria can infect through fecal to oral. *Coliforms* are a natural gut bacteria group in both humans and animals. Most types of *coliforms* do not cause serious illness, but are easy to culture and are therefore used to indicate the presence of fecal originated pathogens. *E. coli* is a type of fecal coliform that is naturally found in the lower intestine, and reproduces quickly. *E. coli* can cause an infection that is transmitted fecal to oral through contamination of food or water (Merck, 2020). *Enterococcus* is another natural occuring gut bacteria. For these bacteria types, the occurrence in the gut microbiome is without harm and instead useful to keep unwanted bacteria in the gut from multiplying.

For *enterococcus* the dangers of free moving contaminated water will cause a risk for serious infections in wounds and blood that the bacteria gets in contact with (Watson, 2020). Much alike, *staphylococcus aureus* is a bacteria that is often found in infections in wounds and on skin.

Furthermore, the bacteria can cause food poisoning and is a hardy bacteria that needs antibiotics to be beaten. For weakened, infections caused by the bacteria can be worsened greatly. The bacteria is normal in almost half the population, but can appear in different forms that will cause infection if foreign. The most common cause of infections by *staphylococcus aureus* is bad sanitation (Statens Serum Institut, September 2016).

Lastly, *pseudomonas aeruginosa* is a bacteria found naturally in soil or water as it is free living. Furthermore, the bacteria can live and grow on fruit and vegetables and infect human through consumption. The bacteria can cause infection, where especially blood infection can be deadly (Struwe, April 2020).

2.3.2 Water vending machines

As the quality for distributing drinking water through community piped water systems services remains questionable, possibly due to outdated piping and bad cleaning, in addition to the both environmental and economical challenge with purchasing new bottled water, other alternatives in terms of water vending machines are in progressive development (Lawson, December 2017).

Since the start 2010s water vending machines, also named water ATMs, become progressively frequent around the world in especially countries such as India, where clean and safe water is uncommon and groundwater is too contaminated to consume (Nest-In, November 2019). The water vending machine operates by refilling bottles with clean water at costs up to a quarter of the price of bottled water. Often the machine is a self-serving kiosk, where payment is through prepaid chips, credit cards or mobile payment. The most common machines offer refilling of bottles ranging in sizes. In addition the water vending machines encourages the reuse of bottles, as well as providing clean water at lower costs at 24-hour operation (figure 2.9). Hereby it allows for a economically and environmentally friendly option to access clean water for urban and rural areas, that are typically battling poverty (Paliwal, June 2015; Lorentz, 2019).

(a) (Rathi, August 2019)

(b) (Lorentz, 2019)

Figure 2.9: *(a) Water ATM located in city for refilling of smaller bottles. (b) Water ATM locatd in rural aral for refilling of larger containers.*

The water ATM was introduced in Tanzania in 2017 by Grundfos at a testing stage by installing 20 stations in one district in the northern region. Although the solution of water ATMs helps facing the issues of waste by reusing water containers, the reusage of bottles will involve a degree of contamination of the bottle and thereby of the refilled clean water before consumption (Lawson, December 2017; Grundfos, n.d.).

2.3.3 Disposal and reuasage of plastic

Generally, the interest in reusage and limiting plastic waste is increasing. However, traditionally trash system are non functioning due to lack of access to some areas both rural and urban for collecting the trash, as well as dealing with the trash once collected. This results in trash being disposed on countrysides, polluting and increasing the amount of microplastics in the environment, and eventually causing contamination of water as well (figure 2.10). Within the past years, petitions in East Africa against one-use plastic are growing and Tanzania has come to ban the use of plastic bags (Woodhouse, July 2018).

(a) (McSheffrey, February 2015)

(b) (kibera$_1$249, n.d.)

Figure 2.10: *The lack of a re-use system, causes trash disposed alongside roads and rivers, polluting the environment.*

In comparison to many developed countries, Tanzania does not have a system for bottle deposits, where the plastic is reused for other purposes. Due to the Tanzanian government not having a waste management system, recycling of plastic is still an undiscovered opportunity. The possibilities for bottle deposits are therefore also limited, and will thereby instead become trash. Following the ban of single use plastic, an increase in demand for environmental friendly alternatives to purchasing new water bottles every time there is a need for water can be seen (Marzo, February 2020).

2.4 KIOO Drinking Water Company

Improvement in the design and functionality of water ATMs is happening all over. A new initiative to the idea of refilling the bottles is to install a cleaning unit before the refilling of bottles and containers, and thereby the use of water ATM will allow for safe reuse of bottles.

KIOO Drinking Water Co. is a Danish owned company located in Tanzanias largest city, Dar Es Salaam. The company produces bottled drinking water, and are responsible for the process of extracting the water, cleaning it, and bottling before selling either at retail or stock within the criteria of the Tanzanian quality terms for drinking water. The company produces bottles in six sizes varying from 0,6 to 18L. The water is extracted from the underground of a local forest reserve, and then purified without using chemicals to maintain high levels of minerals. Instead several stages of filtering and UV irradiation is used to deactivate and remove unwanted bacteria. Right before bottling an ozone generator is used to inhibit growth of harmful microorganisms in the water. Hereby only mechanical cleaning is done and no chemicals are used (Kioo Drinking Water co., n.d.).

As clean water is a product of shortage in Tanzania, reflecting on sanitation and health, KIOO Drinking Water Co. currently supports schools and local communities through micro factories and kiosks located close to costumers. To expand the company's policy of increasing the supply and availability of clean water and recycling, and thereby limiting waste, a cleaning and refilling station is a next step for the company. The general idea will be to offer disinfection and refilling of bottles at a lower price than that of purchasing a new bottle, and approximately bringing down the costs of up to 60% (Kioo Drinking Water co., n.d.).

Design of bottle cleaning and refilling station

The station for cleaning and refilling bottles will consists of a six meters long container with two cleaning and refilling stations, in addition to a kiosk unit selling factory bottled water. The station will be powered by solar power, and doors will allow for covering of sun and rain. Half the container will work as a storage, that is easily accessible for a truck with new supplies. The station is designed by Huse Design (figure 2.11, 2.12 and 2.13).

Figure 2.11: *Design of the kiosk. Made by Huse Design.*

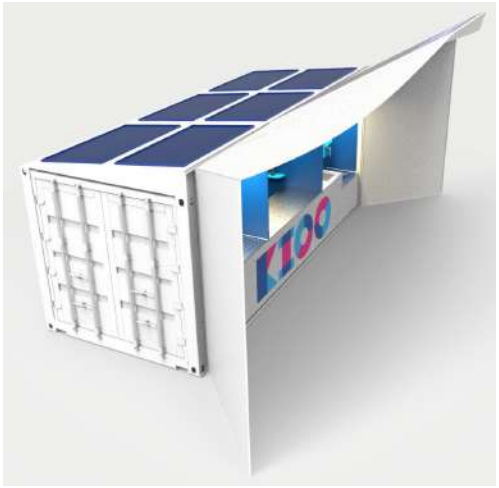


Figure 2.12: *Side view of the kiosk. Solar panels will be installed on the roof for power supply. Made by Huse Design.*

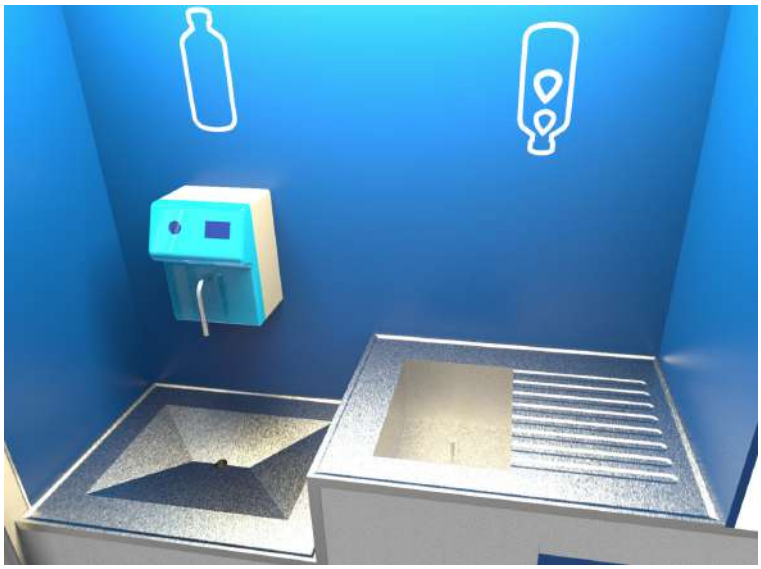


Figure 2.13: *Close up at the cleaning and refilling unit, right and left side of the figure, respectively. Made by Huse Design.*

Location of station

The first station will be located at the university campus in Dar Es Salaam (figure 2.14). Hereby the station will be close to the main office/factory of KIOO Drinking Water co., as well as at a site with many possible users. Although it is not expected that surface water will be a primary source for water at an university campus, a general shortage and lack of access to clean water will remain an issue. It is expected that many university students will be interested in buying clean water at a low cost, reusing bottles and caring for sanitation and their health, as they will be informed of the importance of clean water (Kioo Drinking Water co., n.d.).

Figure 2.14: *Location of the site of the first cleaning station and KIOO drinking water co.*

The pathogenic causes for contaminated water and waterborne diseases 3

Waterborne diseases are the cause for many cases of serious illness and deaths, where especially children and weakened are at high risks due to low immune-system. As previously mentioned (see section 2.1), the diseases are a consequence of an infection by pathogens in forms of either parasites, virus or bacteria. Once the immune system has been compromised by a pathogenic infection, the body's own ability to fight an infection will be compromised as well making it more fragile to other diseases and infections that can be caused by poor sanitation and contaminated water (TeachMePhysiology, N.d).

3.1 Pathogenic microorganisms

The infection, consequential removal and precaution of pathogens is fairly different dependant on the type of pathogenic infection.

Parasites

Parasitic infections are due to parasites invading organ systems and living off the host. The parasites can invade the organ system and produce toxins and cause serious illness. Parasites can be treated with medication as antibiotics that causes the parasites to detach from i.e. the gut, become dissolved and pass through the body naturally (Claire Gillespie, September 2018; TeachMePhysiology, N.d).

Virus

Much alike a parasite, a virus is dependant on a host, and infects by inserting genetic material and taking over the functions of the host cell and disrupting cell function (figure 3.1 (1 and 2)) (Crosta, May 2017). The virus reproduces through the production of new cells with the genetic material of the virus (figure 3.1 (3 and 4)) (TeachMePhysiology, N.d). A virus can be stopped by protein from the host cell, that will interfere with the replication process of the virus. Due to the dependence of a host cell, virus cannot be broken down by medication. Antiviral are used instead for well known virus, where a small dosage of a virus is inserted into the body, where the immune system can prepare itself for getting infected by the virus by shutting down cells compromised by the antiviral with genetic material of the virus (Chow, May 2020).

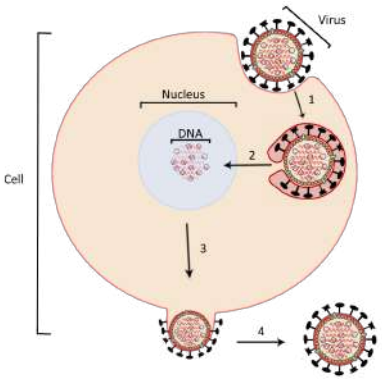


Figure 3.1: *Replication of virus in a host cell (TeachMePhysiology, N.d).*

Bacteria

In contrast, a bacteria is an independent microbiological cell in terms of reproducing, which happens rapidly. Bacteria occur naturally everywhere, and ranges in size and shape. In humans and animals, the majority of bacteria exist in the gut and both harmless and even beneficial to the immune system. Some types of bacteria have parasitic association by causing infections and are categorised as pathogens (Willey et al., n.d.). The pathogen will disrupt the normal function of cells and kill off some tissue when appearing as a foreign cell. This can be due to the bacteria producing toxins that paralyse the metabolic functions of cells or the bacteria overcrowding the host tissues and overwhelming the human immune system. To treat a bacterial infection, antibiotics are used. The antibiotics can block vital processes in the bacteria and thereby stop the reproduction allowing the immune system to fight back (NPS MedicineWise, December 2019). Most bacterial pathogens can be separated into gram-positive and -negative organisms depending on the cell wall, where gram-positive organisms have a layer of peptidoglycan in the cell wall. Using gram-staining, the layer of peptidoglycan will retain a stain, whereas the thin layers in the cell wall of gram-negative-bacteria will turn pink, as the peptidoglycan is found further inside the cell (Steward, August 2019).

The overall structure of bacteria, disregarding size and shape, consists of a wall that contains teichoic acids and endotoxins in the cell capsule of gram positive and -negative organisms, respectively. This causes a negative charge of the bacteria, due to presence of phosphate, a negatively charged component. Furthermore, most bacteria has flagellum that allows for locomotion, and a mitochondrion, that functions as the cells powerhouse (figure 3.2) (Willey et al., n.d.).

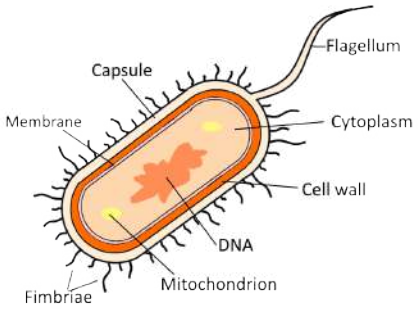


Figure 3.2: *Structure of a bacterial cell (Willey et al., n.d.).*

Another difference between gram positive and -negative organisms is also found in the cell wall, where the presence of an outer lipid membrane makes the gram negative cell wall impenetrable and thereby harder to kill. Many bacteria found in the gut are gram-negative and can thereby be dangerous if consumed, as the immune system would have trouble breaking it down (Steward, August 2019).

Biofilm

An interaction between many microorganisms is called a biofilm, where a solid matrix of microorganisms attached to a surface work together in a force. Biofilms are thereby more resistant to removal by disinfectant, and instead function as a protective habitat for microorganisms such as bacteria (Next Science Group, n.d.). The microorganisms need adenosine triphosphate for survival and growth, which can be found in dead matter within the biofilm, or in material that passes the biofilm. The biofilm will grow concurrently with the growth of microorganisms and attachment of new microorganisms, in addition to the reproduction of new microorganisms. Once matured, the microorganisms will detach, move freely and disperse (figure 3.3) causing further risks of contamination (Hygiena, 2020).

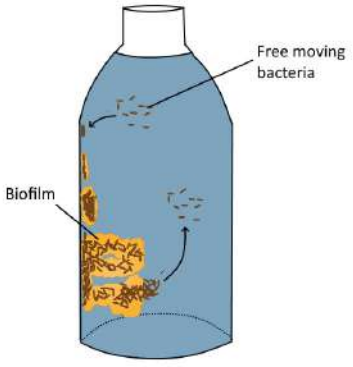


Figure 3.3: *The process of biofilm growth.*

Adenosine Triphosphate

Adenosine Triphosphate, ATP, is an unit of energy used in all living cells. Once the ATP has been used and broken down, the metabolic processes will produce the ATP again (figure 3.4). Thereby microorganisms can consume other cells, and use the ATP for energy. Contaminated water will have high levels of ATP and can thereby act as feeding ground for microorganisms, allowing them to grow and reproduce fast, causing further contamination. ATP is an enzyme, that can be measured both in living and dead cells, however the ATP cannot indicate whether a cell is alive or not (Nave, N.d; Hygiena, 2020).

