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EVALUATION OF NEW METHODS TO DETERMINE ANTIMICROBIAL EFFICIENCY
Exam work at Medibiome AB as part of Vinnova National Project “Innovation mot infektion” focusing on the evaluation of new methods to determine antimicrobial efficiency of disinfectants and cleaning agents

Master’s thesis in Biotechnology

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SUMMARY

The work for this master's thesis, which was performed at Medibiome, was a part of a Vinnova-funded national project “Innovation mot Infektion” for the establishment and evaluation of a draft standard FprEN 16615 in order to evaluate different disinfection and analytical methods. Healthcare associated infections are a global issue and the need for new methods to reduce contamination and evaluate efficiency of disinfection is increasingly recognized in scientific, clinical and political communities.

In order to evaluate the antibacterial efficiency of cleaning and disinfecting products, test floorings were inoculated with bacterial suspensions and an interfering substance, and wiped with a product. A unitary weight was used to standardize the wiping. The bacteria load on the surface was swabbed after wiping and the remaining bacterial load was evaluated after plate cultivation.The ATP bioluminescence analysis was used during a similar procedure to replace the plate cultivation method. The enzyme luciferase provided light emission while degrading the ATP contained into bacteria. The light emission is measured to determine the bacterial load on the surface. The compatibility of wiping materials and cleaning agents was also tested with a test suspension of *S. aureus*. The recovery of *S. aureus* and *P. aeruginosa* after different drying times on surfaces was studied.

The results show that three disinfectants meet the requirements of the standard FprEN 1661: DES45, Rely+On™ Virkon® and Wet Wipe. Input was transmitted to the standard committee, especially regarding the need to sterilize the wipes, to standardize and improve the release of bacteria from the swab, and to decrease the weight of the granite block for more clinical relevance. ATP analysis could be performed without background by using an interfering substance modified with Triton X-100 at 0.2%. It seems to be a promising technique, as it is both fast and cost-efficient but further study needs to be done in order to solve technical issues and standardize the method.

Key words: Healthcare associated infection, disinfection, cleaning, wiping, bacteria, ATP bioluminescence analysis

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PART I

This Master's Thesis Project is a 30 credits exam work performed at Medibiome AB as a part of a Vinnova-funded national project called: “Innovation mot Infektion”. Its goal was the establishment and evaluation of a draft standard FprEN 16615 in order to evaluate different disinfection methods and analytical methods. Healthcare associated infections (HCAIs) are a global issue and the need for new methods to reduce contamination and evaluate efficiency of disinfection is increasingly recognized in the scientific, clinical and political communities.
This exam-work involved partners from industry, institute, and university hospitals. The Karolinska Institut, Gipeco AB, BioThema AB, SP Technical Research Institute of Sweden, and Medibiome AB were the main actors. The overall goals were to find new concepts to reduce the risk of healthcare associated infections.
The main aspects were to set up a novel method to evaluate different disinfectants used at hospitals as part of an international work within CEN, the European Committee for Standardization, and to participate at finalizing the first draft of the Standard FprEN 16615. The evaluation of different disinfectants and cleaning products used in hospitals would provide input on the current efficiency of cleaning and on the necessary improvements to reach a higher efficiency in term of bacterial reduction, especially for the partner on the Vinnova project. Another goal was to determine the clinical relevance of the promising ATP luminescent method run by BioThema®. The survival of bacteria on surfaces and the potential neutralizing effect of wiping materials on chemical compounds have also been investigated to complete the study.

In order to investigate the effect of disinfection, a reliable method is required. A standard FprEN 16615 is under development and run by a working group of CEN in a laboratory in Bonn (Germany), as well as by several voluntary laboratories in Europe. The method is a procedure to analyze the efficacy of disinfectant after wiping. A test flooring is inoculated with a bacterial suspension and wiped with a disinfectant. The amount of surviving bacteria on the surface is sampled after wiping and the remaining bacteria are cultivated. The initial concentration of bacteria on the surface is therefore defined after cultivation.

This method was set up and evaluated during this master's thesis and improvements were suggested. An extended practice of this procedure was necessary in order to understand the practical and financial aspects, and to analyze the clinical relevance. Indeed, the main objectives were to define a reliable method that gives clinically relevant information on disinfectant efficacies. Different disinfectants and cleaning products from industrial partners as Gipeco® have been analyzed, together with the required neutralizers. The different aspects of the standards were questioned and evaluated, i.e. the detection method, the test parameters, the choice of microorganisms. It is also important that the future method remains time and cost effective, and allows for innovation.

According to the draft standard FprEN 16615, the analysis of microorganisms occurs with plate cultivation. The concentration of the remaining microorganism on the surface is determined by sampling of the surface and cultivation. It is the most common method; however, whether or not it really reflects the actual amount of bacteria is questioned. Therefore, this project gave initial input in the evaluation of BioThema's ATP method as a method of analysis. The principle of the BioThema®´s ATP method is to measure intracellular ATP in living bacteria by bioluminescence. The free ATP and the ATP inside mammalian cells is degraded, so the measurement will only consider bacterial ATP. The enzyme luciferase provides light emission while degrading the ATP and a luminometer can measure this light emission. The intensity of the light is proportional to the amount of ATP. A complete understanding of this method is required, so the project includes a visit to BioThema® (Stockholm) and a close partnership with the company.

This project was challenging due to both the incidence of healthcare-associated infection and the international aspects of the European Standard. The balance between literature-based research, experimental work, and critical reasoning was essential for the professional value of this work, as was the favorable work environment provided by Medibiome.

PART II: BACKGROUND

A. Healthcare associated infections

Healthcare associated infections (HCAIs) are infections occurring 48 hours after the admission of a patient to a healthcare facility that were not present or incubating at the time of admission [Kelly 2012, WHO 2009]. HCAIs increase medical costs, length of stay, complication rates, morbidity and mortality [Kelly 2012, Schmidt 2014]. Every day, up to about 10% of hospitalized patients contracts a healthcare associated infection according to the European Centre for Disease Prevention and Control and the World Health Organization [ECDC 2013, WHO 2011]. Worldwide, HCAIs are a frequent flaw in healthcare and approximately 30% of patients in intensive care units in developed countries are infected by them, with an estimated cost in Europe of 7 billions Euros, according to the World Health Organization and the ECDC [ECDC 2013, WHO 2011]. Studies peformed in USA specify that central line-associated bloodstream infections and ventilator-associated pneumonia have the highest rate of deaths, more than 12% [Al-Tawfiq 2014]. The prevalence of HCAIs can be even raised due to the necessity to reduce waiting lists and increase in proximity of patients, both of which increase risk of transmission [Dancer 2008].
Moreover, the emergence of resistance to antibiotic treatments threatens the effective prevention and treatment of HCAIs [Otter 2014, WHO 2014]. The misuse of antimicrobial drugs and inefficient infection control practices can facilitate spreading of antibiotic resistance [WHO 2011]. Indeed, antibiotic exposures favorise the emergence and selection of antibiotic resistance mutations and their expansion when a microorganism survives the drug used [Uhlemann 2014, WHO 2014]. This leaves healthcare workers in high need of alternative solutions.

B. Focus on three bacteria of interest

Different microorganisms are responsible for infection, and some of them are found in the normal flora of the human population. Prions, viruses, bacteria, fungi, protozoa and helminthes can all lead to human infection. Bacteria are one of the main issues in healthcare due to their great ability to survive on surfaces, their potential to divide at a very high rate in good conditions and the formation of biofilm protecting bacteria colonies [McDonnell 2012]. Wonders have been brought on the inoculum size required to initiate a bacterial infection. Some studies show that bacterial load with a value from 1 to 7 log cfu (colony-forming units) have the potential to infect patients [Dancer 2008].

A large survey conducted in 2013 by the European Centre for Disease Prevention and Control [ECDC 2013] revealed that the ten most commonly isolated microorganisms in HCAIs in Europe were *Escherichia coli* (15.9%), *Staphylococcus aureus* (12.3%), *Enterococcus species* (9.6%), *Pseudomonas aeruginosa* (8.9%) *Klebsiella species* (8.7%), *coagulase-negative staphylococci* (7.5%), *Candida* species (6.1%), *Clostridium difficilis* (5.4%), *Enterobacter species* (4.2%), *Proteus species* (3.8%) and *Acinetobacter* species (3.6%).
Three of the four most common pathogenic bacteria are described in the following paragraphs: *Staphylococcus aureus*, *Enterococcus* spp. and *Pseudomonas aeruginosa*.

- *Staphylococcus aureus* is a gram-positive cocci, a component of the normal flora of about 30% of the population and can be transmitted by colonized asymptomatic individuals [Kelly 2012, Wertheim 2005]. However, it can cause infection when present in a wound, surgical site infections or urinary tracts [Dancer 2008]. It can cause skin infections, impetigo, toxic shock syndrome, pneumonia, endocarditis, gastroenteritis, bacteremia and it is also especially troublesome on immunocompromised or diabetic patients [Kelly 2012, McDonnell 2012, Wertheim 2005]. The methicillin-resistant *S. aureus* (MRSA) is called a ‘’ super bug’’ due to its resistance to many

common antibiotics makes it difficult to treat [McDonnell 2012, Norton 2014, Uhlemann 2014]. This resistance seems to spread outside of the healthcare system in community households that serves as a pool for community-associated MRSA (CA-MRSA) diversification, transmission, and infection [Uhlemann 2014]. *S. aureus* is supposed to survive from one week to one year on dry surfaces in hospitals [Kramer 2006] and can be transmitted from contact between individuals, equipment, and various environmental surfaces, such as ventilation grills or furniture in patient rooms [Boyce 2007, Dancer 2008, Schultsz 2003]. Healthcare workers struggle with different types of resistance; an outbreak of mecA-negative borderline resistant *S. aureus* BORSA in a Department of Dermatology in Denmark caused increase in severe skin diseases and hospitalization time [Balslev 2005].

- *Enterococcus* spp. is a gram-positive bacteria group, found as part of the normal flora in the intestine, common in food and environment [Da Silva 2012, McDonnell 2012] and supposed to survive for 5 days to almost 4 years [Kramer 2006]. However, they can be responsible for a wide range of infections, i.e. in wounds, on skin, in the airways, and are responsible of hepatobiliary, intra-abdominal and urinary tract infections, meningitis, infective endocarditis and bacteremia [Kelly 2012, Savini 2013]. Between 2007 and 2011, three hospitals in separate counties in Sweden experienced a large outbreak of vancomycin-resistant enterococci (VRE) *E. faecium* with 760 reported cases of infection [Sivertsen 2014, Söderblom 2010]. Colonization can remain asymptomatic while the bacteria are present in the gastrointestinal tract or on the skin and be transmitted by contact between persons or to the environment [Kelly 2012]. Transmissions have been identified from different media, environmental surfaces and airborne agents [Kelly 2012, Muzslay 2013]. Moreover, drug-resistance starts to be considered a menace [Chan 2011, Savini 2013]. Even if *E. hirae* infections are not widely documented most likely because of misidentification, *E. hirae* has been reported to cause wound infections and gastritis, as well as a few episodes of bacteremia [Chan 2011, Talarmin 2011, Tan 2010].

