Bachelor's thesis

NTNU
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Microbiological Assessment of Disinfection on Indoor Artificial Turf

Mikrobiologisk vurdering av desinfeksjon på innendørs kunstgress

Bachelor's thesis in Chemical Engineering
Supervisor: Ina Merete Stuen
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Institute for materialscience, NTNU
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Preface

This bachelor thesis is written by Chemical Engineering-students at the Department of Materials Science and Engineering at the Norwegian University of Science and Technology, and were executed as a final examination for a project conducted by the Center for Sports Facilities and Technology, SIAT. This thesis was carried out and written from the 5th of January to the 19th of May 2023, and all work was carried out in Flatåshallen and in the laboratories at Kalvskinnet campus at NTNU's Department of Materials Science and Engineering.

The purpose of the bachelor thesis was to evaluate the inhibitory effect the disinfectant, Nüscosept PRO, had on bacterial growth on indoor artificial turf in Flatåshallen. It has been both challenging and educational to work on the task.

We would like to express our gratitude to our internal supervisor Ina Merete Stuen, for giving advice and guidance. We would also like to thank our external supervisor Bjørn Aas for granting us the task and for continuous monitoring and good spirits throughout the testing and writing period. We would like to thank Flatåshallen AS for allowing us to test in their facilities. We would also like to thank Josef Langenwalter and Are Pedersen for an interesting and enlightening meeting, and for sharing their own progress with us. Finally, we would like to express thanks to Hege Sundgård for the help regarding laboratory work and chemical orders.

Norwegian University of Science and Technology

Trondheim, May 2023

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Abstract

In the efforts to exchange inorganic infill with organic infill in artificial turfs due to the inorganic materials negative impact on the environment, another concern is discovered, the possibility of increased bacterial growth. This can lead to growth of indicator organisms that is a risk to players and visitors on the turf, such as *Staphylococcus aureus*. To mitigate the risk of bacterial growth the importance of disinfection and maintenance of the turf are highlighted.

Samples were collected from the indoor artificial turf at Flatåshallen approximately every second day for 6 weeks to examine the microbiological growth. These samples were taken from four different areas on the first half of the turf: the midfield, the sideline, the 16-meter mark and between the 16-meter mark and the midfield. Three parallels with 3M swab-samplers were collected in a 10x10cm area. The samples collected were inoculated on Tryptic Soy Agar and incubated at 36 °C. The Petri dishes were examined after 24 hours and then colony forming units were counted after 48 hours.

The analysis indicated that the disinfectant used on the indoor artificial turf inhibits bacterial growth when applied. With the use of 80 liters of disinfectant when disinfecting, colony forming units counted were below 20 CFU/cm^2 for at least 13 days after application. The results achieved indicated a correlation between bacterial growth and activity levels on the turf. The correlation is attained due to replenishment of biological material from visitors to existing bacteria on the turf.

The assessment of the results from this thesis indicates a need for disinfection of indoor artificial turf in correlation to activity levels. Compared to bacterial growth at other environments, the locker room and campus, the turf is evaluated as generally clean after disinfection.

Sammendrag

I arbeidet med å bytte ut uorganisk fyllmateriale til organisk fyllmateriale i kunstgress grunnet dets miljøfare, oppstår det en ny utfordring, muligheten for økt bakterievekst. Dette kan føre til vekst av indikatororganismer som er en risiko for spillere og besøkende på kunstgressbanen, slik som *S. aureus*. På grunn av risikoen for bakterievekst er viktigheten av desinfeksjon og vedlikehold av kunstgresset fremhevet.

På grunnlag av dette ble det samlet inn prøver fra et innendørs kunstgress i Flatåshallen omtrent annenhver dag i 6 uker for å undersøke den mikrobiologiske veksten. Disse prøvene ble tatt fra fire ulike områder på første halvdel av kunstgresset: midtbanen, sidelinjen, 16-metersmerket og mellom 16-metersmerket og midtbanen. For hvert område ble det samlet tre paralleller med 3M vattpinneprøvetakere i et 10x10cm område. Prøvene som ble samlet ble inokulert på tryptic soy agar og inkubert ved 36 °C. Petriskålene ble undersøkt etter 24 timer og deretter ble kolonidannende enheter telt etter 48 timer.

Analysen indikerte at desinfeksjonsmiddelet brukt på innendørs kunstgress hemmer bakterievekst når det er påført. Ved bruk av 80 liter desinfeksjonsmiddel ved desinfisering, ble det telt kolonidannende enheter under 20 CFU/cm^2 i minst 13 dager etter påføring. De oppnådde resultatene indikerer en sammenheng mellom bakterievekst og aktivitetsnivå på kunstgresset. Korrelasjonen oppnås grunnet påfyll av biologisk materiale fra besøkende til eksisterende bakterier på gresset.

Vurderingen av resultatene fra oppgaven indikerer behov for desinfeksjon av innendørs kunstgress i samsvar med aktivitetsnivå. Sammenlignet med bakterievekst i andre omgivelser, garderoben og skolen, vurderes kunstgresset som generelt rent etter desinfeksjon.

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Abbreviations

NTNU - Norwegian University of Science and Technology

SIAT - Centre of Sport and Technology

EPDM - Ethylene propylene diene monomer

SBR - Styrene-butandiene rubber

TPE - Thermoplastic elastomer

TPO - Thermoplastic olefin

TPV - Thermoplastic vulcanisates

PAH - Polycyclic aromatic hydrocarbons

SVOC - Semi-volatile organic compounds

VOC - Volatile organic compounds

VOX - VO2 max, oxygen uptake

NILU - The Norwegian Institute for Air Research

UV - Ultraviolet

S.aureus - Staphylococcus aureus

BAC - Benzalkonium chloride

QAC - Quaternary ammonium compound

CFU - Colony forming unit

TSA - Tryptic soy agar

OD - Optical density

1 Introduction

One of the most common football turfs used in Norway are artificial turfs [1]. Flatåshallen is an indoor football facility in Trondheim, constructed with an artificial turf. SBR-granules are the most common infill used in artificial turfs. In recent years attempts have been made to substitute the SBR-granules with organic materials due to SBR-granules impact on the environment. There are several organic substitutes being tested, one alternative being olive stones [1]. This infill is currently being tested in Flatåshallen. The use of organic substitutes can lead to potential bacterial growth on the turf. The turfs in indoor facilities, such as Flatåshallen, lack well established regulations on ventilation and maintenance. Visitors leave behind biological material, which give nutrition to the bacteria on the turf. Without the proper regulations of maintenance the bacteria can grow freely and cause infections to players, such as the outbreak of *Staphylococcus Aureus* on American football players [2]. Maintenance is conducted around the facilities in Flatåshallen, such as locker rooms and floors in the indoor arena. The indoor artificial turf is used in the same manner as the other facilities, therefore it is decided that the turf should be held to the same maintenance standards as them.
The purpose of this thesis is to evaluate the disinfection method and maintenance schedule for the turf in Flatåshallen. The evaluation is on a microbiological level, and intends to give a suitable interval for disinfection. Having a consistent maintenance routine is preferable to ensure optimum conditions for sports and other activities. This thesis have been a collaboration with the Centre for Sport Facilities and Technology, SIAT, at NTNU. They wish to obtain an evaluation of the maintenance, as well as how microorganisms affect the turf.

Based on the information above two main research questions have been formulated:

1. **Is there a correlation between bacterial growth and human activity on artificial turfs?**
2. **Based on the bacterial growth, how often is it necessary to disinfect indoor artificial turfs?**

2 Theory

This literature review is included to give relevant background information and understanding regarding this thesis. It includes literature on the evolution and usage of artificial turf, general information about microorganisms and the bacteria *Staphylococcus aureus*. It also includes necessary information regarding microbiological methods and techniques, and statistical methods.

2.1 Artificial turf - evolution and usage

2.1.1 Evolution of generations of artificial turf

Artificial turf is grass made as a replacement for natural grass, used both indoors as well as outdoors [1]. In the 1960s the first artificial turfs were applied. Since then there have been four different generations. The first generation (1G) was made of woven fibers called polyamide. The polyamide mat was placed directly on either concrete, sand or asphalt. The second generation (2G) was introduced in the 1980s. Polyamide was replaced by polypropylene due to polypropylene being a cheaper alternative. The mats used sand as filling material. The sand was applied to stabilize the mat and therefore improved the bounce and rolling of the ball. The sand had its disadvantages in that the players suffered abrasions when they came into contact with the turf. Players in sports like American football and rugby were particularly exposed because of the rough play and more contact with the turf. In the late 1990s a third generation (3G) of artificial turf was developed. In the 3G-turfs the polypropylene was replaced by polyethylene and the filler material was granules instead of sand, both changes more suited for rough contact sports [3]. With the ongoing development of the fourth generation (4G), organic filler materials are introduced. 4G-fields are made of a two-layer grass fiber made of polyethylene. Experimental work on which filler material that is best suited for the fields are still ongoing [1]. Alternatives when it comes to organic filler materials are cork, sand, sugar cane and cellulose. In addition attempts have been made to develop turf without filler material [4]. 4G-fields are still under development and therefore 3G-fields with different filling materials are mainly used in Norway today [1].

2.1.2 Structure of artificial turf

The general structure of the artificial turfs being used today is a multi layer structure consisting of 7 layers. The top layer simulating natural grass is made out of fibers from polyethylene, nylon or polypropylene. The second layer is the filler material, which is placed between the fibers. The filler material can be granules or different organic filler materials. Beneath or at the start of the fibers is a third layer of sand. Beneath the sand is a shock pad or shock absorbing pad. This layer is an attempt to reduce players impact upon landing, as well as prolong the life of the turf [5]. The fifth layer is for leveling and are made out of porous bitmac or a binding layer of stone [6]. Under the leveling layer is a large sub base of compacted crushed stones. Between the bottom layer and the sub base there is a geotextile membrane which helps with the draining. The final layer consists of a formation layer made of compacted ground surrounding the aggregate pipe bedding which surrounds the drainage [5, 6]. The structure of the artificial turf is shown in Figure 2.1.

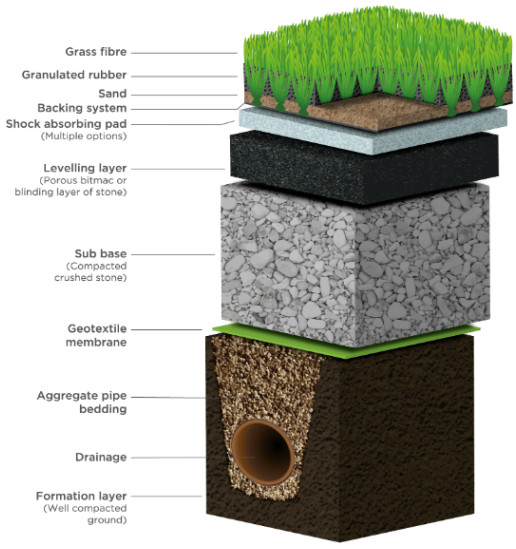

Figure 2.1: A structure of a 3G turf with description of each layer [6].

2.1.3 SBR-granules and its environmental impacts

There are different types of granules and these are styrene-butandiene rubber (SBR), ethylene propylene diene monomer (EPDM), industrial rubber and thermoplastic filling (TPE,TPO,TPV) [7, 4]. SBR-granules are the most used granules on Norwegian artificial turfs and are made out of ground tires. 96% of the artificial turfs in Norway in use

today use SBR-granules, the remaining 4% are divided between sand, olive stone kernels, sugarcanes, cork/coconut, TPE, EPDM and coated sand [8]. SBR has an advantage in it being a cheaper alternative than the other granule types, as well as giving the best playing experience on the turf [4]. The disadvantages with SBR-granules are that they contain environmental toxins and are a source of micro plastics [9, 4]. All the other granule types are also sources of micro plastics. Due to micro plastics being highly damaging to the environment, attempts have been made to find organic alternatives to granules [4]. SBR-granules are a mixed product and consist of zinc, sulphur, lead, copper, black carbon, polycyclic aromatic hydrocarbons (PAH), semi-volatile organic compounds (SVOC) and volatile organic compounds (VOC) [10]. Zinc and sulphur are metals which are described as inhibitory for microbial growth. Organic filler materials lack the ability to inhibit microbial growth, this entails the possibility for more microbial growth on artificial turf with organic filler material [10, 11, 12].

Indoor artificial turf posses another challenge, poor air quality, due to the use of granules [13]. The Norwegian Institute for Air Research (NILU) conducted a study in 2005 which concluded that the use of granules in indoor artificial turf facilities could increase the amount of airborne dust. It was concluded that the concentration of airborne dust inside the facilities was not a notable health hazard, however it was above the national recommendations for indoor facilities. Due to this it is important to reduce peoples exposure to the dust. This has to be done by either replacing SBR-granules or bettering the ventilation in the facilities [14]. The Norwegian Culture department released a guidance in 2007 stating that compounds in granules might give allergies and asthma. Therefore, the department recommended indoor artificial turfs to not use rubber granules [15]. If granule is used in indoor facilities, one should be using a type of granule that do not emit dust, and is easy to clean [16]. In addition, the management of the hall must ensure solutions to prevent plastic-containing filler material ending up in the waste water [15].

2.1.4 Organic filler material - main focus on olive stone

Attempts have been made to replace rubber filler material with organic filler materials due to the environmental impact. Some of the organic filler materials being tested are cork, sugarcane, olive stone and wood fibers [9]. During research and testing of different filler materials it has been discovered that each possess advantages and disadvantages regarding the playing characteristics of the artificial turf [4]. Each filler material has to fulfill a number of qualifications. Centre of Sport Facilities and Technology, SIAT, has divided these qualifications into three main categories, sport, product and environment. The sport category evolves around the user-friendliness of the turf. How the field feels to the athlete and how this can be improved by altering the field and/or the shoes of the athlete. The product category concerns knowledge about the materials used on the field and in the turf. The last category, environment, concerns the environmental impact the different materials will have [17].