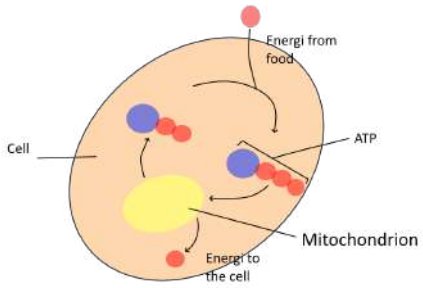


Figure 3.4: *The process of ATP.*

3.1.1 Sanitation and pathogenic removal

Poor sanitation and contaminated water are the main causes for pathogenic infections, despite precautions are fairly simple. Precautions as washing hands and boiling water before use will remove and kill the pathogens before they can cause an infection. It is furthermore possible to use disinfectants to kill the cell before entering the body. Disinfectants have little to no effect on parasites. For virus the disinfectant will inhibit the growth of the disease-carrying microorganisms, whereas it will destroy the bacterial cell and prohibit formation of biofilm. Disinfectants quickly breaks down the cell wall of the pathogenic cells and deactivates replication, and thereby stop the potential danger of infection, using either physical or chemical methods (Nyco, n.d.).

The disinfectant method used for removal of pathogens by KIOO Drinking Water Co. at the cleaning and refill unit needs to be simple and inexpensive, in order for it to be every-man usable and to keep the costs of the cleaning process down. Typical physical methods involve UV light or heat, which will require a further rinse of the bottle. Due to current sewage situation in Tanzania, where tap water may contain pathogens, the station will use water purified by KIOO Drinking Water Co. for cleaning the bottle as well as refilling. Therefore it is desired to use as little clean water as possible for cleaning the bottle. In addition, the cleaning solution used to break down the cell wall of the pathogenic cells, should not contain dangerous chemicals or have the need to be rinsed off the bottle before the bottle being refilled. As a result, the chemical method of Electro Chemical Activated water will be used to disinfect the bottle, where no rinse after use is necessary.

3.2 Electro Chemical Activated water

Electro Chemical Activated water, ECA water, is an oxidising disinfectant, made by treating water and sodium chloride using electrolysis to form hypochlorous acid. The electrolysis causes a non-spontaneous reaction, that is found through both membrane cell (figure 3.5) and single cell technology, causing the compounds to separate into ions where interchange of electrons occurs (HypochlorousAcid.com, n.d.).

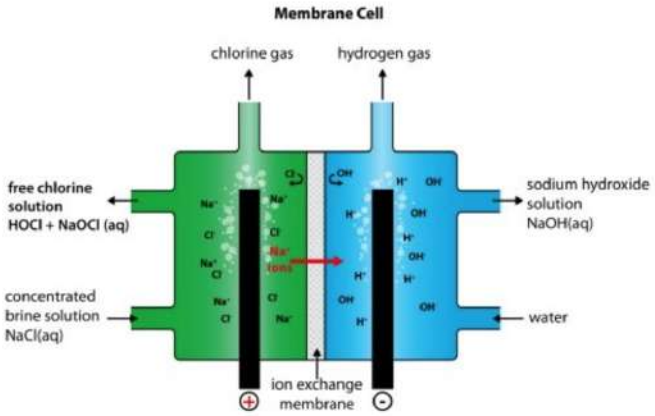


Figure 3.5: *Membrane cell technology used to electrolyse sodium chloride into hypochlorous acid (HypochlorousAcid.com, n.d.).*

The technologies use an anode and a cathode for the following chemical reactions, respectively:

$$2Cl^- \rightarrow Cl_2 + 2e^-$$

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$$

For a membrane cell technology, the following reactions will then happen at the anode and cathode, respectively:

$$Cl_2 + 2OH^- \rightarrow HOCl + HCl^-$$

$$2NaCl + 2H_2O \rightarrow 2Cl_2 + H_2 + 2NaOH$$

The reaction found at the anode, is the one that occurs at single cell technology, where only free chloride is added to the water and therefor not producing sodium hydroxide as a bi-product (HypochlorousAcid.com, n.d.).

Chlorine exist in three different forms that are dependant on the pH-level. At pH below 4 the chlorine will be available as chloride in gas form, at pH between 4-8,5 the chlorine will appear as hypochlorous acid, and at pH above 8 it will be available as hypochlorite (figure 3.6). The sodium hydroxide, NaOH, is an alkali produced bi-product.

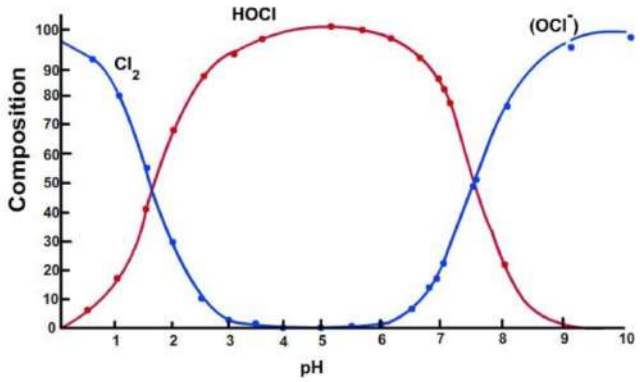


Figure 3.6: *The composition of chlorine at different pH levels (HypochlorousAcid.com, n.d.).*

Hypochlorous acid is the most effective chlorine for disinfectants due to a strong oxidation-potential and neutral charge. In addition, the hypochlorous acid molecules are small and fast moving, making them very effective in terms of deactivating cells quickly (Fooddiagnostics, n.d.).

At high pH levels the hypochlorite will form with sodium to become sodium hypochlorite:

$$Na^+ + OCl^- \rightarrow NaOCl$$

Sodium hypochlorite is often used as either bleach or disinfectant, however not as effective as hypochlorous acid due to it having larger moving molecules and being negatively charged, thereby repelling the bacteria with negative charged surface, unable to enter the bacteria membrane and cause degradation (Lenntech, n.d.).

For the ECA water to be as effective as possible it is therefore important to have a pH level close to 7-8. Furthermore, ECA water is approved by the Danish ministry of victuals and environment as a disinfectant for food industries, herds and storage of fish without a rinse after use, as the product does not impose a health risk (Fødevareministeriet, February 2017). For cleaning purposes ECA water is proved to disinfectant completely at a concentration of 200 ppm with 5 minutes of contact time. In addition, hypochlorous acid is a weak acid causing minimal corrosion and danger for use (Fooddiagnostics, n.d.).

3.3 ECA water deactivating pathogens

Disinfectants do not kill virus, but breaks the wall and proteins within the mitochondrion of the host cell for virus, making it unable to grow and replicate. The strong oxidation potential of ECA water, where the lack of e^- makes the acid unstable, results in the disinfectant making covalent bonds with the cell and breaking the weaker natural bonds. In bacteria, the ECA water also ruptures the outer layers of the bacterial cell and damages the cells protein (figure 3.7). The neutral charge of the ECA water will allow for the disinfectant to break through the negative charged bacterial wall and membrane, and cause microbial degradation by breaking the cell structure and subsequently leak the DNA material of the bacteria (Thompson, April 2012).

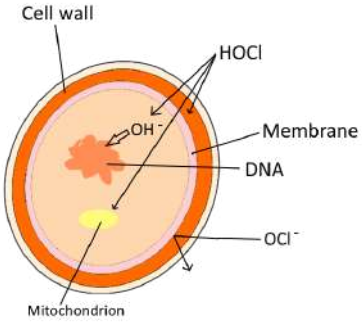


Figure 3.7: *ECA water deactivating bacterial cell. (Hypochlorsyre.com, N.d).*

ECA water is harmless to humans in small concentrations, as human cells have the ability to repair themselves once broken and due to an antioxidant, glutathione, that will be oxidised instead of the cell. Thereby, the oxidation potential of the ECA water will be used, and the waters ability to break down cells and deactivate pathogens will be ineffective. Hence, the ECA water works most efficiently on material surfaces. Furthermore, bacterial cells do not have the ability to repair themselves, stopping the reproduction of bacteria (Hypochlorsyre.com, N.d).

Problem Statement 4

The aim of the project is to investigate if it is possible to clean a contaminated bottle using the disinfectant ECA water and the materials of a cleaning and refill station. The water refilled in the cleaned bottle has to fulfil the requirements for potable drinking water given by the Tanzanian Bureau of Standards, TZS (see section 2.3.1 på side 9). The cleaning unit will allow for contaminated bottles to be reused, followed by refilling with cleaned water, which overall will improve the accessibility of clean water and limit the transmission of waterborne diseases.

To obtain the aim of the project, the following problem statement will be investigated throughout this project:

Under what premises is it possible to fulfil the requirements for potable drinking water by TZS for water refilled in a contaminated bottle, that has undergone cleaning?

The following points will be examined to answer the problem statement:

- Adjustment of concentration of disinfectant, cleaning time, and water pressure.
- Physical adjustments to the nozzle design.
- Measuring viable counts and pathogenic bacteria.
- Measuring ATP present on mouth piece of bottle, thread in bottle cap, and nozzle surfaces.

4.1 Project definition

Improvement of the accessibility of clean water is an improvement of general health. By inflicting refill and cleaning units, bottles or containers that have been exposed to contamination will not transmit the contamination onto refilled clean water since the bottle has undergone cleaning.

Using ECA fluid as disinfectant implies that a cleaned bottle can be refilled directly and limit rinsing of the bottle. Hereby, the amount of wasted water will be limited. As the water used for dilution of the ECA fluid for cleaning and refilling has undergone complete treatment, it is desired to use as little water as possible for the cleaning process. Limiting the wasted water will contribute to reduce the costs, and thereby make the option of cleaning and refilling bottles and containers available for as many users as possible, and hereby contribute to an improvement of general health in addition to promoting re-use of PET-bottles, and limiting pollution of the environment.

Experimental procedure 5

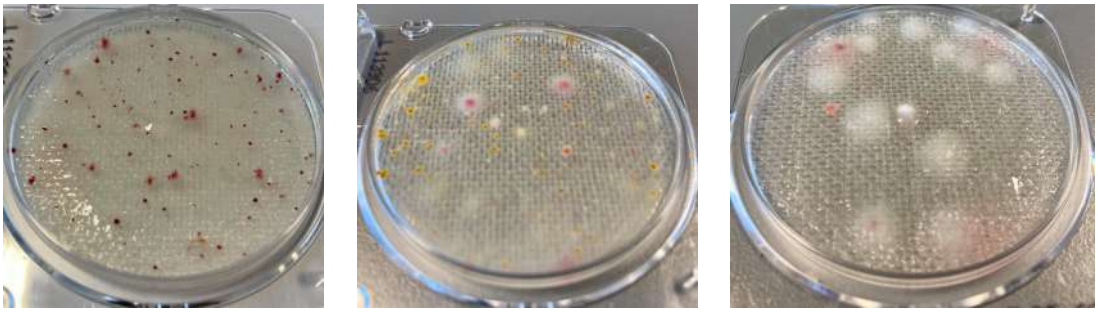
The aim for testing is to find premises where it is possible to fulfil the requirements for potable water stated by TZA for clean water filled into a contaminated bottle, that has undergone cleaning. By varying cleaning time and concentration of ECA water at a chosen water pressure, it will investigated for a combination, where these variables will allow for an adequate decrease in viable counts of bacteria. Furthermore, this combination of cleaning should ensure an absence of pathogenic bacteria, and that the available ATP found on the mouth and cap of bottle as well as the surface area of the nozzle after cleaning is insignificant. As the ECA water will be diluted using pre-cleaned water, it will be aimed to use as little water as possible and thereby low cleaning time. By cleaning time is meant the time for which the nozzle is activated and allows for contact between ECA water and bottle. To keep the costs down for the user of the cleaning station, it will be investigated for a lowering of concentration and cleaning time.

5.1 Methods for testing

To ensure that the viable counts after cleaning the bottle and cap stays within the requirements for drinking water, a quantification of the heterotrophic bacteria will be made. Furthermore, the remaining ATP after cleaning will be measured to secure that new microorganism do not have anything to consume on used surface area nor that virus can transmit between users of the unit. Finally, *coliforms* and *E. coli* tests will be performed to examine the presence of these pathogens.

5.1.1 Determination and quantification of heterotrophic water bacteria

Determination of the presence of heterotrophic water bacteria will be used to indicate the thoroughness of cleaning at a set concentration and cleaning time. A Compact Dry AQ will be used to test the water quality in the cleaned bottle, where 1 mL of water will be put onto the dehydrated plate, turning it gel-like and evenly distributed across the plate. For each test combination, two samples are necessary, as the Compact Dry AQ solution has to incubate at 22 and 37°C for 68 and 48 hours, respectively. After the incubation period, the viable cells will appear in colonies, that can be seperated into three different bacterial types. The heterotrophic water bacteria can be determined as red colonies (figure 5.1 (a)), whereas yeast shows as white-pink colonies (figure 5.1 (b)) and molds will show as white cotton-like colonies (figure 5.1 (c)). The total amount of bacteria can be counted using the 20 1 cm^2 grid on the bottom of the plate. The quantification of heterotrophic water bacteria will be held against the limits of potable drinking water (see section 2.3.1) (R-Biopharm, n.d.*a*).



(a) Heterotrophic water bacteria (b) Yeast (c) Mold

Figure 5.1: *(a) Red colonies indicate presence of heterotrophic water bacteria. (b) White-pink colonies indicate presence of yeast. (c) White cotton-like colonies indicate presence of mold.*

The heterotrophic water bacteria are pigmented using the gram-straining method, where the enzyme peptides, found naturally in the bacteria, is used to die the bacteria. For gram-positive bacteria, a redox-indicator is used to break down the cell wall and allow for the strain to dye the bacteria (R-Biopharm, n.d.*b*).

Some microorganisms will be stressed or chlorine-tolerant and thereby not be detected under the conditions for 48 hour cultivation at 37°C. A longer incubation time at lower temperature is therefore also used for testing to ensure the cultivation of these bacteria. Typically it will be gut bacteria that thrives best at 37°C, and these are the most crucial pathogenic bacteria (Naturstyrelsen, March 2013*a*). At 22°C the viable count will indicate the presence of natural soil and water bacteria, that are rarely pathogenic. However, these can indicate how thoroughly cleaning has been done. Lastly, the colonies cultivated at either temperature can show in colonies differentiating in sizes, which will indicate the presence of formed biofilm. The Compact Dry AQ will not give an indication of dead cells (HyServe, n.d.).

5.1.2 Ultrasnap

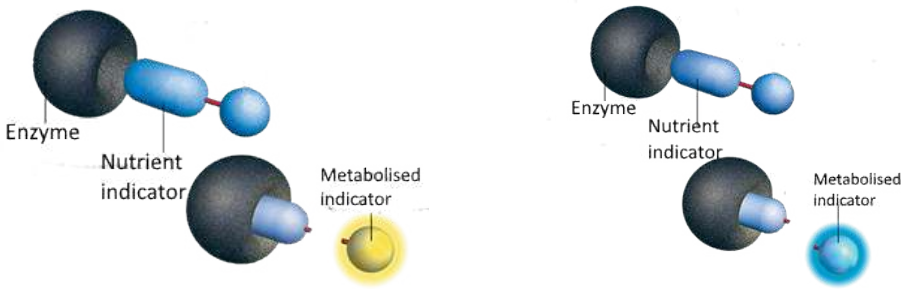
The UltraSnap is a test for counting ATP on surface areas. The UltraSnap consists of a pre-wetted swabber and a test tube with a biochemical in terms of a liquid enzyme. The swabber is used to collect ATP and biofilm on a surface area up to 10 cm^2, before being inserted to the test tube, where the enzyme is released (figure 5.2). The UltraSnap will be paired with an Ensure Touch, that can be adjusted to a certain level of relative light units (RLU), and will then tell within 15 seconds whether or not the collected test pass the chosen maximum level of RLU, that will indicate a growth conditions for new microorganisms. The Ensure touch measure the amount of RLU, and even though there are no direct ratio between RLU and ATP, a greater ATP will givea greater RLU. Since ATP levels do not have official requirements, thresholds based on experience from the food industry will be used (Hyeina, n.d.).