- *Pseudomonas aeruginosa* are gram-negative bacteria present anywhere in the environment, with a predilection for water, but easily found on surfaces [McDonnell 2012, Moore 2011]. It is responsible for a wide variety of opportunistic infections in wounds, pneumonia and urinary tract infections. Some can be lethal, such as neonatal sepsis and chronic lung infections in patients with cystic fibrosis [McDonnell 2012, Moore 2011]. Water systems have been reported to contribute to *P. aeruginosa* transmission in healthcare settings [Loveday 2014]. A study conducted by the University of Chicago revealed that in 7 months, 29% of the patients in Intensive Care Unites were infected with *P. aeruginosa* due to tap water contamination [Trautmann 2001]. A major outbreak of a multi-resistant strain has been reported in a hospital in Japan due to a trans-oesophageal echocardiogram probe used in cardiovascular surgery [Seki 2013]. *P. aeruginosa* produce an extra-cellular matrix to form biofilms and virulence factors that help invasion of tissue and inhibit the immune response [Mann 2012, Moore 2011]. Its ability to form biofilms is a serious issue in healthcare and can be linked to its persistence and to numerous infections [Mann 2012, Moore 2011, Randall 2012]. Biofilms favor the resistance to drugs by protecting the bacterial cells and by facilitating the selection of genetic variants [Mann 2012].

C. HCAIs and Transmission of microorganisms

A large survey conducted in 2013 by the European Centre for Disease Prevention and Control in European hospitals shown that about 19.5% of patients in intensive care units (ICU) suffer from HCAIs [ECDC 2013]. The most common types of HCAIs in ICUs were infections of respiratory tracts, urinary tracts, and surgical sites.

The various ways in which pathogens are transmitted and cause infections are usually complicated and difficult to investigate [Otter 2014]. Studies report numerous different transmission routes, and all have to be taken into consideration to understand the phenomenon of HCAI.

- **Transmission from person to person**

First of all, the transmission between persons can contribute to the spread of microorganisms. The direct transmission between healthcare workers might contribute to the spread within a hospital and to the community [Seibert 2014]. This seems to be enhanced in the case of crowded healthcare center [Aiello 2003]. More conscientious hand-hygiene could limit contamination, but it is difficult in practice to ensure a constant hand cleaning along the day and for each healthcare worker [Pittet 2000, Kim 2003, Seibert 2014]. Healthcare workers may induce transmission of infection from one patient to another via contamination of skin, clothing, or equipment [Seibert 2014]. Detailed outbreak situations have been reported when transmission of staphylococcus occurred between healthcare workers, patients, and people at home [Dancer 2008, Seibert 2014, Blok 2003, Tansel 2003, Eveillard 2004, Calfee 2003]. Due to strong evidence for person-to-person transmission, especially for MRSA, the role of environment and the removal of contamination through cleaning and disinfection can seem a secondary problem [Dancer 2008].

However, studies show that in some cases, even a rise in hand hygiene, and extra care with human contacts are found not efficient enough to reduce infection [Seibert 2014, Siegel 2007, WHO 2009]. Some studies tend also to demonstrate that contact with the contaminated environment can be just as likely to transmit pathogens to the hands of healthcare workers as direct contact with the patient [Guerrero 2012, Stiefel 2011].

- **Environmental transmission**

An increased interest in environmental contamination led to a lot of published articles investigating the routes of transmission [Dancer 2014, Otter 2014, Weber 2013] but the sampling of microorganisms from environmental surfaces vary considerably [Obee 2007]. Theoretical studies that monitor the spread of artificial markers, animal models, and mathematical modeling can provide interesting insight into how surfaces can transmit contamination [Otter 2014]

Infection can be transmitted through contaminated water systems, as it has been reported for *P. aeruginosa* infection in healthcare centers, with contamination apparently centralized in the distal ends of a water system and able to persist for long time [Loveday 2014]. Evidence that MRSA contaminated ventilation grills and generated outbreaks in hospitals has also been presented [Boyce 2007].

Instances of transmissions have also been reported through clothing, bed linens, portable equipment e.g. hydrotherapy equipment or electronic thermometer [Donskey 2013, Sui 2013]. Studies report critical outbreaks of MRSA due to ultrasonic nebulizers [Schultsz 2003] and for multidrug-resistant *P. aeruginosa* due to echocardiogram probe used in cardiovascular surgery [Seki 2013]. In a recent study, seventy reusable surface disinfectant tissue dispensers have been randomly collected from healthcare facilities in Europe and investigated for bacterial contamination, revealing frequent and heavy contamination [Kampf 2014].

Bacteria are capable of surviving a very long time on surfaces, making the contaminated surfaces very good agents for propagation of infections [Exner 2004, Obee 2007]. It is proven than MRSA and VRE can survive for days to weeks on surfaces [Boyce 2007, Kramer 2006]. When sampling is done in healthcare facilities on surfaces near the patients, it is common to find different microorganisms considered responsible for HCAIs [Dancer 1999, Talon 1999, Obee 2007] and the surfaces frequently touched by healthcare workers are usually more contaminated in the rooms of patients infected [Boyce 2007]. According to the literature, the proportion of hospital surfaces contaminated with MRSA varied from 1% to 27% of surfaces in patient rooms [Boyce 2007].

- **Relation between transmission through surfaces and person-to-person transmission**

Healthcare workers can contaminate their hands or gloves by touching those surfaces, and then transmit to other surfaces or patients [Boyce 2007, Dancer 2009, Exner 2004]. Pathogens may also be transferred directly from contaminated surfaces to susceptible patients [Boyce 2007].

For instance, two healthcare workers developed infections caused by community-associated MRSA (CA-MRSA) strains from transmission suspected to come from environmental surfaces, and one of the infected healthcare workers did not have direct contact with patients [Johnston 2006].
Spreading of microorganisms between surfaces is even more difficult to avoid with hand cleaning, as it usually does not seem necessary to clean hands between contacts with two different surfaces if no patient contacts is implied [Seibert 2014, Siegel 2007].

D. Importance of cleaning and disinfection of surfaces

It is considered that microorganisms can spread easily from person to person, and that proper hand hygiene and acute care during patient contact could reduce infections [Seibert 2014, Siegel 2007] and cleaning has been considered efficient enough over long period of time [Donskey 2013]. It has been reported efficient to resolve cases of outbreak of VRE in healthcare facilities [Boyce 2007]. Previously, the lack of published studies assessing the positive effect of routine disinfection for reducing the number of HCAIs centralized the focus on other infection control methods [Exner 2004].

However, more cases report the efficiency of supplementary disinfection compared to routine cleaning for MRSA incidence [Obee 2007], outbreak of *P. aeruginosa* infections in hematology–oncology [Wagenvoort 2000], or outbreaks of *Acinetobacter* [Van Dessel 2002]. Cleaning is sometimes considered insufficient for avoiding the spread of microorganisms and contamination [McDonnell 2012]. Although it can significantly reduce the number of microorganisms, it does not always achieve a greater reduction [McDonnell 2012]. Several studies have also substituted everyday cleaning procedures with products that have acute antimicrobial efficiency in order to successfully block HCAIs [Donskey 2013]. An other study demonstrated that improved terminal cleaning by disinfection can reduce the risk of infection for the next patient [Passaretti 2013].

When it is considered that the routine cleaning of equipment and surfaces does not sufficiently reduce bacterial contamination, improvements in the methods of the healthcare facilities disinfection are needed [Boyce 2007]. The role of cleaners must be taken into serious consideration for the infection control process, and therefore training and an appropriate number of hours for cleaning have to be defined for each healthcare facility. From a financial point of view it is important to consider that investment in effective cleaning processes will reduce the cost of extra medication and extended bed stays [Dancer 2008].
From a drug resistance point of view, cleaning and disinfectant products can also play a dual role, as a concern about products containing, for instance, triclosan has been raised since these products may contribute to antibiotic resistance [Aiello 2003]. Quaternary ammonium compounds, also widely used as a disinfectant in hospitals, could favor antibiotic resistance [Buffet-Bataillon 2012].

E. Assessing proper cleaning or disinfection for surfaces

The Centers for Disease Control and Prevention list the main methods for assessing a proper cleaning as direct practice observation, swabs, agar slides, fluorescent markers, and ATP bioluminescence analysis [Guh 2010].

The common method to assess proper cleaning in hospital is by checking the appearance removal of dirt, even though it can be different to microbiological contamination [Dancer 2008, Obee 2007]. It seems difficult to devise a routine method to determine effectiveness of cleaning or disinfection, even if for training purposes invisible maker tests are sometimes used to reveal unwiped areas [Rutala 2013, Otter 2014]. Appropriate measurement would be necessary, as studies have demonstrated that cleaning is not always performed according to the guidelines [Rutala 2013] and as a low bacterial load on surface could be vector of contamination [Dancer 2009].

Different sampling methods exist to control the bacterial load on surfaces and can be used in healthcare facilities.
A first method is to sample and recover bacteria sampled from surfaces. This can be done using swabs, usually rod-swabs with cotton head. The problem reported with traditional swab methods for surface sampling is the relatively low yield. Studies have shown that improved results with a flocked nylon swab, and even higher recovery with the use of two swabs successively [Hedin 2010]. Contact plates or dip slides can also be used, when a gelified medium used for recovery is in direct contact with the surface [Obee 2007]. Although easy to use, this technique is not suitable for exact discrimination at high concentration of bacteria [Kircheis 2007, Hedin 2010]. The choice of medium for sampling can influence on the recovery but it seems less important than the swabbing technique. Selective media can also be advantageous for discrimination between species but are not as effective for recovery [Obee 2007]. Another parameter that has to be taken into account for sampling in healthcare facilities is the transportation of swabs after sampling. The swab transport devices should allow for the release the maximum of viable bacteria even after several hours, and contradictory studies exist on the best devices to be used [Van Horn 2008].