Olive stone is one of the organic filler materials being tested for use on artificial turf. Olive stone is a waste product from olive oil production [18, 19]. The stones are crushed and reused as filler material on the turf. Figure 2.2 shows what they look like.

Figure 2.2: A picture of olive stones. The stones are light beige, nearly odorless and do not contain or produce dust [19].

Two of the advantages of using olive stone is less refilling and less infill migration due to the stones being heavier than rubber granules [18, 20, 21]. Olive stones are made from bio-based resources and are therefore a sustainable product. In addition, the stones do

not change shape or texture when exposed to varying temperatures [19, 20]. Regardless, there are some uncertainties regarding olive stones which only surfaces after some years of use. This is amongst other things the durability of the product [18]. In addition, players have reported that the olive stones as filler material are more slippery and harder to play on than SBR-granules [22].

2.1.5 Maintenance of indoor artificial turf

Due to the lack of natural moisture such as rain and snow, and UV-radiation from the sun on indoor artificial turf it is necessary to have a proper maintenance and cleaning schedule [23, 24]. In addition, a satisfactory maintenance prolongs the artificial turfs life expectancy. The maintenance keeps the turf soft and a harder turf has a shorter life expectancy than softer turfs [25]. Attempts of replacing SBR-granules as filler material to organic filler materials has introduced problems concerning microbiological growth. This amplifies the need for satisfactory maintenance to prevent too high levels of microbiological growth [12].

A deep cleaning is recommended to be performed 1 to 3 times per year, especially on fields where it is a high activity level. A deep clean on indoor artificial turfs needs to be performed more frequent than outdoor artificial turf due to the particulate matter indoors [26]. The deep cleaning takes between 2 to 5 hours to perform. The activity is performed to remove foreign objects such as garbage, gum and chewing tobacco. In addition, some of the biological material on the turf will be removed such as blood, hair and dead skin cells. For indoor artificial turf the quality of the air will improve due to the deep cleaning removing some of the particulate matter that causes respiratory related health problems [27]. When performing a deep clean a rotating brush with help from the suction turbine of the machine reaches deep down in the turf. [26].

Softening of artificial turf is also a maintenance routine that must be performed. Softening dissolves compressed granule pieces to improve the playing qualities of the turf, as well as reducing the need for refill of filler material. Softening is recommended to be performed 3 to 6 times per month depending on the activity level. The process takes 1 to 1,5 hour to execute [28]. Even if softening is performed maintenance personnel must still

refill granule. The refill is done when necessary to ensure the playing qualities of the turf. In addition, the lifespan of the turf is prolonged with the right amount of refill. The refill is recommended to be performed after a cleanse, and it takes approximately 30 minutes to complete [29].

A final maintenance routine should be controls of air quality inside indoor facilities. This is to prevent respiratory related health problems. Measurements of CO_2, VOX, particulate matter, temperature and moisture help to uncover the need for maintenance of the turf or the ventilation system. It is recommended to either do the measurements on a regular basis or as a scheduled maintenance 2 to 3 times per year. The scheduled maintenance takes approximately 5 to 7 days [30].

It has previously been conducted research on the microbiological growth and methods to disinfect the bacteria on indoor artificial turf. The research suggest that there is a significant bacterial growth on the turf. The disinfectant Nüscosept PRO is documented to be inhibitory to the bacteria and is therefore being used to clean the turf biweekly [12, 31, 32, 33]. Figure 2.3 gives an indication on how the disinfectant is applied to the artificial turf at Flatåshallen.

Figure 2.3: An in action picture of the disinfection device used to apply Nüscosept PRO to the indoor artificial turf at Flatåshallen.

2.1.6 Disinfectant - Nüscosept PRO

Nüscosept PRO is manufactured by Dr. Nüsken Chemie GmbH. It is a liquid disinfection concentrate for professional use [34]. The concentrate is diluted and distributed over the artificial turf in Flatåshallen as a part of the maintenance. Nüscosept PRO contains didecyldimethylammonium chloride and propan-2-ol, these substances are toxic to bacteria at a concentration above 2 mg/L and 5,175 mg/L respectively [35]. In addition, it contains benzalkonium chloride (BAC) which is a quaternary ammonium compound (QAC) [35]. BAC is widely used in disinfectants for use on cationic surfaces and in health care facilities [36]. The disinfectant is safe to use inside indoor facilities considering the active substances in its solution. These active substances could cause build up of organic substances on the turf. The disinfectant used in Flatåshallen is a 0,5% dilution and due to the low concentration a build up on the turf is not an issue. The undiluted product have been tested against athletes foot diseases and for hand wash products. These tests give no indication of health problems correlated to the disinfectant [37].

2.2 Microorganisms

2.2.1 Generally about bacteria

Microorganisms are organisms so small the human eye can not see them in their natural habitat. *Bacteria* is one of three domains in the world of microbial life, the two others are *Archaea* and *Eukarya*. Bacteria are divided into two groups, gram-positive and gram-negative. Bacteria are capable of transferring their DNA between each other. This characteristic helps develop the bacteria, and ensures survival in new environments. Transferring DNA also helps with transferring antibiotic resistance, this is a major complication regarding treatment of infections. As of today, the only applied treatment for bacterial infections is antibiotics. Scientist are developing alternatives, however they are not promising against gram-positive cocci, such as *Staphylococcus aureus* [38].

When bacteria are grown in laboratory cultures we can describe their general growth with a growth curve. The nutrients are limited in a batch culture therefore the bacteria can not grow indefinitely. A typical growth curve consists of a lag-, an

exponential-, a stationary- and a decline phase. The lag phase is the initial phase when the sample first is plated on to the growth media. The bacteria do not immediately grow when plated due to it needing to adjust to its new environments. When the adjustments are executed the exponential phase starts. During this phase the bacteria doubles in population at regular intervals. The exponential phase continues till it no longer is sustainable growth conditions. When the growth stagnates the bacteria enters the stationary phase. In this phase there is no increase or decrease in population. The bacteria prepares for maintenance and survival, and eventually nutrients run empty and the bacteria enter the decline phase. The amount of bacteria decreases [38]. A general growth curve is shown in Figure 2.4.

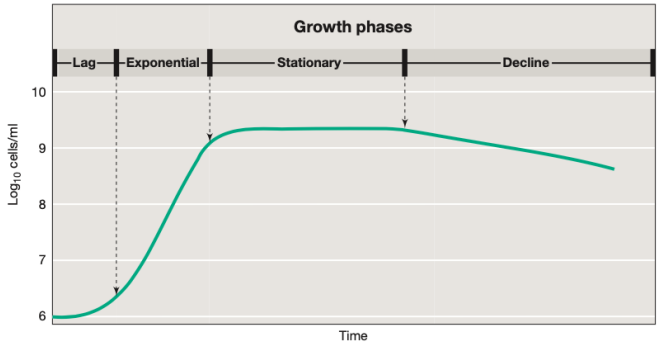

Figure 2.4: A graph showing a general growth curve for bacterial growth with its growth phases. The amount of bacteria is plotted against the time of the growth period.

For a typical bacterial growth curve the lag phase lasts approximately 8 hours, the exponential phase lasts approximately 15 hours, the stationary phase lasts approximately 6 hours and the decline phase lasts approximately 12 hours [39]. The phases duration will depend on which bacteria it is and type of nutrition used. Most bacteria grow between 5 °C and 60 °C, and their fastest rate of growth is around 37 °C [38]. A general growth medium used to grow most bacteria is TSA. The media contains casein degraded by enzymes and flour from soybeans [40]. TSA is a non selective and a non differential media, and supports growth of a wide variety of microorganisms [41]. One liter of TSA contains 15 g agar and 30 g tryptic soy broth [42].

2.2.2 *Staphylococcus aureus*

Staphylococcus aureus can be found on the skin and in the throat of humans [43]. Since the bacteria already exists on humans, it does not always cause infections. However, if the bacteria enters the bloodstream or internal tissue through a cut or any other injuries it can cause serious infections [44]. *S.aureus* infections can cause toxic shock syndrome, atopic dermatitis, arthritis, the Kawasaki syndrome, and other related disorders [43, 44]. It is important to note that most cases of these infections occur in people with multiple risk factors for infection [44]. Taking that into account, the risk of serious infections in healthy active people is low. Despite the low risk, most *S.aureus* isolates are resistant to penicillin [44]. This resistance stem from the presence of the *mec* gene. The *mec* gene encodes PBP-2a, a penicillin-binding-protein [45, 44]. PBP-2a will continue to build the cell wall even in the presence of antibiotics, consequently helping the bacteria infection grow [45]. *S.aureus* have been adapting and becoming resistant to every new antibiotic since the discovery of penicillin, which makes treating infections caused by *S.aureus* difficult [43]. Additionally, *S.aureus* is able to form biofilm, a microbial community where cells are embedded in a protective extracellular polymeric matrix, when attached to a host [46]. The genes *icaADBC*, *hla*, *clfA* and *dltA*, need to be present for biofilm formation [47]. The formation of biofilm will increase tolerance to antibiotics and disinfectants, as well as expedite the progression of chronic diseases [46, 36]. Due to this persistent resistance, it is vital to prevent infection and treat them correctly.

2.3 Microbiological methods and techniques

2.3.1 Sample collection method

There are several different ways to collect bacterial samples from the environment around us. Some that are commonly used are the wipe-rinse, contact plate and swabbing methods. The wipe-rinse method involves using a moistened, sterilised cloth to collect samples. The cloth is steadily pressured into the surface, then folded so that the contaminated side is inside the fold. Pressure is then applied to the surface two consecutive times. The cloth is then placed into a glass container and transported to the laboratory. In the laboratory the cloths are rinsed with 400 mL buffered solution and placed in an ultrasonic bath

for 2 minutes. Dilutions from the container are then plated on medium and incubated. The method is time consuming, it has however a generally high collection efficiency of microorganisms. The cloth has a tendency to hold on to the collected microorganisms making it hard to release them and giving a lower recovery efficiency than originally collected. The cloths cover a greater area than any competing sample collection methods [48].

The contact plate method involves utilizing contact agar plates, often called Rodac plates, to collect microorganisms in sample areas [48, 49]. The contact agar plates are plates with the agar built above the plate's boundary to easily reach the surface of the sample area. The plates are pressed down on the surface for 3 to 5 s, then incubated [49]. The contact plate method has a high recovery and repeatability efficiency for flat and firm surfaces [50].

Swabbing method involves leading a swab over the sample area to collect microorganisms. The swab is either moistened manually or pre-moistened in its container. The sample is transported to the laboratory and plated on agar plates. For plating the sample both the pour-plate and the spread-plate method can be utilized [49]. A benefit of the swab-rinse method is that the swab can cover small and uneven surfaces [50]. Its limitations are that the swab collects less sample compared to other sample collection methods, and it has a tendency to retain collected organisms [48].

2.3.2 Methods for inoculation of samples

When evaluating and analysing cells under a microscope there is no differentiating between viable and non-viable cells. Culture media can be utilized to perform viable counts, and with this method it is possible to distinguish between non-viable and viable cells. To perform a plate count two methods are most common to use: the spread-plate method and the pour-plate method. The spread-plate and the pour-plate method are techniques for plating liquid samples containing bacteria [38]. When performing the spread-plate method a given volume of the sample, often 0,1 mL, is pipetted on to the plate and spread out with a Drigalski spatula [51, 52]. The Drigalski spatula can be made out of metal or glass, and is a cylindrical rod bent in a triangle shape at the end. The pour-plate method

is also a method used to plate liquid bacteria samples. A given volume of the bacteria sample, often 0,1 or 1 mL, is added to an empty Petri dish. Liquid agar at approximately 50 °C is then poured over the sample and mixed together [38]. The pour-plate method is often used for CFU counting in fluids, however the process and preparation is time consuming [53].

2.3.3 Quantification methods for bacterial cultures

Microscopic counting, also called total count, is an example of a quantification method. [54]. Samples dried on slides or liquid samples are observed under a microscope and the total count of microbial numbers are recorded. Samples can be stained to differentiate viable and non-viable cells. When counting cells in liquid samples a counting chamber, often called a Hemocytometer, with a precise volume is used. Total cells in a sample are calculated as shown in Equation 2.1. Total represent the total cells in the sample, and V is the original sample volume [55].

$$Total = \frac{Total\,cells\,counted * Dilution\,factor * 10,000\,cells/mL}{Number\,of\,squares\,counted} * V \tag{2.1}$$

Another limitation other than not being able to differentiate viable and non-viable cells without staining is the difficulty being precise with the method. Small cells are often hard to count under a microscope, which can lead to counting errors [38].

Another counting method is counting of CFU, often called plate count method. This is a manual counting method. CFU, colony-forming units, is a measurement used for determining number of viable cell colonies [54, 56]. It is often measured in CFU/mL or CFU/cm^2, where these refer to CFU units per mL or cm^2. This is either the volume of the sample or the size of the sample area [57]. The bacterial count per mL can be found as shown in Equation 2.2 and the bacterial count per cm^2 can be found as shown in Equation 2.3 [40, 58].

$$CFU/mL = \frac{Average\,CFU\,/\,plate * Dilution\,factor}{Volume\,of\,sample\,[mL]} \tag{2.2}$$

$$CFU/cm^2 = \frac{Average\,CFU\,/\,plate * Dilution\,factor}{Area\,[cm^2]} \tag{2.3}$$

The plate count method is primarily used with samples with a high number of microorganisms. In addition, it is mostly used with samples containing microorganisms that do not grow well in liquid media. The original sample is diluted into tubes, thereafter the aliquots are plated by either the pour-plate or spread-plate method. The colonies observed on the plates after incubation are then counted. The typical counting range are 25 to 250 CFU and 30 to 300 CFU [56, 59]. Sources of error when counting is that colonies close to each other may merge and that neighbor colonies may inhibit growth. In addition, since the method is a manual method there is the risk of human error causing a counting error [59].

