MICROBIOLOGICAL RESULTS OF PIP HEALTHCARE CLEANING IN A CLINICAL ENVIRONMENT

STUDY

And Microbiological Analyses By The

UNIVERSITY OF GHENT

Field Trials And Direct Testing By

LOKEREN GENERAL HOSPITAL

This Study Has Been Funded In Part By A Grant From

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With Data Analyses By The University Of Ghent Consultancy Avecom
And In Cooperation With Chrisal N.V.

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STUDY AUTHORS AND PARTICIPANTS

Prof. Willy Verstraete, University of Ghent

Established in 1817, the University of Ghent is one of the leading institutions of higher education and research in the Low Countries and is the biggest in Belgium. The University has some 26,000 students and 5,450 staff members. The University, and its hospital, in cooperation with the Lokeren General Hospital and others and with resources provided by the Government of Belgium and total access to the materials, staff and data of the solutions provider, used Avecom to assist in the analysis of the over 4,000 test data sets generated during this study.

Koen Van Landeghem, Lokeren General Hospital

The general hospital of Lokeren (AZ Lokeren) is a regional hospital providing a broad range of healthcare services to the inhabitants of Lokeren itself, as well as the surrounding communities. Since January 2005, the Lokeren General Hospital is also an active partner of the University Hospital of Ghent. The hospital has a capacity of 170 beds, with a staff of 350, of which 50 physicians. The Lokeren Hospital donated a great deal of resources for this study due to the interest in the potential benefits of the probiotic solutions studied.

Dr. ir. Wim De Windt, Avecom

All microbial analyses were performed by the Laboratory of Microbial Ecology and Technology at Ghent University in collaboration with Avecom, a consultancy group originally developed by the University which does analytical and other studies.

Dr. Robin Temmerman, Chrisal

Chrisal originally established in 1989 to develop specialized cleaning solutions and products for the food and healthcare industries, has been working for years to develop a new technology to combat the rising crisis of resistant bacteria due to the overuse of antibiotics and disinfectants. Further, the key goal was to establish a methodology to allow immune deficient people to live normal lives in their own homes. Once Chrisal's patented break-through in probiotic PIP cleaning was developed, Chrisal has offered its solutions to hospitals to combat nosocomial infections.

As secondary hospital infections cost many thousand of lives and close to a hundred million Euros each year just in Belgium alone, let alone the hundreds of thousands of lives and billions of Euros in the rest of Europe, The Government of Belgium provided a grant for a significant portion of the study as well as the Lokeren General Hospital provide its resources without payment in order to establish if the Chrisal PIP probiotic range of cleaning solutions would be a viable solution to the growing crisis of secondary (nosocomial) infections in hospitals, nursing homes and other medical facilities.

INTRODUCTION TO THIS STUDY

The intense use of disinfectants and antibiotics in hospitals has resulted in a number of highly resistant micro-organisms, which become increasingly involved in nosocomial infections of patients.

Especially Methicillin Resistant *Staphylococcus Aureus* (MRSA) and *Clostridium* are currently a huge problem in hospitals, causing severe illness and death among hospital residents. The currently used cleaning products and disinfectants are no longer effective in removing these and other (opportunistic) pathogens from hospitals.

A new range of cleaning products was developed in Belgium based on probiotic bacteria to solve this problem that now has grown to crisis proportions. In initial tests, these products appeared to be extremely effective, but before allowing expectations to rise that this could be a break-through solution to the product, rigorous testing was needed.

The organization that developed these solutions under the leadership of Corrie Gielen was Chrisal, a company in microbiological healthcare cleaning materials and systems. The company called its solutions, "Probiotics In Progress – PIP" (Chrisal PIP Healthcare® products). As the government and the hospital had a great interest in seeing if these products really could provide the required solutions, and the company itself wanted to verify whether these products actually provide an efficient alternative to 'regular' cleaning and disinfection products in a hospital environment, a formal study of the PIP products was formulated.

Under the direction of the University of Ghent and in cooperation with the AZ Lokeren hospital and Avecom, a series of study steps were formulated to properly verify these PIP solutions in a clear and independent basis. The first step was a large study as a preliminary test case in the hospital utility rooms of the AZ Lokeren hospital (done in September of 2006). This large scale study had to demonstrate that these new PIP Healthcare products are indeed capable of managing problems with (opportunistic) pathogens, especially MRSA and *Clostridium*. The concept of PIP is that of microbial management, with the aim of establishing a healthy and stable beneficial microbial community to control the environment treated, instead of trying for the unreachable and dangerous goal of disinfectants of an absolute and unconditional sterility.

In this study, during **PHASE-1**, a complete floor (a full level) of the **AZ Lokeren** hospital was cleaned for one full month with Chrisal's PIP Healthcare products and this segregated whole floor was microbiologically monitored by **Ghent University** and **Avecom**.

A full comparison was made with between the new PIP Healthcare products and the regular cleaning and disinfection products and procedures normally used by the hospital.

During **PHASE-2**, which was scheduled only "if" the initial phase was successful, to greatly expand the study, nearly the complete AZ Lokeren hospital was then cleaned with PIP Healthcare products; again with comparison to the regular cleaning and disinfection carried out with the normal cleaning products used by hospitals on the Floors of the Hospital that were used as a **"CONTROL"** for the test trials.

Due to the success of **PHASE-1**, the full study was then continued through and into **PHASE-2** for the full hospital and then beyond the initial study and used in additional buildings.

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PROJECT DESCRIPTION

SECTION

1. PRODUCT INFORMATION

This part of the report provides a brief overview of the general concept of microbiological cleaning, as developed by the company Chrisal. The mode of action and safety of the PIP products, their advantages over disinfection, as well as an overview of the products used during this study is presented.

A. CONCEPT

A broad range of pathogenic (= disease causing) micro-organisms cause numerous health problems to humans and animals. Some examples are for instance *Campylobacter, Candida, Clostridium, E. coli, Legionella*, *Listeria* (Fig 1)*, Salmonella*, *Staphylococcus* (MRSA) and *Streptococcus.* In addition to the dangers induced by these organisms in each of our personal environment, they are also responsible for a large number of economic losses due to increased animal mortality (breeding programs), reduced productivity (food industry) and increased health care costs (hospital bacterium, dust mite). Using antibiotics and disinfectants, these problems could easily be managed during the past decades. However, the past years a rapidly increasing resistance against these "miracle agents" has been noticed in all sectors, to such an extent that a radical new approach is eminent.

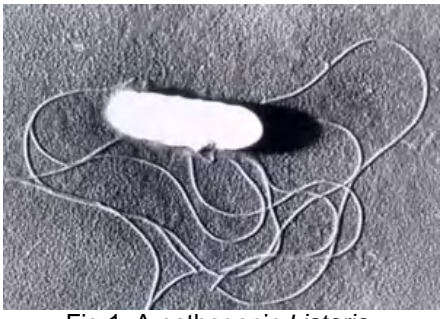


Fig 1. A pathogenic *Listeria.*

By the creation of the **PIP (*Probiotics In Progress)*** products that are the subject of this study, Chrisal has offered an apparent innovative and sustainable solution to resistance problems. These products rely on the concept of 'microbial management', in which no longer complete sterile environments are desired (and in fact, have proven ant productive in the end), but instead, a stable and healthy microbial community is created. This can be achieved by means of probiotic micro-organisms (Fig. 2). These are safe and useful bacteria or yeasts that are already known and exploited for years in food and healthcare industry because of their health promoting properties to humans and animals. By means of extensive research and validation tests, Chrisal succeeded in applying this probiotic concept to environmental applications. All PIP products contain probiotic bacteria as a crucial ingredient, which possess the unique property of sporulation. This process makes it possible for these bacteria to survive harsh conditions and regain their activity as soon as environmental parameters improve. Without this feature it would be impossible to implement probiotics into cleaning products for environmental or industrial process applications.