However, the conventional techniques had some issues. For example, the bacterial count on plates is made from colony-forming units, which could be formed from one cell to several hundred [Sutton 2011]. Major limitations to the plate counts are upper limit and lower detection limit. The upper limit, generally around 250 cfu, is due to the competition for space and nutrients with a higher bacteria load, which can require serial dilution [Sutton 2011]. The lower detection limit can be seen as the limit at which the count is considered reproducible, generally around 25 cfu. Moreover it increases the difficulty of attributing a value when the plates do not have any cfu [Sutton 2011]. Conventional techniques can also be considered are time consuming [Carrascosa 2012, Amodio 2014].

The need for more accurate and faster technique leads to the emergence of new technology. The food industry has to evaluate cleanliness with standardized and fast techniques, and ATP bioluminescence analysis has been used since more than a decade in the ood industry [Carrascosa 2012, Amodio 2014, Shama 2013]. This technique appears promising and its effectivity in hospital cleaning procedure has been evaluated [Amodio 2014, Shama 2013].

Living organisms, such as bacteria, use Adenosine triphosphate (ATP) for intracellular energy transfer. The principle of the ATP analysis method is to use bioluminescence to measure intracellular ATP in living bacteria [Lundin 2000]. With oxygen and magnesium, luciferin is converted to oxyluciferin and ATP is converted to adenosine monophosphate, releasing a pyrophosphate and with emission of light in a specific wavelength [Shama 2013]. A luminometer can measure this light emission and the intensity of the light in RLU (relative light unit) is proportional to the amount of ATP [Lundin 2000, Shama 2013, Amodio 2014]. Moreover with some systems, the free ATP and the ATP inside mammalian cells can be degraded so the measurement will only consider bacterial ATP [Lundin 2000].
The general conclusion of the numerous studies that have been carried out in the past decade is that ATP bioluminescence analysis is a promising technique that can quickly provide a standardized and sensitive measure of bacterial load on surfaces [Amodio 2014, Shama 2013]. Precise measurements can be obtained from surfaces by using ATP Kits with swabs or a more rough idea can be obtained using rapid ATP monitoring systems [Shama 2013, Lundin 2000]. Comparison of measurements before and after cleaning appears to be an especially useful tool for education of healthcare workers [Amodio 2014, Rutala 2013]. However, it is still under development, and some issues have to be solved, especially the reproducibility and the need to standardize the correspondence between the colony-forming units and RLU [Amodio 2014, Shama 2013]. Chemicals, microfiber products, and certain plastics can interfere with the enzyme and cause fluctuating ATP, leading to false results [Shama 2013, Mulvey 2011].

F. Overview of disinfection methods for surfaces

Disinfection is commonly performed in hospitals with disinfectant products, however automated devices with 'non-touch principles' are also used to prevent or stop infections.

- **Disinfectant compounds**
  A wide range of disinfectants are available on the market, and the most common active compounds are [Rutala 2013]:
  - Alcohol: Bactericidal, tuberculocidal, fungicidal and virucidal, alcohol disinfectants are easy to use, fast-acting, and do not leave a toxic residue. However, they are not sporicidal, lack cleaning properties and their inflammability and evaporability can damage some instruments. They are mostly used for small surfaces.
  - Chlorine: Broad-spectrum bactericidal, tuberculocidal, fungicidal, virucidal, sporicidal and active against biofilms, chlorine disinfectants are fast-acting and relatively stable. However, they can cause irritation and burns when greater than 5%, and are not compatible with all surfaces, as they can corrode metal and discolore fabrics.
  - Improved Hydrogen peroxide: Bactericidal, virucidal, mycobactericidal, safe for users and the environment, compatible with most surfaces, Improved Hydrogen peroxide is usually more expensive, however.
  - Iodophores: Bactericidal, mycobactericidal, virucidal, iodophores require prolonged contact to kill fungi and do not have sporicidal action. They are more commonly used as antiseptics than disinfectants.
  - Phenolic: Bactericidal, tuberculocidal, fungicidal, virucidal, phenolic disinfectants are usually inexpensive. However they do not have a sporicidal action, can irritate tissue and can be absorbed by porous materials.
  - Quaternary ammonium: Bactericidal, fungicidal, Quaternary ammonium is reported to have a virucidal activity against enveloped viruses like HIV, they are also good cleaning agents compatible with most surfaces and with a persistent antimicrobial activity. However, they are not sporicidal, generally not tuberculocidal, and not virucidal against non-enveloped viruses.

- **Automated devices with 'non-touch principles'**

Automated devices with 'non-touch principles' can be used instead of disinfection with products, like hydrogen peroxide vapor or aerosol devices and Ultra Violet radiation devices. They have been reported to be effective on *C. difficilis* spores and can also prevent the risk of incomplete cleaning by healthcare workers, as they are supposed to decontaminate all exposed surfaces in the room [Weber 2013]. However, the systems are expensive and have operational constraints (transport of the system, removal of patients from the room) and do not replace cleaning [Rutala 2013].

Hydrogen peroxide vapor has been reported to reduce transmission or infection due to microorganisms in several outbreak cases. However, this system takes four times longer than routine cleaning or disinfection [Otter 2014, Rutala 2013].

At certain wavelengths, UV irradiation can break the molecular bonds in DNA. UV radiation devices are considered to be advantageous for different applications, such as control of legionellosis, disinfection of air, surfaces, and instruments. Their efficacy varies with e.g. intensity, exposure time, and placement. Their performance differs according to studies, but a log reduction between 2 and 4 is generally reported and with a sporicidal action. However, their effectiveness is reduced when surfaces were not in direct exposure [Rutala 2013, Weber 2013].

G. Development of a standardized method for evaluation of disinfection

The CEN (*Comité Européen de Normalisation* in French) the European Committee for Standardization is an association of the National Standardization Bodies of 33 European countries. It is one of three European Standardization Organizations that have been officially recognized by the European Union and by the European Free Trade Association for generating voluntary standards CEN [CEN 2014].

Standardization can bring together research, innovation and business in order to raise significant economic benefits with increased productivity and innovative efficiency [CEN 2014]. A European Standard (EN) is a technical document created by consensus and used as a guideline. Standards have to support innovation by providing basis for developing solutions. Industries and private or public organizations can ensure that they fulfill the EU requirements for safety by using harmonized standards [CEN 2014].

The redaction and approval of a standard is a long process [Hatto 2011, CEN/TC278 2012]: During the optional *Preliminary stage*, a potential new subject is defined by the technical committee, together with a program, time plan, and necessary resources. The process always contains a *Proposal stage* of maximum 6 weeks that confirms the need for the future standard through votes and recruitment of participants for the future work group. The redaction starts properly at the *Drafting Stage,* while parts are attributed to working groups with experts that write a draft standard. This *Drafting Stage* can last 12 months maximum, and can include an optional but important *Committee Stage* when the draft standard is reviewed by all the Technical Committee members that will give their comments. Following, the *Enquiry Stage* will last 8 months and will see the public release of the draft standard to all the stakeholders, who are then invited to send their comments. Then, during the *Approval Stage* of 8 more months, the comments are consulted and a final draft standard is written and sent to *the National Standard Organization* that will give or deny its 'Formal vote' for approval of the draft standard. After corrections based on editorial comments, the new EN standard can be published during the *Publication Stage* in 31 countries in Europe.

In Europe, it is generally considered that the assessment of a specific disinfectant requires several tests [Pitten 2003]. In phase 1 tests, the efficacy of a product is demonstrated in a suspension with microorganisms. In phase 2 the product is tested with interfering substances (step 1) and in simulation of real conditions (step 2). Phase 3 tests include field trials or clinical tests [Pitten 2003]. Currently, no established European standard proposes a method to evaluate the efficiency of disinfection with wiping as it is performed in hospitals. The difficulty of setting up a clinically relevant method and the wide range of parameters that have to be taken into account make the elaboration of this standard complicated.
The draft standard FprEN 16615 "Chemical disinfectants and antiseptics — Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test) — Test method and requirements (phase 2, step 2)" is under development and aims to fulfill the need of a standardized and adequate method.

The principle is to analyze the efficacy of disinfectant use with a wipe to remove bacteria from a contaminated source while the bacteria are spread by the wiping motion. Four different test fields are drawn on the surface from the beginning to the end of the wiping motion and analyzed separately by sampling and plate cultivation. Different requirements in terms of bacterial load are defined by the draft standard for the four test fields in order to assess the antimicrobial activity.

PART III: MATERIAL AND METHODS

A. Study with the draft standard FprEN 16615

- **Test solutions**

Two cleaning agents were tested at the concentration required for professional use: Hågeren Allrent (Gipeco®) 50 ml to 10L and Chick Allrent (Gipeco®) at 100 mL to 10 L. Three ready-to-use alcohol based products were selected: DES45, DES70 and Bacticid. One peroxygen-based disinfectant, Rely+On™ Virkon® Powder, was also tested and used as a solution of 1 gram of powder diluted in 100 mL as recommended by the manufacturer. Also, Wet Wipe Chlorine disinfection wipes impregnated with chlorine compound were tested.
Hard water was used to dilute non-ready-to-use products and for the Water Control. It was prepared with 6 ml of Solution A (19.84 g/l magnesium chloride ($MgCl_2$) and 46.24 g/l calcium chloride ($CaCl_2$)) and 8 ml of Solution B (35.02 g/l sodium bicarbonate ($NaHCO_3$)) in 1 L water at pH 7.0 ± 0.2.

- **Test organisms**

Two microorganisms were studied, *Enterococcus hirae* CCUG 32258 (ATCC 10541) and *Staphylococcus aureus* CCUG 10780 (ATCC 6540). The working cultures for the bacteria suspensions were produced from fresh subcultures of streak plates from frozen culture. Bacteria suspensions were maintained at 20 ±1°C in a water bath until use.
For bacteria culture, Trytpic soy agar (TSA) plates were chosen and a solution of general diluent (Tryptone (1.0 g /l), Sodium chloride (8.5 g/l), and water (1 L)) was used for bacterial suspension, dilution and preparation of other solutions.