A final counting method is with a spectrophotometer. Light at 540 nm, green light, is sent through the sample. The turbidity of the sample is examined in optical density, OD. The optical density is often proportional to cell number, therefore the turbidity is a good substitute for total viable count. A standard curve relating cell number to turbidity must be made before the test can be run due to error at high cell density. At high density the correspondence between cell number and turbidity deviates from linearity, therefore a standard curve needs to be designed. The method is very time efficient and easy to perform, however it also has its flaws. Microorganisms do not always grow evenly in liquid samples. To achieve a total cell mass of a sample it is necessary to shake or stir the sample to maintain an even distribution of cells in the liquid during analysis. In addition, this method is used primarily on pure cultures, and will be less exact for mixed cultures [38].

2.3.4 Aseptic technique

Aseptic techniques are important to ensure that the cultures and the samples are not contaminated, in addition to not infect workers. Flaming is a technique used when caps and lids are removed from liquid cultures. The technique does not sterilize however it creates a warm air convection. This convection prevents entrance of particles into the opening [41]. Another technique is the correct use of Petri dishes to prevent contamination of the media and culture. The Petri dish lid allows access to some air, however it simultaneously prevents contamination by other microorganisms. When the lid is removed it should be held over the plate as a shield. A Petri dish lid should never be placed on a bench top, and plates should never be left uncovered. In addition, it is important not to walk around the room when plates are open. Finally, when plating it is important to work rapidly as well as carefully. It is also important to keep a clean workspace, therefore washing the workspace with disinfectant before and after use is highly important [41].

2.3.5 Cleanliness scale

Microbiological standards have been introduced for surface hygiene. A standard method for environmental sampling has not been discussed, but the efficiency of disinfection has previously been assessed. It is assumed that most contamination comes from hand hygiene, and the risk of acquiring infection from environmental surfaces is small. However, assessments show that there is clinical evidence identifying connections between poor hygiene on surfaces and transmission of microorganisms causing infections. Given the surface at hand, the cleaning method will range from different levels of disinfection to soap and water cleaning. There are several studies documenting the need for environmental disinfection to prevent or control infections. Two microbiological standards for surface hygiene in hospitals have been proposed: identification of an indicator organism that is a risk for patients, and quantitative assessment of all organisms found in the area. There has not been proposed any specific methods to perform these standards [60].

A study carried out in the UK in intensive care units and high dependency units suggested that often the only method for evaluating cleanness is visual assessment [60]. In other studies it has been discovered that 82% of hospital areas were assessed as visually clean,

while 30% of the same areas were assessed as clean using microbiological techniques. The criteria for a clean surface which was practicable in a hospital was < 2.5 CFU/cm^2. It was suggested that surfaces with 40 CFU/cm^2 were unsanitary. It is no specification for the count of viable microorganisms and count of indicator organisms [61].

2.4 Statistical Methods

Statistics is used to validate data. It helps tackle uncertainty and different variables. There are different methods for validating data, depending on what type of data is available and what type of validating is needed [62]. Dispersion in statistics means how much measured values differ from a fixed value. The most common dispersion method is standard deviation, which measure how dispersed the data is in relation to the mean [63]. A high standard deviation means a wide spread in the data range and can indicate an out liner or an inadequate amount of data points. Standard deviation are calculated as shown in Equation 2.4.

$$Standard\,deviation = \sqrt{\frac{\Sigma(x - \overline{x})^2}{n - 1}} \tag{2.4}$$

Regression analysis is a statistical tool for the investigation of relationships between variables [64]. A regression of the effect one variable have on another is called a simple regression, this is unrealistic when in reality there are more variables to consider [64]. A regression analysis can communicate a correlation between two or more variables. For the analysis to be admissible it needs to be a clear correlation and several data points. However, a regression analysis is still just an estimate and is not conclusive.

3 Materials and Methods

The materials and methods used in this thesis are presented in this section. The method was developed and tested from the 30th of January to the 21st of February. The finalised method was preformed from the 21st of February to the 31st of March. During the testing period the artificial turf in Flatåshallen was cleaned with 20 liters of disinfection on the 20th of February, and with 80 liters of disinfectant on the 18th of March. Below the preparation of the media is presented, as well as the execution of the sample collection and application.

3.1 Equipment and chemicals

Table 3.1 shows the materials and chemicals used to preform the method in this thesis. The table includes the producer and the product number of each material and chemical.

Table 3.1: A list of the materials and chemicals used in this thesis, including their producer and product number.

Material and chemicals	Producer	Product number
Tryptic Soy Broth (TSB)	Sigma-Aldrich	22092-500g
Agar	VWR International AS	20767.298
Autoclave	TOMY Digital Biology	TOMY SX-700E
Incubator	Termaks	B 9051
3M Swab-Samplers 10 mL	3M Norge	RS96010LET
3M Swab-Samplers 1 mL	3M Norge	RS9604LET

3.2 Methods

3.2.1 Preparation of media

Tryptic Soy Agar was prepared by dissolving 30 g Tryptic Soy Broth and 15 g agar in 1000 mL of deionized water. The solution was added to a Pyrex bottle and put to heating and stirring. When the solution was dissolved the bottle was autoclaved at 121 °C for 15 minutes. Thereafter, Petri dishes were filled up to 1/3 of their volume with the solution. The Petri dishes were left on a sterilised bench without their lids for 20 minutes. After the agar had set, the Petri dishes were flipped with their lid on. The dishes were stored

on a sterilised bench in a open plastic bag over night. When the condensation was gone the dishes were stored in a cooler at 4 °C in a closed plastic bag for later use. The method of making the media was inspired by former laboratory work in microbiology [65, 42].

3.2.2 Sample collection from artificial indoor turf

The samples were collected from four different areas on an indoor artificial turf at Flatåshallen. The areas were at the sideline nearest the entrance, at the 16-meter mark, between the 16-meter mark and the midfield and the midfield. Figure 3.1 illustrates the sample collection areas. All the samples were collected at the same side of the field. From every area it was taken 3 parallels.

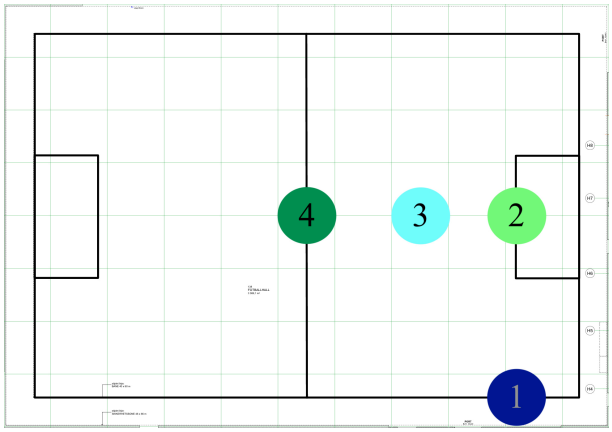

Figure 3.1: The test sample locations shown on the floor plan of Flatåshallen. Sample area 1 is the sideline by the entrance, area 2 is 16-meter mark, area 3 is between the 16-meter mark and the midfield, and area 4 is the midfield.

The swabs used were 3M swab-samplers as shown in Figure 3.2. The swabs were brushed over the turf in a specific pattern as shown in Figure 3.3 in a 10x10cm area. The samples were transported to the laboratories at Kalvskinnet for application to the medium immediately after collection.

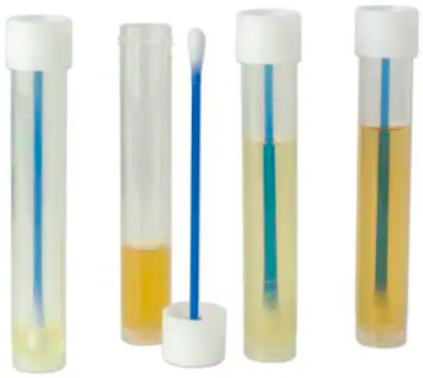

Figure 3.2: 3M swab-samplers used for sample collecting [66].

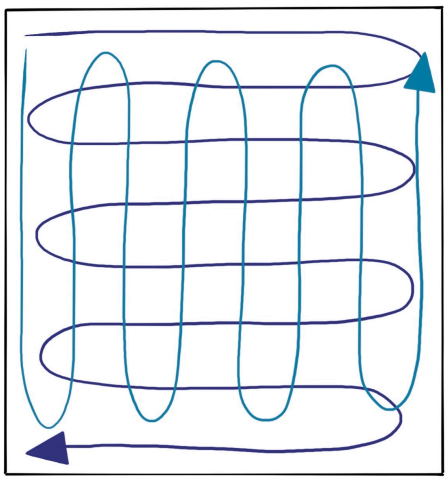

Figure 3.3: The pattern the 3M swab samplers were brushed over the turf.

3.2.3 Sample collection from a locker room and Kalvskinnet campus

Samples were collected from a locker room in Flatåshallen and school environments at Kalvskinnet campus at the Norwegian University of Science and Technology. Four locations in the locker room were selected: bench, floor, door handle and shower button. From every area it was taken 3 parallels. Four locations in school environment were also selected: door handle at the second floor male toilets, floor at the laboratory, elevator button on the first floor and buttons on the water station in the cafeteria. The swabs that were used were the same as at the artificial turf, and the motion the swabs were brushed in were the same. The size of the area varied depending on the object being analysed. The size of the area covered by the swab on the floor in the laboratories, the bench and floor in Flatåshallen were 10x10cm. The size of the area covered on the door

handle in Flatåshallen and the door handle at school were 2x5cm. Lastly, the size of the area covered on the shower button and the buttons on the water dispenser were 2x1cm, and on the elevator button it was 5x5cm.

3.2.4 Sample application and counting of bacterial colonies

It was taken three samples out of each parallel. This resulted in application to 36 agar plates total. Each test sample were thoroughly mixed between each application. 75 µL of the samples were applied to the agar plates by the help of a pipette. A Drigalski spatula made out of glass were sterilized with spirit and a flameboy, then used to spread the solution over the media. When the bacterial suspension had settled, the plates were incubated at 36 °C for 48 hours. All the plates were controlled after 24 hours, and after 48 hours the bacterial colonies on the plates were counted and listed in an Excel sheet. The plates were counted with a marker and a click counter. The plates were also counted after another 48 hours at the beginning of the thesis till the 10th of March. Thereafter, the plates were counted after 24 hours and 48 hours of incubation.

4 Results and discussion

The results and the discussion from microbiological testing of the artificial turf in Flatåshallen are presented in this section. The results and the discussion from microbiological testing from the locker room and on campus are also included. All the samples were collected during a time period of 7 weeks. The equation needed to calculate CFU/cm^2 is explained in Section 2.3.3. The statistical method used for calculating standard deviation is explained in Section 2.4. The results and evaluation of the results from the turf in Flatåshallen are shown in Section 4.1. The results and evaluation of the results from the locker room in Flatåshallen and Kalvskinnet campus are shown in Section 4.2. An evaluation of the methods used is presented in Section 4.3. Lastly, recommendations for further research linked to the thesis are shown in Section 4.4.

4.1 Results from the artificial turf

4.1.1 Quantification of bacterial colonies

The bacterial count from every agar plate was recorded in a table during testing. The original table was separated into four tables for clarity. Tables A1.1, A1.2, A1.3 and A1.4 in Appendix A1 show the complete results. Table 4.1 is include here as an example and shows the count from sample area 1. It was collected three parallels from each area, and it was retrieved three samples from each parallel. The sample dates are to the left. The *Checked* column next to the sample dates shows how many days after inoculation the bacteria were counted. The thick black line after the 10th of March indicates a shift in counting dates. Under the *Number of bacteria colonies* cell the identification numbers that mark every agar plate are presented. The three numbers represent sample area, swab number and agar plate number, respectively. For example 1,1,1 means sample area 1, swab number 1 and plate number 1. The red cells indicate an overgrown plate where counting was impossible, the numbers are estimates made at the end of the testing period based on similar plates. The bacterial count after two days of incubation was used for further calculations in Section 4.1.3.

Table 4.1: Bacteria colonies counted on agar plates from sample area 1 throughout the experiment. The *Checked* column represents the days after inoculation the bacteria were counted. The identification number at the top represents sample area, swab number and agar plate number, respectively. The red cells are estimated numbers due to overgrowth.