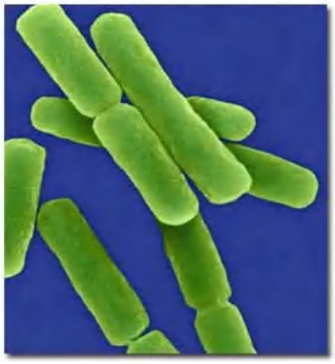


Fig 2.
Probiotic PIP bacteria

MODE OF ACTION: COMPETITIVE EXCLUSION AND QUORUM SENSING

Bacteria, especially pathogens, have a strong tendency to develop resistance to any substance that might be detrimental or lethal to them. This phenomenon is currently flagrant in case of antibiotics and disinfectants. In order to avoid such resistance development, none of the PIP products has any direct biocidal action towards other organisms. The mechanism of action is based on the principle of **"COMPETITIVE EXCLUSION"**, combined with an influence on the "**QUORUM SENSING"** communication between pathogenic organisms.

THE PROBLEMS WITH DISINFECTANTS: Especially in case of disinfectants, an important disadvantage of disinfectants is the unspecific action of these agents, killing both beneficial (good) and harmful (bad) micro-organisms. This results in an open surface, subject to fast re-colonization by harmful (pathogenic) and opportunistic bacteria. Hence, disinfection results in a fast – but also very short and unstable reduction of the number of micro-organisms. Because of the current resistance problems, continuously increasing concentrations and frequencies of disinfectant have to be applied, which is very detrimental to humans and the environment because of their aggressive chemical nature.

THEREFORE THE QUESTION:

Why are these problems not relevant with the probiotic PIP products?

The idea behind **COMPETITIVE EXCLUSION** is that during the cleaning procedure a layer of probiotic bacteria is placed on the treated surface, therefore, immediately occupying the 'field', the area treated, with beneficial (good) bacteria. These probiotic bacteria act like allied "solders" that overwhelm the area and that will consume all of the available food sources (including dead organic matter by means of necrotrophy), leaving nothing behind for potential pathogenic invaders requiring space and food. The probiotic PIP bacteria are formulated to be extremely efficient and outdo all other (pathogenic) bacteria. Additional to competitive exclusion, also, most important, **QUORUM SENSING** between pathogenic bacteria is influenced. This is an extremely fast way of communication between bacteria, making use of signal molecules. When the probiotic PIP bacteria are applied to a surface, this immediately results in the fact that pathogenic bacteria, by means of quorum sensing, are communicated about this unfavorable condition, causing them to go into an inactive metabolic state.

The PIP approach has the main advantage that it provides a stable solution to problems with pathogens, without any resistance build-up. The only demand set by this method is that the frequency of cleaning is kept constant. Using the PIP solution requires that PIP be applied at least once every three days (72 hours). However, cleaning on at least a daily basis is absolutely necessary for any hospital, medical facility, restaurant, food processing plant, etc. Therefore, this requirement is already evident for any hospital environment. It should be noted that after PIP cleaning, the total number of micro-organisms on the surface will not necessarily be higher; as **the good bacteria simply replace the bad ones**. And also that the total count may not be reduced, of course.

The following table presents a conclusive comparison between disinfection and PIP cleaning:

DISINFECTION	PIP CLEANING
- 50/50 ratio of good/bad bacteria	+ 95/5 ratio of good/bad bacteria
- Short effect (unstable effect)	+ long lasting effect (stable effect)
- Resistance problems	+ no resistance possible
- Detrimental / unsafe products	+ harmless / safe products
- Chemical / environment unfriendly	+ biological / environment friendly
- Aggressive	+ neutral

b. SAFETY ASPECTS

PIP products are demonstrated to be completely safe to use. Several reasons are:

- The probiotic bacteria used in the PIP products are members of the genus *Bacillus* and belong to **biosafety class 1, as listed by the American Type Culture Collection (ATCC). The following table presents all four bio-safety classes:**

CLASS	DESCRIPTION	RISK
1	NON-PATHOGENIC MICRO-ORGANISMS	NONE
2	Micro-organisms and parasites that may cause disease, but with an unlikely spread and for which efficient prophylaxis or treatment exists.	Low
3	Micro-organisms and parasites that are able to spread and cause disease, but subjective to efficient prophylaxis or treatment	Average
4	Micro-organisms and parasites with large scale spreading and serious illness, for which no prophylaxis or treatment exists.	High

- **A NUMBER OF PROBIOTIC *BACILLUS* SPECIES HAVE BEEN GRANTED THE GRAS (GENERALLY RECOGNIZED AS SAFE) LABEL BY THE FOOD AND DRUG ADMINISTRATION (FDA) AND CAN AS SUCH BE USED FOR HUMAN PURPOSES WITHOUT ANY HAZARD**.
- **THE PIP BACTERIA BELONG TO THE GROUP OF SPORULATING PROBIOTICS, OF WHICH OVER HUNDRED COMMERCIAL PHARMACEUTICAL AND NUTRITIONAL PRODUCTS ARE AVAILABLE FOR HUMAN ORAL CONSUMPTION**. A regular dose of these preparations is 10 billion bacteria per day, which is about 10.000 x more concentrated than the PIP products.

- Additional to the safety classification by ATCC, the producer of Chrisal's PIP bacterial strains performed a large number of toxicity tests to guarantee the safety of PIP bacteria. **No single toxic effect** from any of PIP's *Bacillus* strains was ever detected.
- In addition to all the testing done in this study, Chrisal itself, in collaboration with external and accredited laboratories, performed an ongoing series of multiple safety tests, all of which data has been available to the study group and others. In all these tests, all the PIP products were certified as safe to use.
- In view of antibiotic resistance, *Bacillus* strains are Gram-positive organisms, which have much less tendency to develop, acquire or transfer antibiotic resistance. Although certain *Bacillus* strains are intrinsically resistant to certain cephalosporin, macrolide and quinolone antibiotics, from scientific literature, it can be concluded that in all the history of research and studies through to this moment, no *Bacillus* strains are known to transfer this antibiotic resistance to other organisms, neither *in vitro* nor *in vivo*.
- Members of the genus *Bacillus* are used intensively in different kinds of industries because of their high enzyme production capacity. Examples are in food preservation, as well as in washing powders, waste water treatment, and other such usese…

IN CONCLUSION

The probiotic PIP bacteria are perfectly safe to use. These organisms have been officially classified as "save organisms" and have been used for decades without any negative effect. During the course of this specific study patients were not washed directly with these products and so did not come into contact with the cleaning products themselves. However, a direct contact with the PIP bacteria was possible through the treated surfaces in the patent areas. Given the fact that the PIP beneficial bacteria replace pathogenic bacteria, the only result of a patient's contact with any of these surfaces treated with PIP is a lower chance of contact with pathogens.

C. PRODUCT RANGE

During the course of this study, the following **PIP Healthcare® products** were used:

- **PIP FLOOR CLEANER NFG**: This floor cleaner is a probiotic bacteria containing product, with a neutral composition suitable for all kinds of floors. The chemical composition is consultable in the MSDS file on demand; the number of probiotic bacteria is 30 million CFU/ml, with a dilution factor depending on the type of application (average of 2%). Dilution has to be done using water of approximately 40°C.
- **PIP INTERIOR (ALL-PURPOSE) CLEANER**: This product has a neutral composition, making it suitable for all kinds of materials and surfaces. The chemical composition is again available through the MSDS file on demand; the bacterial composition is equal to the above mentioned Floor Cleaner.
- **PIP DAILY SANITARY CLEANER**: This cleaner is suitable for all kinds of sanitary installations and contains a higher concentration of probiotic PIP bacteria. This in order to compensate for the increased washout because of the running water in the installation. The bacterial concentration of the sanitary cleaner totals 50 million CFU/ml.
- **PIP ALLERGY FREE SPRAY**: This product has been developed to render any kind of textile free of pathogenic bacteria, as well as dust mite allergens. The product contains 50 million CFU/ml of PIP bacteria and has to be sprayed on the textile during 3 seconds.

The formulation and dilution factors for each of the above products have been calculated in such a way that the final concentration of probiotic bacteria on the treated surfaces equals as much as possible the concentration of residual micro-organisms before application. By means of precision pumps mounted on the cans, a reproducible dosage could be obtained throughout the study.