- **Wiping procedure**

A test flooring was inoculated with a bacterial suspension in general diluent mixed with an interfering substance, and then was wiped using a wipe soaked with product (see Figure 1). The interfering substance for simulating a dirty condition was freshly prepared in the laboratory with bovine albumin (3 g/l) and sheep erythrocytes (3 ml/l) in general diluent. After the wiping and the contact time *t*, the amount of survival bacteria *Na* on the surface was carefully sampled with swabs, and the bacteria were spread on TSA plates and cultivated. The initial bacterial load *Na* on the surface was therefore defined after cultivation by counting the colony-forming units (cfu).
Two controls were performed: a Water Control where water replaced the product during wiping, and a Dry Control where the inoculum of bacteria was not wiped but let dry. The Dry Control *Dc0* was sampled just after visible dryness of the inoculum and the Dry Control *Dct* was sampled after sampling of the flooring wiped with disinfectant and the contact time *t*.
The sampling method implied the use of two swabs. A neutralizer was used during the sampling to avoid the remaining effects of the disinfectant during the cultivation. First, a dry swab was used to rub the test field, then it was soaked in a solution of neutralizer before performing a second swabbing with the same

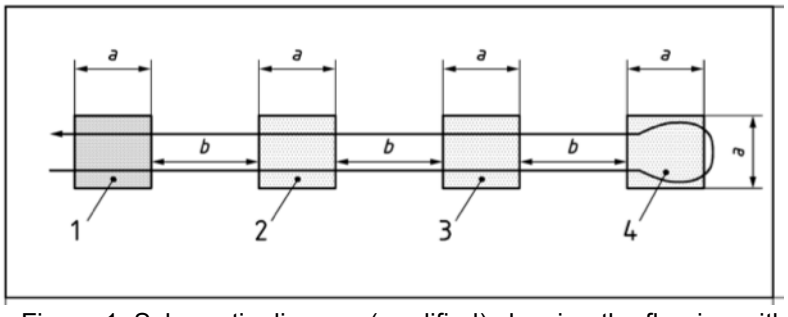

Figure 1: Schematic diagram (modified) showing the flooring with the four test fields (1, 2, 3, 4) and the direction of the wiping.

swab. The swab was then cut and the extremity remained in a solution of neutralizer. A second swab was used to dry the surface and then the extremity was cut and put into the solution of neutralizer together with the first swab. Each one of the different test fields drawn on the surface was sampled separately and the bacteria load was analyzed separately on at least two TSA plates for each test field.

This part of the experiment that tested the efficiency of the disinfectant, including the Water Control, Dry Control and the cultivation, will be called in this report Wiping Procedure (WIP).

- **Validation test**

In order to assess the validity of the experiment, Validation Tests (VAL) were performed before the Wiping Procedure. The test Control B assessed the non-toxicity of the neutralizer by cultivation of bacteria with the neutralizer, and the test Validation C assessed the efficiency of the neutralizer to neutralize the product by cultivation of bacteria in contact with both the products and the neutralizer.
Different neutralizers were tested in order to ensure an efficient and non-toxic neutralization of the test products. Neutralizer P80 (Polysorbate 80 (3%), saponin (3%), histidine (0.1%) and cysteine (0.1%)) and neutralizer Dey-Engley (Casein enzymic hydrolysate (5 g/l), Yeast extract (2.5 g/l), Dextrose (10 g/l), Sodium thioglycollate (1 g/l), Sodium thiosulphate (6 g/l), Sodium bisulphite (2.5 g/l), Lecithin (7 g/l), Polysorbate 80 (5 g/l), Bromocresol purple (0.02 g/l)) were tested for the cleaning agents from Gipeco® and the disinfectants. Two neutralizers were specifically tested for Wet Wipe Chlorine Disinfection, the Neutralizer A (Sodium Thiosulfate 5 g/L, Polysorbat 80 30 g/L, Lecithin 3 g/L, Saponin 30 g/L, Histidine 1 g/L) and the Neutralizer B (Natrium Thiosulfate 10 g/L, Polysorbat 80 30 g/L, Lecithin 3 g/L).

- **Assessment of efficiency and evolution of the requirements**

This method is described in the draft standard FprEN 16615 and is still under development by the redaction committee. After collecting the comments from different laboratories, the redaction committee provided an updated draft of the standard prEN16615. The new draft FprEN 16615 was released the 2014-04-07. The major modifications are regarding:

- The solution used to sample the Dry Controls: neutralizer used instead of general diluent, so it is similar to the solution used for the sampling of the Water Control and the Products.
- Initial concentration of bacteria in the test suspension: increased.
- Basic limit in bacterial load for the water control on test field 2-4: lowered
- Requirement for the maximum of bacterial load for the product on test fields 2-4: increased.

In order to assess both the validity of the experiments and the bactericidal efficiency of the test product, basic limits and requirements are defined in the standard FprEN 16615 and presented table 1. The bacterial load is expressed as the number *N* of colony-forming unit (cfu) per ml of suspension, or as logN.

Table 1: Basic limits and requirements according to the draft standard FprEN 16615

	Bacterial load N required
Initial bacterial suspension	1.5×10^9 cfu/ml < N < 5.0×10^9 cfu/ml
Dry control (Dc0 and Dct)	6.75×10^6 cfu/ml < N < 2.5×10^8 cfu/ml ($6.83 \leq \log N \leq 8.35$)
Water control (test fields 2-4)	N > 10 cfu/ml (log N > 1)
Product (test field 1)	At least log Reduction 5 compare to Dct (logDct – logN > 5)
Product (test fields 2-4)	N < 50 cfu/ml (log N < 1.70)

B. Study with the ATP bioluminescence analysis method

- **Test solution**

A cleaning product from Gipeco®, Hågeren Allrent, was used at the concentration 50ml for 100ml. Hard water was used to dilute Hågeren Allrent and for the Water Control.

- **Test organism**

Staphylococcus aureus CCUG 10780 (ATCC 6540) was used and the working cultures for the bacteria suspensions were produced from fresh subcultures of streak plates using frozen ceramic beads. Bacteria suspension was maintained at 20 ±1°C in a water bath until use. For bacteria culture, Trytpic soy agar (TSA) plates were chosen and a solution of General diluent (Tryptone (1.0 g /l), Sodium chloride (8.5 g/l), and water (1 L)) was used for bacterial suspension and dilution.

- **ATP bioluminescence analysis during wiping procedure**

This ATP analysis method could be used to analyze the efficacy of disinfectant after wiping. Four different test fields were drawn on the surface from the beginning to the end of the wiping motion and analyzed separately. The wiping was performed with a granite block (unitary weight) in order to standardize the applied strength.
A test flooring was inoculated with a bacterial suspension in general diluent and mixed with an interfering substance and wiped with a wipe soaked with product. The interfering substance was a solution of bovine albumin, sheep erythrocytes and 0.2% Triton freshly prepared in laboratory and tested before use to ensure that the luciferase would not react the components, creating false positives. After the wiping and the contact time *t*, the free ATP and the mammalian ATP were degraded using ATP eliminating reagent spread with a swab on the test fields. Then the ATP inside the remaining bacteria was extracted by the Extractant B/S spread with the same swab. For the swabbing, sterile ATP-free swabs provided by BioThema® with a knitted flat head that absorbs ATP were used. The bacterial ATP trapped on the swab was measured using a luminometer after the release of the ATP into a solution of ATP Reagent HS (High Sensitivity) containing the enzyme luciferase that reacts with ATP to produce luminescence. The addition of an ATP standard containing a known amount of ATP was used as a reference to determine the precise amount of bacterial ATP released.
Two controls were carried out: a Water Control where water replaced the product during wiping, and a Dry Control where the inoculum of bacteria was not wiped but let dry. The Dry Control *Dc0* was sampled just after visible dryness of the inoculum and the Dry Control *Dct* was sampled after sampling of the flooring wiped with disinfectant and the contact time *t*.

- **Required pre-tests**

Prior to the experiments, it was important to test the background of the different solutions, including the reagents and the interfering substance, to evaluate the amount of false positives. Measurements of extracellular ATP, intracellular and extracellular ATP and bacterial ATP were conducted, especially on the interfering substance.

C. Compatibility study of the wiping material and products

The study on the potential inhibition of the active components was realised for Gipeco® by studying the cleaning agents Hågeren Allrent and Chick Allrent with the Pulp:PET wipe recommended by the standard (55% pulp and 45% PET) and with the Gipeco Wipe (80% PES and 20% PA). The effect of the wiping material was studied by soaking the wipes with a test solution for half an hour, then stomaching them to extract the solution. The solution was then added to a suspension of *Staphylococcus aureus* CCUG 10780 (ATCC 6540) for 10 minutes before being neutralized by the addition of a neutralizer. A control was done by suspension of the *S. aureus* with a test solution that had not been in contact with a wipe. The bacterial load of the initial bacteria suspension, the remaining bacterial load with the control *Nc* and the remaining

bacterial load with test solution soaked with the material *Nm* were assessed by bacteria cultivation on TSA plates.

D. Long term survival of *S. aureus* on surface

Twelve surfaces were inoculated with 50 µl of bacterial suspension of *Staphylococcus aureus* CCUG 10780 (ATCC 6540) (suspension about 3 x 10^8 cfu/ml and concentration on the flooring 1.5 x 10^7 cfu/ml) and sampled with the 2 different methods, cotton swabs and dip slides, at 24h, 44h and again after week of drying time. The reference used is the initial concentration on the flooring, evaluated by plate inoculation with the initial bacterial suspension and after 24h cultivation.

E. Short term survival of *P. aeruginosa* on surface

The survival of *P. aeruginosa* was studied in comparison with *S. aureus*, used as reference.

- **Test *organism***

Staphylococcus aureus ATCC 6540, *Pseudomonas aeruginosa* ATCC 15692, and ATCC 15442 were used and the working cultures for the bacteria suspensions were produced from subculture of streak plates from frozen ceramic beads. Bacteria suspension was maintained at 20 ±1°C in water bath until use. For bacteria culture, Trytpic soy agar (TSA) plates were chosen, and a solution of General diluent (Tryptone (1.0 g /l), Sodium chloride (8.5 g/l), and water (1 L)) was used for bacterial suspension and dilution.

- **Comparison with *S. aureus***

In the first part of the study, four surfaces, two for each bacteria *S. aureus* ATCC 6540 and *P. aeruginosa* ATCC 15442*,* were inoculated with 50 µl of bacterial suspension with 10% of interfering substance according to inoculation procedure the standard FprEN 16615. After one hour of dryness, the surfaces were sampled with swabs according to the sampling procedure in the standard FprEN 16615 and the bacterial load was evaluated after plate inoculation and 24h cultivation. The reference used is the initial concentration on the flooring, evaluated by plate inoculation with the initial bacterial suspension and after 24h cultivation.

- **Comparison between the two strains of *P. aeruginosa***

In a second part of the study, four surfaces, two for each strains *of P. aeruginosa,* were inoculated with 50 µl of bacterial suspension with 10% of interfering substance according to inoculation procedure the standard FprEN 16615. After one hour of dryness, the surfaces were sampled with swabs according to the sampling procedure in the standard FprEN 16615 and the bacterial load was evaluated after plate inoculation and 24h cultivation.