Figure 5.2: *The swabber used to collect ATP for the Ultrasnap test.*

Although the ATP test cannot confirm the presence of pathogens, it is a good indicator of general cleanliness, as it will count the amount of ATP present.

5.1.3 Colilert

Coliforms and *E. coli* are bacterial pathogens and naturally found in the gut, where especially *E. coli* cause serious infection in a foreign environment due to quick reproduction (Verhille, January 2013). *Coliforms* are however not directly dangerous, but they are easy to culture and can be used to indicate the presence of other fecal pathogens, that are dangerous. A colilert test, that tests for the presence and amount of *coliforms* and *E. coli*, will be used as an indication to fulfil the drinking water requirements, where absence of specific pathogens is necessary (see section 2.3.1). The colilert test uses two nutrient-indicators that can be metabolised by their individual enzyme found in *coliforms* og *E. coli* respectively. If the enzyme is present, the nutrient indicator will turn either yellow or fluorescent once metabolised from their respective enzyme (figure 5.3 (a) and (b)) (Idexx, n.d.).



(a) Metabolism of *coliforms*. (b) Metabolism of *E. coli*.

Figure 5.3: *The nutrient-indicators of Coliforms and E. coli being metabolised by their respective enzyme (Idexx, n.d.).*

Once the nutrient indicators have been added to the water sample and placed in a incubation tray, the tray has to be stored at 35°C for 24 hours to allow for the metabolism to take place. The colouring from the *coliforms* enzyme can be seen without further needs if present, whereas the flouroscent from the *E. coli* enzyme needs UV-light to be observed (Idexx, n.d.).

5.2 Experiment description and materials

The experiment will be carried out using a testing station (figure 5.4 (a)), that has been installed with a sink with two different nozzles; one for cleaning the bottle and one for cleaning the bottle cap (figure 5.4 (b) and (c), respectively). Two different nozzles are necessary as the cleaning purposes for the bottle and cap are different, since the bottle needs cleaning both inside and outside on the thread. Therefore, the nozzle unit for cleaning the bottle has a bowl function, that will be filled up with water and thereby allowing the thread to come in contact with cleaning fluid, in addition to spraying water upwards into the bottle. The nozzle for cleaning the cap solely sprays water upwards. Furthermore the station has a dosage pump installed.

The diluted ECA water will spray water upwards into the bottle and kill the bacteria and rinse it out as the water flows out of the bottle. To assure that the measurements are comparable despite changing variables as concentration, cleaning time, and water pressure the same procedure and testing methods will be used for all experiments (appendix A). The nozzles are initial products, where altering will be performed.

(a) Cleaning station.

(b) Nozzle for bottle.

(c) Nozzle for cap.

Figure 5.4: *Testing station.*

5.2.1 The pump

To control the concentration of ECA water used for each cleaning, a DDC/DDA dosage pump from Grundfoss is used. The ECA water has a concentration of 4500 ppm, from which the pump can dose 16,8 mL per pulse. The pump is installed to use 1L of water per pulse. This allows for a maximum concentration of 75,6 ppm. The dosage per pulse is adjustable and allows for variations in the concentration (Grundfoss, n.d.).

5.3 Testing strategy

The experimental procedure will function as a pilot survey and be progressive and empiric, where the bottles will be tested under contaminated stages until consistent result of meeting the requirements for potable drinking water is fulfilled. By adjusting the variables cleaning time, concentration of ECA water, water pressure, and physical adjustments to the nozzle through an iteration method, determination of bacteria will be the main method used to track the progress, and examine if it is possible to lower cleaning time and concentration. Furthermore, initial test conditions will be found to illustrate the initial state of a clean and a contaminated bottles condition.

The main tests will be performed using a 0,6 L PET bottle, that will be contaminated using sewage water to ensure a worst-case-scenario of the presence of pathogens in the bottle. Concluding tests will be performed on bottles varying in size and shape to verify that the drinking water criteria can be met for other bottles and thereby fulfil the idea of reusage. The sewage water was taken from the treatment plant in the East of Aalborg on 17^{th} of October at an early cleaning stage before sedimentation has taken place.

The report of the test program will be split into four parts; Limitations and initial conditions, Preliminary tests, Calibrations and following outcome, and Concluding tests.

Limitations and initial conditions 6

The experiment will have some limitations due to materials, time and expenses. The limitations and possible consequence are stated below. One of the consequences of the limitations is the necessity for assumptions. These are also stated below. Furthermore, initial conditions of the experiment will be tested for. The initial conditions will be used to show the effectiveness of the cleaning optimising process.

6.1 Test limitations

A limitation is found in the dosage pump, as it allows for a maximum of 16,8 mL per pulse, concluding in a concentration of ECA water of 75,6 ppm. The aim of the project is to investigate whether lower concentrations than the ones used for validating the method will be adequate for cleaning the bottles. The validating concentration was 200 ppm and it is suggested by Hygiena (2020) that a concentration as low as 10 ppm should be sufficient. Thereby, it is aspired that a concentrations of maximum 75,6 ppm will be adequate. The variation in concentration will be adjusted using a dosage pump and validated using chloride measuring strips.

The testing period will be affected by limitations in both expenses and time. The expenses related to testing equipment will result in the Compact Dry AQ being used as the main method for testing, as the costs involved with this method is inferior to those involved with other methods. However, since the Compact Dry AQ tests takes 68 hours to complete for each test stage, and results are observed to identify future modifications, it is pursued to keep the contamination time for the testing to a minimum without compressing the growth and replication of biofilm and bacteria in the bottle (see section 6.4). In addition, only one bottle produced by KIOO Drinking Water Co. in size 0,6 L is available for the entire testing period.

The testing station will be located in Aalborg, Denmark, at floor level, which will indicate a large water pressure at tap outlet. As this differs to the circumstances in Tanzania, where a maximum of 2,6 bar is possible, the pressure will be adjusted and limited. A pressure reducing valve will be installed, as soon as it is available. In the mean time, which will involve the preliminary testing, a water flow of 10,6 L per minute will be used, as this corresponds to approximately 2 bar (AEL heating, n.d.).

The Compact Dry AQ test for microorganisms as viable counts of heterotrophic water bacteria, and can differentiate between bacteria, yeast and mold. Unfortunately, the test does not differentiate between the type of bacteria, and it can therefore not tell if there is a presence of i.e. specifically *E. coli* but indicate if there is a presence of bacteria both pathogenic and non-pathogenic.

Thereby, it is necessary with further tests, like the colilert test, to ensure that there are not present pathogens before fulfilling the complete requirements for potable drinking water.

The fluid used to contaminate the bottles will consist of 20 mL sewage water and tap water for the remaining space in the bottle in order to have sufficient sewage water for all tests in the standard bottle size 0,6 L. This will equal a dilution of a factor 30. It is assessed that this contamination level will equal the worst cases of contamination or surpass the levels of contamination in water used for consumption purposes in Tanzania.

6.2 Assumptions

Replication of bacteria in the collected sewage water will occur until an equilibrium between one bacteria dies for each new one that is produced. This means that the concentration of the sewage water will intensify from when it was collected and until equilibrium is reached. Since the sewage water is stored at maximum 5°C, it can be assumed that replication of bacteria within the stored sewage water is limited. For testing purposes it is assumed that the changes in concentration will be indifferent for the results.

Aalborg municipality, where the testing takes place, allows for a pH interval of 7-8,5 (Aalborg Kommune, 2014). ECA is created at pH 8, which allows for the hypochlorous acid to last longer and to limit corrosion of the equipment that comes in contact with the fluid. As a pH of 8 is the upper limit of hypochlorous acid, it is assumed that the pH of the tap water does not surpass the given interval.

Lastly, since not all bottle variation produced by KIOO Drinking Water Co. are available for testing, it is assumed that the bottles available and used for testing can represents all bottle sizes. The concentration will be adjusted for each bottle size and equal to a dilution of factor 30.

As it is not possible to test for the presence of virus in a water sample, it will be assumed that the presence herefore follows that of bacteria. The spreading of viruses can occur through surfaces touching, and it is therefore necessary to ensure that the transmission of pathogens between bottles and cleaning units does not occur. The measurements for ATP will be used to indicate how well the surfaces have been cleaned during the cleaning of the bottle, and it is assumed that the presence of ATP can resemble how thoroughly cleaning has been done.

6.3 Interpretation of heterotrophic water bacteria results from Compact Dry AQ

The colonies displayed on the Compact Dry AQ are the viable cells that are capable of growing after the disinfectant has been used. The colonies therefore gives an indication of the general contamination level and overall quality of the water. As the water samples for all tests are taken as single samples for water in cleaned bottles, and the Compact Dry AQ plates allow for up to 300 viable colonies cultivating per plate, there will be uncertainties involved when examining the results and comparing viable counts to illustrate development. To account for the uncertainties, the variability of the results will be displayed as a shade surrounding the counted colonies on the graphic visualisation of the test results, where an interval of ± 10 viable counts will be used. General variations of ± 10 is accepted as insignificant (Greenwood, August 2020).

6.4 Initial test condition

The initial test condition will be used as comparison to demonstrate the efficiency of cleaning a contaminated bottle.

As it is allowed for a viable count of microorganisms of up to 20 per mL in tap water at the receiver in Aalborg municipality (Aalborg Kommune, 2014), an initial test will be taken of clean tap water, that has only been in contact with a clean non-used bottle. The results will indicate whether there will be a transfer of microorganism from the tap water used for cleaning and to the bottle, from where a sample is taken.

Another sample will be taken to illustrate the condition of a contaminated bottle filled with clean tap water, which will be used to resemble reality. There is a wide difference in the time taken for pathogenic growth, replication and dispersion of biofilm at 10 minutes and four days respectively of contamination of the bottle (Britannica, n.d.*a*). To avoid compressing the reality of replication and growth, there will be made a comparison between 10 minutes and four days of contamination time. The comparison will be used to identify the difference in viable count and to assure that 10 minutes of contamination will surpass the drinking water requirements, and thereby represent severe contamination and keeping testing time to a minimum.

A sample will be taken of the diluted contaminated water. This will be used as a comparison to the contaminated bottle with clean water to show the effect of replacing the contaminated water with clean without cleaning the bottle, as well as the dangers involved with not having access to clean water.

Lastly, the amount of ATP on the mouth and cap of a bottle with regular use and the surfaces of the cleaning unit will also be tested as an initial condition for comparison for how well the cleaning unit works to avoid transfer of bacteria from theses surfaces before and after cleaning.

Results

The precise viable counts for all initial test can be found in appendix B.1.

The initial test for a clean bottle shows close to no heterotrophic water bacteria present, and it can thereby be assumed that the tap water used for both cleaning and refilling the bottle is without microorganisms.

The contaminated bottle with clean water as 10 minutes contamination shows a presence of up to 200 heterotrophic water bacteria per mL, which reveals the problematic of refilling used contaminated bottles with clean water, as the bacteria will travel form the bottle sides to the water fast, and the necessity of sanitation. Comparing 10 minutes to four days of contamination time shows a large difference of both heterotrophic water bacteria and small amounts of yeast of over 100 per mL. However, since the presence of bacteria for both contamination of 10 minutes and four days exceed the drinking water limit by far, a contamination time of 10 minutes will be used without compressing the growth and replication of bacteria and biofilm.

The contaminated water shows a presence of bacteria exceeding the plates limit of 300, which reveals the risks involved with lacking access to clean water.

The ATP levels of a bottle mouth used for regular use exceeds a level of 2200 RLU, which is a very critical level that allows for ideal growth conditions for future bacteria and transmitting diseases between bottle mouth, cleaning station, and future users.

Preliminary tests 7

The idea of preliminary testing is to test whether the idea of cleaning a contaminated bottle before refilling with clean water using ECA water will work in terms of deactivating bacteria and showing less heterotrophic water bacteria present in the water than what was found in section 6.4. Furthermore it is desired to validate the results and assess for adjustments that can improve further tests.

7.1 Introductory test of using ECA water as disinfectant

The introductory testing will be used to find a co-relation between cleaning time and concentration level of ECA water. As the general idea is to lower costs of getting clean water and make it more accessible, the testing will focus on lowering the cleaning time and concentration compared to the validation tests of ECA water and to stay within the drinking water requirements (see section 2.3.1) (Hygiena, 2020).

Considerations and procedure for the test

The variables; cleaning time and concentration of ECA water, will be adjusted accordingly with a pressure equal to a flow of 10,6 L per minute, where the cleaning time will be tested as the first variable (figure 7.1 (1)). Once a lowered cleaning time has been found, where drinking water criteria is complied, the concentration will be tested as a variable (figure 7.1 (2)). A lowered cleaning time will imply that the surface area of the bottle has shorter contact time with the ECA water and thereby that some biofilm may be able to resist the disinfectant and not be rinsed out. Furthermore, a lowered concentration will mean that there is less hydrochloric acid present to deactivate the bacteria. It is desired to find a combination of cleaning time and concentration of ECA water that is adequate to deactivate enough bacteria to subceed the criteria for potable drinking water.

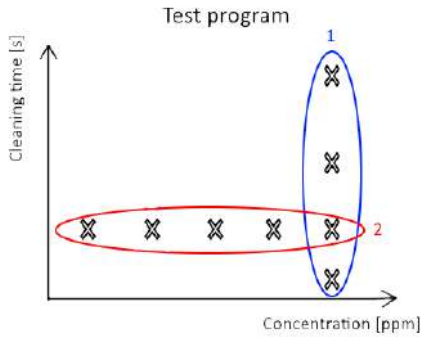


Figure 7.1: *The cleaning time is first tested as a variable. Hereafter the concentration is tested as a variable at time chosen from the first test.*

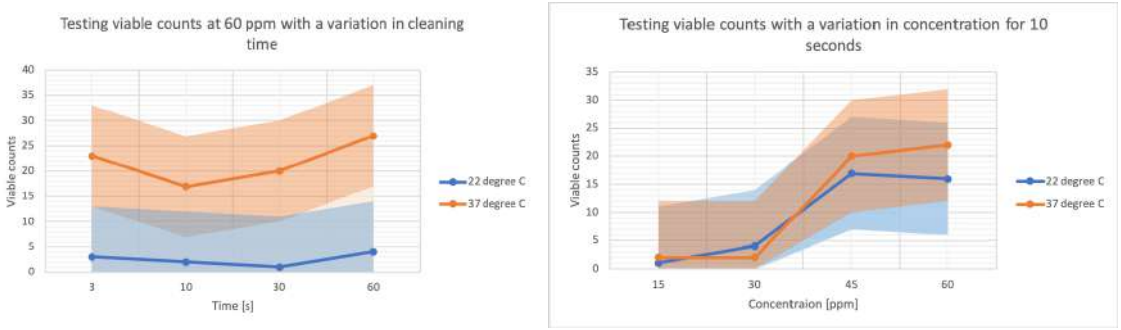
Results

At the chosen flow from tap of 10,6L per minute, the water splashing from the nozzle will only in the first 10-15 seconds have enough pressure to reach the bottom of the bottle when the bottle is vertical, and hereafter barely reach half way up the bottle, as a damming of water is created (figure 7.2).

Figure 7.2: *A water damming is created right under the label when cleaning time exceeds 10-15 seconds.*

The pressure from the valve is bigger once released and then decreases to constant stage, hence a short cleaning time of maximum 10 seconds should be used for further testing. This is reflected in the results, where the amount of heterotrophic water bacteria increases when 30 seconds of cleaning time is tested. It is also assessed that three seconds will be too short for cleaning (figure 7.3 (a)).

With the results from time as a variable in mind, 10 seconds of cleaning time has been used for testing a variation in the concentration. However, there has been found no reasonable tendency in the results of varying the concentration, as the amount of heterotrophic water bacteria increases along with an increase in concentration, whereas the opposite would be expected (figure 7.3 (b)).