Photograph of the PIP Products Tested in this Study

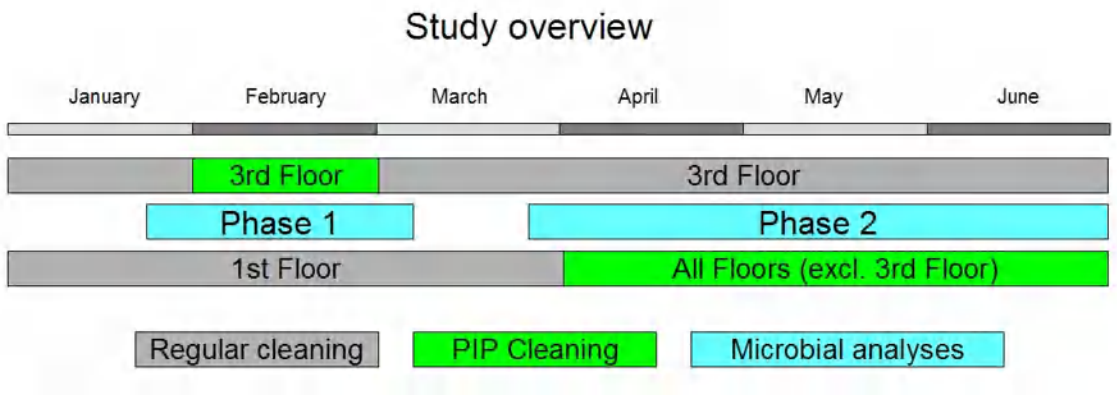
2. STUDY PROTOCOL

The study, reported in this document, followed on a preliminary test that was carried out in September 2006 to verify the potential of the **PIP Healthcare®** products for microbiological cleaning of a clinical environment. That test provided such positive results that it was immediately decided to perform a large scale study in order to demonstrate the efficiency of the PIP products, compared to classical cleaning procedures.

BECAUSE THIS STUDY ALSO IMPLIED THE CLEANING OF PATIENT ROOMS, THE ETHICS COMMITTEE (REGISTRATION NUMBER 0G217) OF THE AZ LOKEREN HOSPITAL HAD TO APPROVE THIS STUDY, WHICH WAS DONE ON THE 9TH OF JANUARY 2007, AFTER CAREFULLY EVALUATING THE DOSSIER. THIS STUDY HAS BEEN GRANTED THE FOLLOWING CLINICAL TRIAL NUMBER: B2652006814

THE OVERALL STUDY PRESENTED IN THIS REPORT COMPRISES TWO PHASES:

- **PHASE 1**: Instead of one utility room, **THE ENTIRE THIRD FLOOR** of the AZ Lokeren hospital was treated with Chrisal's PIP products and compared to the first Floor, harboring an equal patient type.
- **PHASE 2**: Following the first Phase, a buffer period of one month with overall regular cleaning was inserted. Subsequently, the entire hospital was cleaned with the PIP products, except for the third Floor now serving as a control.



The next part of this report provides an overview of the study protocol, addressing the following items:

- Location: information on AZ Lokeren, trial hospital for this study
- Cleaning schedule: according to which time schedule and hygienic guidelines was cleaned during the course of this study.
- Microbial analyses: which micro-organisms were screened for and which sampling procedure was applied.

- Patient monitoring: How were patients monitored during the course of the study?
- Report: How were the results interpreted and processed into this report.

A. TRIAL HOSPITAL: AZ LOKEREN

The general hospital of Lokeren (AZ Lokeren) is a regional hospital providing a broad range of healthcare services to the 37.500 inhabitants of Lokeren itself, as well as the surrounding communities. Since January 2005, AZ Lokeren is also an active partner of the university hospital of Ghent. The hospital has a capacity of 170 beds, with a staff of 350, of which 50 physicians.

THE FOLLOWING DIVISIONS AND SERVICES ARE PART OF THE HOSPITAL'S SYSTEMS:

- **SURGERY**
- **INTENSIVE AND MEDIUM CARE**
- **INTERNAL MEDICINE**
- **MATERNITY**
- **PAEDIATRICS**
- **DAY CARE**
- **EMERGENCY UNIT**
- **MEDICAL LABORATORY**
- **MEDICAL IMAGERY**
- **PALLIATIVE CARE**

PHASE 1 of the study was performed at the **1st and 3rd Floor** of the hospital, which Harbour the internal medicine department. Both floors have a capacity of 31 beds **for patients suffering from diseases of the heart, digestive tract, longs, joints, skin and illnesses such as diabetes.** Because of the same kind of patients on both Floors, the microbiological load is assumed to be similar and representative for this study. This was already verified during the preliminary study and the microbiological analyses preceding the actual trial.

During phase 2, nearly the entire hospital was subjected to PIP cleaning, comprising different pathologies and patient types. This facilitated the evaluation of PIP cleaning under different microbiological loads.

B. CLEANING SCHEDULE

During **PHASE 1** of this study, two similar floors (geriatrics) of the AZ Lokeren hospital were chosen:

- The **1st floor served as control**, with regular cleaning.
- The **3rd floor was subjected to PIP cleaning**.

During **PHASE 2** of the study, after one month of overall regular cleaning, PIP cleaning was expanded:

- The **3rd floor served as control**
- The **rest of the hospital was subjected to PIP cleaning** [excl. Operation units (completely) and Intensive Care, Maternity, Radiology (only the floor was PIP cleaned)]

Although not presented in detail, **cleaning schedules** as designed by AZ Lokeren were identical for all Floors and **remained unaltered** during the course of the study. Only a replacement of the regular products by **PIP Healthcare®** products was done; except for those on the control Floor. By means of precision pumps mounted on the cans, a reproducible dosage could be obtained throughout the study.

- On weekdays the complete Floor was cleaned following a strict schedule. All floors, sanitary and furniture were cleaned.
- During weekends, only patient rooms were cleaned completely following the weekday schedule. Central hall and general areas were not cleaned.

Special cleaning protocols exist for contaminated rooms (e.g. hepatitis, MRSA,…), mostly describing a disinfection step each day of the patients stay, followed by a thorough disinfection of all surfaces and furniture in these rooms after discharge of the patient. Although Chrisal's PIP products have been developed as an alternative to disinfectants, the ethics committee decided not to omit disinfection protocols in case of contaminated patients. Although encountered with low frequency, each disinfection step that occurred during the study was registered.

The most important aspect of this study is that **all cleaning procedures remained the same** during this study; only the products were replaced by those of Chrisal. Doing so, it became possible to obtain a reliable comparison between the performance of regular cleaning and disinfection products with that of Chrisal's **PIP Healthcare®** products.

C. MICROBIAL ANALYSES

All microbial analyses were performed by the Laboratory of Microbial Ecology and Technology (Ghent University), in collaboration with the consultancy company Avecom. The applied sampling and analyses protocols in this study are equal to those of the preliminary test case in the utility rooms. These protocols proved to be efficient and reproducible.

SAMPLING PROCEDURE:

Samples were taken **23 hours after cleaning** by means of sterile swab plates of 30 cm², moisturized by means of 3 ml of sterile physiological solution/swab. After 3 minutes of contact with the surface, each plate was transferred to a sterile Petri dish and transported to the lab for microbial analyses. Each sampling was performed in triplicate in order to deliver statistically significant quantification. Upon arrival, swab plates were immediately placed on selective growth media for three minutes, after which these media were incubated at the proper temperature and atmosphere. After the correct incubation time for each of the organisms to determine, colonies on all plates were manually counted.

THE FOLLOWING SELECTIVE GROWTH MEDIA WERE USED:

1. **TRYPTICASE SOY:** Non selective medium for the determination of the **total count** of bacteria on the sampled surfaces. All colonies were counted and provide information on the amount of PIP bacteria that remain on the treated surfaces.

2. **MCCONKEY**: Elective medium for the quantification of **coliform bacteria**, with *E. coli* as type organism. On this medium, all colonies were counted. This provides information on the fecal contamination of the sampled surfaces.

3. **BAIRD PARKER***:* Selective medium for the determination of ***Staphylococcus aureus***. Positive counts are visible as brown, halo-surrounded colonies. These counts provide information on the potential MRSA load on the sampled surfaces.

4. **CLOSTRIDIUM DIFFICILE AGAR**: Selective medium for the detection of ***Clostridium difficile*****.** Positive counts are visible as grey-white coloured colonies. These organisms are detected after anaerobic incubation.

During PHASE 1 of this study, only media 1, 2 and 3 were used; during PHASE 2 also *Clostridium* was determined.