PART IV: RESULTS

In order to investigate the antimicrobial effect of test products with wiping, experiments were performed according to the draft standard FprEN 16615 on a flooring with 4 test fields. The bacteria load on surfaces or in suspension was defined after plate cultivation and colony-forming unit (cfu) counts. Neutralizers were used to avoid remaining effects of the disinfectants after sampling on the test fields. Part A presents the results from the Validation tests conducted to evaluate the toxicity and the neutralization efficiency of the selected neutralizers. Part B presents the antimicrobial effects of the test products according to the requirements of the standard FprEN 16615. The part C contains the outcomes obtained with the ATP bioluminescence analysis. Part D. exposes the findings related to the compatibility study of the chemical compounds in the cleaning agents and the material of the wipes. Part E and F present the data for the survival of bacteria on surfaces, for *S. aureus* and for *S. aureus* and two strains of *P. aeruginosa*, respectively.

A. Validation tests of the neutralizers for the studies with the standard FprEN 16615

Neutralizers were used to avoid remaining effects of the disinfectants and cleaning agents after sampling on the test fields. Validation tests were performed to select the neutralizers that are not toxic and possess neutralization efficiency. The criteria for a non-toxic and efficient neutralizer is that there are more than 50% bacterial survivors of the bacterial inoculum after neutralization. Four neutralizers were tested, the Neutralizer P80 and Dey Engley for the products Hågeren, Chick, DES45, DES70, Bacticid and Rely^{+}On™ Virkon®, and the Neutralizers A and B for the product Wet Wipe. The results for the selected neutralizers that met the criteria are presented in the table 2, the initial bacterial inoculum (BI) is used as a reference.

Table 2: Toxicity test and efficacy test performed with the selected neutralizers Neutralizer P80 for Hågeren, Chick, DES45, DES70, Bacticid and Rely^{+}On™ Virkon® and the Neutralizer A for Wet Wipe. Initial bacterial inoculum BI is used as a reference.

Test suspensions	**Initial bacterial inoculum BI (cfu/ml)**	**Bacterial load on test suspension (cfu/ml)**	**Percent survivors**	**Pass the limit >50% of bacterial inoculum?**
Toxicity test with *S. aureus* – Control B				
Neutralizer P80 + BI	1.44×10^3 1.56×10^4	1.60×10^3 1.44×10^4	> 100% 92%	Yes
Neutralizer A Wet Wipe + BI	1.78×10^2	2.00×10^2	> 100%	Yes
Neutralization efficacy test with *S. aureus* – Validation C				
Neutralizer P80 + Hågeren	1.44×10^3 1.56×10^4	1.47×10^3 1.46×10^4	> 100%	Yes
Neutralizer P80 + Chick	1.44×10^3 1.56×10^4	2.37×10^3 1.33×10^4	> 100%	Yes
Neutralizer P80 + DES45	3.12×10^3	1.56×10^3	50%	Yes
Neutralizer P80 + DES70	2.70×10^3	2.15×10^3	80%	Yes
Neutralizer P80 + Bacticid	2.70×10^3	2.35×10^3	87%	Yes
Neutralizer P80 + Rely^{+}On™ Virkon®	2.70×10^3	2.10×10^3	94%	Yes
Neutralizer A Wet Wipe + Wet Wipe	1.78×10^2	2.55×10^2	> 100%	Yes

Toxicity test with *E. hirae* – Control B				
Neutralizer P80 + BI	1.90 x 10^3	1.70 x 10^3	90%	Yes
Neutralization efficacy test with *E. hirae* – Validation C				
Neutralizer P80 + Hågeren	1.90 x 10^3	2.15 x 10^3	> 100%	Yes
Neutralizer P80 + Chick	1.90 x 10^3	1.73 x 10^3	91%	Yes
Neutralizer P80 + DES45	2.48 x 10^3	2.77 x 10^3	> 100%	Yes
Neutralizer P80 + DES70	1.52 x 10^3	0.950 x 10^3	63%	Yes
Neutralizer P80 + Bacticid	1.52 x 10^3	1.05 x 10^3	70%	Yes
Neutralizer P80 + Rely$^+$On™ Virkon®	1.52 x 10^3	1.30 x 10^3	86%	Yes

B. Antimicrobial effects of the products according to the standard FprEN 16615

'FprEN 16615 standard or 4-field test' was run to evaluate the bactericidal effect of two cleaning agents, four disinfectants, and the Wet Wipe Chlorine Disinfection. The procedure analyzed the efficacy of disinfectant after wiping on a surface with 4 test fields drawn from the beginning to the end of the wiping motion. A known amount of bacteria is inoculated on the test field 1, and after wiping the bacterial load on each test field was sampled and analyzed separately after inoculation on plates and 24h - 48h incubation.
Two controls were performed: a Water Control where water replaces the product during wiping, and a Dry Control where the inoculum of bacteria was not wiped but let dry.
For the product to meet the requirements of the standard FprEN 16615, basic limits and requirements must be met (Table 3).

Table 3: Basic limits and requirements

	Bacterial load (N) required
Initial bacterial suspension	1.5 -5 x 10^9 cfu/ml
Dry control (Dc0 and Dct)	6.75 x 10^6- 2.5 x 10^8 cfu/ml (Log 6.83 - log 8.35)
Water control (test fields 2-4)	> 10 cfu/ml (> log 1)
Product: test field 1	At least log 5 reduction compared to Dct (logDct – logN > 5)
Product: test fields 2, 3, 4	< 50 cfu/ml (< log 1.7)

The first criterion for a product to reach the requirement is to present a log 5 reduction on the first test field compare to the load on the Dry Control Dct as logDct – logN > 5. The log reduction with the Dry control allow to compare the activity of the product compare to not wiping at all, when the surface is let dry.
The second criterion for a product to meet the requirement is the presentation of an average of bacterial load on the test field 2, 3, 4 lower than 50 cfu/ml.

Three out of five tested disinfectants; DES45, Rely$^+$On™ Virkon®, and Wet Wipe Chlorine Disinfection, met the requirements of FprEN 16615 but not the disinfectants Bacticid and DES70 nor the cleaning solutions Chick and Hågeren (Table 4 and 5).

Table 4: Log reduction for test field 1 and average load for the test field 2, 3, 4 for the disinfectants DES45, DES70, Bacticid and Rely+On™ Virkon®, and the Wet Wipe Chlorine Disinfection.

Disinfectants	***Bacteria***	**Log R1 (Product)**		**Test field 2, 3, 4**	
		Log Reduction	**Pass (> Log 5)**	**Bacterial load (log CFU/ml)**	**Pass (< Log 1.70)**
DES45	*S. aureus*	5.03	Yes	0.99 (1.22, 0.82, 0.92)	Yes
	E. hirae	6.39	Yes	0.37 (0.70, 0.40, 0.00)	Yes
DES70	*S. aureus*	4.07	No	0	Yes
	E. hirae	4.44	No	0	Yes
Bacticid	*S. aureus*	4.04	No	0	Yes
	E. hirae	5.95	Yes	0	Yes
Rely+On™ Virkon®	*S. aureus*	> 7.14	Yes	0	Yes
	E. hirae	> 7.35	Yes	0	Yes
Wet Wipe	*S. aureus*	>7.40	Yes	0	Yes

Table 5: Log reduction in test field 1 and average load for the test field 2, 3, 4 for the cleaning agents Chick and Hågeren for the wiping performed with the PET wipe recommended by the standard and the Gipeco Wipe.

Cleaning agents	Bacteria	Log R1 (Product)		Test field 2, 3, 4	
		Log Reduction	Pass (> Log 5)	Bacterial load (log CFU/ml)	Pass (< Log 1.70)
Pulp:PET wipe + Chick	*S. aureus*	3.59	No	2.02 (2.15, 1.87, 2.03)	No
	E. hirae	4.28	No	0.43 (0.88, 0.00, 0.40)	Yes
Pulp:PET wipe + Hågeren	*S. aureus*	3.49	No	2.56 (2.81, 2.83, 2.04)	No
	E. hirae	4.01	No	2.17 (2.34, 1.94, 2.22)	No
Gipeco Wipe + Hågeren	*S. aureus*	4.22	No	0.47 (0.0, 0.70, 0.70)	Yes

C. Results of the ATP bioluminescence analysis

Tests of the background of different solutions used during the ATP Bioluminescence analysis were conducted and several wiping procedures were run. All the solutions presented a very low background, around 1 to 3 RLU according to the requirement for the ATP analysis method. The challenging solution to be tested was the interfering substance containing sheep erythrocytes that presented highly variable measurements with higher background. A solution of interfering substance was tested by adding Triton X-100 at 0.2% and no background was detected, allowing the use of this modified interfering substance for the wiping procedure.
However, the experiments of wiping procedures did not give any quantitatively valuable results.

D. Results of the compatibility study product by the wiping material

The compatibility study of the active components in the cleaning agents Hågeren Allrent and Chick Allrent was carried out with the Gipeco Wipe (80% PES and 20% PA) and the Pulp:PET wipe recommended by the standard (55% pulp and 45% PET).
The initial bacterial load gives the concentration in the suspension before addition of tested solution. The control is conducted with a test solution that has not been in contact with any wipe. The effect is Inh = (Nm – Nc) / Nm, with *Nc* the bacterial load in the control suspension (cfu/ml) and *Nm* the bacterial load with the suspension that has been in contact with the wipe material.

The antimicrobial activity of Hågeren at 50 ml/10L was inhibited by 55% with the pulp:PET wipe and by 15% with the Gipeco Wipe and for the Chick at 50 ml/10L the antimicrobial activity was inhibited by 96% with the pulp:PET wipe and 100% with the Gipeco Wipe (see Table 6).

Table 6. Inhibition effect Inh of the pulp:PET wipe and Gipeco Wipe on the Gipeco® cleaning agents Hågeren and Chick. The initial bacterial load gives the concentration in the suspension before addition of tested solution. The control is realized with a test solution that has not been in contact with Wipe or wipe. The inhibitory effect is Inh = (Nm – Nc) / Nm

Product	Tested material	Concentration	Initial bacterial load (cfu/ml)	Bacterial load with control *Nc* (cfu/ml)	Bacterial load with material *Nm* (cfu/ml)	Inhibitory effect *Inh*
Hågeren Allrent	Pulp:PET wipe	Non-diluted	75	10	35	71%
		50 ml/10L	75	25	55	55%
	Gipeco Wipe	Non-diluted	63	43	29	none
		50 ml/10L	63	69	81	15%
Chick Allrent	Pulp:PET wipe	Non-diluted	$2x10^5$	0	0	0
		50 ml/10L	$2x10^5$	15	790	96%
	Gipeco Wipe	100 ml/10L	63	0	62	100%
		50 ml/10L	63	0	32	100%

E. Long term survival of *S. aureus* on surface

The survival and recovery of *S. aureus* was studied after long drying time using two different sampling techniques, cotton swabs and dip slides.
The results show that the log cfu of bacterial load for *S. aureus* on surfaces after one week with an initial concentration of 6.75 is 5.37. The dip slides showed surfaces covered with bacteria colonies, at a density too high to allow the counting. However, this method could be used at lower bacterial concentration, as it is very convenient to use. The equivalence in cfu for surfaces covered of bacteria is a bacterial load superior or equal to log 6.