(a) Time as variable

(b) Concentration as variable

Figure 7.3: *Viable counts when testing the variables cleaning time and concentration.*

The graphs are based on the count of heterotrophic water bacteria appearing on the Dry Compact AQ after incubation time (appendix B.2).

From initial conditions without cleaning, an improvement of up to 99% is found by using 15 ppm at 37°C, and 27% for 22°C cultivation. Hereby, the results of the introductory tests show that it is possible to reduce the amount of viable counts at a lowered cleaning time and a lowered concentration of ECA water to stay within the criteria for potable drinking water.

Identification of problems and improvements for next stage

The lack of tendency in varying the concentration can have multiple causes. The nozzle used is fairly unstable, resulting in a lot of variation in the positioning of the bottle and perhaps not cleaning all of the bottles surface area. Furthermore, the taps pressure was based on an average flow, where the tap was turned off and on in-between each test. A more stable nozzle with four thin plates to press the bottle onto is installed for future tests with the general idea that less variation in the positioning of the bottle will give more identical cleaning (figure 7.4). A pressure reducing valve is installed to ensure a fixed pressure, and limit pressure as variable.

Figure 7.4: *A new design for the nozzle, that allows for more steady positioning of the bottle.*

Lastly, an error was made when adjusting some of the concentrations, that were tested from low to high. The error consists of not allowing enough water to flow through the unit before cleaning with a new concentration. The first tests were taken after using a maximum concentration of 75 ppm for testing time as a variable. The water used for cleaning therefore had some of the high concentration, which is noted in the results of the lower concentrations. In the same way, the results of higher concentrations may reflect the lower concentrations that have not yet been replaced by higher concentrations in the pipes of the unit.

7.2 Examining tendency in viable counts and available ATP

The following tests will use the improvements of a stabled nozzle, fixed pressure, 10 seconds of cleaning time to certify that concentrations at and below 30 ppm are sufficient to meet drinking water criteria for potable water, as the lowest viable counts at 37°C were found at this concentration. In addition, the two concentrations will be used to show a relation between concentration and presence of heterotrophic water bacteria.

Furthermore, Ultrasnap will be used to test for available ATP at the surface area of the bottle mouth, thread of cap, and of the cleaning unit, from where bacteria and virus could transmit between users.

Considerations and procedure for the test

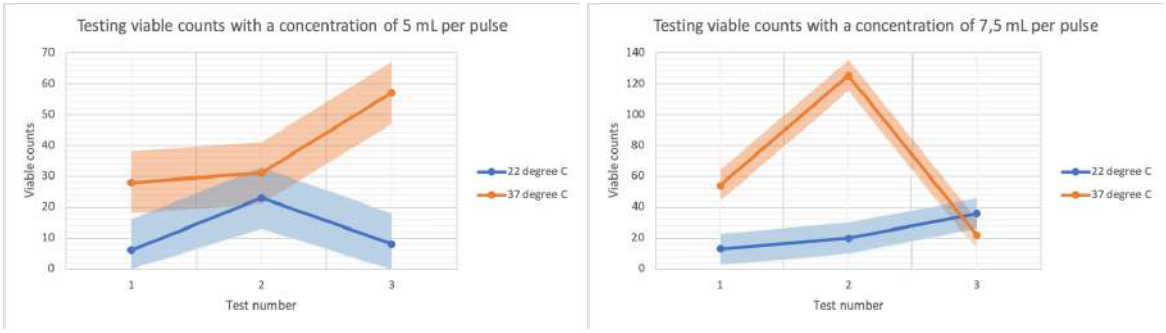
With the pressure reducing valve installed, a set pressure of 2 bars is set. Using the results from section 7.1 it is found that the results does not vary in conjunction with a change in concentration. However, using a cleaning time of more than 10 seconds concludes in the water jet not reaching the bottom of the bottle. A more stable nozzle unit has been installed, which limits the effect of the bottles positioning and thereby the cleaning will be more alike for each test. Lastly, concluding from the preliminary tests, a concentration of 5 and 7,5 mL per pulse, equal to approximately 15 and 30 ppm, of ECA water at 10 seconds cleaning will give a results within the criteria for potable water. The effect of ECA waters concentration will need to be reevaluated, and the effectiveness of 30 ppm will need to be validated.

Lastly, the Ultrasnap will test for how well filling the bowl with used ECA water kills and removes bacteria off the surface areas affected by the contaminated bottle by counting the presence of ATP (figure 7.5).

Figure 7.5: *The nozzles cup filled with water and allow ECA fluid to clean the mouthing piece of the bottle.*

Results

No tendency was found in relation to amount of bacteria and concentration nor steadiness at given concentration (figure 7.6 and appendix B.3), where the viable counts found at 30 ppm exceed the criteria for potable water.



(a) Concentration of 5 mL per pulse

(b) Concentration of 7,5 mL per pulse

Figure 7.6: *Viable counts when testing the variables cleaning time and concentration.*

When comparing the average results of viable counts at 22 and 37°C to that found in section 7.1, an increase of 51 and 62,5%, respectively, is found for 15ppm, whilst an increase of 475 and 3250%, respectively, is found for 30 ppm. This is despite the adjustments to the nozzle and instalment of a pressure reducing valve. This shows a large variation in results, and it is therefore necessary to make control tests for one concentration to investigate if it is possible to find a smaller deviation in the results.

When testing for available ATP in the cap, bottle mouth and surface area of nozzle, it was found that the level of ATP was within the limits (RLU beneath 20) for all surfaces at a concentration of 30 ppm for 10 seconds of cleaning time (appendix B.4). In comparison to the initial conditions, the cleaning of a bottle will successfully limit the risk of transmitting pathogens between user of the unit.

Testing improvements for next stage

It is assessed that the deficiency in the results tendency is due to the positioning of the bottle when activating the nozzle, as this is the only parameter that may vary between each trial, as it is assured that the other parameters are identical under each trial. This is due to the nozzle still being fairly unstable, as it only has two legs and thereby wiggles easily (figure 7.7). Furthermore, the issue with a water barrier being created, and the water jet not reaching the bottle bottom, reoccurs although the cleaning time was decreased to 10 seconds. This coincides with the results being close to a not cleaned bottle, if the water damming has terminated the water jets possibility to clean the entire bottle. Further lowering of the cleaning time is therefore necessary to avoid a damming to occur.

Figure 7.7: *The cup of the nozzle balances on two legs, which makes it fairly unstable.*

7.3 Testing for the efficiency of ECA water at 30 ppm

Further improvements from the previous tests involves changing the cleaning time to 5 seconds, where it is reassured that the bowls will be filled with water and that bottle mouth is cleaned, without a water damming occurring. To prevent the unsteadiness of the nozzle to have an effect, the positioning of the bottle when cleaned will be made identical by tilting the bottle to one side. This also assists the water jet in reaching the bottom of the bottle in each test for the full cleaning time.

Considerations and procedure for the test

As there is no tendency and little resemblance in the results from section 7.1 and 7.2, but an idea from single samples that the requirements for potable water can be met, the efficiency of cleaning the bottle will be examined by taking ten identical tests. The tests will be taken, where the bottle is tilted as far as the nozzle allows whilst cleaning (figure 7.8). The tests will be taken with a pressure of 2 bar with a concentration of 30 ppm at 5 seconds of cleaning time. The variations between each test has thereby been limited as much as possible.

(a) The nozzle can be tilted to the sides.

(b) Water jet reaches the bottles bottom when tilted.

Figure 7.8: *By tilting the nozzle and bottle, the water jet will spray in the opposite site of the water damming, and reach to the bottles bottom.*

Results

With an average of 18,3 and 3 in presence of heterotrophic water bacteria and a spread of 8 and 3 at 37 and 22°C, respectively, the requirements for potable water, which is viable counts of maximum 100 and 50 at 37 and 22°C, respectively, are met (figure 7.9 and appendix B.5).

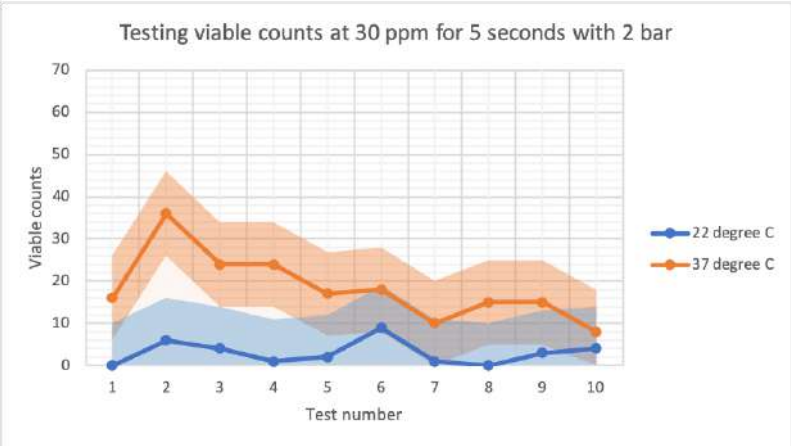


Figure 7.9: *Viable counts at 30 ppm for 5 seconds of cleaning time.*

The average viable count found for the 10 tests shows an improvement of 92,4 and 96,6% for 22 and 37°C when comparing to the initial condition of the bottle before cleaning. Overall, the averages of the 10 tests differs from the previous results found, when making three similar tests, whilst cleaning with a concentration of 30 ppm, with a decrease of 73 and 64%, respectively. The difference between the two set-ups, that could cause the improvement is found in tilting the bottle and decreasing cleaning time, and thereby allowing for the water jet to reach the bottom of the bottle.

Testing improvements for next stage

The bottle requires tilting in order for the water jet to reach the bottom of the bottle due to water damming. It is expected that lowering the cleaning time will result in an increase in viable counts due to the results found in section 7.1. Therefore, further optimisation of the unit is necessary. Since tilting the bottle forced a pressure increase in one side of the nozzle, and thereby allowed for complete flushing of the water in the other side of the bottle mouth, it will be investigated whether a larger fixed pressure will be sufficient to break through the water damming or if further adjustments to the nozzle is necessary.

7.4 Investigating the possibility of breaking through the water damming

Visual tests will be performed to test the pressures influence on breaking through the barrier created by the water damming and allow the water jet to reach the bottom of the bottle without tilting the bottle during cleaning.

Considerations and procedure for the test

It is possible to examine the effect of an increase in the fixed pressure up to 5 bar, as this is the maximum of the pressure reducing valve. No matter the positioning of bottle, the water jet should still reach the bottom of the bottle and ensure for all surface area within the bottle to be in contact with the ECA water. Bottles in different sizes and materials (1 L PET, 0,6 L PET, and 0,45 L glass bottle) will be tested as representatives of the bottles supplied by KIOO Drinking Water Co.

Results

A water damming will occur regardless the pressure of the water, when cleaning the 0,6 L and 1 L PET bottles. In comparison, a water damming does not occur when cleaning the 0,45 L glass bottle (figure 7.10).

Figure 7.10: *When cleaning the glass bottle, a water damming does not occur.*

The difference between the PET bottles and the glass bottle is found in the shaping of the two bottle types, where the glass bottle is both shorter and has a larger mouth than the PET bottles (figure 7.11). Hereby the exchange ability of the water with jet streaming water in and gravity flowing water out is greater than with a smaller bottle mouth. Furthermore, the shorter length of the bottle allows for the water jet to reach the end.

(a) Sizes of bottle mouths.

(b) Length of 0,6 L PET and glass bottle.

Figure 7.11: *Difference in size of mouth pieces of PET bottles and the 0,45L glass bottle, and in length of a 0,6L PET bottle and a 0,45L glass bottle.*

Testing improvements for next stage

To avoid the water damming creating a barrier for the water jet to go through in the PET bottles, a temporary plastic pipe is installed. The pipe will prolong the water jet past the water damming, and ensure that the entire surface area inside the bottles gets cleaned (figure 7.12).

(a) Pipe to prolong the water jet.

(b) Entire surface area of bottle being cleaned

Figure 7.12: *A pipe is installed to extend the water jet further in the bottle and break through the water damming, that allows for the entire surface area of the bottle to be cleaned.*

Calibrations and following outcomes 8

With a prerequisite that the water jet reaches the bottle bottom, which is found possible in previous tests with an installed pipe to prolong the water jet, it is expected that the drinking water requirements of potable water can be met with a cleaning time of 5 seconds. Further improvements will be tested to find a suitable concentration with the new instalment. A water pressure of 2,6 bar will be used for further tests, as it has been informed that this is the preferred pressure for the cleaning unit on site in Tanzania (Kioo Drinking Water co., n.d.).

8.1 Testing the significance of ECA water and its concentration

Results found in section 7.1 when testing of varying the concentration showed no tendency that reflects the variation in concentration in the viable count. Testing for concentration as a variable will therefore be repeated with the new instalments.

The concentration will vary between 0 and 75 ppm at 2,6 bar with a pipe extending the water jet into the bottle, will be made to show the effect of using ECA water for cleaning.

Considerations and procedure for the test

The concentrations will vary from 0 to 75 ppm with intervals of 15 ppm, and it is expected that since the installed pipe ensures that the bottom of the bottle is reached by the water, a decrease in the viable counts will be found as the concentration increases. The tests will be identical in terms of the contamination and cleaning procedure. The test without ECA water (0 ppm concentration) will be performed to validate that ECA water has an effect on the viable count, and that a rinse is not adequate for cleaning.

Results

At concentrations of 0 and 15 ppm, the plate has turned yellow indicating that presence of yeast, whereas the plates with concentration higher than 30 ppm are completely clean disregarding the heterotrophic water bacterial colonies. This is the case at both 22 and 37 °C (figure 8.1 and 8.2, respectively). The expected decrease in viable counts as the concentration increases is not completely clear at 37°C, as it only for concentrations of 60 and 75 ppm that a decrease is found. Furthermore the viable counts exceed the criteria stated for potable water at concentrations lower than 60 ppm, which contradicts with previous found results. For incubation at 22°C the level of bacteria is close to none as soon as some ECA water is used, and high when there is no ECA water, however still passing the requirements for potable water (figure 8.3 and appendix B.6).

Figure 8.1: *Results of presence of bacteria at 22°C at variation in concentration, from highest to lowest in left to right.*

Figure 8.2: *Results of presence of bacteria at 37°C at variation in concentration, from highest to lowest in left to right.*

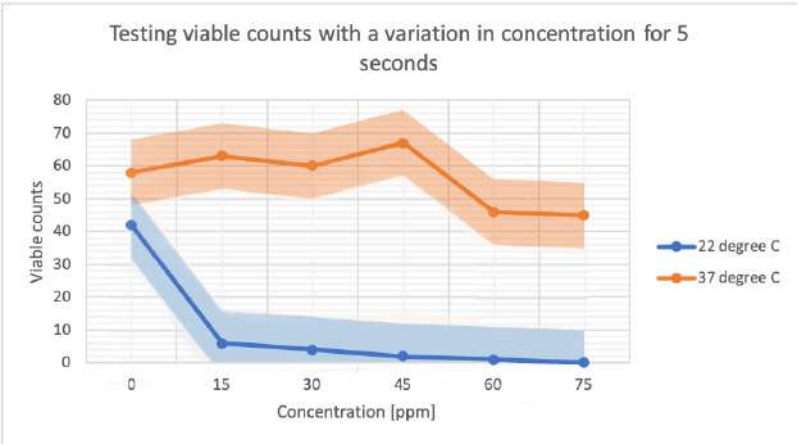


Figure 8.3: *Viable counts at 37 and 22°C along the concentration variation.*

When comparing the results to previous found when investigating concentration as a variable, the results differs within the interval of 98% as a decrease to an increase of 400% for 15 ppm . When comparing the results found for 30 ppm with that found in previous tests, where a concentration of 30 ppm har been tested specifically, the results of viable counts vary from previous found within an interval of a decrease of 83% to an increase of 227%. The results found here are thereby very irregular when compared to previous found results, and may not be a suitable representation of concentration as a variable.