Sample date	Checked	Number of bacteria colonies								
		1,1,1	1,1,2	1,1,3	1,2,1	1,2,2	1,2,3	1,3,1	1,3,2	1,3,3
21-Feb	day 2	1	3	3	1	3	0	6	0	962
	day 4	1	3	6	2	3	0	7	0	962
23-Feb	day 2	2	4	6	11	7	5	2	1	8
	day 4	3	7	7	13	7	5	2	1	8
25-Feb	day 2	6	1	877	3	8	61	4	15	13
	day 4	7	1	877	5	8	66	11	24	21
02-Mar	day 2	37	11	13	546	18	24	124	95	110
	day 4	41	16	18	547	22	26	133	109	121
04-Mar	day 2	63	3262	2560	216	55	53	639	88	113
	day 4	74	3262	2560	234	79	76	647	92	120
06-Mar	day 2	19	33	46	2814	18	280	23	21	689
	day 4	28	40	54	2814	27	285	29	25	689
08-Mar	day 2	435	99	3500	2471	100	35	25	31	31
	day 4	438	114	3500	2471	100	44	33	35	33
10-Mar	day 2	42	1170	48	29	29	25	695	89	66
	day 4	42	1170	48	29	29	27	695	92	70
13-Mar	day 1	44	41	51	58	61	3784	28	37	38
	day 2	57	47	67	81	91	3784	55	66	69
15-Mar	day 1	37	56	38	35	69	138	86	71	69
	day 2	48	78	56	57	89	154	122	96	115
17-Mar	day 1	26	36	22	74	100	89	29	95	32
	day 2	37	49	36	95	114	104	37	97	40
19-Mar	day 1	34	13	17	5	2	1	2	0	2
	day 2	34	13	17	5	2	1	3	5	4
21-Mar	day 1	57	50	58	43	42	56	19	28	35
	day 2	66	62	71	50	57	69	30	49	41
23-Mar	day 1	27	25	23	33	33	169	21	60	40
	day 2	41	39	34	58	54	184	63	88	81
25-Mar	day 1	3	8	4	6	5	3	25	50	37
	day 2	10	17	7	27	19	24	32	59	39
27-Mar	day 1	3	3	4	7	5	7	11	3	5
	day 2	8	4	5	12	46	9	13	9	7
29-Mar	day 1	39	74	185	21	19	16	11	10	13
	day 2	51	83	1230	33	30	31	16	25	32
31-Mar	day 1	36	37	28	216	620	287	19	60	41
	day 2	61	46	43	224	665	305	24	67	63

4.1.1.1 Evaluation of colony counting method

Overgrown plates were difficult to count since the bacteria colonies grew on top of and into each other [59]. There is no guarantee the group counted every plate correctly. However, the more bacteria grown on the plates, the less effect incorrect number of colonies have. There were also different bacteria that migrated over the whole plate, these were counted as one. There were many cases of bacteria spreading and affecting other

bacteria. However, no effort was made to dilute the samples further due to the varying bacteria count. This was decided in case of the loss of data from plates with smaller bacteria count.

When combining the standard plate count method with proper incubation time, the method was less time consuming and there were less overgrown plates. In hindsight, the group would have used a plate light to better view the bacteria colonies during the counting. Other than this observation this counting method was superior to the other counting methods, due to the time aspect of the thesis. If the group had more time at hand more quantification methods could have been presented. However, considering the quantity of the samples and time at hand the standard plate counting method was the most optimal method.

4.1.2 Bacteria appearance on agar plates

Figure 4.1 shows plate number 3,1,1 from the 2nd of March, and is an example of a plate that is overgrown. The number of bacteria colonies was estimated based on the count of similar plates. Figure 4.2 shows every plate from sample area 2 collected on the 2nd of March. The picture was taken 2 days after inoculation and is an example of the diversity of growth.

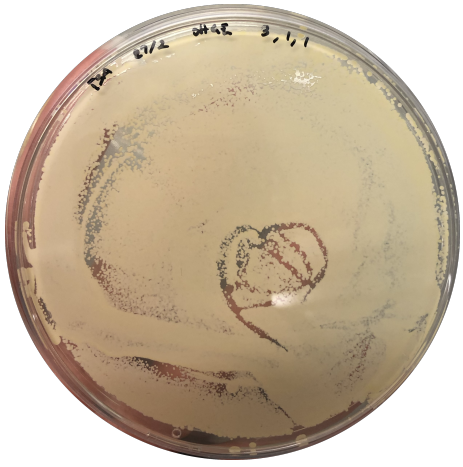

Figure 4.1: Plate number 3,1,1 from the 2nd of March is an example of an overgrown plate, where the bacteria count was estimated and not counted.

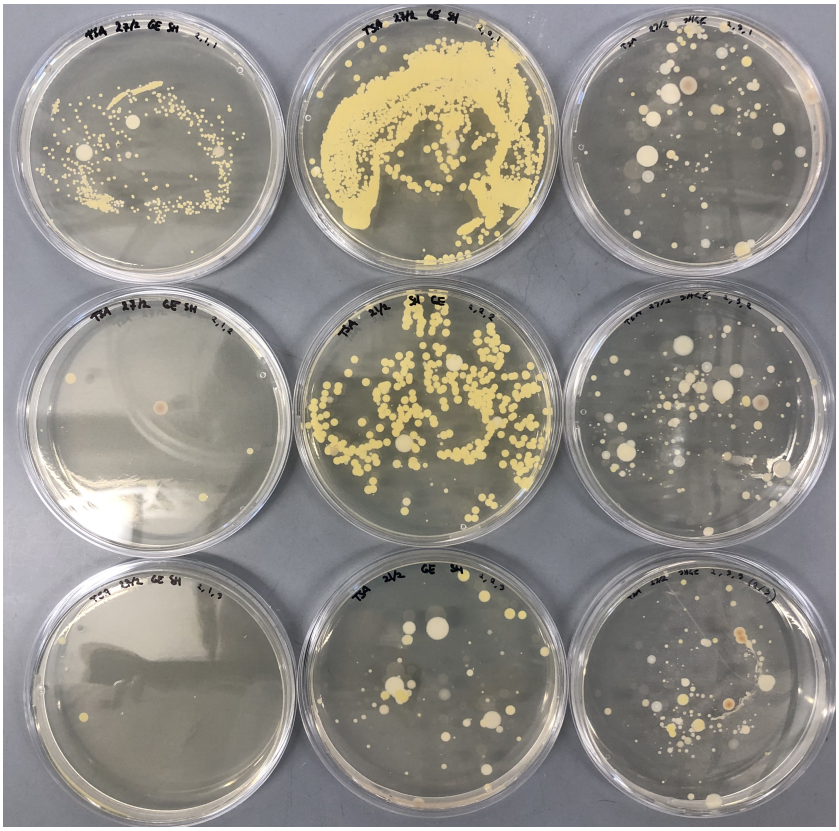

Figure 4.2: All nine agar plates from sample area 2 from the 2nd of March. The left row; 2,1,1, 2,1,2 and 2,1,3. The middle row; 2,2,1, 2,2,2 and 2,2,3. The right row; 2,3,1, 2,3,2 and 2,3,3

4.1.2.1 Evaluation of results on Petri dishes

Figure 4.2 exhibit the various samples from parallels collected from area 2 on the turf. The figure gives an indication of the variation of bacteria on the turf and the variation of amount collected. As can be observed by the colour of the colonies there are several different types of bacteria on the Petri dishes. The thesis analysed the general bacteria count, therefore there are no further analysis of which bacteria are on the Petri dishes. The figure also gives an indication of varying amount of bacteria collected for each parallel. Limitations of sample collecting are discussed in 4.3.1.3, and as discussed here the sample collecting method could affect the sample amount collected. The limitations could explain why each parallel from the area collected a varied bacteria amount.

The varied bacteria amount from each sample from each parallel could also be a result of uneven growth of bacteria in liquid. Attempts of shaking and stirring the sample were made to achieve an even growth when transferring the liquid to the agar [38]. In some cases the attempts might not have given the wanted results. According to the plates in Figure 4.2 there is reason to assume that parallel 1 and parallel 2 did not achieve enough stirring before inoculation.

Lastly, Figure 4.1 gives an example of an overgrown plate. The overgrown plates, marked with red in Appendix A1, had too many or intertwining colonies obstructing correct bacteria count. Removing these plates from the data set would cause a sizeable error. Therefore the bacteria count was estimated at the end of the sampling period to ensure the most accurate estimates.

4.1.3 Calculations of colony forming units

The samples from the turf were collected from the 21st of February to the 31st of March. Figures 4.3, 4.4, 4.5 and 4.6 show the calculated CFU/cm^2 after two days of incubation from the four sample areas on the turf in four separate graphs. The location of the sample area is illustrated with a diagram in the top right corner of each Figure. The vertical lines on each column represents the standard deviation from that specific date.

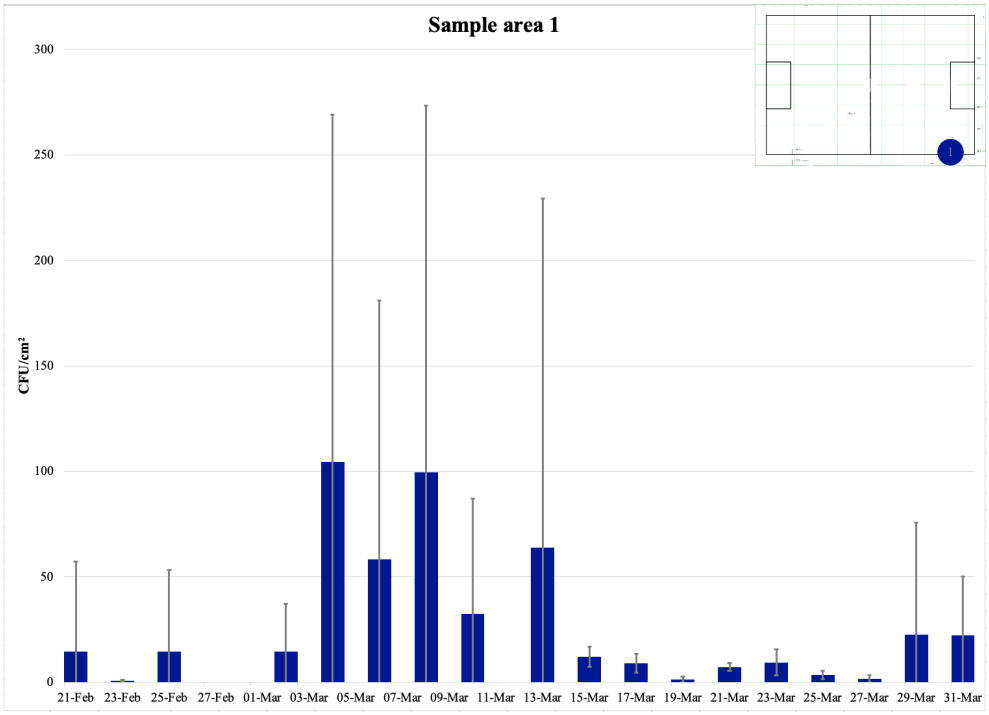

Figure 4.3: This chart shows the calculated CFU/cm^2 as columns with standard deviation as vertical lines from sample area 1, as well as a diagram in the upper right corner of where sample area 1 is located on the turf.

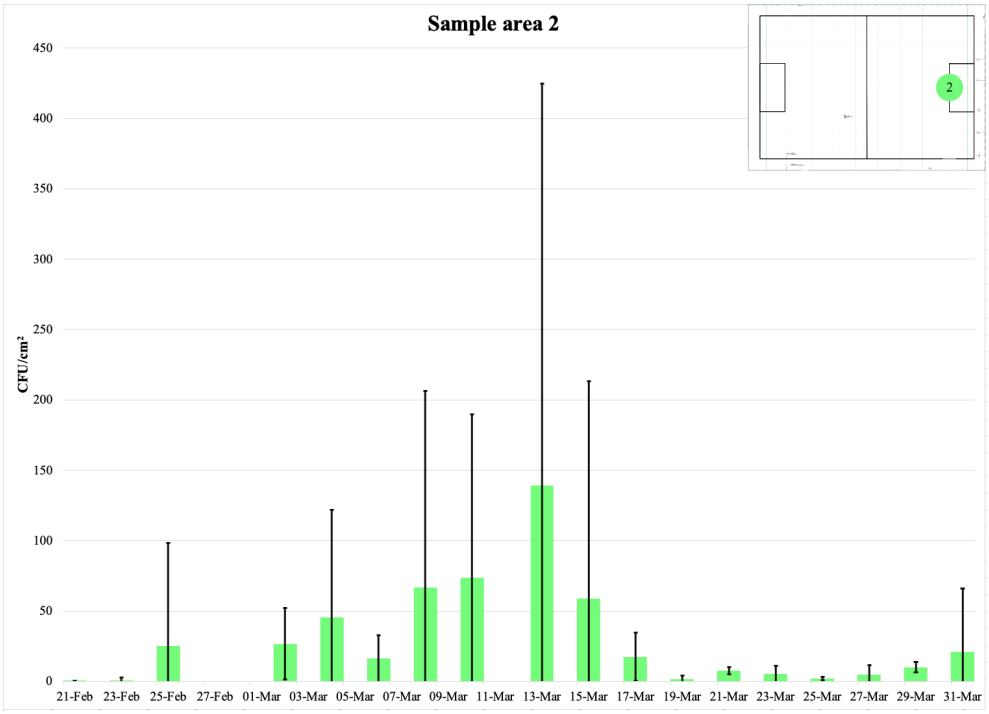

Figure 4.4: This chart shows the calculated CFU/cm^2 as columns with standard deviation as vertical lines from sample area 2, as well as a diagram in the upper right corner of where sample area 2 is located on the turf.