Table 7: Bacterial load of *S. aureus* after drying on surface for one hour, 24 hours and one week.

	Initial bacterial inoculum	Average on surface after the drying time *t*		
		t=1 hour	t=24 hours	t = 1 week
Load *log(cfu/ml) with cotton swab*	6.75	6.14	5.79	5.37
Load *log(cfu/ml) with Dip Slides*	6.75	> 6	> 6	> 6

F. Short term survival of *P. aeruginosa* on surface

The survival of *P. aeruginosa* has been studied on dry surface in comparison with *S. aureus*, used as reference. The result of the first study shows that *P. aeruginosa* survives equally well as *S. aureus* after one hour of drying on a flooring.
The second experiment comparing the survival of the strain ATCC 15692 and the strain ATCC 15442 revealed empty plates for both of the strains after one hour drying.

Table 8: Bacterial load of *S. aureus* ATCC 6540 and *P. aeruginosa* ATCC 15442 after drying on surface for one hour.

Microorganism	**Initial bacterial inoculum Log(cfu/ml)**	**Average load on surface after one hour Log(cfu/ml)**
S. aureus ATCC 6540	6.75	5.70
P. aeruginosa ATCC 15442	6.38	5.60

PART IV: DISCUSSIONS

A. Choices of disinfectants

- **Concentration of alcohol in alcohol-based products**

It is interesting to note that DES45 meets the requirements while DES70 and Bacticid do not. DES45 contains about 45% alcohol, and DES70 about 50 to 80% alcohol and Bacticid 72%. It could be expected that the highest concentration of alcohol would have the better effect, as has been assumed in the past [McDonnell 1999]. The bacterial load on the first field is higher for Bacticid and DES70 than for DES45. However, DES70 and Bacticid have no detected bacteria on the fields 2,3,4 while DES45 presents a bacterial load of 0.99 and 0.37 for *S. aureus* and *E. hirae.* In a paper describing a method to evaluate cleaning and disinfectant action of surface disinfectants [Walder 1989], it is explained that a high concentration of alcohol can denature on blood protein and therefore increase the fixation of the bacteria on a surface. As the bacteria are inoculated together with an interfering substance of sheep blood, the denaturating effect could explain the difference between the disinfectant with lower alcohol concentration DES45 and the disinfection with higher alcohol concentration as DES70 and Bacticid. This result emphasizes the importance of mimicking the clinical conditions while testing disinfectants.

Furthermore, DES45 contains a surfactant cocamidopropylamine oxide, a tertiary amine oxide classified as nonionic surfactant with amphiphilic compounds [Cosmetic Ingredient Review 2008, Gaysinsky 2007]. Nonionic surfactants can increase the antimicrobial properties of a product by modifying the availabilities of the antimicrobial compounds, due to their low emulsification ability and a good adsorption on surfaces [Adewale 2013, Gaysinsky 2007, Nabel 2012].

- **Importance of the disinfection techniques**

Three tested disinfectants meet the requirements of FprEN 16615: DES45, Rely^{+}On™ Virkon®, and Wet Wipe Chlorine Disinfection.
DES45 is a ready-to-use, alcohol-based product that can be directly applied to surfaces or spread onto a wipe before wiping.
Rely^{+}On™ Virkon® is a peroxygen-based product provided as powder and diluted in water. Once dissolved, the solution of Rely^{+}On™ Virkon® must be used within a week and extra care should be taken while manipulating the product due to its toxicity. It can be applied directly to the surface or spread onto a wipe before wiping.
Wet Wipe Chlorine Disinfection is available on the market in sealed packages containing pre-impregnated wipes and a pouch of surfactant. By briefly applying high pressure on the package, the pouch of disinfectant will break inside the package and the surfactant will spread all over the wipes. The wipes are single use.

The practice of the method in the laboratory brought up several concerns about the practical aspect of disinfection in healthcare facilities. While performing the first wiping procedures, the wipes were a source of contamination as they are provided in regular packaging and not sterile. Therefore, sterilization in autoclave was necessary. Furthermore, an in-house test was conducted by the cleaning staff at Medibiome in real conditions with a clean mop. It was shown that the mop itself was contaminated with microorganisms and was spreading them to previously non-contaminated surfaces on the floor.
The wiping technique should be conducted with extra care to allow for easy disinfection and also to ensure that cleaning is not a source of contamination. Re-use of contaminated wipes or use of clean wipes still contaminated after washing seems to be a common risk in healthcare facilities. As both the existing literature and our study demonstrate, species like *S. aureus* can survive a very long time on surfaces [Kramer 2006], so the risk of contamination from surfaces should not be neglected.
Therefore, the concept of single-use pre-wet wipes appears to be a good alternative technique. The contamination can be avoided, and the post-treatment with washing is not needed. A recent study

emphasized the value of ready-to-use disinfection wipes [Wiemken 2014] which are considered to be easier and quicker to use, and possibly cost saving.

Furthermore, it has been shown that the wiping material could decrease the efficiency of the products. Indeed, the wipe recommended by the standard contains 45% Polyethylenterephthalat, is considered to have specific chemical binding properties on some products like quaternary ammonium [El Ola 2004, Mohamed 2012]. The study evaluating the compatibility of the material used for wiping showed that the inhibition of the material can vary from 15 to 100%. Using a ready-to-use wet wipe would avoid this issue.

- **Wiping procedure with water**

While running the wiping procedure according to the standard FprEN 16615, a Water Control was conducted at the same time as the wiping with the product in order to control the performance of the mechanical action. The wiping and sampling were performed in the same way as in the product test, except that the product test was replaced with sterile water. The standard required a minimum average of bacterial load of 10 cfu on the test fields 2,3 and 4 for the Water Control. It is therefore interesting to analyze the antimicrobial effect of the wiping with water. It was found that the log reduction on the test field 1 is usually between 3 and 4 [*data on file*]. This emphasizes the importance of the mechanical action of wiping and by analogy the choice of wiping material.

- **Validation tests**

Neutralizers were used to avoid any remaining effects of the test products after sampling. Validation tests assessed the non-toxicity and sufficient neutralization efficiency of the selected neutralizers, with more than 50% bacterial survivors of the bacterial inoculum. The same neutralizer was also chosen for the tests performed with DES45, DES70, Bacticid, Rely^{+}On™ Virkon® and the cleaning agents Hågeren and Chick from Gipeco®, to facilitate the performance of the experiment while testing several products at the same time. Extra care should be taken during the Validation tests as it has been established that neutralizers can lead to false results with overestimation of disinfectant actions [Fernandez-Crehuet 2013].

B. Input to the standard FprEN 16615

The standard FprEN 16615 must be clinically relevant in order to provide an appropriate evaluation of disinfectant products and therefore to participate in the necessary improvements of methods to reduce the incidence of HCAIs. However, it should also allow or even favor innovative products. The experiments should also be clearly described, easily performed at a reasonable cost and duration, and as close as possible to the practice in healthcare facilities.

Different aspects of the method described by the standard FprEN 16615 have been evaluated with greater focus on practicality and clinical relevance. The main aspects have been summarized and transmitted to the committee responsible of the redaction of the standard FprEN 16615.

Editorial inputs have been proposed in order to point out redactional mistakes or misleading information.

- **Focus on the wiping material**

The repeated practices and discussions about the standard methods provided technical inputs to the standard committee. Special attention was given to the wipes. Indeed, the wipes were a central part of the experiment as they carried the product to be tested and/or the Water Control. Experiments at Medibiome showed that the wipes could be a source of contamination as they are provided in regular, non-sterile packaging. Even if the microorganisms can be easily differentiated on a plates, the growth competitivity between species can decrease the number of cfu on a plates and give false results. The sterilization of wipes in autoclave was carried out prior to the experiment, and should be required by the standard.

Furthermore, the results from the compatibility study of the wiping material with the products demonstrated that the material could inhibit the action of the antimicrobial components while in contact and thus probably during wiping. Further studies would be necessary to evaluate the influence of the inhibition during the wiping, but it can be already stated that manufacturer should advise as to the type of fabric to be used during the test and during the real cleaning or disinfection practice.

- **Single sampling of the field 2, 3, 4.**

It is important to keep in mind reduction of workload when considering the price of tests and their accessibility, strongly linked to innovation. According to the standard, the fields 2, 3 and 4 have to be sampled and analyzed separately for the cfu counts. However, the results are summarized as a mean value for the three last fields, and the requirements from the standard are stated for this mean value. It would be sufficient to sample the test fields 2, 3, and 4 in one unique test tube at one time using two swabs. Sampling separately does not add any further information as to the disinfectant meets the requirements or not according to the FprEN 16615 standard, as it is the mean value on the test fields that matters.

- **Maximizing bacteria recovery**

After sampling with two swabs from the flooring, mixed by mechanical agitation (e.g. vortex) is referred but without a required time. This step would be essential to standardize since bacteria recovery varies depending on how the vortex is performed in time and force. During this project, 2 min of vortexing was performed, as it is more effective, but adding a sonication step would also be a good option [Bjerkan 2009]. Also, studies have shown that the recovery of bacteria is improved with flocked nylon swabs instead of cotton swabs [Hedin 2010].

- **Problematic use of the granite block**

During the wiping procedure, a unitary weight (granite block, around 2.5 kg) was used to standardize the wiping and was intented to simulate the average pressure when wiping floors or benches. However, an in-house test was performed by the cleaning staff at Medibiome and no such pressure was applied when cleaning. Tests have been conducted and 1.5 kg seems more accurate than 2.5 kg. This step is very important for the clinical relevance of the whole procedure, and decreasing the weight of the granite block seems to be mandatory. Moreover, the high log reduction with the Water Control could be due to the higher strength applied during the mechanical action.
It is also difficult for a laboratory to find a granite block with the appropriate dimensions. Furthermore, the granite block is very difficult to manipulate due to both its dimensions and weight, leading to less reproducibility for the experiments and an increase the workload.