Testing improvements for next stage

The necessity of ECA water is shown in the test at 22°C, where a large decrease in viable counts is found by adding the ECA water. Furthermore it can be seen as the Compact Dry AQ plates will lose the indication of yeast at concentrations higher than 30 ppm.

The contradiction of results found at 37°C could be due to inferior single samples, that should however still be able to meet the criteria for potable water, or the ECA water could possibly be closing in on an expiration date.

8.2 Validating the ECA water used for testing

Since the ECA water used for all tests was opened to free air in the beginning of test in start October, there is a possibility that the solution has exceeded a potential expiration date. A test will be performed using clean ECA fluid and half diluted, equivalent to a concentration of 4500 and 2250 ppm, followed by confirmation of the concentration using chloride strips compared to an unopened container of ECA water (figure B.39 in appendix B.7).

Considerations and procedure for the test

The results found in section 8.1 does not coincide with the results found in section 7.3 for incubation at 37°C, where a much lower mean value was found for test at 30 ppm than at 75 ppm when retesting for variation in concentration. Using clean ECA water, it will be examined whether removal of all bacteria present is possible at incubation at 37°C, or if the ECA water has passed expiration date and thereby cannot remove all microorganisms. A half diluted ECA water tests will be performed in case the expiration date has been exceeded, to see if it will be reflected in the decrease of concentration.

Allowing for free air to be in contact with the ECA water for a time span of three months, it is possible that airborne pathogens may have used up some of the oxidation potential of the solution. Before testing viable counts, the concentration of ECA water of the opened bottle was compared to an unopened using chloride strips. It was found that both bottles surpassed the capacity of the strips, which means that the clean ECA water used still has a very high concentration close to the initial.

Results

There is found a complete absence of viable counts present in the water sample once the bottle has been cleaned with either full or half diluted concentration at both 37 or 22°C (appendix B.7). In accordance with the results of the testing the amount of chloride using strips, it is assumed that the used ECA water should be working as expected.

8.3 Testing for the efficiency of ECA water at 30 ppm with new instalments

Five identical tests are take at 30 ppm at 2,6 bar with 5 seconds of cleaning time, as this was the found necessary concentration to avoid cultivating yeast, and has previously given results within the potable water requirements.

Considerations and procedure for the test

Following the results found in section 8.2, the ECA water remains valid, therefore it will be tested if inferior single samples may be the cause of the contradiction results found in section 8.1. Five identical tests will be taken to find a mean and deviation, this will be compared to previous found mean value (see section 7.3). By comparing mean values before and after the pipe was installed, it will it is possible to examine the effectiveness of the adjustments made to the nozzle. Furthermore, by performing several identical tests, the possibility of an inferior single sample will be limited off. Based on an overall evaluation of previous results, it is assessed that a concentration of 30 ppm should be sufficient to met the criteria for potable water without cultivating yeast.

Results

With a mean value of 6,8 and 0,6 in viable counts and a spread of 1,5 and 0,9 at 37 and 22°C, respectively, the requirements for potable water are met for the five tests (figure 8.4 and appendix B.8).

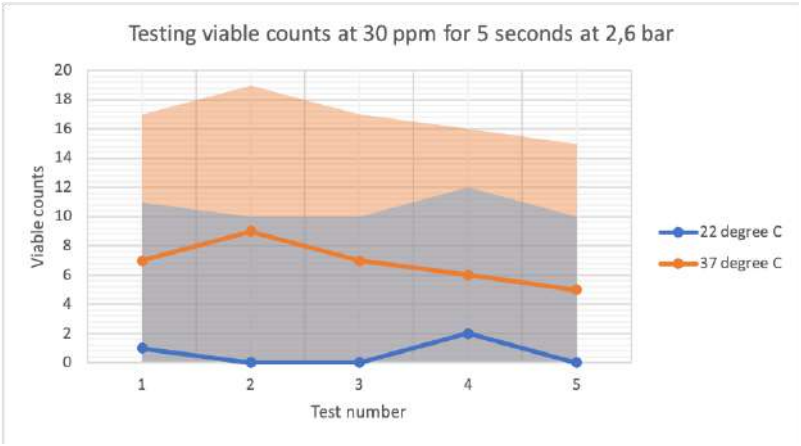


Figure 8.4: *Viable counts at five identical test at 30 ppm.*

To illustrate the effect of the new initiatives in terms of the pipe to prolong the water jet, an average of the five identical tests has been compared to average of the ten identical test, where an improvement of 63 and 93% is found as a decrease of viable counts for 22 and 37°C.

In comparison to initial conditions of fresh water put into a contaminated bottle, the viable counts have decreased with 97 and 99% for 22 and 37°C, respectively. It is therefore assessed that a concentration of 30 ppm with 5 seconds of cleaning time at 2,6 bar using a nozzle with a bowl and pipe to extend the water jet into the bottle is sufficient to pass the criteria for viable counts for potable water. The found conditions will be used for further and concluding tests.

8.4 Testing the effect of a cleaned bottle on water with varying retention times

Once the bottled has been cleaned, the time before consuming the water will vary. This can depend greatly on the bottle size, where storage time and temperature could have an effect on the condition of the water in the cleaned bottle. The importance of storage time will be tested in the interval of one minute to one day of storage at room temperature of approximately 20°C. The importance of storage time will be tested at 30 ppm, 5 seconds of cleaning time and 2,6 bar.

Considerations and procedure for the test

Since the water circulates within the bottle when cleaned, and the water drips out of the bottle, it is expected that most of the deactivated bacteria is loosened from the bottle sides and removed through the rinse when cleaned. Furthermore a circulation of the water will occur when refilling the bottle. Remaining bacteria would hereby be distributed out in the water from the bottle sides. In case all bacteria has not been deactivated, these will have the opportunity to regrow during the storage time and feed off remains.

Results

The viable counts in storage time varying from one minute to one hour are similar and stays well within the limits of potable drinking water (figure 8.5 and appendix B.9). The results are similar to those found in previous tests, and indicates that if the water is consumed within one hour of refilling, it will as safe as if consumed directly after consumption.

For storage of one day, the viable counts barely remain within the requirements. Especially the bacteria that are cultivated at 22°C have increased drastically. Since the water was stored at room temperature close to 22°C, the bacteria that are chlorine-tolerant of stressed will have had ideal growth conditions during storage time before cultivation in oven.

Figure 8.5: *Viable counts after storage time varying from one minute to one day.*

8.5 Testing for viable counts in bottle of regular use

As a comparison to worst case scenario where diluted waste water has been used to ensure contamination, a sports bottle is tested both before and after cleaning.

Considerations and procedure for the test

The sports bottle is a regular 1 L hard plastic bottle, that has been produced for multiple use (figure 8.6). The bottle has undergone light cleaning using soap since being taken into use. The bottle has not undergone contamination besides that of regular use, where clean tap water has refilled the bottle several times.

(a) Side view.

(b) Top view.

Figure 8.6: *Sportsbottle used for testing viable counts.*

To identify the condition of water stored in bottle, the water will be tested after three cases; seven hours of storage, representing a condition of the water consumed in the same bottle at the end of normal school/work day. Right after refilling of the bottle, to represent a condition of fresh water filled into a not-cleaned bottle. Refilled after cleaned, to represent a condition of fresh water filled into a cleaned bottle. The last case can be compared to the worst case scenario contamination and cleaning of the bottles in this trial.

Results

The viable counts after seven hours of storage was above maximum for the Compact Dry AQ plates, and thereby potentially as contaminated as when the bottles have been contaminated using diluted sewage water. For the case of fresh water in the non-cleaned bottle, the same case happened for the cultivation at 37°C, whereas the viable count at 22°C was close to the results of a cleaned bottle. Once the bottle had been cleaned and refilled with fresh water, the viable counts for both 37 and 22°C were close to none (figure 8.7 and appendix B.10). When comparing the seven hour old water in a used bottle to fresh water filled in a cleaned bottle, an improvement in terms of decrease in viable counts was found to 100 and 97% 22 and 37°C, respectively. Furthermore, the results found are very similar to the mean values found in section 8.3, indicating that despite level of contamination before cleaning, levels of viable counts that fulfil the potable water requirements at 37 and 22°C can be achieved.

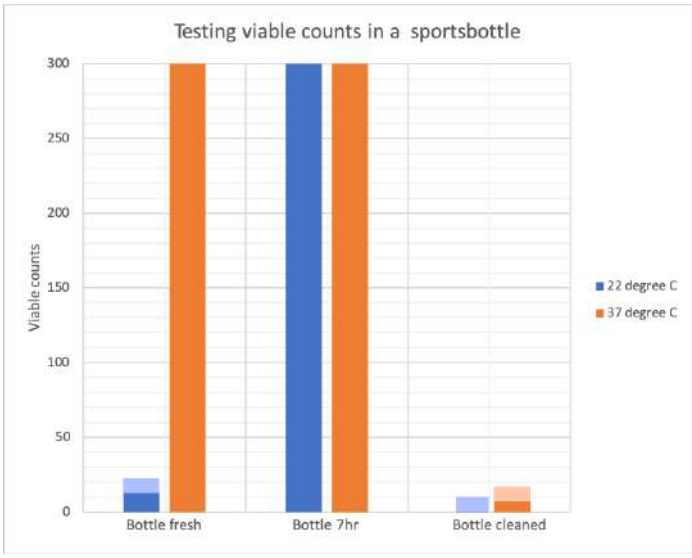


Figure 8.7: *Viable counts found in a sports bottle used for regular use with 7 hour old water and fresh water and after cleaning.*

Concluding tests 9

Following the results found in section 8.3, where a concentration of 30 ppm with 5 seconds of cleaning time at a pressure of 2,6 bar resulted in an average of 6,8 and 0,6 in viable counts at 37 and 22°C respectively, concluding tests will be performed to examine if the found conditions works for a variation of bottle types. Furthermore it will be examined that no dangerous pathogens are present, and that the risk of transmitting a virus from one user of the cleaning station to the next is insignificant under the found conditions.

9.1 Testing for viable counts using the found conditions on bottles varying in type and size

The found conditions of 30 ppm for 5 seconds at 2,6 bar will be tested on a 0,45 L glass bottle, a 1 L PET bottle, and a 5 L PET bottle for viable counts. The 5 L PET bottle is not a product of KIOO Drinking Water Co. The contamination of the bottles will consist of 1:30 of sewage:tap water.

Considerations and procedure for the test

Due to roughness of PET bottle surfaces, bacteria will have better growth conditions on the surface type, as the biofilm matrix will have a structural context to stick to. Furthermore it was found in section 7.4 that the size of the bottle had an influence in whether the water could reach the bottom of the bottle, as well as breaking the problematic water damming in earlier test stages. It will be examined if the found cleaning conditions are adequate for bottles different to the 0,6 L PET bottle from KIOO Drinking Water Co. There will be taken two samples from each bottle size to verify the results and make sure that the found viable counts do not resemble a single sample.

Results

For all samples taken from the three different bottles, the viable counts stay within the drinking water requirement for potable water (figure 9.1 and appendix B.11). Furthermore, the results have an average of 6,8 and 8,6, and a spread of 5,3 and 12,8 for 37 and 22°C respectively, and are thereby fairly close. In addition, the average of the different bottles viable counts is for 37°C identical to that found in section 8.3 for 0,6 L PET bottle with the same conditions. The 5 L PET bottle stands out in the results with a high viable count in cultivation at 22°C. This could be due to the shape of the bottle, that consists of a handle in the top of the bottle as a part of the container, where cleaning is limited. As the contaminated water did not reach this surface area, this is not reflected in the cultivation at 37°C, where the gut-bacteria typical found in sewage water has best grow conditions.

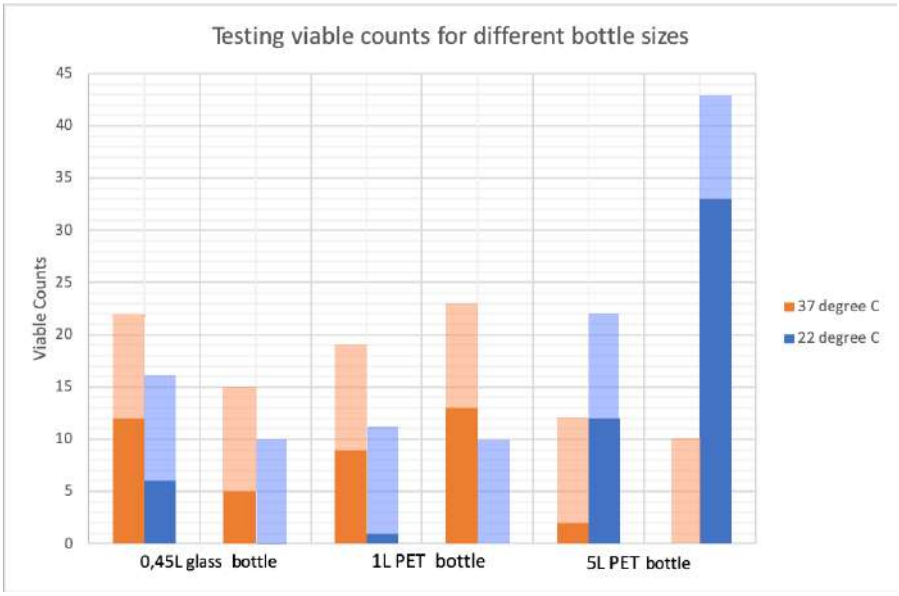


Figure 9.1: *Viable counts in bottles varying in size. There are two samples from each bottle.*

9.2 Testing for *coliforms* and *E. coli* on varying bottle types presented by KIOO Drinking Water Co.

Testing the found condition on a 0,45 L glass bottle, 0,6 L PET bottle, 1 L PET bottle for *coliforms* and *E. coli*. The test will be performed using the colilert test (see section 5.1.3).

Considerations and procedure for the test

As the Compact Dry AQ only indicates the presence of bacteria, colilert test will be used to test for the presence of *coliforms* and *E. coli* bacteria. As an amount of up to 50 bacterial colonies are allowed for drinking water at incubation of 37°C, it will be tested whether these bacteria are non-pathogenic, or if *coliforms* and *E. coli* are present, as these bacterial types are highly found in the sewage water used for contaminating the bottle. Furthermore, a direct criteria of meeting the drinking water requirements is an absence of *coliforms* and *E. coli*.

Following the idea in section 9.1, the growth conditions found on a PET surface may potentially differ to those on a glass surface, the colilert tests will therefore be performed on both PET bottle in size 0,6 L and 1 L, and the 0,45 L glass bottle.

Results

For neither of the three bottles *coliforms* or *E. coli* was found in the water samples. For the 0,6 L PET bottle, further time was added to allow for further metabolism of the indicator-nutrients, however without results indicating *coliforms* or *E. coli* (figure 9.2).

(a) As the incubation tray has not turned yellow, there is an absence of *coliforms*.

(b) As the incubation tray has not turned fluorescent, there is an absence of *E. coli*.

Figure 9.2: *The colilert test showed an absence of coliforms and E. coli in samples taken from the three bottle types. (b) shows the sample from 0,6 L PET bottle under UV light.*

In addition to the viable counts, found in section 8.3, staying within the criteria for potable water, the absence of *coliforms* and *E. coli* is also a fulfilment of the requirements. Furthermore, this absence indicates a general absence of pathogens, hence the found conditions should be sufficient to meet all the requirements for potable drinking water stated by TZS.

9.3 Testing for available ATP on surface area after cleaning contaminated bottle

To secure insignificant risks of transmitting diseases between users of the cleaning station, measurements of RLU will be used to ensure that the surfaces in contact with the bottles will have ATP levels that can be categorised as approved (Hyeina, n.d.).