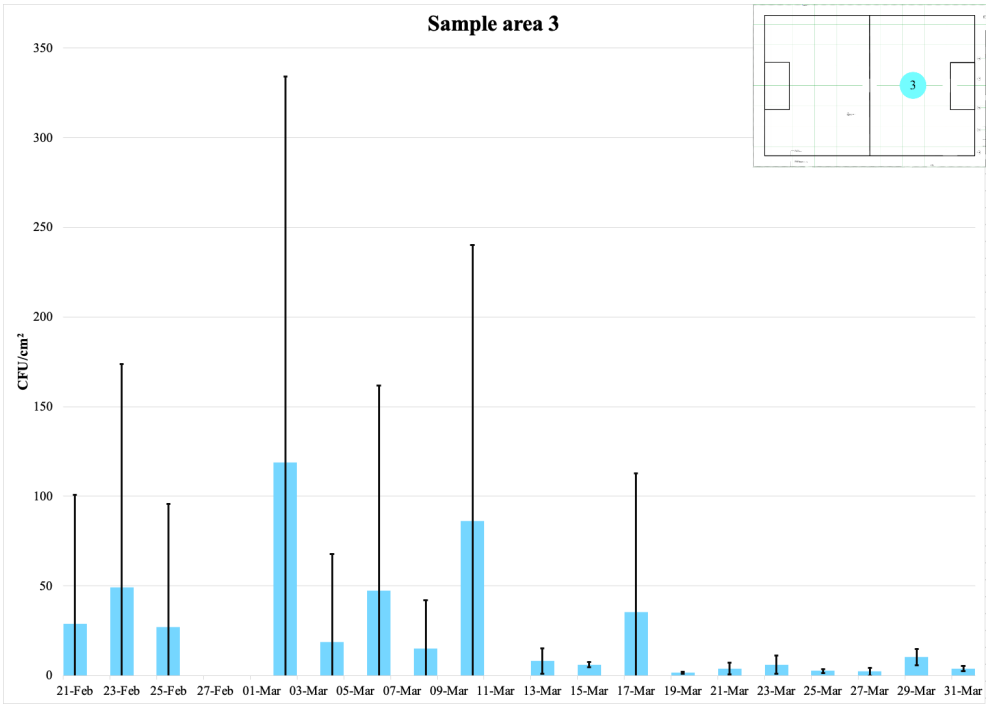

Figure 4.5: This chart shows the calculated CFU/cm^2 as columns with standard deviation as vertical lines from sample area 3, as well as a diagram in the upper right corner of where sample area 3 is located on the turf.

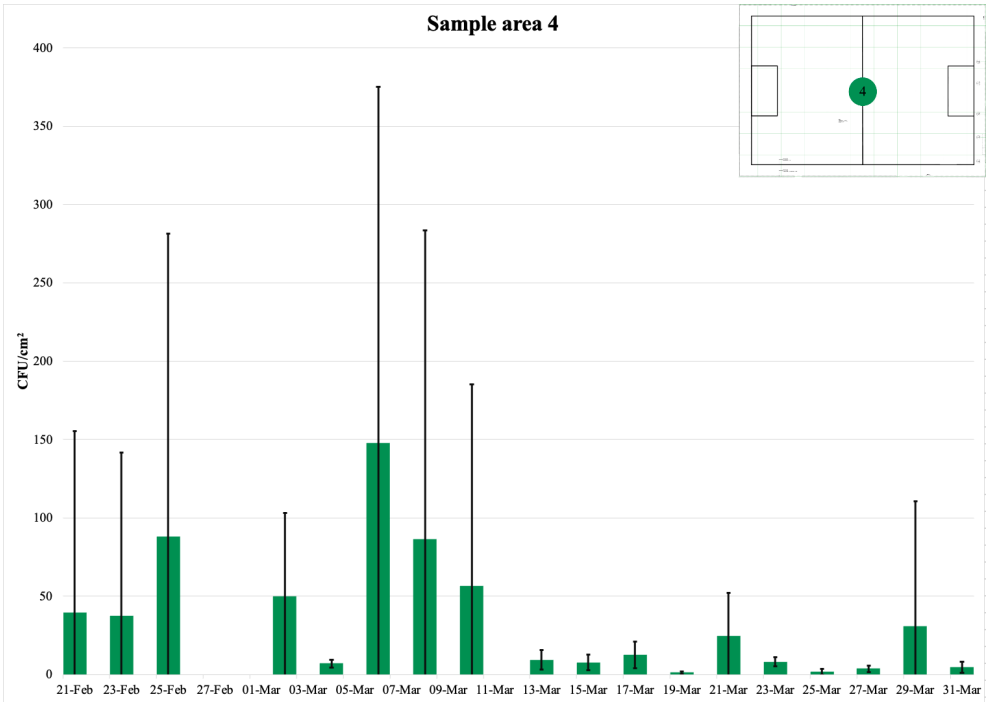

Figure 4.6: This chart shows the calculated CFU/cm^2 as columns with standard deviation as vertical lines from sample area 4, as well as a diagram in the upper right corner of where sample area 4 is located on the turf.

Figure 4.7 shows the CFU/cm^2 graph from all sample areas in one chart. The y-axis shows CFU/cm^2 and the x-axis shows the time period. This graph highlights the differences between each sample area.

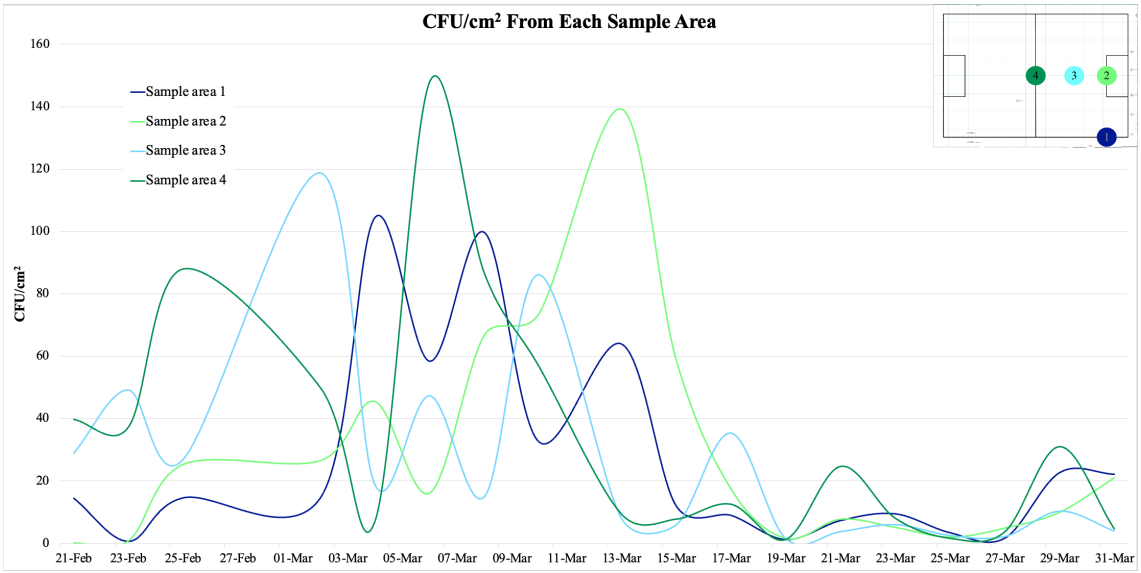

Figure 4.7: CFU/cm^2 from all four sample areas in one chart to illustrate differences, with a diagram in the upper right corner of where each sample area are located on the turf.

4.1.3.1 Evaluation of the standard deviation

A large standard deviation indicates a wide spread of data, ideally the standard deviation is minor [62]. The standard deviation in Figures 4.3, 4.4, 4.5 and 4.6 from the turf and Figure 4.9 and 4.10 from different environments are varying, and some are inordinately high. All the charts indicates the need for more parallels. The standard deviation is as high or higher than the results, which indicates a wide spread between samples [62]. Ideally more samples should have been taken from both the locker room and the school environments to prevent high standard deviation on each sample locations. The charts from the turf has in general a more moderate standard deviation, however there are sample dates indicating the need for more samples. There will always be a standard deviation when analysing data sets, in spite of that the goal will always be to achieve a minor standard deviation [62]. The charts showing the results from the turf have a high standard deviation in various bars, and on the basis of these results it is evaluated a need for more samples.

It is important to see the achieved results in context with the behaviour of microorganisms and the limitations of the sample collections. A varying bacterial count is normal considering the unpredictable behaviour of bacteria correlated to uneven growth conditions [38]. Various faults in the sample collection could also potentially cause variations in bacteria collected from the turf. Figure 4.7 illustrates bacterial growth from each area on the turf. The chart illustrates the variation of bacterial growth in each location, and supports the statement of varied bacterial growth.

4.1.4 Visitors compared to CFU

The average number of visitors in Flatåshallen is recorded every hour. Figure 4.8 shows the correlation between registered visitors and bacterial growth. The grey columns show the number of people plotted against the left y-axis. The teal graph shows the average CFU/cm^2 from all four sample areas and is plotted against the right y-axis. The two green dots represent when disinfection is preformed. On the 20th of February, 20 liters of disinfectant was used. On the 18th of March, 80 liters of disinfectant was used. The x-axis shows the time period.

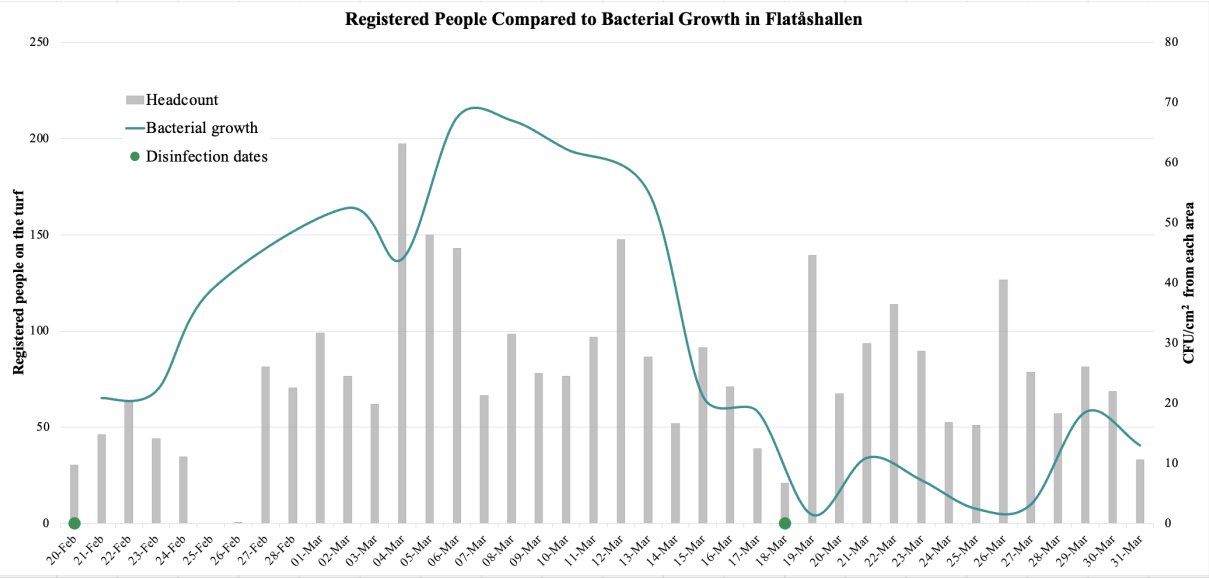

Figure 4.8: This figure shows registered people in Flatåshallen as grey columns plotted together with the average CFU/cm^2 from the four sample areas as the teal graph. Green dots represent disinfection dates.

4.1.4.1 Evaluation of the sample period

Figure 4.8 shows a sampling period of 6 weeks, and when analyzing and reviewing the results the group found that it would be beneficial to have a longer sampling period to achieve a clear tendency. To achieve a clear tendency on the graph it is important to have a high amount of samples. Due to the thesis length the group had time limitations regarding the sample collecting. Beneficially the samples should have been collected in the period with the highest activity on the indoor artificial turf, which is the winter season approximately from late September to early April. During the sampling period there were two holidays, winter break and Easter. The activity during each holiday is lower than on regular weeks. In addition, the turf was closed during Easter therefore it was not conducted any sampling. Due to these holidays the average use of the turf could not be tested. Since the period was 6 weeks the group lost 2 weeks of research. Lastly, the amount of disinfectant used on the turf was changed during the testing period. 20 liters of disinfectant were used on the first disinfection on the 20th of February, and approximately 80 liters were used on the second disinfection on the 18th of March. The change in amount of disinfectant changes the affect on the turf and the graph does not show an average. Due to the changes during the testing period and the holidays it is concluded that the testing period should have been longer to achieve a clear tendency of the effect of the disinfectant.

Table 4.2 shows a section of interest from Figure 4.8, highlighted dates represent disinfection dates, and dashes mean no samples were collected on these dates. The full table is shown in Table A3.1 in Appendix A3.

Table 4.2: A section of interest from Figure 4.8. This table shows the shift in CFU/cm^2 after disinfection on the 18th of March.

Dates	Registered people	CFU/cm^2
15-Mar	91,5	21
16-Mar	71,0	-
17-Mar	39,0	19
18-Mar	21,0	-
19-Mar	139,5	1
20-Mar	67,5	-
21-Mar	93,5	11

4.1.5 Evaluation of overall results from the turf

Figure 4.8 indicates that the growth is fluctuating in the period between disinfection and maintenance. When comparing activity on the turf to the bacterial growth a connection between the two can be observed. The correlation stems from bacteria needing biological material to grow, without this the amount of bacteria decreases [38]. Typical bacteria have an exponential phase of 15 hours and a stationary phase of 6 hours. Without replenishment of biological material during the exponential or stationary phase the bacteria will eventually die [39]. Therefore, when there are few visitors leaving behind biological material on the turf the bacteria will slowly die due to lack of sustainable growth conditions. The bacteria will survive approximately a day on the turf without replenishment of biological material [38]. The temperature inside Flatåshallen can also affect the bacterial growth. Most bacteria have a optimum temperature at approximately 37 °C, and can survive a temperature between 5 °C and 60 °C [38]. Inside the hall the general temperature is about 12 °C, and the bacteria will survive in these conditions. However, the temperature inside the hall is colder than the optimum temperature and will therefore prevent the bacteria from thriving in the conditions given.