- **Dirty conditions with interfering substance**

According to the standard, the experiments have to be performed in clean conditions (with an inoculum of bacteria and bovine albumin) and dirty conditions (with an inoculum of bacteria mixed with an interfering substance of sheep erythrocytes and bovine albumin). The utilization of sheep erythrocytes is ethically debatable, hard to standardize and the preparation of the interfering substance is time consuming. However it is known that erythrocytes facilitate the survival of bacteria on surfaces and that it is therefore important to test disinfectant with an interfering substance containing blood [Pitten 2003, Walder 1989]. The presence of blood on surfaces due for instance to blood spill in healthcare facilities is possible [Chitnis 2004]. Moreover, the clean condition would not bring any relevant information on the efficiency of the product compare to the dirty conditions. It would be interesting to require the experiment to be run in dirty condition only to save products, materials, time and therefore to reduce the cost.

- **Study with *P. aeruginosa***

The draft standard requires the use of three bacteria *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, and *Enterococcus hirae* ATCC 10541 and one fungi, *Candida albicans* ATCC 10231. *P. aeruginosa* is described as more sensitive to dryness on surfaces. The rate of survivors on dry surfaces can be low and therefore the standard FPrEN 16615 recommended to inoculate them with glycerol to improve their survival. The study aimed to compare the survival of *P. aeruginosa* on surfaces, using *S. aureus* as a reference and performed without glycerol. In the first part of the study, the Medibiome strain demonstrated a good ability to survive after one hour drying. However, no bacteria were detected after swabbing and plate inoculation on the second part of study where the two strains of *P. aeruginosa* where studied. This result could be due to either a dysfunction during the experiments or the sensitivity of the two strains of *P. aeruginosa* to different environmental conditions.

C. Evaluation of the ATP bioluminescence analysis

One goal of this project was to determine if the ATP bioluminescence analysis could be used as an option to evaluate the bacterial load on surfaces.
The purpose of this study was to evaluate the feasibility of the ATP analysis as an alternative method to the draft standard FprEN 16615. In the future, this method could be used to replace the traditional culture plate method with counting of the colony-forming units. In order to adapt it to the standard FprEN 16615, a protocol has been written and the interfering substance has been tested to be sure that the background is not reacting with the enzyme luciferase.

- **Proposed benefits**

As its preparation time is short, the ATP analysis method is quicker and cost-efficient. The media and materials consumption are very low and there is no need to perform plate inoculation and 24h - 48h cultivation. Also, there would be no need to use neutralizers which could lead to false positive results [Fernandez-Crehuet 2013]. The estimated working time with the ATP bioluminescence analysis for one study with 3 microorganisms and one product would be about six hours, while it would take four working days with the plate cultivation method.

- **Troubleshooting**

However, it was found that further work must be done to adapt the ATP analysis for intended application. Troubleshooting was done involving several experiments, with the result in that some issues were solved.
The cfu measurement given by the luminometer can appear too low while measuring bacteria suspension. Indeed, the measurements given by the machine depend on parameters that are not adjusted properly and the values in RLU given by the luminometer should be used and converted into amount of ATP in pmol or cfu, assuming that a bacterial cell contains 2 amol of ATP, according to the *Instruction for Uses* of *BioThema Luminescent Assay Intracellular ATP Kit HS.*

The main issue identified at the beginning was due to the measurement of the background that appeared higher than the expected measurement value. Indeed, the sheep erythrocytes in the interfering substance for dirty conditions contain proteins that react with luciferase. One proposal was to use only bovine albumin but at higher concentration instead of a mix of bovine albumin and sheep erythrocytes, but it seemed more interesting to perform the experiments according to the draft standard FprEN 16615. A solution of interfering substance was tested by adding Triton X-100 at 0.2% and no background was detected. The aggregation of erythrocytes was avoided while adding Triton X by vortexing 1 - 2 minutes and mixing with a pipette at each step.

Another issue was the limit of blank measured higher than the expected measurement value. It appeared that the delayed luminescence due to the environment created a background that was detected by the luminometer, although it was not an issue with the luminometer used at BioThema®. In order to avoid delayed luminescence, the cuvette has to be manipulated with a naked hand (no glove) or with forceps, and the cuvette should remain about 30 seconds in the luminometer before performing the measurement so the delayed fluorescence decreases. It is important to perform several blanks with all the solution and also empty cuvettes to make sure that the measured values are correct.

While performing the wiping procedure using ATP bioluminescence analysis, the measurement values always appeared too low while measuring ATP released from a swab after swabbing a test field. For the Dry Control, the cfu defined with the ATP analysis were log 2 – 3 too low compare to plate cultivation.

This issue could not be solved during the project. However, some explanations are proposed:

- The maximum detection value could be lower than expected.

- The swabbing could be not efficient enough at high bacteria concentration, so the swabbing could be performed with two swabs and the measured values added.
- The lysis could be incomplete at high bacteria concentration, and an increase in the concentration or volume of the lysis reagent could be necessary.
- Adjustment of ATP standard for high bacteria concentration.
- Some reagents could be actually toxic for the bacteria on surface.
- After swabbing, the surface remains slightly damp, so a dilution factor might be necessary.

PART VI: CONCLUSION

Input has been transmitted to the partners, first as editorial and technical input to the draft standard FprEN 16615, and also as evaluation of the antibacterial efficiency of the disinfectants DES45, DES70, Bacticid, Rely+On™ Virkon®, Wet Wipe Chlorine Disinfection and of the cleaning agents Hågeren and Chick (Gipeco®) towards the bacteria *S. aureus* and *E. hirae*, together with the validation results for the neutralizers.

The project aimed to give an input to the draft standard FprEN 16615. Indeed, this standard should describe a method offering an appropriate evaluation of disinfectant products. The clinical relevance of the described method could support the selection of disinfection methods adapted to the healthcare facilities. An expectation is that it will therefore contribute to the improvement of methods to reduce the incidence of HCAIs. During the project, the draft standard FprEN 16615 was evaluated with a greater focus on the practicality and clinical relevance of the method. A summary of the main input was transmitted to the committee responsible for the redaction.

The evaluation of the test products show that three disinfectants meet the requirements of FprEN 16615: DES45, Rely^{+}On™ Virkon®, and Wet Wipe Chlorine Disinfection. The results have been communicated in particular to the Karolinska Institute in Stockholm, in order to provide more reliable data on the efficiency of their cleaning and disinfecting methods in healthcare facilities.
The intended use of cleaning agents is different than that disinfecting products. Cleaning should remove dirt and debris, and is usually assessed visually in hospitals [Dancer 2008, Obee 2007, Rutala 2013]. However, it has been shown that improved cleaning can reduce incidence of HCAIs [Boyce 2007, Dancer 2008]. The cleaning agents from Gipeco® Hågeren and Chick did not meet the requirements but did present an antimicrobial activity higher than the water control. The standard FprEN 16615 is addressed not to cleaning agents but disinfectants. It was however interesting to evaluate the antimicrobial activity of these products. Tests have been conducted with non-diluted Chick and no bacterial load was detected. It could be interesting for Gipeco® to require more tests in order to define the minimum concentration of product that meets the requirements of the draft standard FprEN 16615.

The compatibility study between the wiping material and the chemical compounds emphasized that the wiping material is not always compatible with the chemical compound. The result is that some compounds may not reach the set requirements of the standard that could have been possible if the wipe had been composed with other compatible material. Also, manufacturers of both wipes and cleaning/disinfectant products should be aware of the compatibility and inform consumers.
The study of survival of bacteria emphasize the importance of considering surfaces as a source of transmission, as *S. aureus* can survive a long time on dry surfaces, for example [Kramer 2006]. Further study with *P. aeruginosa* will be interesting to compare the survival of different strains used in laboratories and evaluate disinfectants with a clinically relevant strain.

A first evaluation of the ATP bioluminescence analysis method as a future alternative to bacteria culture and colony-forming units counting has also been performed. The evaluation confirms the general conclusion in published reviews: this method is extremely promising as it is both time and cost efficient, can have a very low detection limit, and can be used for surfaces. However, some issues remain to be solved and further experimentation would be both interesting and fruitful.

REFERENCES

Adewale A. et al., Antibacterial Activities of Nonionic and Anionic Surfactants From Citrullus lanatus Seed Oil, Journal of Microbiology (2013) pages 205-208

Aiello A.E. et al., Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community, The Lancet Infectious Diseases (2003) pages 501–506

Al-Tawfiq J. A. et al., Healthcare associated infections (HAI) perspectives, Journal of Infection and Publication (2014) pages 339-344

Amodio E. et al., Use of ATP bioluminescence for assessing the cleanliness of hospital surfaces: A review of the published literature (1990–2012), Journal of Infection and Public Health, (2014) Pages 92–98

Balslev U. et al., An outbreak of borderline oxacillin-resistant Staphylococcus aureus (BORSA) in a dermatological unit, Microbiology Drug Resistance (2005) pages 78-81

Bjerkan G. et al., Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro, Acta Orthopeadica (2009) pages 245-250

Blok H.E. et al., Role of Healthcare workers in outbreaks of methicillin-resistant Staphylococcus aureus: a 10-year evaluation from a Dutch university hospital, Infection Control and Hospital Epidemiology (2003) pages 679-685

Boyce JM et al., Environmental contamination makes an important contribution to hospital infection, Journal of Hospital Infection (2007) pages 50-54

Buffet-Bataillon S. et al., Emergence of resistance to antibacterial agents: the role of quaternary ammonium compounds—a critical review, International Journal of Antimicrobial Agents (2012) pages 381-389

Calfee DP et al., Spread of methicillin-resistant Staphylococcus aureus (MRSA) among household contacts of individuals with nosocomially-acquired MRSA, Infection Control and Hospital Epidemiology (2003) pages 422–426

Carrascosa C. et al., Monitoring of cleanliness and disinfection in dairies: Comparison of traditional microbiological and ATP bioluminescence methods (2012) pages 368–373

CEN European Committee for Standardization, Who we are, www.cen.eu, online 28 Aug. 2014

CEN/TC 278 The standard making process, A step-by-step guide for people involved in the standardization work of CEN/TC 278, (2012)

Chan TS et al., Enterococcus hirae-related acute pyelonephritis and cholangitis with bacteremia: an unusual infection in humans, Kaohsiung Journal Medical Sciences (2011) pages 111–114

Chitnis V. et al., Practical limitations of disinfection of body fluid spills with 10,000 ppm sodium hypochlorite (NaOCl), American Journal of Infection Control (2004) pages 306-308

Cosmetic Ingredient Review Expert Panel, Amended final report of the safety assessment of cocamidopropylamine oxide, International Journal of Toxicology (2008)