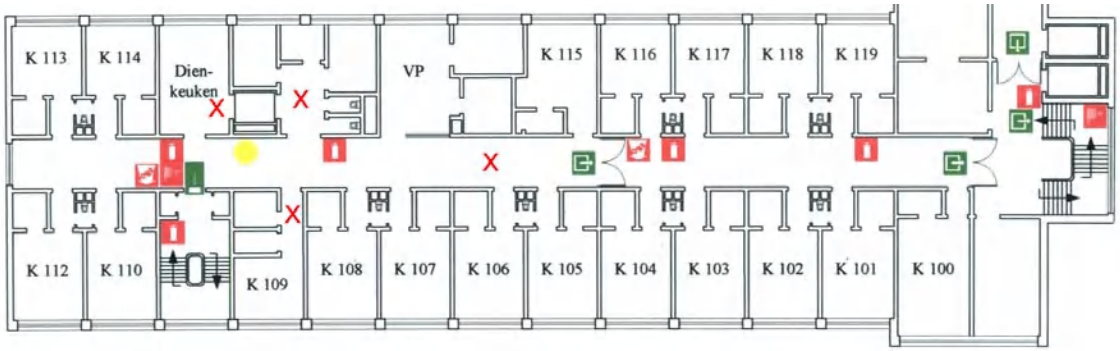
PHASE 1: SAMPLING POINTS AND FREQUENCIES

Sampling points were at all times identical for both the 1st and 3rd floor. Each sampling day, 5 or 6 points were sampled, of which **4 fixed points** and 1 or 2 variable locations. The fixed points were always floor samples, whereas the **variable points** comprised a broad range of samples such as a lavatory, sink, shower, table, bed, mattress, tray…

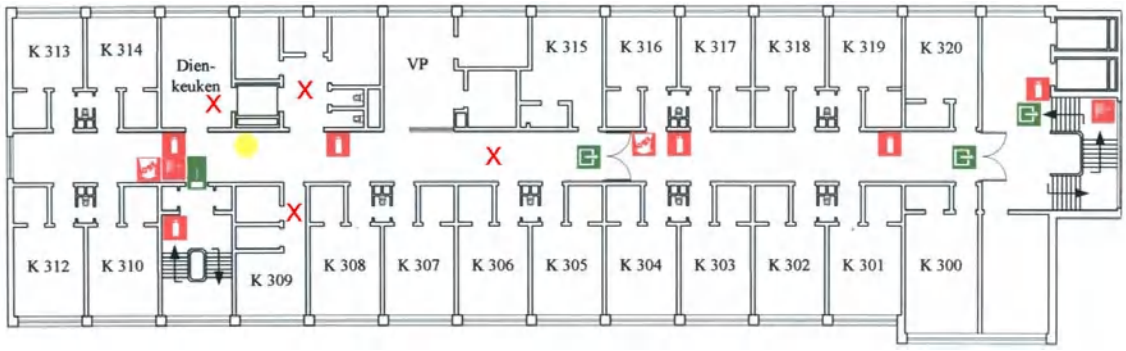
Fixed sampling points at the 1st and 3rd floor: (Marked with a red x on the maps below)

- Hall, at the centre of the 1st and 3rd floor
- Room 109/309, at the entrance to the room and lavatory
- Kitchen, in front of the service elevator
- Utility room, in the middle of the room

1st floor:



3rd floor:



VARIABLE SAMPLING POINTS AT THE 1ST AND 3RD FLOOR:

Variable points were taken each time at a different location, but were always identical for both Floors. The following table gives an overview of all variable locations sampled during the course of this study (the exact locations can be determined on the above displayed maps).

Sampling day	Variable location	Type of sample
1	Rooms 110/310	Floor
	Room 309	Mattress
2	Rooms 112/312	Floor
3	Rooms 113/313	Floor
4	Rooms 114/314	Floor
5	Rooms 115/315	Floor
6	Rooms 108/308	Floor
7	MRSA Rooms 115/314	Floor
	MRSA Rooms 115/314	Service tray
	Hallway chair	Leather surface
8	Rooms 109/309	Mattress
9	Rooms 116/316	Bed pushing bar
	Rooms 116/316	Sink in lavatory
10	Central desk	Floor

The actual cleaning with Chrisal's PIP products started on **Tuesday the 6th of February 2007 at 8 am**. From that day onward, samples were taken daily during the first week, and twice a week during the following weeks (on Mondays and Thursdays). **Sampling time was 7.30 am, just before the start of the next cleaning procedure.** This allowed those of us in the study group to determine the minimum effect of the PIP products.

PHASE 2: SAMPLING POINTS AND FREQUENCIES

Between **PHASE 1 and 2** a buffer period was inserted during which the entire hospital was again cleaned using regular cleaning products. From **April 11th** onward, **PHASE 2** started, with PIP cleaning of the entire hospital, except for the 3rd floor (serving as control) and a few critical divisions.

During this PHASE, only fixed sampling points were chosen, that were sampled each Tuesday and Thursday at 7.30 am, before the start of the next cleaning round. Additional to **PHASE 1**, also *Clostridium* was monitored during **PHASE 2.** The following sampling points were chosen:

1. **Emergency**
2. **Maternity**
3. **1st Floor**
4. **2nd Floor**
5. **3rd Floor (= control Floor; regular cleaning)**
6. **4th Floor**
7. **5th Floor**

NOTE - ALL SAMPLES WERE TAKEN ON THE FLOOR IN THE MIDDLE OF THE CENTRAL HALLS OF THESE DIVISIONS.

D. PATIENT MONITORING:

Because the applied PIP bacteria have no history of any pathology (class 1 organisms, see above), no specific parameter is available to monitor with patients. Furthermore, the actual concentration of PIP bacteria the patients might have had contact with, is not higher compared to the residual microbiota previously present. Only the percentage of pathogens is lower during the study.

Considering the safety of the applied bacteria and the very low dosage to which patients are exposed, a close clinical monitoring of patients was not done. However, prior to the start of this study, all physicians and nursery staff were briefed on the upcoming trial. This facilitated a proper diagnosis of potential complaints of patients and the verification whether these were due to the patients' reason for internalization or due to the study.

In order to inform patients, a brochure was distributed explaining the ongoing study and the potential visit of a laboratory technician for sample taking. Also, the necessary contact information was provided in case additional questions should arise.

E. REPORT

All communications and reports were facilitated by the laboratory, in collaboration with Avecom as a consultancy company. The obtained results were provided immediately to the hospital and to Chrisal, in order to evaluate the proceeding of the study concerning efficiency and safety to the patients and personnel.

After finishing the study, Avecom, on the University's behalf, collected all of the generated data during the study in order to prepare the final report (i.e. the present document).

CONCLUSION

This study was financed in part by the Belgium Government, in part by the fact that in the interest of public safety, the hospital did not charge fees for its services and the remainder by the company Chrisal, who had initiated the study in order to validate the potential of its new range of probiotic cleaning products, to establish a healthy and stable microbiota in a clinical environment. In order to assure an independent report and to obtain reliable results, all microbial analyses, data processing and reporting has been handled and processed by the Ghent University and Avecom.

A presentation and discussion of the obtained results can be found in parts 2 and 3 of this report.

PART 2

STUDY PROJECT

RESULTS – PHASE 1

SECTION

1. INTRODUCTION

This part of the report presents the results obtained during PHASE 1 of the study.

First, an overview is given of the microbiological results of the four **fixed sampling points**. These are presented as bar plots over time, with three graphs for each of the sampling points: total count, coliform bacteria and *Staphylococcus aureus.* Further on in this report, the total number of *Staphylococcus aureus* measured by plate counting on Baird-Parker agar is referred to as total MRSA, although stricto sensu MRSA refers to 'Methycillin Resistant *Staphylococcus aureus*'. Each graph contains the number of colony forming units per square meter of surface (= **CFU/m²**) of both the 1st (regular cleaning = control) and 3rd floor (PIP cleaning).

Second, microbiological results of the **variable sampling points** are presented by means of tables. Each sampling point has its own table containing the results for the total count, coliform bacteria and *Staphylococcus aureus.* These results are presented as the number of colony forming units per square meter of surface (= **CFU/m²**) of both the 1st (regular cleaning = control) and 3rd floor (PIP cleaning).

All **results are the average values of triplicate sampling and analysis**. These threefold analyses provide standard deviations, demonstrating the statistical significance of each measurement. These deviations are presented by means of error flags in the graphs.