The samples the graph in Figure 4.8 are based on were collected approximately every second day. When designing the graph the slope are adjusted to give an even line between each sample collected. The graph can therefore only be analysed as an estimate of the bacterial growth on the turf, and can only be used to indicate tendencies regarding the bacterial growth, not confirm them. From the 12th to the 19th of March it appears that the bacterial growth decreases. As stated the samples are collected

approximately every second day. The dates between the 15th and the 21st are closer documented in Table 4.2 to highlight the increase in visitors after disinfection, yet a decrease in bacteria count. On the 12th it was about 150 visitors on the turf and it can observe that the graph and the samples collected on the 13th are affected by the amount of visitors. The following days, 13th and 14th, the amount of visitors are lower which affect the amount of bacteria collected on the 15th by decreasing the bacteria amount. The amount of visitors on the 15th and 16th are approximately the same as the two previously dates, this causes a similar bacteria count on the 17th as on the 15th. On the 18th disinfection was conducted, and on the 19th samples were collected and the bacteria count was low. The amount of visitors were low on the two previous days and the bacterial count would decrease nonetheless. These results indicates that bacterial growth on the turf correlates to the activity levels on the turf. In addition, since samples were collected every second day the graph does not show the days between testing, and only creates a slope connecting the sample dates. The group can therefore not say what the bacteria count is in between the sample dates. However, by observing visitor numbers there is reason to assume the bacteria count would stay up. The Figures A2.1 and A2.2 in Appendix A2 show the complete overview of the plates from the 17th and 19th of March and illustrates the shift in growth after disinfection.

In Figure 4.8 the effect of the disinfectant can be exhibited after the disinfection on the 18th of March. As explained and showcased in the previous paragraph the bacteria count correlates to the activity levels on the turf. By comparing samples collected on the 12th of March and the 19th of March, with the same amount of visitors, the bacterial amount collected on the 19th of March is lower than that on the 12th of March. There is a slight increase in bacterial growth from the 19th of March to the 21st of March. This indicates that the disinfectant has a positive effect on the inhibition of bacteria. Continuously high amounts of visitors in further dates accompanied by low bacterial counts again show the effect of the disinfectant.

4.2 Results from the locker room at Flatåshallen and Kalvskinnet campus

4.2.1 The locker room at Flatåshallen

Samples from the locker room were collected on the 12th of April, from four different areas. These four areas were in the middle of the longest bench, the door handle of the locker room entrance, the button on the middle shower and the middle of the floor. The areas of sampling were 10x10cm, 2x5cm, 1x2cm and 10x10cm, respectively. Figure 4.9 shows the calculated CFU/cm^2 with standard deviation and an average from the turf. Table 4.3 shows the same data as the Figure, with precise numbers. The results are used for comparing CFU values to the CFU values found on the turf, and are further commented in Section 4.2.3.

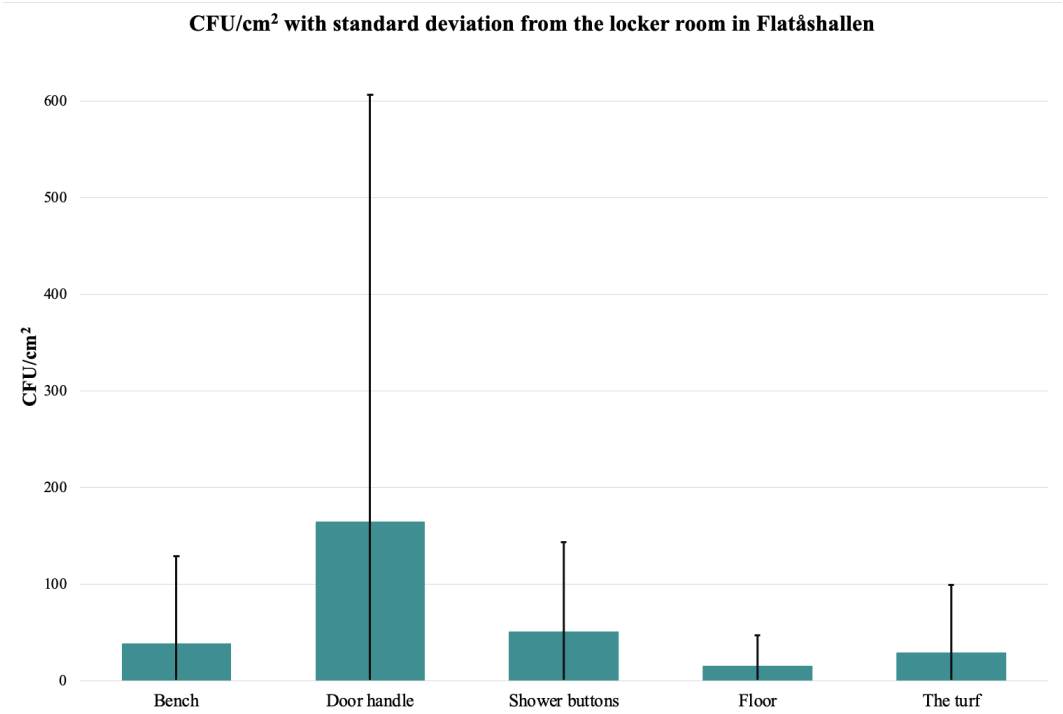

Figure 4.9: This chart shows the calculated CFU/cm^2 from four different areas in the locker room at Flatåshallen with standard deviation as black lines. An average from the turf is also included for comparison.

Table 4.3: This table shows the results from samples collected in the locker room at Flatåshallen. The first column is the sample area, second column is the average counted number of bacteria colonies, the third is the CFU/cm^2, and the last column is the associated standard deviation.

Sample area	Bacteria growth	CFU/cm^2	Standard deviation
Bench	289	39	91
Door handle	123	165	442
Shower button	8	50	93
Floor	111	15	32

4.2.2 Kalvskinnet campus

Samples from Kalvskinnet campus were collected on the 12th of April, from four different areas. These four areas were the door handle on the inside of the men's toilet on the second floor, the floor of the laboratory on the third floor, the buttons on the water dispenser in the cafeteria and the buttons to the elevator on the first floor. The areas of sampling were 2x5cm, 10x10cm, 1x2cm and 5x5cm, respectively. Figure 4.10 shows the calculated CFU/cm^2 with standard deviation and an average from the turf. Table 4.4 shows the same data as the Figure, with precise numbers. The results are used for comparing CFU values to the CFU values found on the turf, and are further commented in Section 4.2.3.

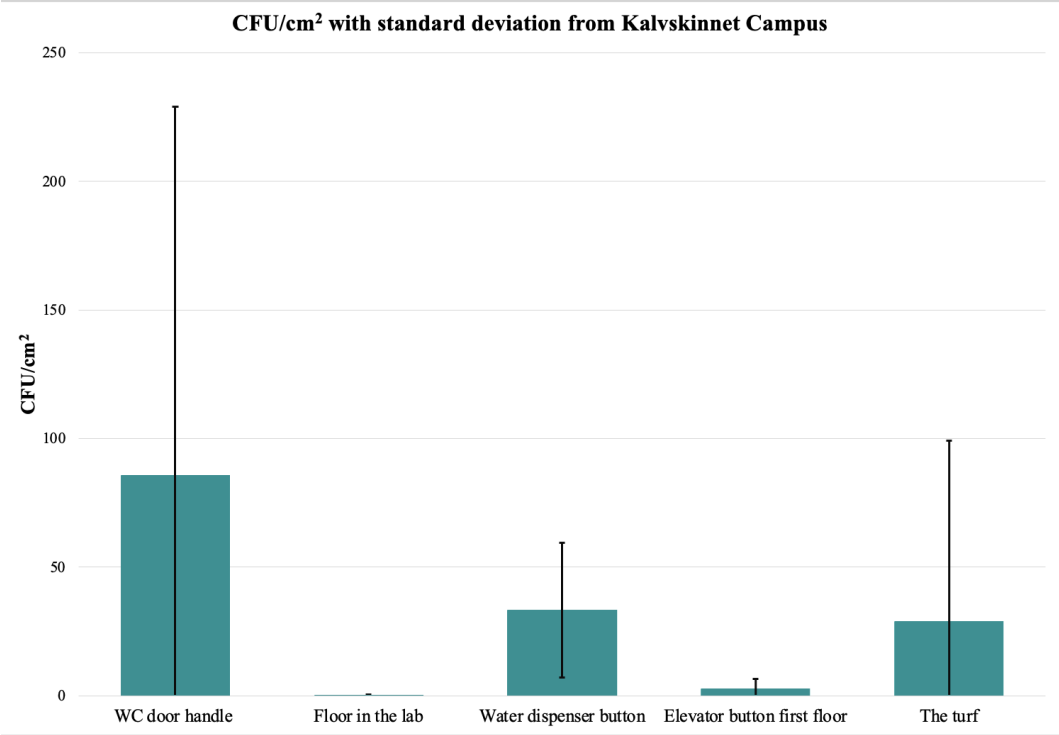

Figure 4.10: This chart shows the calculated CFU/cm^2 from four different areas at Kalvskinnet campus with standard deviation as black lines. An average from the turf is also included for comparison.

Table 4.4: This table shows the results from samples collected at Kalvskinnet campus. The first column is the sample area, second column is the average counted number of bacteria colonies, the third is the CFU/cm^2, and the last column is the associated standard deviation.

Sample area	Bacteria growth	CFU/cm^2	Standard deviation
WC door handle	64	86	143
Floor in the lab	2	0,3	0,2
Water dispenser buttons	5	33	26
Elevator button first floor	5	3	4

4.2.3 Comparison of results from the turf, the locker room and Kalvskinnet campus

A CFU count between 25 and 250 or 30 and 300 is a typical counting range, numbers above or below this are considered a source of error [56, 59]. Most of the data gathered are between the stated range and are evaluated as appropriate for the analysis. Previous studies conducted on the cleanliness of sterile hospital environments stated that 2.5 CFU/cm^2 was the limit for practice [61]. This statement is conflicting with the typical counting range, however the studies are focused on sterile environments in hospitals. These are environments demanding little to no bacteria due to infection risk to their patients. Due to the strict demand in hospitals the CFU count range is lower than in general everyday environments [61]. When evaluating the CFU counts achieved on the turf, some of the counts are below the limit of 2.5 CFU/cm^2 which can be observed in Figure 4.8 and Table A3.1. However, some of the highest counts are close to 60 CFU/cm^2. Since the analysed surface is artificial turf where physical activity is preformed the limit for cleanliness is raised compared to sterile hospital environments. There is no fixed upper CFU count limit on unclean surfaces, however previous studies have suggested that 40 CFU/cm^2 is unsanitary [60, 61]. Visitors on the turf are in closer contact with the surface than what users of the other facilities being sampled are. Taking these numbers and the amount of exposure to the surface into account it is evaluated a need for cleaning indoor artificial turf to maintain a lower CFU count than 40 CFU/cm^2. Comparing the results of the other environments with the results from the turf, as shown in Figures 4.9 and 4.10, it can be observed that the CFU count on the turf are quite average to other environments, which proves that the disinfection of the turf helps to maintain a cleanliness equal to other cleaned and disinfected environments used on a daily basis.

4.3 Method validation

4.3.1 Sample collection

4.3.1.1 Quantity of samples collected

Microorganisms are unpredictable and can behave different depending on their environment [38]. To ensure a good analytic standard several samples and parallels must be collected to account for the unpredictability of microorganisms. When initial sample collection started the group acknowledged the necessity for several parallels from each sample area. This is due to uncertainty when collecting samples [38, 62]. The swab is not able to cover the whole area of the 10x10cm frame, therefore different parallels could potentially collect different microorganisms. Taking one sample from each area will therefore not give thorough enough results.

When developing a solid method for sample collection and inoculation, the group found that the variation of colony count from each parallel from each sample area were extensive. It was therefore necessary to inoculate three individual samples from each parallel from each of the four areas on the turf. Figure 4.2 in the results indicates the varying colony count in each sample collected from parallels in area two on the turf. The figure demonstrates reasoning for our demand for increased amount of samples from each parallel.

4.3.1.2 Amount of activity during sample collecting

Bacteria survives on the turf when given continuous access to biological material [38]. When collecting samples after high activity levels, more bacteria will be collected due to it being more viable bacteria on the turf surviving on biological material from visitors than with minor activity levels on the turf. There is a difference between collecting viable bacteria surviving on biological material than collecting the biological material directly from the visitors. Results will show higher levels of bacterial growth on the Petri dishes if biological material from visitors are collected, rather than if only bacteria surviving on the biological material are collected. This occurs when samples are collected during activities such as football tournaments and kindergarteners play around the sample taker.

4.3.1.3 Limitations of sample collecting

The sample collecting was preformed by two different individuals and the areas each individual collected samples from varied. This can cause different execution by each individual applying different pressure on the turf when collecting the samples. When applying more pressure more microorganisms are collected. The turf is also uneven, potentially leading to different amount of bacteria collected for each sample.

The samples were collected only on the surface of the turf. There were no collection of microorganisms deeper down in the turf. Therefore, the results given only indicates the bacteria on the surface of the turf. The way the fibers of the turf are located compared to the motion of the swab can also affect how deep down in the turf it goes. These aspects affect the amount of bacteria collected.

It has been used two different types of swabs when collecting samples on the turf and other environments. The first type had 10 mL of broth in its container. The second swab had 1 mL of broth. The use of different swabs may cause a varying execution. However, with the correct calculations the change in swabs is close to insignificant.