Considerations and procedure for the test

The surface area of the nozzles used for the cap and the bottle will be examined after cleaning a contaminated bottle under the given conditions of 30 ppm and 2,6 bar for 5 seconds cleaning time. This will indicate the risks of transmitting diseases between users, where RLU measurements categorised as approved will indicate insignificant risks. Furthermore, the thread on both the cap and bottle will be tested, as well as the remaining mouth piece of the bottle. This will indicate whether biofilm and other pathogens can use the thread as a habitat due to narrow spaces, and if the circulation effect of the nozzle bowl collecting the water is adequate or a water jet is necessary for thorough cleaning.

Results

For the thread on the bottle and cap, as well as the mouth piece, 30 ppm for 5 seconds will be sufficient for ATP levels to be categorised as approved. However, for the surface area of the nozzle, some fluid remains between each cleaning, which concludes in the bacteria not being rinsed away, and the ATP levels are categorised as cautious.

When increasing the concentration to 45 ppm, the levels decrease and a category of accepted can be reached (appendix B.12), however it was noticed that less fluids remained in the cup before using the swabber. The results found on the bottle and cap corresponds with the results found in earlier test stages (see section 7.2), where it was possible to get an acceptable results for the bowls surface as well. This could be due to a cleaning time of 10 seconds, whereas this test is based on 5 seconds. The doubling of cleaning time could conclude higher amounts of ATP from the bottle being rinsed off the surface area of the nozzle. A small incline of the bowls horizontal surface towards the centre hole, could possibly help future test by allowing the water to flow out of the bowl before the next user and thereby restrain gathered water used for cleaning from being a possible source of transmission, whereas it now is completely horizontal. However, the bottle does not come in contact with the small amounts of collected water in the bowl, as it is placed on the four thin plates, which are raised above the bottom of the bowl (figure 9.3).

(a) Sideview of the nozzle bowl with a completely horizontal bottom.

(b) The risen four plates ensures that the bottle does not touch the bottom area of the bowl.

Figure 9.3: *The design of the current nozzle bowl.*

Discussion 10

Overall, the results throughout the experimental process have shown little tendency. Large deviation found both as decrease and increase in viable counts was found despite making continuous adjustment to improve the cleaning conditions, such as adjustments to the nozzle. Despite initiatives that should make the test as identical as possible and an accepted interval of ±10 in viable counts, uncertainties have had a significant impact on the results. This was found in lack of tendency when adjusting concentration, and large deviations between tests made on different dates, causing uncertainties that questioned the methods and set up used for contaminating and cleaning the bottle. The initiatives and following uncertainties will be discussed and evaluated.

10.1 The evolution of the nozzle

Throughout the experimental process, physical adjustments have been made to the nozzle design (figure 10.1). The process is significant in terms of the results, that should reflect when a new nozzle design is taken into use, either as a progress or as en increase in viable counts.

(a) Initial nozzle (b) Second nozzle design (c) Third nozzle design (d) Final nozzle design

Figure 10.1: *(a) Initial nozzle design, that remains to be used for cleaning the bottle cap. (b) Second nozzle design, where a bowl has been added. (c) Third nozzle design with more stability for positioning the bottle. (d) Final nozzle design, where a pipe has been added to prolong the water jet.*

The first draft of the nozzle design was without a cup and thereby without the ability to clean the outside of the bottles mouthing piece. This was added to the second design. However the cup consisted of two large surface that the bottle should push down onto to enable the water jet. In addition to a stability problem of the bottle, the large surface involved a large risks of transmitting pathogens between users. Furthermore a water damming was found when enabling the water jet for longer time periods, which was reflected in results that used more than ten seconds of cleaning time.

The idea for the third nozzle design was therefore to make four slim lines that the bottle should be in contact with, having as smaller surface area for transmitting pathogens. This would also allow for more water to run out of the bottle, and thereby a greater water exchange and hopefully less water damming inside the bottle. With four contact points, the bottle would be more stable when pressed down to enable the water jet from the nozzle.

However a water damming would still occur even at shorter time intervals, causing no improvements in the viable counts. It was found that even though the surfaces had contact with four points to stabilise the bottle, the nozzle was still fairly unstable due to two legs of the bowl, that allows for shifting from side to side. The cleaning was therefore very individual for each test, as lack of stability caused many variations in the results as the directions of the water jet caused some surface area to not be cleaned. To avoid the damming it was found that a tilting of the nozzle would be sufficient, however not ideal as the positioning had to be very precise to work, and not ideal for every-man use.

For the final design, a pipe was installed to the nozzle to extend the water jet past the water damming, and thereby allow for cleaning of the bottom of the bottle without specific positioning of the bottle being necessary. A degree of coincidence still affects the results, despite the pipe ensuring for the water jet to reach the bottles bottom, however the results are within range of previous found. The installed pipe is still a draft version, as it is made of plastic, where a final version will be made of stainless steel following the design of the cleaning unit. To ensure that the water jet is not directly hitting one point only, a rotating water spreader should be installed at the end of the pipe and thereby secure that all surface area inside the bottle is struck by ECA water. Further improvements could be found by tilting the bottom surface of the nozzles bowl, thereby ensure it being emptied between each user, and avoid transmitting pathogens in the fluid collected here.

10.2 Water quality in Tanzania and resembling contaminated water

As previously mentioned, the water quality of Tanzania is unknown, and will vary greatly within the country and depend on the origin of the water.

Using diluted sewage water, the idea was to resemble worst case scenario of contamination. It is assumed that this contamination will exceed that of water used for consumption purposes in Tanzania, however this is an uncertainty. On the contrary, the resemblance could illustrate a much worse case of contamination than found in Tanzania, concluding in excessive cleaning. Since the same level of viable counts was found after cleaning a sportsbottle, that had not been contaminated with diluted sewage water, the found combination of 30 ppm for 5 seconds at 2,6 bar is within reasonable means.

The sewage water collected on October 17^{th} was collected at an early treatment stage, where sedimentation had not yet taken place. During storing of the sewage water, sedimentation took place in the container resulting in the sewage water to be more concentrated during final experiments, despite the same dilution was used throughout the entire experimental process. This may have had an influence on an increase in viable counts found in final experiments.

10.3 Inconsistency of results

An inconsistency was found in the results, where viable counts for tests with a varying concentration and same cleaning time did not differ or were found to follow an opposite trend than expected. Despite diligent efforts and adjustments that should make the tests circumstances as identical as possible, conjectured results was found on several test causing pondering of the reason herefor.

The reason for inconsistency could be found related to limitations and assumptions made for the project, or it due to aimlessness of the water jet during cleaning. Ideally the water jet should ensure that the entire surface area of the bottle has contact with ECA water and thereby is cleaned, however it is uncertain if this is the case. Lastly, a reason for inconsistency may be due to coincidences that are not to be controlled, nor describable, but however causes wondering related to causes that could be optimised.

An initial test was made to find the viable counts in the water delivered by Aalborg municipality, where no viable counts was found. However, the experiments were not all performed on the same day nor the same time of the day, and the viable counts in the tap water used for both cleaning and refilling may have had viable counts within the municipality's limit of up to 50, and the results could thereby be affected by this.

Despite addressing the possible uncertainties related to testing single samples, with allowing a variation of ± 10 in viable counts, better representation of the different tested variations could be made by collecting and testing more samples from the same test case. The effect of allowing an interval of ± 10 in viable counts was found to have a certain impact when the improvements of conditions from initial to test results were compared. Here differences of up to 22% between counted results for identical tests, could be reduced to 14% by manipulating the results. As a contradiction the difference in results could also increase to 29%.

10.4 Uncertainties of the testing methods

Whilst viable counts are not directly health related themselves, they can be used to identify a level of concern based on the effect that colonies have on recovery of *coliforms*. Therefore in tests showing an absence of the indication bacteria *coliforms* and *E. coli* high levels of viable counts can be due to extended residence time, high temperatures, or mild contamination within the network (Naturstyrelsen, March 2013*b*), and not necessarily dangerous.

Pathogens have different sensitivity to the disinfectant depending on the type. Whilst both viable counts and the colilert tests give an indication of the general cleaning, viral pathogens are often more chlorine-resistant and thereby less likely to be deactivated from the ECA water. Furthermore, *E. coli* is sensible to chlorine, which can conclude in wrong interpretation of the results and thereby evaluate water quality safer than to be true (Naturstyrelsen, March 2013*b*).

10.5 Different requirements for bottle in different sizes

In the initial stages of the experimental procedure, it was assumed that the found conditions would work for bottles in all sizes produced by KIOO Drinking Water Co. This was tested for bottles up to 5 L, where the found conditions for water pressure, concentration of ECA water and cleaning time assured for potable drinking water requirements to be met although an increase in viable counts was found. However, KIOO Drinking Water Co. produces containers up to 18 L. Further testing of larger containers is necessary to find adequate cleaning time, disinfectant concentration, and water pressure to clean larger containers. This will potentially be reflected in the amount of water used for cleaning of each container, and thereby the possible related costs.

10.6 The recyclability of PET bottles

The experimental procedure was performed using PET bottles and a glass bottle produced by KIOO Drinking Water Co. The PET bottles consists of thin plastic, and are thereby at risk of deforming when pressure is applied to push the nozzle during cleaning (figure 10.2). This is especially an issue with the 1 L bottle, and it could therefore be the case for the bottles larger than this, as well as the structure of the bottle is less robust.

Figure 10.2: *Deformation of the 1 L PET bottle during cleaning.*

The deformation of the PET bottles results in the bottles having a limited amount of times to be reused. It is therefore arguable how suitable the PET bottles are for being reused, despite lasting up to 10 times of cleaning. The bottle most suitable for unlimited reuse are the glass bottles, and it should therefore be pursued to make them more desirable for the user.

10.7 Alternative methods for disinfection

ECA water has been chosen as disinfectant, as it does not contain dangerous chemicals, does not require an after-rinse, it is easily accessible, does not corrosion of material with a pH of 8, and can be produced on site (Fooddiagnostics, n.d.).

ECA water will denature the protein within the cell, in addition to breaking the cell wall with a strong oxidation potential. Much alike, detergent is a physical disinfection method that breaks up the lipids surrounding the protein in the membrane and thereby killing the cell.

Boiling of fluids has the same effect as using alcohol, where the protein within the cell is denatured. In contrast, UV light will destroy the DNA, and thereby making genetic changes and deactivating the cell (Nyco, n.d.).

Whilst bleach and alcohols are harmful to consume, and will need to be rinsed after disinfecting the bottle, having the bottles surface be in contact with boiling water or using UV light is non-dangerous consumption-wise. However, using UV-light will also require further rinsing to rinse away the deactivated pathogens, and boiling water does require some degree of precaution to avoid burn-related dangers.

In terms of effectiveness, as some pathogens are more sensible to some disinfectant methods than others, ECA water is not the most effective. For most effective, boiling for at least one minute or UV light are the best methods (Thompson, April 2012). If using UV light as a disinfectant, the requirements for potable water will be more strict (Nyco, n.d.), as presence of either *coliforms* or viable counts will indicate insufficient treatment.

Conclusion 11

In the face of climate change, the livelihood of many Tanzanians is worsening, where inequality, poverty, and the lack of access to clean water is increasing. Moreover, medication to treat diseases related to bad sanitation and clean water is unavailable for the majority. Initiatives in improving the accessibility of clean water and inducing reuse of plastic bottles and containers in terms of installing water refilling stations is slowly increasing, leaving the main risks for waterborne disease to be found in used containers transmitting diseases to clean water.

The following problem statement was therefore stated: *Under what premises is it possible to fulfil the requirements for potable drinking water by TZS for water refilled in a contaminated bottle, that has undergone cleaning?*

The premises necessary for meeting the requirements are tested and consists of the disinfectant to be ECA water at a concentration of 30 ppm for five seconds of cleaning time at a water pressure of 2,6 bar. It was found that the premises concluded in an improvement of viable counts of 97% and 99% for 22 and 37°C, respectively, when comparing to initial conditions of fresh water put into a contaminated bottle. Furthermore an absence of *Coliforms* and *E. coli* is concluded, which indicates an absence of pathogens.

For the cleaning unit, a nozzle with a bowl installed is used to ensure that the mouth piece of the bottle also gets cleaned sufficiently, as the bowl gets completely filled during each cleaning. Furthermore, a pipe, that prolongs the water jet emerging from the nozzle, is installed, which ensures that the disinfectant reaches the bottom of bottle, for bottles types ranging from 0,45 to 5 L. The ECA water that is filled in the bowl also disinfects the surface area of the bowl once emptied, and hereby makes the risk of transmitting diseases between users of the unit insignificant.

It can be concluded that it is possible to create a unit that both disinfects and refills bottles that have been contaminated to a level surpassing or equal to that found in Tanzania, where the refilled water in the bottles meets the requirements for potable drinking water stated by TZS. The conclusion is however drawn as a conjecture, as large variations in results were found between the different tests.

Further development of the nozzle is require to ensure that no water is collected in the bottom of the bowl between users, in addition to manufacturing the pipe in steel possibly with a sprinkler-function at the end that spreads the water inside the bottle, in contrast to one water jet centring the disinfectant and relying on rinsing to be sufficient contact between ECA water and surface area.

Perspectives 12

The project revolves around the idea of it being a possibility to clean and refill water bottles, so that the water can fulfil the requirements for potable drinking water stated by TZS. Due to questionable quality of tap water in Tanzania, pre-treated water will be used for both diluting the disinfectant, ECA water, and for refilling. The ambition behind the cleaning unit is to improve general health by making clean water more accessible, in addition to an environmental aspect of promoting reusage of plastic. The environmental aspect of reusage will be brought into perspective in terms of creating waste water from pre-treated water, along with implementing the concept of a cleaning unit in Tanzania, and eventually expanding to other developing countries.

12.1 Reuse of water used for cleaning

The ECA water will be diluted using treated water to clean the contaminated bottles. For five seconds of cleaning time with a water pressure of 2,6 bar, 0,725 L of clean water will be used. To carry on the idea of promoting reusage and limiting waste, this water has the opportunity to be collected and reused. As the water was clean before use, and has ECA water added, that should deactivate the pathogens present, the water should be suitable for reusage. Collection of the water and use for cleaning purposes on site, of i.e. the stations areas, or open for a sanitation area could be options.

12.2 Implementing the unit in Tanzania, and globally

Installing and implementing the station with a cleaning and refilling unit in Tanzania will differ to the test location in Denmark both related to the user phase, surrounding components, and competitive alternatives. The first station will be installed at a busy university campus, where the importance of clean water is common knowledge and there is a lot of costumer potential. Advertisement of the concept is therefore a limited need, and contamination of the bottles in need of cleaning may be limited as well. As found in the testing stage, the retention time of clean water in a cleaned bottle has an influence in the viable counts when surpassing one hour. As the average temperature in Tanzania is higher than the found temperature when testing for retention time, it is a prospect that the viable counts will have improved conditions for growth and thereby exceed the results found in this report. The principal of filling a cleaned bottle with clean water, with low viable counts, will therefore be an issue if the water cannot be categorised as potable due to high viable counts within a short time period after cleaning.

When implementing the idea of a cleaning and refilling station in rural and less populated areas, the rentability of the unit will differ to those of units located in populated areas. The storage of water used for refilling will need to be reevaluated, as less user may be result in less water being purchased. Furthermore, the users in rural areas will most likely use large containers to collect the water, and thereby cause a variation the in the use of the station, concluding in practical challenges. First of all, the storage of water in the container will need to be both sanitary and at low temperatures to avoid the station facing problems of existing water towers, where the water becomes non-potable during storage. Moreover, poverty may force potential users, that are typically found in rural areas, to skip the cleaning step and ignore the health risks, and instead incline towards solely refilling.