2. MICROBIAL ANALYSES

a. Fixed sampling points

The first section of the results part of this report presents the obtained microbiological data from the fixed sampling points. Because these points were followed in time, a graphical representation is possible. For each of the sampling points, three graphs are given corresponding to the total count, coliform count and *S. aureus* count. Values are averaged over triplicate analyses and present the results from the 1st (= control) floor (black bars) and the 3rd (= PIP) floor (green bars). All fixed samples were taken on the floor.

Important remark:
Day 1 in the graphs corresponds to the starting situation just before the start of PIP cleaning. Day 2 is the first measurement after PIP cleaning.

i) Sampling point 1: Central hall

A logical fixed sampling point was the central hall, serving as a passage to all medical personnel, patients and visitors. This sampling point is most likely subject to the highest microbial load of the entire floor, with high a potential of cross-contamination.

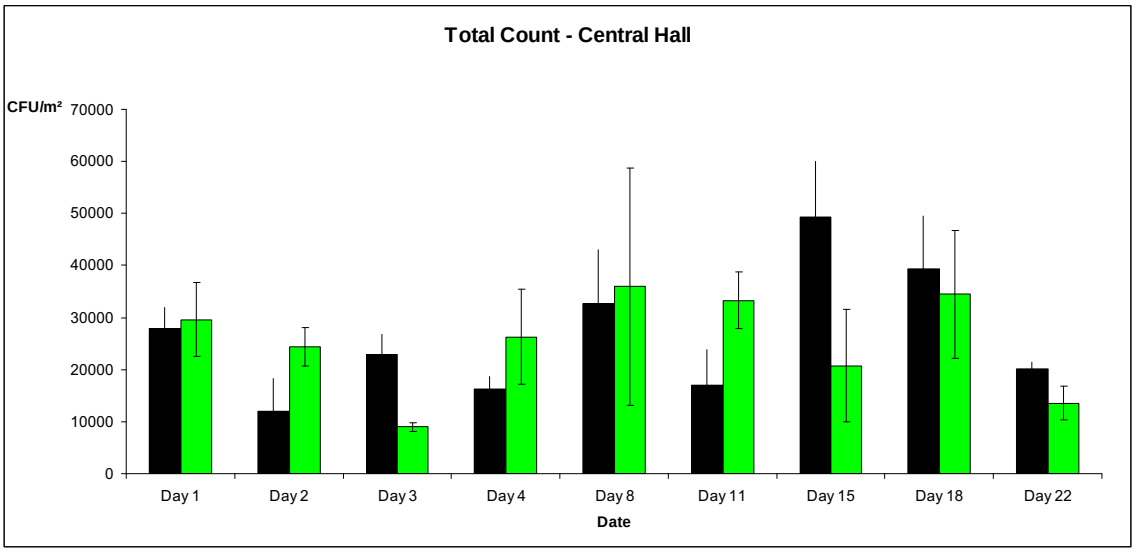


Fig 1. Total count in the central hall of the 1st floor (control, black bars) and 3rd floor ('PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

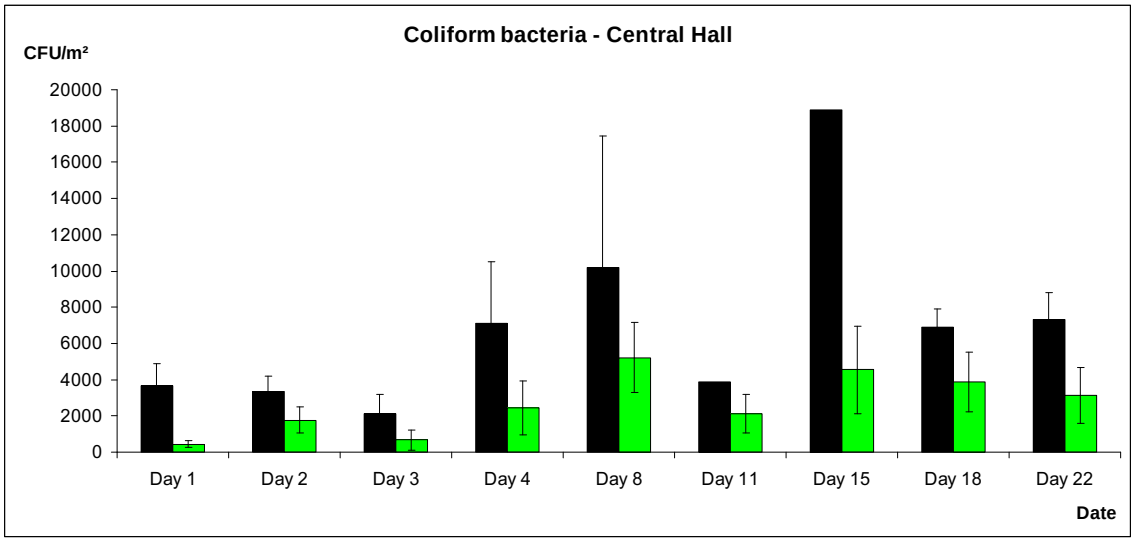


Fig 2: Coliform count in the central hall of the 1st floor ('control', black bars) and 3rd floor ('PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

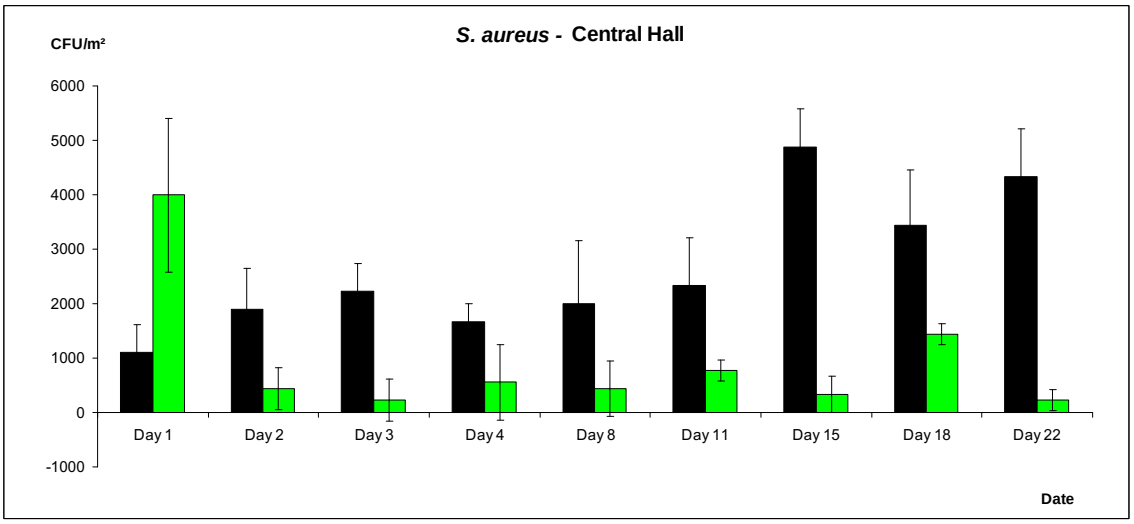


Fig 3: Total *Staphylococcus aureus* count (MRSA) in the central hall of the 1st floor ('control', black bars) and 3rd floor ('PIP' cleaning, green bars).

Cleaning with PIP products from Chrisal started on Day 2.

CENTRAL HALL CONCLUSIONS:

- Cleaning of the central hall floor of the hospital with **PIP Healthcare®** cleaning products significantly altered the microbial community.
- **Total count did not statistically change**, which means that the total number of bacteria on the surface is not necessarily higher during PIP cleaning (Fig. 1).
- After PIP cleaning, the number of **coliform bacteria was on average 60 % lower** compared to regular cleaning (Fig 2).
- The number of **MRSA bacteria was on average 78 % lower** compared to regular cleaning on Floor 1 (Fig 3).
- These lower numbers of coliform and MRSA bacteria did not demonstrate any significant fluctuations, indicating **a stable PIP effect**.
- It is obvious from this study that since PIP treatment started, there were no situations where coliform or MRSA numbers in the PIP-cleaned hall surpassed the numbers of the hall on control Floor 1. Before PIP cleaning started, this was not necessarily true, as can be observed from the MRSA number (3996 +/- 1413 CFU/m^2 on Floor 3 compared to 1110 +/- 509 CFU/m^2 on Floor 1). These results indicate that **PIP cleaning creates a safer microbial environment**.