The samples were collected on the field-half nearest the entrance. The other field-half was not tested. The field-half nearest to the entrance is more in use than the opposing half, and has in general more visitors walking on the turf due to the location of the entrance. This proposes that the field-half nearest the entrance will collect more biological material from visitors. Therefore, the samples being collected on this field-half will contain more bacteria than on the other field-half. However, this does not propose an error in the thesis. This thesis wants to analyse the effectiveness of the disinfectant, and sampling the areas with the most bacteria serves as the superior test of the disinfectant. It shows the largest bacteria amount the disinfectant can inhibit, and gives the most accurate indication of the effectiveness of the disinfectant.

4.3.1.4 Sample collection from the locker room and Kalvskinnet campus

Three parallels were taken when collecting samples from the locker room in Flatåshallen and on different locations around Kalvskinnet campus. These samples were meant to be used as comparisons for the samples collected on the turf. These places have a high traffic of people and are cleaned on a regular basis same as the turf. To achieve a higher quality comparison it should have been collected more samples from each location. In addition, the samples were collected in the middle of the day without any information of when it was last cleaned. The group had no information of the cleaning schedule and therefore the microbiological material on the sample areas could potentially be at their highest, lowest or at an average at the time of the sample collection. For that reason, the samples are an indication of the microbiological material in and around the locker room and the school environments, and are only used as comparison to the turf samples. The samples collected from these locations should therefore not be used as results by themselves since they do not give an adequate enough insight of the sample locations.

4.3.2 Laboratory work

4.3.2.1 Sterile environment during laboratory work

An important aspect of working with microbiology is keeping a sterile environment, as well as using aseptic techniques. This is to keep the samples free of contamination from outside environments [41]. While pouring the agar into the Petri dishes they were openly placed on sterile counter tops and left to solidify for 20 minutes after. Afterwards the plates were stored in a cooler. These activities could potentially have caused contamination of the Petri dishes. However, when the plates were used they were examined for possible contamination and disposed of in cases of contamination.

Occasionally while plating there were several students walking past the work area, this might cause contamination of the samples. The possibility of contamination from students is minimal, however it is worth mentioning as a possible source of error. Further on, the Petri dishes were always kept closed after inoculating the samples and the tubes containing the sample were closed after every use. A flameboy was also used on the spatula between every spread to maintain the sterile environment [41]. There is a

possibility contamination occurred even though every precaution were followed, therefore it is mentioned as a potential source of error.

4.3.2.2 Reasoning for sample collecting method and plating method

There are several different types of sample collecting methods, as well as plating methods. The sample collecting method chosen was with a swab. The wipe-rinse method and the press plate method were considered, however when collecting on an uneven surface using a cloth or a Rodac plate are not optimal solutions [48, 50]. With a swab, the collector are able to collect in between the fibers with more ease than with a cloth [50]. The swabs were used on all sample collections to compare the samples without any inconsistencies. A limitation of the swab is that it collects less microbiological material than its competitors. On a 10x10cm area the swab will not be able to cover the whole area. As documented in other studies, use of swabs are optimal in small areas, such as buttons and door handles [48]. In conclusion, using swabs as a sample collecting method has advantages and disadvantages, however compared to other sample collecting methods this was the most optimal for indoor artificial turf.

The spread-plate method was chosen due to its time saving aspect, and the method being widely used to quantify bacteria in a sample [53]. During the thesis approximately a thousand samples needed inoculating, to do this in the most effective and time saving manner the spread-plate method was chosen. The method is quicker to preform than the pour-plate method. The pour plate method is optimal for more sensitive samples, which are samples with a lower bacterial count. In addition, with the pour plate method the bacteria becomes embedded to the agar and can not be further analysed [53]. Since the spread plate method is less time consuming and is used to quantify bacteria in a sample, this method was the optimal method for the thesis.

4.3.2.3 Reasoning for chosen incubation time and temperature

Different incubation times were tested. After 48 hours the plates were often overgrown or had a high number of colonies, therefore it was decided that the plates were to be counted after 24 hours and 48 hours of incubation. The plates were incubated at 36 °C, which is the approximate body temperature of human beings [38]. The temperature was chosen to

see what the bacteria existing on the turf could potentially survive and how much harm they could cause if exposed to humans. In addition, 36 °C is the optimum temperature for most bacteria [38]. Therefore, when assessing quantity of all organisms found on the turf this temperature is favorable. A previous thesis have documented indications of *S.aureus* on the turf [32]. This is the indicator organism that is a risk for visitors for the surface analysed. The optimum temperature for *S.aureus* is 37 °C, therefore incubating the samples at 36 °C is appropriate when analysing the possibility of *S.aureus* on the turf.

4.4 Recommendation for further research

The recommendations for further research proposed are a continuation of the work and methods already developed in this thesis. It is recommended to redo the method with a longer sample period to achieve a clear result without holidays and unequal amount of disinfectant disturbing the results. To clearly see the disinfectants effect the exact same amount of intervals between the disinfection should be conducted. This is to achieve an equal evaluation every week of sampling.

Previous theses have covered microbiological growth on indoor artificial turf and the types of organisms found on the turf. One thesis found indications of bacteria on the turf and in this thesis it is assumed that their results were adequate [33]. However, further analysis with a high quantity of samples is needed to correctly assume bacteria growth on the turf.

Lastly, it is recommended that the effect of the disinfectant is tested. Considering the hazardous level of the chemicals used in the disinfectant it is important to evaluate the long term effect it could have on the turf and the visitors using the turf. The potential organic build up over time should be further researched. During this testing it could potentially be interesting to view the effect the disinfectant has on outside artificial turf as well.

5 Conclusion

To conclude, this thesis conducted numerous microbiological sample collections to evaluate the efficiency of the disinfectant used on the indoor artificial turf at Flatåshallen. During the sample period there was significant microbiological presence on the turf. The highest bacterial growth found was 67 CFU/cm^2 and the lowest was 1 CFU/cm^2. By comparing the results from the turf with results from other environments around Kalvskinnet campus and a locker room at Flatåshallen, as well as other previous studies conducted at hospitals, it has been evaluated a need for disinfection when there are high activity levels on the indoor artificial turf. This indicates a correlation between activity and bacterial growth, however to get a more unambiguous result testing needs to be conducted over a longer time period. Based on our research a disinfection schedule should follow the activity levels on the turf. Due to the difference in applied amount of disinfectant the most suitable interval is unclear.

There are minor uncertainties concerning the sampling method, plating method and counting method of the thesis. The uncertainties are concerning whether there are better methods to utilize for this analysis compared to the ones at hand, the limitations of the methods chosen are minor and therefore considered insignificant. The results achieved are indications of bacterial growth on the artificial turf, and should be evaluated as indications not definite results.

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Appendix

A1 The bacteria count from the four sample areas on the turf

Table A1.1: Bacteria colonies counted on agar plates from sample area 1 throughout the experiment. The Checked column represents the days after inoculation the bacteria were counted. The identification number at the top represents sample area, swab number and agar plate number, respectively.

Sample date	Checked	Number of bacteria colonies								
		1,1,1	1,1,2	1,1,3	1,2,1	1,2,2	1,2,3	1,3,1	1,3,2	1,3,3
21-Feb	day 2	1	3	3	1	3	0	6	0	962
	day 4	1	3	6	2	3	0	7	0	962
23-Feb	day 2	2	4	6	11	7	5	2	1	8
	day 4	3	7	7	13	7	5	2	1	8
25-Feb	day 2	6	1	877	3	8	61	4	15	13
	day 4	7	1	877	5	8	66	11	24	21
02-Mar	day 2	37	11	13	546	18	24	124	95	110
	day 4	41	16	18	547	22	26	133	109	121
04-Mar	day 2	63	3262	2560	216	55	53	639	88	113
	day 4	74	3262	2560	234	79	76	647	92	120
06-Mar	day 2	19	33	46	2814	18	280	23	21	689
	day 4	28	40	54	2814	27	285	29	25	689
08-Mar	day 2	435	99	3500	2471	100	35	25	31	31
	day 4	438	114	3500	2471	100	44	33	35	33
10-Mar	day 2	42	1170	48	29	29	25	695	89	66
	day 4	42	1170	48	29	29	27	695	92	70
13-Mar	day 1	44	41	51	58	61	3784	28	37	38
	day 2	57	47	67	81	91	3784	55	66	69
15-Mar	day 1	37	56	38	35	69	138	86	71	69
	day 2	48	78	56	57	89	154	122	96	115
17-Mar	day 1	26	36	22	74	100	89	29	95	32
	day 2	37	49	36	95	114	104	37	97	40
19-Mar	day 1	34	13	17	5	2	1	2	0	2
	day 2	34	13	17	5	2	1	3	5	4
21-Mar	day 1	57	50	58	43	42	56	19	28	35
	day 2	66	62	71	50	57	69	30	49	41
23-Mar	day 1	27	25	23	33	33	169	21	60	40
	day 2	41	39	34	58	54	184	63	88	81
25-Mar	day 1	3	8	4	6	5	3	25	50	37
	day 2	10	17	7	27	19	24	32	59	39
27-Mar	day 1	3	3	4	7	5	7	11	3	5
	day 2	8	4	5	12	46	9	13	9	7
29-Mar	day 1	39	74	185	21	19	16	11	10	13
	day 2	51	83	1230	33	30	31	16	25	32
31-Mar	day 1	36	37	28	216	620	287	19	60	41
	day 2	61	46	43	224	665	305	24	67	63

Table A1.2: Bacteria colonies counted on agar plates from sample area 2 throughout the experiment. The Checked column represents the days after inoculation the bacteria were counted. The identification number at the top represents sample area, swab number and agar plate number, respectively.

Sample date	Checked	Number of bacteria colonies								
		2,1,1	2,1,2	2,1,3	2,2,1	2,2,2	2,2,3	2,3,1	2,3,2	2,3,3
21-Feb	day 2	1	1	1	1	3	1	0	0	0
	day 4	1	1	1	2	3	2	0	0	0
23-Feb	day 2	4	43	2	1	1	1	3	2	1
	day 4	7	46	3	1	1	1	4	2	1
25-Feb	day 2	2	1	11	2	5	4	8	30	1647
	day 4	2	1	12	4	5	4	10	31	1647
02-Mar	day 2	557	5	3	339	388	60	136	183	120
	day 4	557	5	4	342	401	79	204	250	217
04-Mar	day 2	113	125	103	63	65	102	66	1793	637
	day 4	140	154	134	71	76	106	86	1795	655
06-Mar	day 2	167	67	93	40	435	24	101	49	115
	day 4	176	77	94	44	435	31	105	55	123
08-Mar	day 2	3100	1170	47	7	8	23	43	51	60
	day 4	3100	1170	53	7	9	24	52	52	61
10-Mar	day 2	42	111	23	1537	37	55	2431	45	698
	day 4	49	114	24	1537	39	57	2431	45	699
13-Mar	day 1	55	40	52	32	70	6147	17	2938	10
	day 2	60	75	64	37	24	6147	28	2938	23
15-Mar	day 1	19	24	41	13	8	3530	16	31	51
	day 2	34	48	70	15	13	3530	72	80	114
17-Mar	day 1	30	45	26	37	52	60	119	421	184
	day 2	53	63	51	49	76	85	156	439	210
19-Mar	day 1	3	1	1	1	0	9	42	16	16
	day 2	3	2	2	1	1	9	56	19	17
21-Mar	day 1	34	48	43	24	32	37	63	65	67
	day 2	44	59	49	39	44	46	73	96	74
23-Mar	day 1	5	9	8	26	13	10	26	37	19
	day 2	9	15	9	155	23	25	32	49	29
25-Mar	day 1	8	7	10	18	29	22	3	6	3
	day 2	12	12	13	25	25	29	5	8	6
27-Mar	day 1	150	12	7	8	16	8	35	26	25
	day 2	161	20	8	10	19	9	47	29	28
29-Mar	day 1	88	69	91	47	38	42	88	42	44
	day 2	108	86	113	66	41	53	103	51	51
31-Mar	day 1	10	1053	24	29	21	24	43	56	50
	day 2	15	1053	31	31	29	41	68	75	75

Table A1.3: Bacteria colonies counted on agar plates from sample area 3 throughout the experiment. The Checked column represents the days after inoculation the bacteria were counted. The identification number at the top represents sample area, swab number and agar plate number, respectively.