In many developing countries, refill stations, called water ATMs, have gained a lot of success in terms of improving the accessibility of clean water and having a large user interface. In addition, the environmental awareness is increasing, causing an increase in the interest of reusing bottles, plastic, with more, both in developing and develop countries. Therefore there is potential of implementing an option for safe refilling of water bottles, whether it being PET or more reusable ones, for both individuals or in industries, especially in countries where tap water is not an option. The main criteria to fulfil is ensuring that the water stays within the requirements for potable water. Many countries that uses chemical treatment for water, have made an association between the smell of chlorine and clean water (Abildgaard, December 2017). The method of using ECA water for disinfecting bottles will thereby use an existing association to promote the idea of clean and safe water.

References

Aalborg Kommune (2014), 'Kontrol af drikkevand'.
URL: *https://www.aalborg.dk/media/2018656/kontrol-af-drikkevand-2014.pdf, accessed 26.12.2020*

Abildgaard, I. (December 2017), 'Vi drukner vores tørst i unødigt flaskevand'.
URL: *https://samvirke.dk/artikler/vi-drukner-vores-toerst-i-unoedigt-flaskevand, accessed 26.12.2020*

AEL heating (n.d.), 'Guide to water flow rates and pressure'.
URL: *https://www.aelheating.com/blog/guide-water-flow-rates-pressure/, accessed 26.12.2020*

Arnfield, A. J. (November 2020), 'Koppen climate classification'.
URL: *https://www.britannica.com/science/Koppen-climate-classification, accessed 26.12.2020*

Britannica (n.d.*a*), 'Growth of bacterial populations'. Last accessed 29.12.2020.
URL: *https://www.britannica.com/science/bacteria/Growth-of-bacterial-populations*

Britannica, (N.d*b*), 'Tanzania'.
URL: *https://www.britannica.com/place/Tanzania/Settlement-patterns, accessed 28.12.2020*

British Geological Survey (2000), 'Groundwater Quality: Tanzania'.
URL: *https://core.ac.uk/download/pdf/78863684.pdf, accessed 26.12.2020*

Chiara, M. (November 2018), 'Re-interpreting the Relationship between Water and Urban Planning'.
URL: *https://www.foodandmigration.com/water-urban-planning-pastore/, accessed 26.12.2020*

Chow, D. (May 2020), 'Why are viruses hard to kill? Virologists explain why these tiny parasites are so tough to treat'.
URL: *https://www.nbcnews.com/science/science-news/why-are-viruses-hard-kill-virologists-explain-why-these-tiny-n1202046, accessed 26.12.2020*

Claire Gillespie (September 2018), 'What Are Intestinal Worms?'.
URL: *https://www.healthline.com/health/intestinal-worms, accessed 26.12.2020*

Crosta, P. (May 2017), 'What to know about viruses'.
URL:
$https://www.medicalnewstoday.com/articles/158179what_are_viruses, accessed$26.12.2020

EEPCO (December 2009), 'Baseline Survey for ISSUE 2 Program'.
URL: *http://www.eepco-tz.org/2009/baseline-survey-for-issue-2-program/, accessed 26.12.2020*

Eric Sturdza Investments (April 2020), 'Eric Sturdza Investments builds three new clean water wells in Tanzania'. Last accessed 28.12.2020.
URL: *https://ericsturdza.com/insights/3-wells-tanzania/*

Fooddiagnostics (n.d.), 'ECA Vand'.
URL: *https://www.fooddiagnostics.dk/eca-vand/, accessed 26.12.2020*

Fødevareministeriet (February 2017), 'Approval of the product Toucan ECA'.
URL:
https://www.fooddiagnostics.dk/seekings/uploads/Approval$_T$*oucan*$_E$*CA.pdf, accessed*26.12.2020

Government Portal Content Committee (October 2015), 'Urban Water Supply and Sewerage'.
URL: *https://www.tanzania.go.tz/home/pages/506, accessed 26.12.2020*

Greenwood, J. (August 2020), 'What is total viable count in water testing and why is it important?'. Last accessed 29.12.2020.
URL: *https://www.wcs-group.co.uk/wcs-blog/total-viable-count-in-water-testing*

Grundfos (n.d.), 'Grundfos Holding A/S, Global Partnerships'.
URL: *https://waterinstitute.unc.edu/files/2018/11/03*$_S$*E04*$_M$*arket* − *Based*$_A$*pproach*$_S$*ustainable*$_W$*ater*$_S$*olutions.pdf, accessed*26.12.2020

Grundfoss (n.d.), 'DDA'.
URL: *https://product-selection.grundfos.com/dk/products/dosing-pumps-digital/dda?tab=documentation, accessed 26.12.2020*

Hyeina (n.d.), 'ATP is measured in RLU's (relative light units). '.
URL: *https://www.hygiena.com/rlulimits-food.html, accessed 26.12.2020*

Hygiena (2020), 'Frequently asked questions'.
URL: *https://www.hygiena.com/frequent-asked-questions-food-and-beverage.html, accessed 26.12.2020*

HypochlorousAcid.com (n.d.), 'Hypochlorous Acid '.
URL: *https://www.hypochlorousacid.com/hocl-chemistry, accessed 26.12.2020*

Hypochlorsyre.com (N.d), 'Om Hypochlorsyre'.
URL: *https://da.hypochlorousacid.com/about, accessed 26.12.2020*

HyServe (n.d.), 'Compact Dry AQ'.
URL: *https://hyserve.com/files/CompactDry-AQ*$_E$*N.pdf, accessed*26.12.2020

Idexx (n.d.), 'Colilert'.
URL: *https://www.idexx.com/en/water/water-products-services/colilert/, accessed 26.12.2020*

kibera$_1$249(*n.d.*), ''.
URL:*https://www.pinterest.dk/pin/785244885006632372/, accessed 26.12.2020*

Kioo Drinking Water co. (n.d.), 'Kioo Drinking Water co.'.
URL: *https://kioowater.com/, accessed 26.12.2020*

Kjellén, M. (n.d.), 'Water provisioning in Dar Es Salaam, Tanzanina: the public - private interface'.
URL: *http://hydrologie.org/ACT/Marseille/works-pdf/wchp6-5.pdf, accessed 26.12.2020*

Lawson, M. (December 2017), 'Water ATMs: How Technology is Improving Water Governance in Tanzania'.
URL: *https://nextbillion.net/water-atms-how-technology-is-improving-water-governance-in-tanzania/, accessed 26.12.2020*

Lenntech (n.d.), 'Disinfectants Sodium hypochlorite'.
URL: *https://www.lenntech.com/processes/disinfection/chemical/disinfectants-sodium-hypochlorite.htm, accessed 26.12.2020*

Lorentz (2019), 'Sustainable water provision'.
URL: *https://partnernet.lorentz.de/files/lorentz$_c$asestudy$_d$rinking − water$_k$enya$_e$n.pdf*, *accessed*26.12.2020

Makoye, K. (February 2012), 'Climate Change Triggers Disease Risk in Tanzania'.
URL:
http://www.ipsnews.net/2014/02/climate-change-triggers-disease-risk-tanzania/, accessed 26.12.2020

Makoye, K. (March 2017*a*), 'Survey finds most Tanzanians go hungry, despite government denials'.
URL: *https://www.reuters.com/article/tanzania-hunger-idUSL5N1GJ5CP, accessed 20.12.2020*

Makoye, K. (n.d.), 'Most Tanzanians rely on unsafe water despite government efforts'.
URL: *https://cn.reuters.com/article/instant-article/idUSKBN0TF22420151126, accessed 26.12.2020*

Makoye, K. (September 2017*b*), 'Tanzanian city gets new sewage scheme to curb disease, ocean pollution'.
URL:
https://www.reuters.com/article/us-tanzania-pollution-water-sanitation/tanzanian-city-gets-new-sewage-scheme-to-curb-disease-ocean-pollution-idUSKCN1BM134, accessed 20.12.2020

Makoye, K. (September 2017*c*), 'Tanzanian city gets new sewage scheme to curb disease, ocean pollution'.
URL:

https://www.reuters.com/article/us-tanzania-pollution-water-sanitation/tanzanian-city-gets-new-sewage-scheme-to-curb-disease-ocean-pollution-idUSKCN1BM134, accessed 26.12.2020

Marzo, L. (February 2020), 'Recycling: Tanzania's growing opportunity'. Last accessed 28.12.2020.
URL: *https://furtherafrica.com/2020/02/19/recycling-tanzanias-growing-opportunity/*

MCC (May 2019), 'Improving Water Supply in Dar es Salaam, Tanzania'.
URL: *https://www.mcc.gov/resources/doc/evalbrief-052019-tanzania-water-dar-es-salaamevaluation-questions, accessed 26.12.2020*

McSheffrey, E. (February 2015), 'Tanzania'.
URL: *https://elizabetharoundtheworld.com/tanzania/, accessed 26.12.2020*

Merck (2020), 'Coliforms, E. coli Enterobacteriaceae'. Last accessed 29.12.2020.
URL: *https://www.merckmillipore.com/DK/en/products/industrial-microbiology/culture-media/culture-media-for-food-and-beverage-industry/dehydrated-culture-media/enrichment-isolation-differentiation-by-organism/coliforms-e.coli-and-enterobacteriaceae/RaGb.qB.O6oAAAFAqBE.1Zwo,nav?Referrer URL=https%3A%2F%2Fwww.google.com%2F*

Ministry of Foreign affairs of Denmark (2020), 'Current and future challenges and opportunities in Tanzania'.
URL: *https://um.dk/en/danida-en/strategies%20and%20priorities/country-policies/tanzania/current-and-future-challenges-and-opportunities-in-tanzania/, accessed 20.12.2020*

NASA (n.d.), 'The Effects of Climate Change'.
URL: *https://climate.nasa.gov/effects/, accessed 28.12.2020*

Naturstyrelsen (March 2013*a*), 'Håndtering af overskridelser af de mikrobiologiske drikkevandsparametre'.
URL: *https://naturstyrelsen.dk/media/nst/66817/kogevejledning%202013.pdf, accessed 26.12.2020*

Naturstyrelsen (March 2013*b*), 'Koge vejledning'. Last accessed 29.12.2020.
URL: *https://naturstyrelsen.dk/media/nst/66817/kogevejledning%202013.pdf*

Nave, R. (N.d), 'Adenosine Triphosphate'.
URL: *http://hyperphysics.phy-astr.gsu.edu/hbase/Biology/atp.html, accessed 26.12.2020*

Nest-In (November 2019), 'Why are Water ATMs Crucial for Smart Infrastructure Development?'.
URL: $https://medium.com/@nestin_tsl/why-are-water-atms-crucial-for-smart-infrastructure-development-664cc8d753c1, accessed 26.12.2020$

Next Science Group (n.d.), 'What is a biofilm?'.
URL: *https://biofilm.healthcare/the-problem-of-biofilm/, accessed 26.12.2020*

NPS MedicineWise (December 2019), 'Antibiotics, explained'.
URL: *https://www.nps.org.au/consumers/antibiotics-explained, accessed 26.12.2020*

Nyco (n.d.), 'Disinfecting Sanitizing, Educational'. Last accessed 29.12.2020.
URL: *https://www.nycoproducts.com/resources/blog/types-of-disinfectants-how-to-make-the-best-choice-for-your-facility/*

Outwater, A., Pamba, S. and Outwater, A. (2013), 'Risks, exposure, effects on health and control approaches in Tanzania'.
URL: *https://www.constructedwetlands.net/IR3-Water-related%20Diseases$_F$IN.pdf*, *accessed*26.12.2020

Paliwal, A. (June 2015), 'Water ATM'.
URL: *https://www.downtoearth.org.in/coverage/water-atm-39930, accessed 26.12.2020*

Prüss-Ustün, A., Wolf, J., Corvalán, C., Bos, R. and Neira, M. (2016), 'Preventing disease through healthy environments'.
URL:
https://apps.who.int/iris/bitstream/handle/10665/204585/9789241565196$_e$ng.pdf?sequence = 1, *accessed*26.12.2020

Prüss-Üstün, A., Bos, R., Gore, F. and Bartram, J. (2008), 'safer water, better health'.
URL:
https://apps.who.int/iris/bitstream/handle/10665/43840/9789241596435$_e$ng.pdf?sequence = 1, *accessed*26.12.2020

R-Biopharm (n.d.*a*), 'Compact Dry AQ'.
URL: *https://food.r-biopharm.com/products/compact-dry-aq/, accessed 26.12.2020*

R-Biopharm (n.d.*b*), 'Compact Dry katalog'.
URL: *https://www.viams.net/wp-content/uploads/2020/03/katalog-compact-dry.pdf, accessed 26.12.2020*

Rathi, V. (August 2019), 'Chandigarh smart city limited to set up 20 water ATMS'.
URL: *https://www.urbannewsdigest.in/2019/08/chandigarh-smart-city-limited-to-set-up-20-water-atms/, accessed 26.12.2020*

Shore, R. (2020), 'Water in crisis - Tanzania'.
URL: *https://thewaterproject.org/water-crisis/water-in-crisis-tanzania, accessed 20.12.2020*

Statens Serum Institut (September 2016), 'MRSA'. Last accessed 29.12.2020.
URL: *https://antibiotika.ssi.dk/resistens-i-bakterier-og-svampe/viden-og-raad-om-mrsa*

Steward, K. (August 2019), 'Gram Positive vs Gram Negative'.
URL: *https://www.technologynetworks.com/immunology/articles/gram-positive-vs-gram-negative-323007, accessed 26.12.2020*

Struwe, S. (April 2020), 'Pseudomonas'. Last accessed 29.12.2020.
URL: *https://denstoredanske.lex.dk/Pseudomonas*

Tanzania Bureau of Standards (n.d.), 'Standards catalogues'.
URL: *https://www.tbs.go.tz/catalogues, accessed 26.12.2020*

TeachMePhysiology (N.d), 'Pathogens'.
URL: *https://teachmephysiology.com/immune-system/infections/pathogens/, accessed 26.12.2020*

The United Nations (2020), 'Sustainable Development Goals'. Last accessed 20.12.2020.
URL: *https://www.un.org/sustainabledevelopment/sustainable-development-goals/*

The united republic of Tanzania, ministry of Water (October 2015), 'Guidelines ofr the preparation of water safety plans - resilient to climate change for rural water supply services'.
URL: *https://www.who.int/globalchange/resources/wash-toolkit/guidelines-wsp-tanzania-rural.pdf?ua=1, accessed 28.12.2020*

Thompson, K. M. (April 2012), 'The Science Of Disinfectants'.
URL: *https://www.cmmonline.com/articles/the-science-of-disinfectants, accessed 26.12.2020*

USAID (January 2012), 'Climate Change Adaptation in TANZANIA'.
URL:
https://www.climatelinks.org/sites/default/files/asset/document/tanzania$_a$*daptation* fact$_s$*heet*$_j$*an*2012.*pdf*, *accessed*26.12.2020

Verhille, S. (January 2013), 'Understanding microbial indicators for drinking water assessment: interpretation of test results and public health significance '.
URL:
https://www.ncceh.ca/sites/default/files/Microbial$_I$*ndicators*$_J$*an*$_2$013$_0$.*pdf*, *accessed*26.12.2020

water.org (2020), 'Tanzania's water and sanitation crisis'.
URL: *https://water.org/our-impact/where-we-work/tanzania/, accessed 20.12.2020*

Watson, S. (2020), 'Enterococcus Faecalis'. Last accessed 29.12.2020.
URL: *https://www.healthline.com/health/enterococcus-faecalisprevention*

Willey, Sherwood and Woolverton (n.d.), 'Bactrial Cell Structure'. Last accessed 29.12.2020.
URL:
https://www.bellarmine.edu/faculty/dobbins/Secret%20Readings/Lecture%20Notes%20313/Ch03.pdf

Woodhouse, J. (July 2018), 'Geomorphology support for the city of Dar es Salaam'.
URL: *https://www.jbaconsulting.com/knowledge-hub/geomorphology-dar-es-salaam/, accessed 26.12.2020*

World Health Organisation (June 2019), 'Drinking-water'.
URL: *https://www.who.int/news-room/fact-sheets/detail/drinking-water, accessed 20.12.2020*

World Health Organisation (May 2017), 'Diarrhoeal Diseases'.
URL: *https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease, accessed 26.12.2020*

Experimental procedure A

For each stage a procedure will be used to ensure equal primary conditions. The bottle will be in contact with contaminated water for 10 minutes for all tests before being cleaned and refilled with tap water, where the contaminated water will have the same concentration of sewage water of 20 mL sewage water and remaining tap water. The bottle will be shaken to ensure an even distribution of the sewage water in the mixed fluid.