It can be concluded that in the central hall, a location with high potential of cross-contamination due to busy passage of both hospital personnel and patients, **PIP-based cleaning resulted in a stable reduction of coliform and MRSA bacteria, thereby resulting in a healthier microbiological environment.**

II) SAMPLING POINT 2: ROOMS 109/309

Patient rooms 109 and 309 on the 1st and 3rd floor, respectively, were selected for continuous microbiological survey during the study. No specific type of patients was placed in these rooms. However, it was avoided to harbour MRSA infected patients in one of these rooms because this might impair the stability of microbiological data obtained.

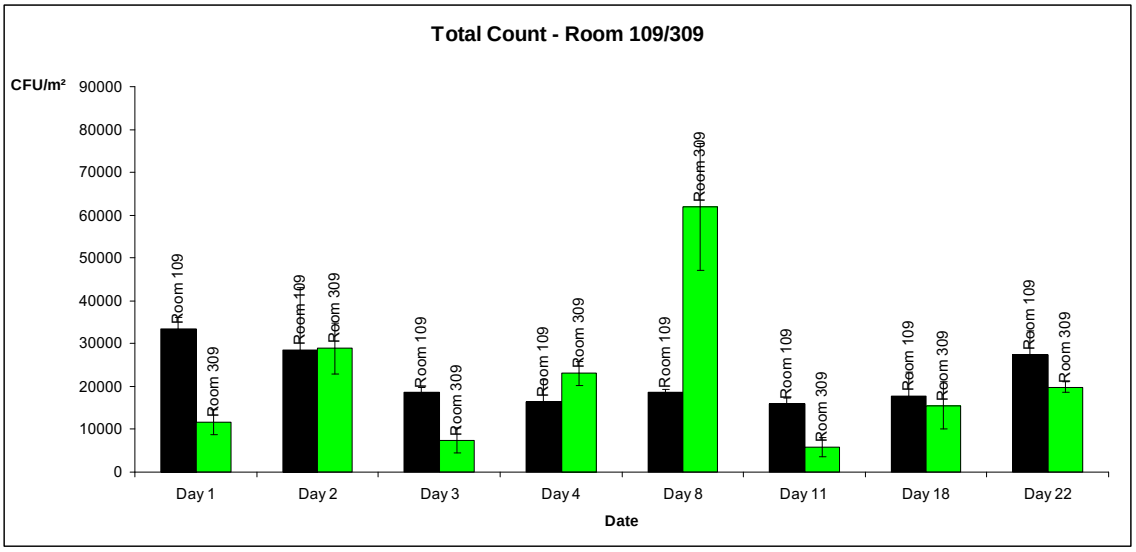


Fig 4: Total count in Room 9 of Floor 1 (Room 109 'control', black bars) and Floor 3 (Room 309 'PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

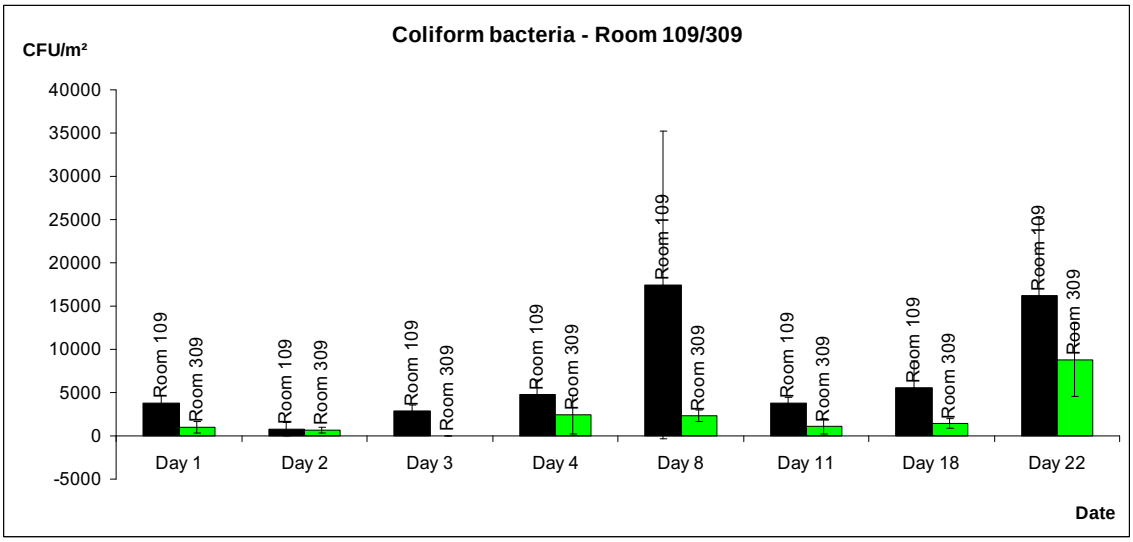


Fig 5: Coliform count in Room 9 of Floor 1 (Room 109 'control', black bars) and Floor 3 (Room 309 'PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

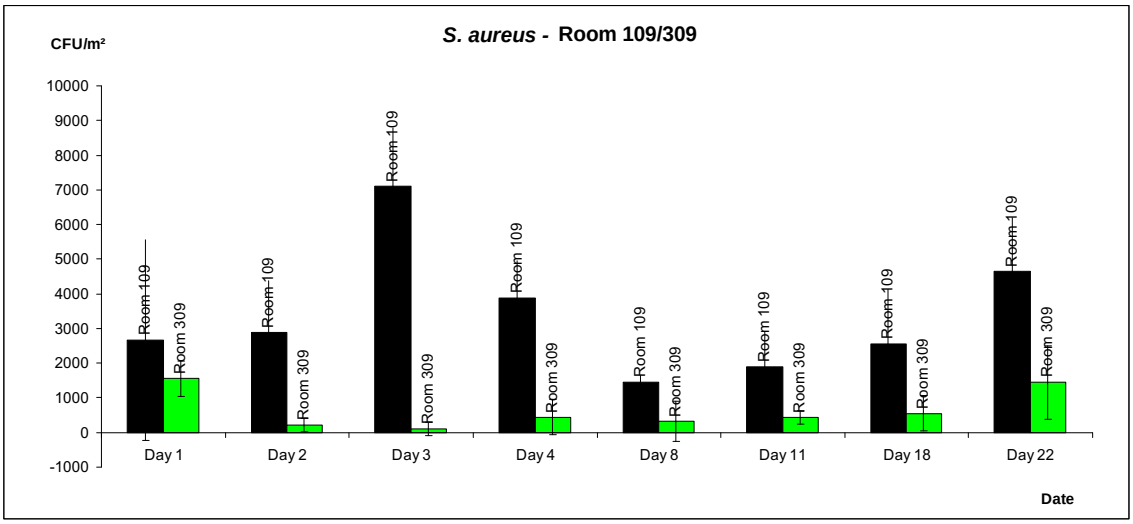


Fig 6: ***Staphylococcus aureus*** **count (MRSA)** in Room 9 of Floor 1 (Room 109 'control', black bars) and Floor 3 (Room 309 'PIP' cleaning, green bars).
Cleaning with PIP products from Chrisal started on Day 2.

PATIENT ROOM CONCLUSIONS:

Daily cleaning of patient room 309 with Chrisal **PIP Healthcare®** cleaning products:

- Did not influence the total count of the bacteria (Fig. 4)
- Resulted in a **lower number (- 64%) of coliform bacteria** compared to the control room (Fig. 5).
- Resulted in **78% less MRSA** over time, compared to regular cleaning (Fig. 6).
- **Total count fluctuated** during the test; this could possibly be related to bacteria originating from 'variable' sources such as the patients in the room.
- **Coliforms** exhibited an average viable cell number that was **64 % lower** in the patient room 309 in comparison to room 109. However, the number of coliforms remained equal in room 309 over time, due to the low starting number at day 1.
- The lower coliform and MRSA numbers associated with PIP-based cleaning **remained stable** and did not exhibit sudden fluctuations.