Sample date	Checked	Number of bacteria colonies								
		3,1,1	3,1,2	3,1,3	3,2,1	3,2,2	3,2,3	3,3,1	3,3,2	3,3,3
21-Feb	day 2	1626	0	1	0	326	0	0	0	1
	day 4	1626	0	1	0	326	0	0	0	1
23-Feb	day 2	2	0	428	0	4	6	45	2830	2
	day 4	2	1	428	0	4	7	45	2830	2
25-Feb	day 2	43	0	167	3	7	1	27	0	1572
	day 4	43	0	167	4	8	3	27	1	1572
02-Mar	day 2	5000	3	1442	184	903	254	78	77	71
	day 4	5000	5	1442	192	904	256	82	83	75
04-Mar	day 2	27	22	1120	22	20	6	19	19	18
	day 4	27	22	1120	22	20	6	22	22	21
06-Mar	day 2	37	39	2642	47	72	52	70	107	127
	day 4	39	46	2642	56	81	74	71	112	127
08-Mar	day 2	409	8	13	6	16	6	527	26	4
	day 4	409	8	17	8	17	9	527	32	6
10-Mar	day 2	404	61	2295	3000	8	6	15	12	10
	day 4	407	66	2295	3000	8	10	18	12	10
13-Mar	day 1	8	26	21	51	154	58	39	34	11
	day 2	15	41	32	87	187	83	45	42	16
15-Mar	day 1	32	21	46	42	27	30	34	25	26
	day 2	51	45	65	50	33	35	52	36	35
17-Mar	day 1	486	19	21	15	1751	17	0	3	7
	day 2	498	25	30	23	1753	33	6	8	11
19-Mar	day 1	6	16	7	4	8	10	9	12	11
	day 2	8	19	18	5	8	13	12	13	12
21-Mar	day 1	13	14	11	43	36	13	7	4	67
	day 2	18	20	13	48	45	17	10	4	80
23-Mar	day 1	65	34	19	94	16	14	14	25	15
	day 2	97	49	33	120	20	22	20	28	19
25-Mar	day 1	11	8	1	6	6	13	5	3	4
	day 2	16	16	13	18	20	34	34	8	14
27-Mar	day 1	1	4	1	16	7	37	12	10	17
	day 2	2	6	3	18	13	56	15	14	21
29-Mar	day 1	94	84	90	49	45	40	43	34	30
	day 2	135	112	120	64	55	54	49	58	51
31-Mar	day 1	23	22	22	32	21	24	6	10	28
	day 2	30	28	30	48	29	36	12	19	37

Table A1.4: Bacteria colonies counted on agar plates from sample area 4 throughout the experiment. The Checked column represents the days after inoculation the bacteria were counted. The identification number at the top represents sample area, swab number and agar plate number, respectively.

Sample date	Checked	Number of bacteria colonies								
		4,1,1	4,1,2	4,1,3	4,2,1	4,2,2	4,2,3	4,3,1	4,3,2	4,3,3
21-Feb	day 2	1	1	2	0	1	70	0	2610	0
	day 4	1	2	2	0	1	70	0	2610	1
23-Feb	day 2	31	52	36	1	5	3	2364	12	17
	day 4	32	52	38	1	6	3	2364	13	18
25-Feb	day 2	5	5	1177	18	4395	19	2	192	127
	day 4	6	6	1177	19	4395	19	2	192	127
02-Mar	day 2	424	313	9	325	348	501	62	67	1330
	day 4	425	313	9	386	408	524	69	76	1330
04-Mar	day 2	31	38	29	64	68	84	61	47	59
	day 4	32	38	31	66	71	84	68	53	62
06-Mar	day 2	80	81	5431	909	87	754	57	1407	1180
	day 4	93	93	5433	915	103	766	68	1407	1180
08-Mar	day 2	40	44	4500	186	986	35	15	13	22
	day 4	41	53	4500	186	986	36	16	20	23
10-Mar	day 2	13	64	15	23	566	19	2952	27	142
	day 4	14	64	15	23	568	20	2952	29	144
13-Mar	day 1	50	48	32	7	37	15	70	78	87
	day 2	59	57	42	9	65	24	125	127	132
15-Mar	day 1	31	15	57	34	25	34	46	55	46
	day 2	40	23	70	40	34	40	145	75	62
17-Mar	day 1	155	138	186	24	19	27	16	97	49
	day 2	164	145	203	47	36	37	31	118	70
19-Mar	day 1	20	12	4	4	2	4	8	3	8
	day 2	24	15	10	6	4	7	10	3	9
21-Mar	day 1	385	149	153	22	46	26	515	28	35
	day 2	533	214	199	30	62	33	515	36	47
23-Mar	day 1	31	38	32	11	13	23	58	45	31
	day 2	83	77	71	34	30	39	84	80	51
25-Mar	day 1	2	0	1	3	1	2	12	9	13
	day 2	3	0	1	11	3	6	28	24	39
27-Mar	day 1	13	40	18	5	19	6	21	29	22
	day 2	18	45	24	5	24	11	34	46	47
29-Mar	day 1	58	67	1820	7	25	11	17	22	28
	day 2	71	74	1820	7	36	11	21	25	31
31-Mar	day 1	19	83	22	21	21	23	16	19	8
	day 2	37	102	32	25	34	31	21	25	13

A2 All incubated agarplates from the 17th and 19th of March

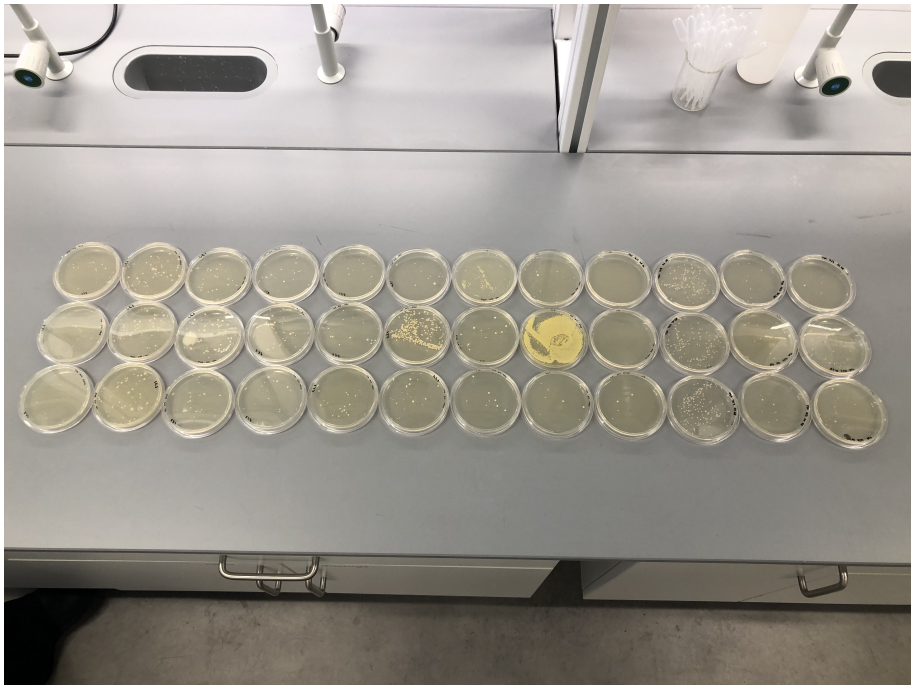


Figure A2.1: This picture shows the complete set of agar plates from the 17th of March, the day before disinfection with 80 liters of disinfectant.

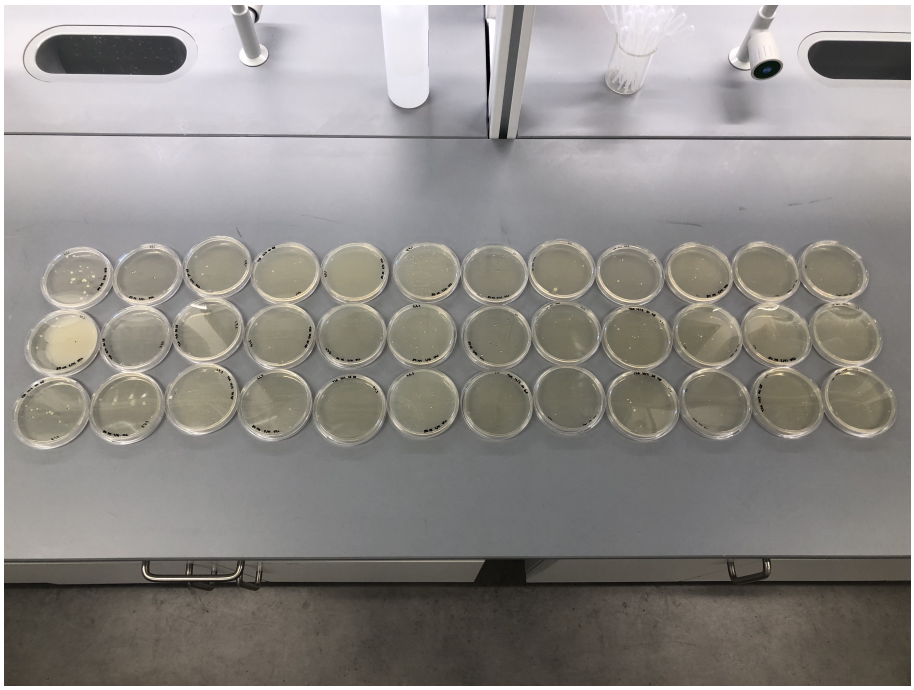
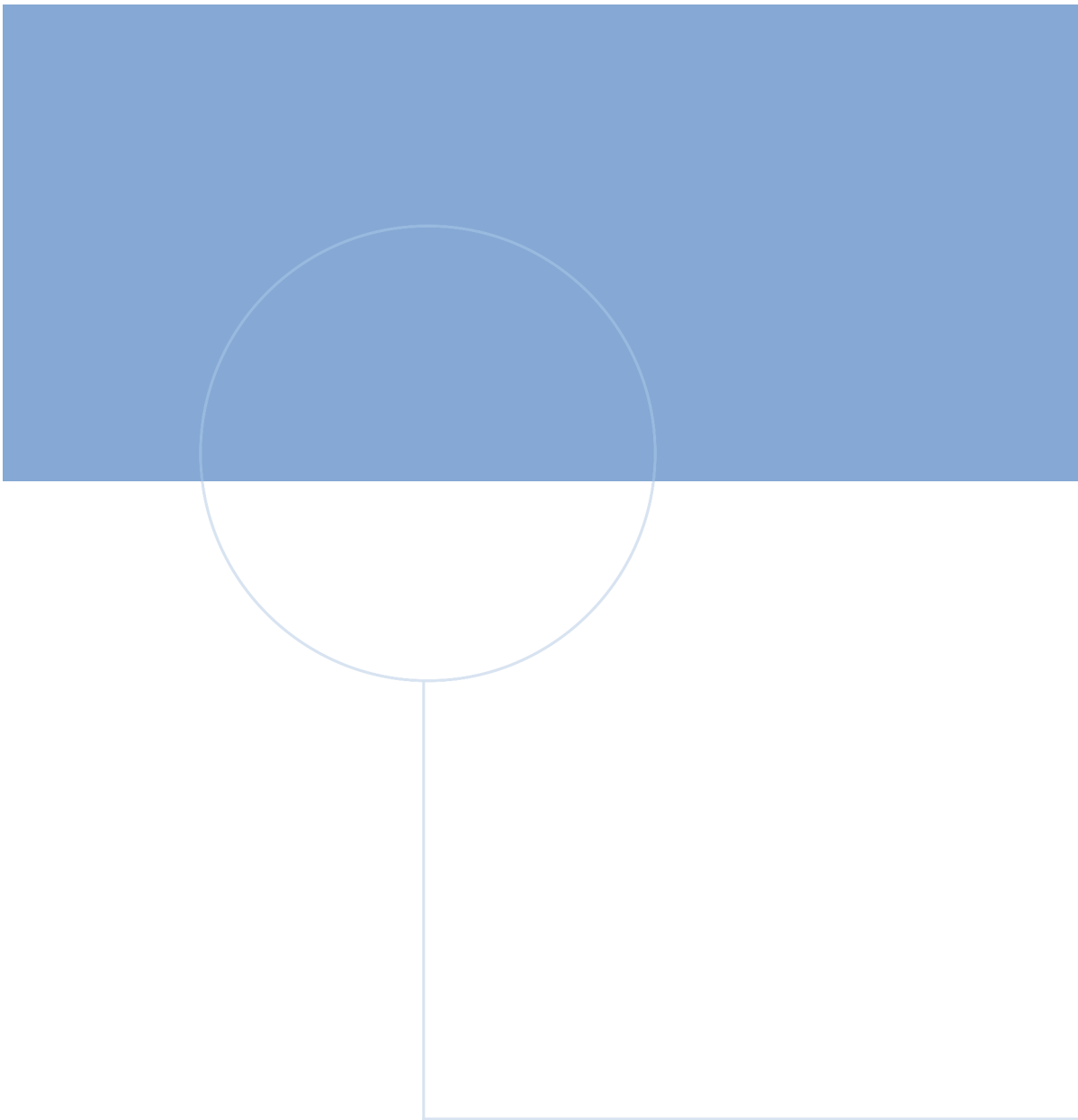


Figure A2.2: This picture shows the complete set of agar plates from the 19th of March, the day after disinfection with 80 liters of disinfectant.

A3 Complete table of registered people and CFU count

Table A3.1: This table shows the data used to make Figure 4.8. The highlighted dates represent disinfection dates, and dashes mean no samples were collected on these dates.

Dates	Registered people	CFU/cm^2
20-Feb	30,5	-
21-Feb	46,0	21
22-Feb	63,0	-
23-Feb	44,0	22
24-Feb	34,5	-
25-Feb	0,0	39
26-Feb	0,5	-
27-Feb	81,5	-
28-Feb	70,5	-
01-Mar	99,0	-
02-Mar	76,5	52
03-Mar	62,0	-
04-Mar	197,5	44
05-Mar	150,0	-
06-Mar	143,0	67
07-Mar	66,5	-
08-Mar	98,5	67
09-Mar	78,0	-
10-Mar	76,5	62
11-Mar	97,0	-
12-Mar	147,5	-
13-Mar	86,5	55
14-Mar	52,0	-
15-Mar	91,5	21
16-Mar	71,0	-
17-Mar	39,0	19
18-Mar	21,0	-
19-Mar	139,5	1
20-Mar	67,5	-
21-Mar	93,5	11
22-Mar	114,0	-
23-Mar	89,5	7
24-Mar	52,5	-
25-Mar	51,0	2
26-Mar	126,5	-
27-Mar	78,5	3
28-Mar	57,0	-
29-Mar	81,5	19
30-Mar	68,5	-
31-Mar	33,0	13



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