The Compact Dry AQ will be used for determining the presence of heterotrophic water bacteria, where the below listed experimental procedure will be followed.

1. Ensure correct concentration from dosage pump, and check using strips
2. Ensure correct pressure settings at pressure reducing valve
3. Contaminate bottle for a time interval of 10 minutes with 20 mL sewage water and remaining tap water
4. Empty bottle from contaminated water
5. Clean at chosen time-interval (contact time)
6. Refill with fresh tap water
7. Take two 1 mL samples from water in bottle and apply to two Compact Dry AQ (see figure A.1)
8. Put samples in oven for 48 and 68 hours at respectively 37 and 22°C
9. Count colonies from each sample

(a) Taking a 1 mL water sample from refilled bottle.

(b) Applying 1 mL water sample to Compact Dry AQ.

Figure A.1: *Collecting sample for Compact Dry AQ.*

Experimental results B

B.1 Initial test conditions

	Viable counts	
	22°C	37°C
Clean water	1	0
Contaminated bottle clean water 4 days contact time	150	+300
Contaminated bottle clean water 10 min contact time	67	250
Contaminated water	+300	+300

(a) 22°C (b) 37°C

Figure B.1: *Viable counts for clean tap water.*

(a) 22°C (b) 37°C

Figure B.2: *Viable counts for 4 day contaminated bottle and clean water contact time.*

(a) 22°C (b) 37°C

Figure B.3: *Viable counts for 10 min contaminated bottle and clean water contact time.*

(a) 22°C (b) 37°C

Figure B.4: *Viable counts for contaminated water.*

B.2 Introductory tests of using ECA water as disinfectant

		Viable counts	
		22°C	37°C
Time [s]	60	3	23
	30	2	17
	10	1	20
	3	4	27
Concentration [mL per pulse]	12,5	16	22
	10	17	20
	7,5	4	2
	5	1	2

B.2.1 Testing for contact time at concentration of 15 mL per pulse.

(a) 22°C

(b) 37°C

Figure B.5: *Viable counts for contact time of 1 min.*

(a) 22°C

(b) 37°C

Figure B.6: *Viable counts for contact time of 30 sec.*

(a) 22°C (b) 37°C

Figure B.7: *Viable counts for contact time of 10 sec.*

(a) 22°C (b) 37°C

Figure B.8: *Viable counts for contact time of 3 sec.*

B.2.2 Testing for concentration as variable at 10 seconds of cleaning time.

(a) 22°C (b) 37°C

Figure B.9: *Viable counts for concentration of 12,5 mL ECA water per pulse.*

(a) 22°C (b) 37°C

Figure B.10: *Viable counts for concentration of 10 mL ECA water per pulse.*

(a) 22°C (b) 37°C

Figure B.11: *Viable counts for concentration of 7,5 mL ECA water per pulse.*

(a) 22°C (b) 37°C

Figure B.12: *Viable counts for concentration of 5 mL ECA water per pulse.*

B.3 Examining tendency in viable counts

	Viable counts	
	22°C	37°C
Concentration 5 mL per pulse	6	28
	23	31
	8	57
Concentration 7,5 mL per pulse	13	54
	20	125
	36	22

(a) 22°C (b) 37°C

Figure B.13: *Viable counts for concentration of 5 mL ECA water per pulse for 10 seconds of cleaning time.*

(a) 22°C (b) 37°C

Figure B.14: *Viable counts for concentration of 5 mL ECA water per pulse for 10 seconds of cleaning time.*

(a) 22°C

(b) 37°C

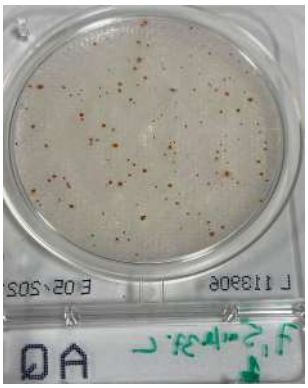
Figure B.15: *Viable counts for concentration of 5 mL ECA water per pulse for 10 seconds of cleaning time.*

(a) 22°C

(b) 37°C

Figure B.16: *Viable counts for concentration of 7,5 mL ECA water per pulse for 10 seconds of cleaning time.*

(a) 22°C



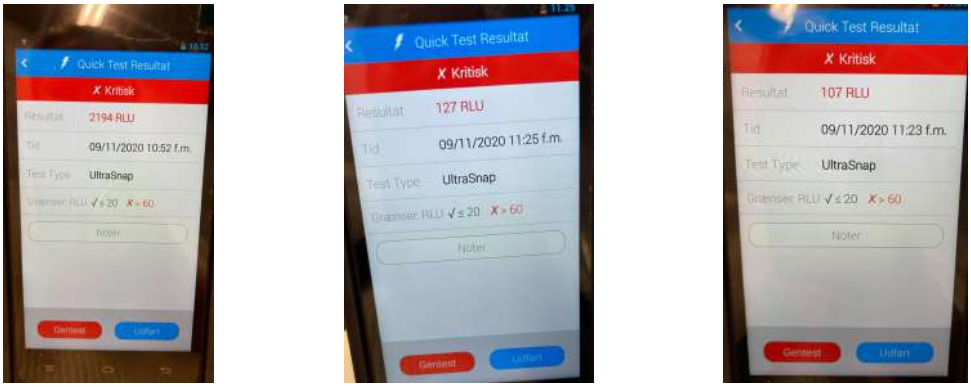
(b) 37°C

Figure B.17: *Viable counts for concentration of 7,5 mL ECA water per pulse for 10 seconds of cleaning time.*

(a) 22°C (b) 37°C

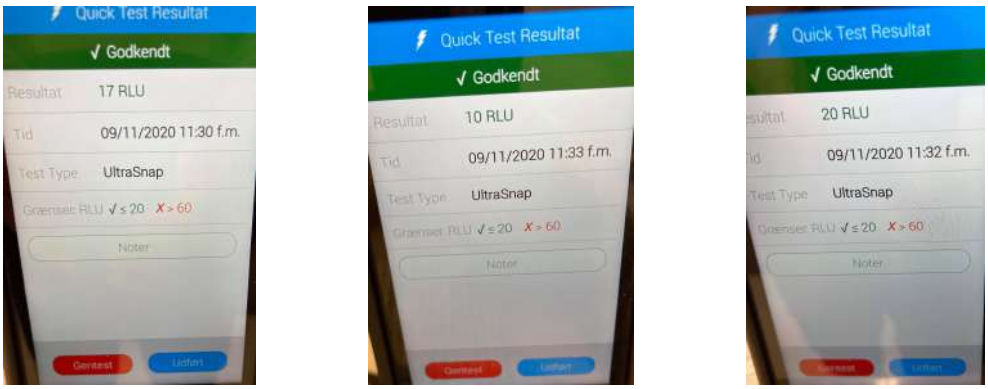
Figure B.18: *Viable counts for concentration of 7,5 mL ECA water per pulse for 10 seconds of cleaning time.*

B.4 Examining available ATP



(a) Critical - mouth piece (b) Critical - cap (c) Critical - nozzle surface

Figure B.19: *Testing for ATP.*



(a) Approved - mouth piece (b) Approved - cap (c) Approved - nozzle surface

Figure B.20: *Testing for ATP.*

B.5 Testing the efficiency of ECA water at 30 ppm

	Viable counts	
	22°C	37°C
1	0	16
2	6	36
3	4	24
4	1	24
5	2	17
6	1	18
7	9	10
8	0	15
9	3	15
10	4	8

(a) 22°C (b) 37°C

Figure B.21: *Viable counts for test 1 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.22: *Viable counts for test 2 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C

(b) 37°C

Figure B.23: *Viable counts for test 3 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C

(b) 37°C

Figure B.24: *Viable counts for test 4 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C

(b) 37°C

Figure B.25: *Viable counts for test 5 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.26: *Viable counts for test 6 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.27: *Viable counts for test 7 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.28: *Viable counts for test 8 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.29: *Viable counts for test 9 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.30: *Viable counts for test 10 for 5 seconds of cleaning time at 30 ppm.*

B.6 Testing the significance of ECA water and its concentration

	Viable counts	
	22°C	37°C
0 ppm	42	58
15 ppm	6	63
30 ppm	4	60
45 ppm	2	67
60 ppm	1	46
75 ppm	0	45

(a) 22°C (b) 37°C

Figure B.31: *Viable counts for Concentration of 0 ppm per pulse for 5 seconds of cleaning time.*

(a) 22°C (b) 37°C

Figure B.32: *Viable counts for Concentration of 15 ppm per pulse for 5 seconds of cleaning time.*

(a) 22°C (b) 37°C

Figure B.33: *Viable counts for Concentration of 30 ppm per pulse for 5 seconds of cleaning time.*

(a) 22°C

(b) 37°C

Figure B.34: *Viable counts for Concentration of 45 ppm per pulse for 5 seconds of cleaning time.*

(a) 22°C

(b) 37°C

Figure B.35: *Viable counts for Concentration of 60 ppm per pulse for 5 seconds of cleaning time.*

(a) 22°C

(b) 37°C

Figure B.36: *Viable counts for Concentration of 75 ppm per pulse for 5 seconds of cleaning time.*

B.7 Validation of ECA water used for testing

	Viable counts	
	22°C	37°C
4500 ppm	0	0
2250 ppm	0	0

(a) 22°C (b) 37°C

Figure B.37: *Viable counts for Concentration of 4500 ppm.*

(a) 22°C (b) 37°C

Figure B.38: *Viable counts for Concentration of 2250 ppm per pulse.*

Figure B.39: *Chloride level of ECA water in opened and unopened container, respectively*

B.8 Testing the efficiency of ECA water at 30 ppm with new instalments

	Viable counts	
	22°C	37°C
1	1	7
2	0	9
3	0	7
4	2	6
5	1	5

(a) 22°C

(b) 37°C

Figure B.40: *Viable counts for test 1 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C

(b) 37°C

Figure B.41: *Viable counts for test 2 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.42: *Viable counts for test 3 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.43: *Viable counts for test 4 for 5 seconds of cleaning time at 30 ppm.*

(a) 22°C (b) 37°C

Figure B.44: *Viable counts for test 5 for 5 seconds of cleaning time at 30 ppm.*

B.9 Testing the effect of a cleaned bottle on water with varying retention times

	Viable counts	
	22°C	37°C
1 min	1	8
10 min	0	6
1 hour	1	7
1 day	100	46

(a) 22°C (b) 37°C

Figure B.45: *Viable counts for cleaning of 1 min at 30ppm.*

(a) 22°C (b) 37°C

Figure B.46: *Viable counts for cleaning of 10 min at 30ppm.*

(a) 22°C (b) 37°C

Figure B.47: *Viable counts for cleaning of 1 hour at 30ppm.*

(a) 22°C (b) 37°C

Figure B.48: *Viable counts for cleaning of 1 day at 30ppm.*

B.10 Testing for viable counts in bottle of regular use

	Viable counts	
	22°C	37°C
7 hour old water contaminated bottle	+300	+300
fresh water contaminated bottle	+300	13
fresh water clean bottle	7	0

(a) 22°C (b) 37°C

Figure B.49: *Viable counts for 7 hours old tap water in used sportsbottle at 30 ppm for 5 seconds of cleaning.*

(a) 22°C (b) 37°C

Figure B.50: *Viable counts for new tap water in used sportsbottle at 30 ppm for 5 seconds of cleaning.*

(a) 22°C (b) 37°C

Figure B.51: *Viable counts for fresh tap water in cleaned bottle at 30 ppm for 5 seconds of cleaning. OBS: labelling of the sample is incorrect.*

B.11 Testing for viable counts using the found conditions on bottles varying in size

	Viable counts	
	22°C	37°C
1 L PET bottle	1	9
	0	13
5 L PET bottle	12	2
	33	0
0,45 L Glas bottle	6	12
	0	5

(a) 22°C (b) 37°C

Figure B.52: *Viable counts for 1 L PET bottle at 30 ppm for 5 seconds of cleaning.*

(a) 22°C (b) 37°C

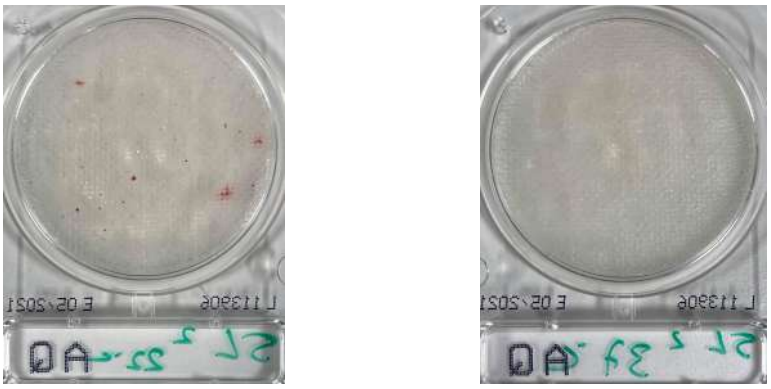
Figure B.53: *Viable counts for 1 L PET bottle at 30 ppm for 5 seconds of cleaning.*

(a) 22°C (b) 37°C

Figure B.54: *Viable counts for glas bottle at 30 ppm for 5 seconds of cleaning.*

(a) 22°C (b) 37°C

Figure B.55: *Viable counts for glass bottle at 30 ppm for 5 seconds of cleaning.*



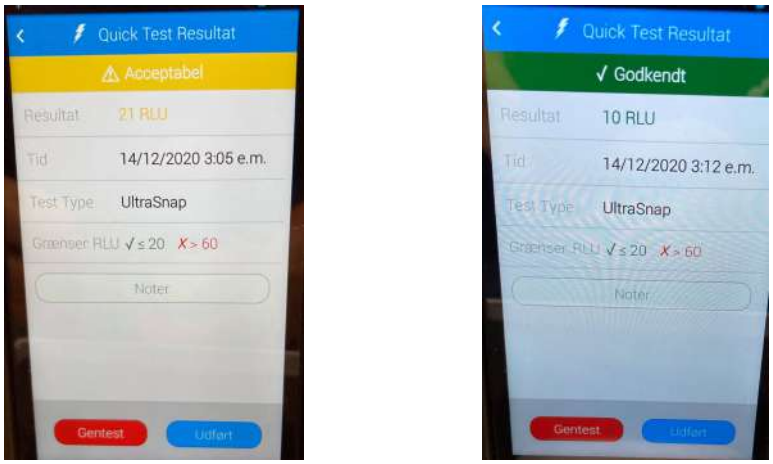
(a) 22°C (b) 37°C

Figure B.56: *Viable counts for 5 L PET bottle at 30 ppm for 5 seconds of cleaning.*

(a) 22°C (b) 37°C

Figure B.57: *Viable counts for 5 L PET bottle at 30 ppm for 5 seconds of cleaning.*

B.12 Testing for available ATP on surface area after cleaning contaminated bottle



(a) Nozzle with 7,5 mL per pulse giving a RLU of 21, being acceptable, however not approved

(b) Nozzle with 10 mL per pulse giving a RLU of 10, being approved

Figure B.58: *ATP for nozzle surface cleaned for 5 seconds.*