It can be concluded that in the patient rooms, a location with a high possibility of cross-infection to other patients, **PIP cleaning resulted in a large and stable reduction of pathogenic coliform and MRSA bacteria, thereby creating a healthier microbiological environment.**

III) SAMPLING POINT 3: KITCHEN

Although the hospital has one main kitchen on the ground level preparing all meals, each floor is equipped with a small kitchen in order to do some final handlings of the food before distribution to the patients. Also, the hospital staff consumes their meals in this kitchen. The samples were taken in front of the service elevator door, through which all food is delivered and waste is sent back to the main kitchen. Important remark: these kitchens were only cleaned once a week, on Wednesday, meaning that samples taken on Thursday should demonstrate a lower microbial contamination, compared to those taken on Mondays.

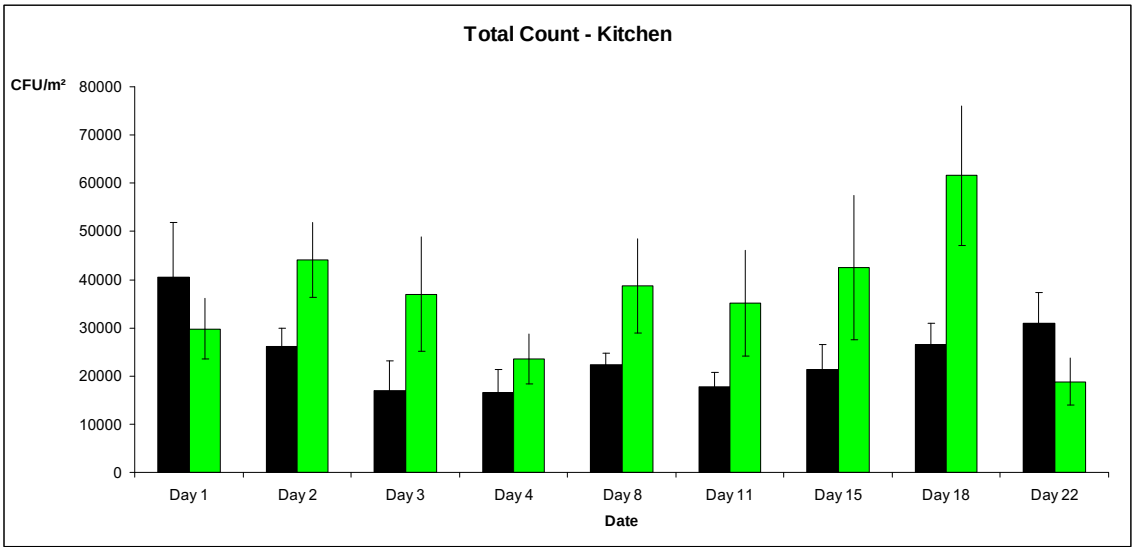


Fig 7: Total count in the kitchen of the 1st floor (control, black bars) and the 3rd floor ('PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

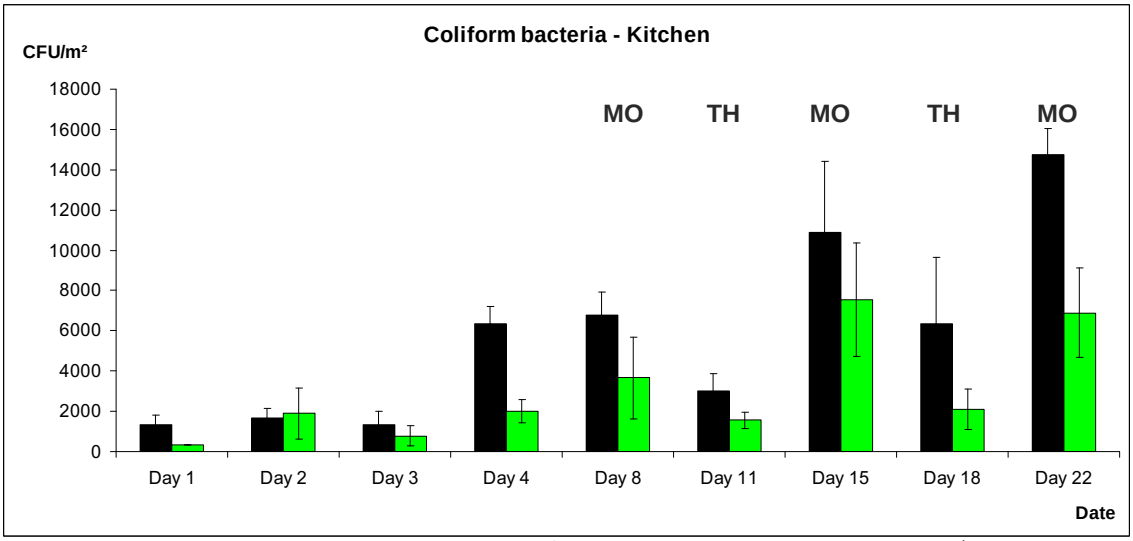


Fig 8: Coliform count in the kitchen of the 1st floor (control, black bars) and the 3rd floor ('PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

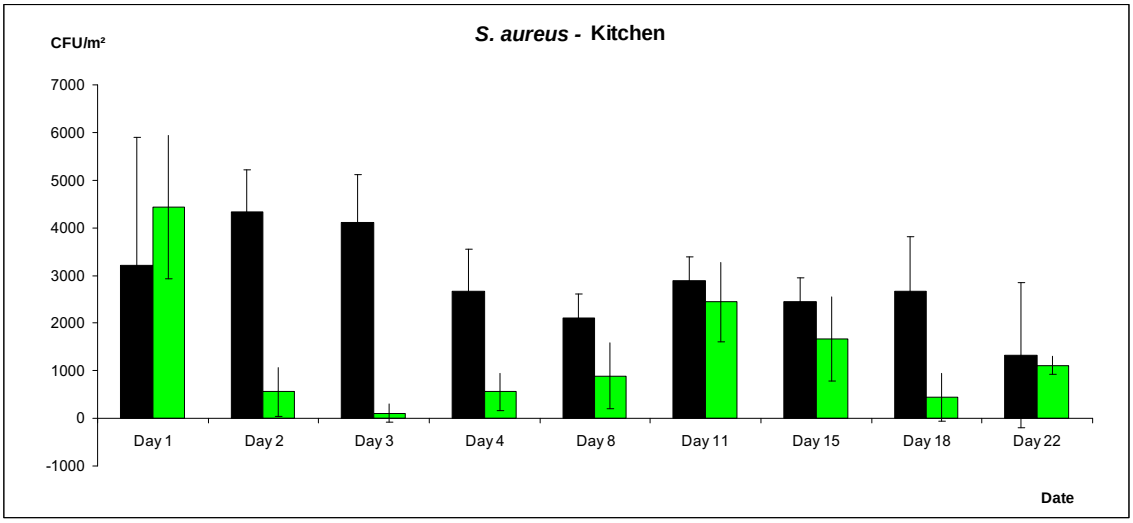


Fig 9: ***Staphylococcus aureus*** **count (MRSA)** in the kitchen of the 1st floor (control, black bars) and the 3rd floor ('PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

KITCHEN CONCLUSIONS:

The kitchen was cleaned only once a week with PIP cleaning products, and resulted in the following:

- **Total count was higher** on the 3rd Floor (Chrisal) compared to the control Floor (Fig. 7).
- The average **number of coliforms** on the 3rd Floor was **46 % lower** than the average coliform number on the 1st Floor.
- The number of viable **MRSA bacteria was about 48 % lower** on the Chrisal Floor, compared to the control Floor.
- In contrast to the central hall and patient rooms, **significant fluctuations** in MRSA and coliform numbers occurred, **both on the control floor and PIP floor**, indicating that a weekly application of PIP cleaning cannot guarantee a stable and healthy environment and that the cleaning frequency needs to be increased.
- Compared to the results of the central hall and patient rooms, pathogen reduction by PIP products was lower in the kitchen. This indicates that daily PIP cleaning is required to obtain high pathogen reduction.

PIP cleaning of the kitchen on the 3rd Floor resulted in a **lower number of pathogenic bacteria**. However, because of the low cleaning frequency, this effect is insufficiently stable to guarantee a safe environment. **Daily PIP cleaning is advised** in order to obtain a stable and healthy microbiota.

IV) SAMPLING POINT 4: UTILITY ROOM

Each Floor is equipped with a utility room, serving as a collecting point for temporary storage of all medical waste or biologically contaminated equipment. Also, showers and toilets are accessible through the utility room. These rooms were already used in a preliminary study to verify the potential of the Chrisal products compared to disinfection and regular cleaning.