Da Silva N., Microbiological Examination Methods of Food and Water: A Laboratory Manual; Chapter: Enterococci, CRC press (2012) pages 137-149

Dancer S.J., Mopping up hospital infection. Journal of Hospital Infection (1999) pages 85-100

Dancer SJ et al., Measuring the effect of enhanced cleaning in a UK hospital: a prospective cross-over study BMC Medicine (2009)

Dancer SJ, Importance of the environment in meticillin-resist- ant Staphylococcus aureus acquisition: the case for hospital cleaning. Lancet Infection Disease (2008) pages 101-113

Donskey C.J., Does improving surface cleaning and disinfection reduce health care-associated infections?, American Journal of Infection Control, (2013) pages S12-S19

ECDC European Centre for Disease prevention and Control, Press release: Each day, one in 18 patients in European hospitals has a healthcare-associated infection: ECDC estimates (2013)

El Ola S. et al., Unusual polymerization of 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride on PET substrates, Polymer (2004) pages 3215-3225

Eveillard M et al., Carriage of methicillin-resistant Staphylococcus aureus among hospital employees: prevalence, duration and transmission to households. Infection Control and Hospital Epidemiology (2004) pages 114–120

Exner M. et al., Household cleaning and surface disinfection: new insights and strategies, Journal of Hospital Infection, (2004) pages 70-75

Fernanandez-Crehuet M. et al., Specificity of the neutralizers as the cause of errors in evaluating disinfectant efficacy: an assessment of triclosan, Letters in Applied Microbiology (2013) pages 517-525

Gaysinsky V., Emulsions and microemulsions as antimicrobial delivery systems, Doctoral Dissertations Available from Proquest, (2007)

Guerrero D.M. et al., Acquisition of spores on gloved hands after contact with the skin of patients with Clostridium difficile infection and with environmental surfaces in their rooms, American Journal of Infection Control (2012) pages 556-558

Guh A et al., Environmental Evaluation Workgroup: Options for evaluating environmental cleaning, Centres for Disease Control and Prevention (2010)

Hatto P., The Standards Development Process, Nanotechnologies standardization committees, Standardization seminar (2011)

Hedin G. et al., Reduction of bacterial surface contamination in the hospital environment by application of a new product with persistent effect, Journal of Hospital Infection (2010) pages 112-115

Johnston C.P. et al., Epidemiology of community- acquired methicillin-resistant Staphylococcus aureus skin infections among healthcare workers in an outpatient clinic, Infection Control and Hospital Epidemiology (2006) pages1133-1136

Kampf et al., Poorly processed reusable surface disinfection tissue dispensers may be a source of infection, BMC Infectious Diseases (2014)

Kelly K.N. et al., Hospital-acquired infections, Surgery Oxford (2012) pages 640-644

Kim PW et al., Rates of hand disinfection associated with glove use, patient isolation, and changes between exposure to various body sites, American Journal of Infection Control (2003) pages 197-103

Kircheis U. et al., Testing the suitability of different types of dip slides for rapid self-control of processed endoscopes Hygiene and Medizine (2007) pages 382–388

Kramer A, How long do nosocomial pathogens persist on inanimate surfaces? A systematic review, BMC Infectious Disease (2006)

Loveday H.P. et al., Association between healthcare water systems and Pseudomonas aeruginosa infections: a rapid systematic review, Journal of Hospital Infection (2014) pages 7-15

Lundin A., Use of Firefly Luciferase in ATP-Related Assays of Biomass, Enzymes, and Metabolites, Method in Enzymology (2000)

Mann E. E. et al., Pseudomonas biofilm matrix composition and niche biology FEMS Microbiology Revuem (2012) pages 893–916

McDonnell et al., A Practical Guide to Decontamination in Healthcare, John Wiley & Sons (2012)

McDonnell, Antiseptics and Disinfectants: Activity, Action, and Resistance, Clinical Microbiology (1999) pages 147-179

Mohamed N. H. et al., Surface modification of poly(ethylene terephthalate) fabric via photo-chemical reaction of dimethylaminopropyl methacrylamide, Applied Surface Science (2012) pages 261–269

Moore N. M. et al., Epidemiology and Pathogenesis of Pseudomonas aeruginosa Infections, Clinical laboratory science (2011) pages 43-46

Mulvey D. et al., Finding a benchmark for monitoring hospital cleanliness. Journal of Hospital Infection (2011) pages 25-30

Muzslay M. et al., Dissemination of antibiotic-resistant enterococci within the ward environment: The role of airborne bacteria and the risk posed by unrecognized carriers, American Journal of Infection Control (2013) pages 57-60

Nabel A. et al., Environmentally Friendly Nonionic Surfactants Derived from Tannic Acid: Synthesis, Characterization and Surface Activity, Journal of Surfactants and Detergents (2012) pages 433-443

Norton A., Homes now 'reservoirs' for superbug MRSA, Medical Xpress (2014)

Obee P. et al., An evaluation of different methods for the recovery of meticillin-resistant Staphylococcus aureus from environmental surfaces, Journal of Hospital Infection (2007) pages 35-41

Otter J. A. et al., A guide to no-touch automated room disinfection (NTD) systems, Decontamination in Hospitals and Healthcare (2014) pages 413–460

Passaretti C.L. et al., An evaluation of environmental decontamination with hydrogen peroxide vapor for reducing the risk of patient acquisition of multidrug-resistant organisms, Clinical Infection Disease (2013) pages 27-35

Pitten F.A. et al., A standardized test to assess the impact of different organic challenges on the antimicrobial activity of antiseptics Journal of Hospital Infection (2003) pages 108–115

Pittet D. et al., Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Lancet (2000), pages 1307-1312

Randall J.O. et al., Bacterial Genomics in Infectious Disease and the Clinical Pathology Laboratory, Archives of Pathology & Laboratory Medicine, (2012) pages 1414-1422

Rutala W.A. et al., Disinfectants used for environmental disinfection and new room decontamination technology, American Journal of Infection Control, (2013) pages S36–S41

Savini V. et al., Enterococcus hirae: a zoonotic microorganism in human umbilical cord blood, World Journal of Microbiology Biotechnology (2013) pages 1423-1426

Seibert D. J. et al., Preventing transmission of MRSA: A qualitative study of health care workers' attitudes and suggestions, American Journal of Infection Control, (2014) pages 405–411

Schmidt et al., The role of continuous microbial debulking in the hospital environment and its effect on reducing hospital-acquired infections (HAI), In Decontamination in Hospitals and Healthcare, Woodhead Publishing (2014) pages 232-253

Schultsz C., et al. Ultra-sonic nebulizers as a potential source of methicillin- resistant Staphylococcus aureus causing an outbreak in a university tertiary care hospital, Journal of Hospital Infection (2003) pages 269-275

Seki M. et al., Nosocomial outbreak of multidrug-resistant Pseudomonas aeruginosa caused by damaged transesophageal echocardiogram probe used in cardiovascular surgical operations, Journal of Infection and Chemotherapy (2013) pages 677-681

Shama G., The uses and abuses of rapid bioluminescence-based ATP assays International Journal of Hygiene and Environmental Health (2013) pages 115–125

Siegel J. et al., Healthcare Infections Control Practices Advisory Committee. Management of multidrug-resistant organisms in health care settings 2006, American Journal of Infection Control (2007) pages 165-193

Sivertsen A. et al., A Multicentre Hospital Outbreak in Sweden Caused by Introduction of a vanB2 Transposon into a Stably Maintained pRUM-Plasmid in an Enterococcus faecium ST192 Clone, Plos-One (2014)

Söderblom T. et al., Surveillance and outbreak reports: Alarming spread of Vancomycin resistant enterococci in Sweden since 2007, Eurosurveillance, (2010)

Stiefel U et al., Contamination of hands with methicillin-resistant Staphylococcus aureus after contact with environmental surfaces and after contact with the skin of colonized patients, Infection Control and Hospital Epidemiology (2011) pages 185-187

Sui W. et al., Comparing the transmission potential of Methicillin-resistant Staphylococcus aureus and multidrug-resistant Acinetobacter baumannii among inpatients using target environmental monitoring (2013) pages 411-415

Sutton S., Accuracy of plates counts, Journal of Validation technology (2011) pages 42-46

Talarmin J.P. et al., Relapse of Enterococcus hirae prosthetic valve endocarditis, Journal of Clinical Microbiology (2011) pages 1182–1184

Talon D. et al., The role of the hospital environment in the epidemiology of multi-resistant bacteria, Journal of Hospital Infection (1999) pages 13-17

Tan C.K. et al., Bacteremia caused by non- faecalis and non-faecium enterococcus species at a Medical center in Taiwan, 2000 to 2008, Journal of Infection (2010) pages 34–43

Tansel O. et al., A methicillin-resistant Staphylococcus aureus outbreak in a new university hospital due to a strain transferred with an infected patient from another city six months previously, New Microbiology (2003) pages 175–180

Trautmann M. et al., Tap water colonization with Pseudomonas aeruginosa in a surgical intensive care unit and relationship to Pseudomonas infections in ICU patients, Infection Control and Hospital Epidemiology (2001) pages 49-52

Uhlemann A-C. et al., Molecular tracing of the emergence, diversification, and transmission of S. aureus sequence type 8 in a New York community, Proceedings of the National Academy of Sciences (2014) pages 6738–6743

Van Dessel et al., Outbreakof a susceptible strain of Acinetobacter species 13 (sensu Tjernberg and Ursing) in an adult neurosurgical intensive care unit, Journal of Hospital Infection (2002) pages 89-95

Van Horn K.G. et al., Comparison of 3 swab transport systems for direct release and recovery of aerobic and anaerobic bacteria, Diagnostic Microbiology and Infectious Disease (2008) pages 471-473

Wagenvoort J. et al., Better environmental survival of outbreak vs. sporadic MRSA isolates, Journal of Hospital Infection (2000) pages 231-234

Walder M. et al., A method to evaluate the cleaning and disinfectant action of surface disinfectants, Journal of Hospital Infection (1989) pages 149-159

Weber D.J. et al., Self-disinfecting surfaces: Review of current methodologies and future prospects, American Journal of Infection Control, (2013) pages S31–S35

Wertheim H. et al., The role of nasal carriage in Staphylococcus aureus infections, Lancet Infection Disease (2005) pages 751–762

WHO World Health Organization, Antimicrobial resistance: global report on surveillance (2014)

WHO World Health Organization, Report on the burden of endemic healthcare associated infection worldwide (2011)

WHO World Health Organization. A guide to the implementation of the WHO multimodal hand hygiene improvement strategy, (2009)