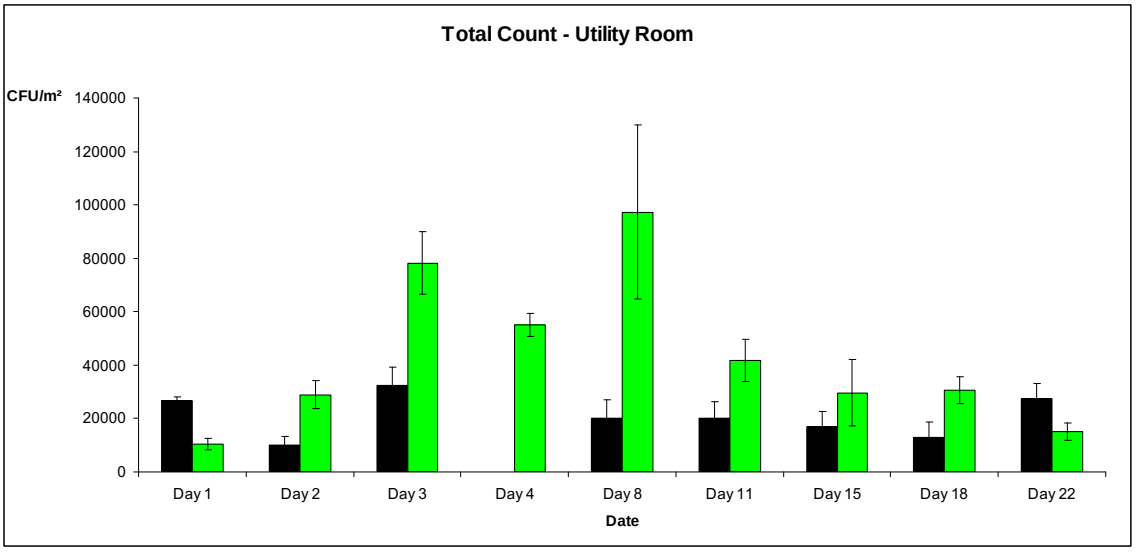


Fig 10: **Total count** in the Utility Room of the 1st floor (control, black bars) and the 3rd floor ('PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

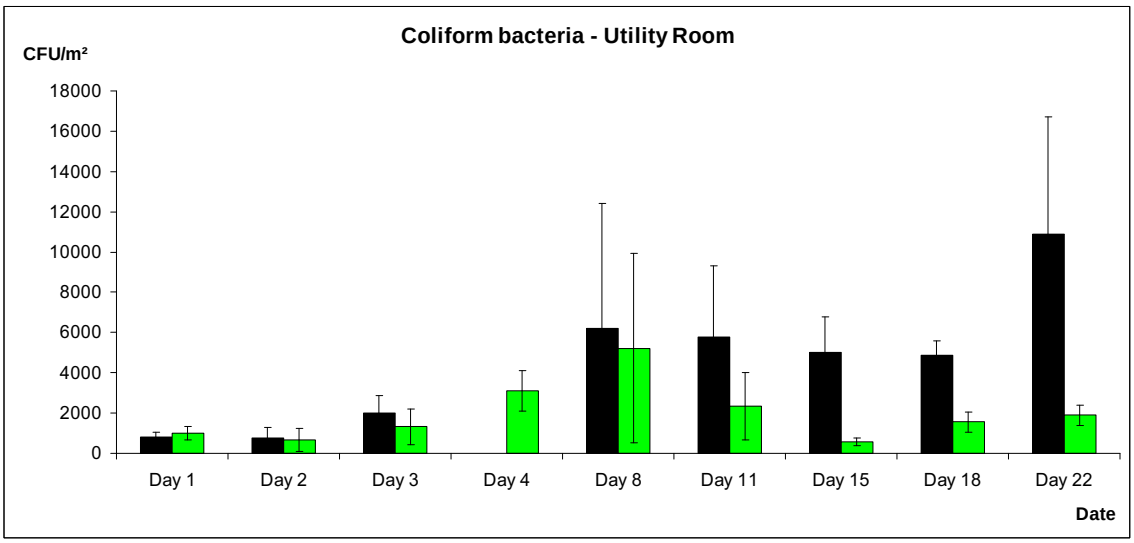


Fig11: Coliform count in the Utility Room of the 1st floor (control, black bars) and the 3rd floor ('PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

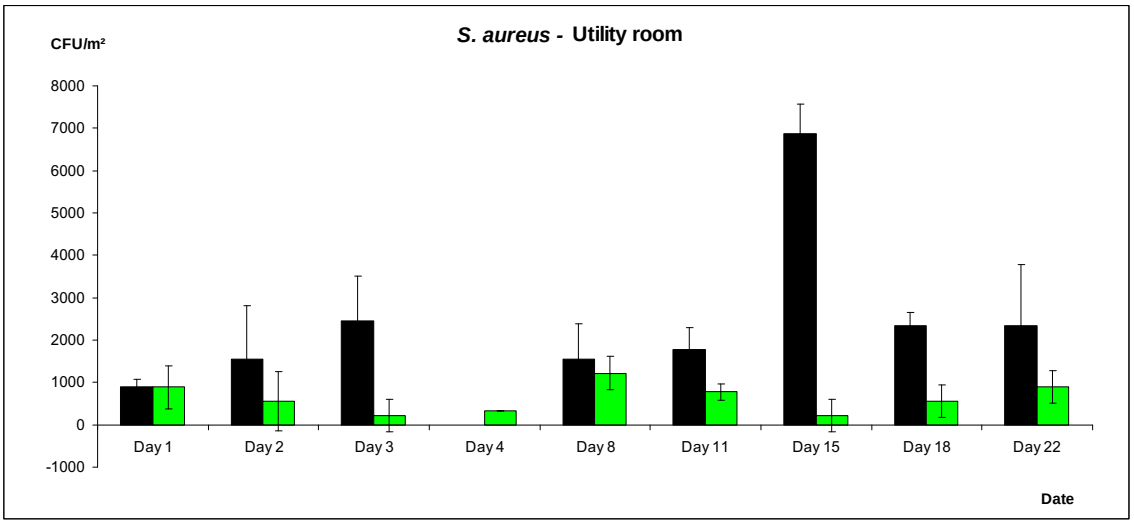


Fig 12: *Staphylococcus aureus* count (MRSA) in the Utility Room of the 1st floor (control, black bars) and the 3rd floor ('PIP' cleaning, green bars). Cleaning with PIP products from Chrisal started on Day 2.

UTILITY ROOM CONCLUSIONS:

- During PIP cleaning, the **total viable cell count** in Utility Room on the 3rd Floor (PIP) was on average **43 % higher** than on the 1st Floor (Fig. 10).
- **Coliform bacteria** were on average **51 % lower** on the 3rd Floor (Fig. 11)
- **MRSA bacteria** were on average **67 % lower**, compared to Floor 1 (Fig 12).
- **Some fluctuations** during PIP cleaning were observed for all cell counts; these may have several reasons:
  - The UR is a heterogeneous environment with strongly fluctuating degrees of contamination (waste storage, shower/toilets)
  - At the start of the cleaning procedure, the maintenance carts are prepared and loaded with PIP products in these rooms (some spilling may occur at the place of sampling, altering the concentration of PIP bacteria on the surface)
  - At the end of the cleaning procedure, all the dirty water is collected and removed through these rooms; spilling might enrich the pathogenic numbers

This study shows that PIP cleaning on a daily basis results in lower coliform and MRSA numbers that were rather stable despite the strongly fluctuating conditions of the Utility Rooms. **It can be concluded that PIP cleaning is able to manage constantly changing bacterial populations, resulting in lower numbers of pathogenic genera and species.**

B. VARIABLE SAMPLING POINTS

In contrast to the fixed sampling points, all being floor samples, the variable sampling points were randomly chosen and represent different types of surfaces, such as beds, mattresses, lavatories and other furniture. Because these samples were only taken once, no graphical presentation over time is possible. Therefore, for each of the variable points, a table compares the obtained microbiological values of both Floors, for each of the three types of organisms (total count, coliform count, *S. aureus*).

I) SAMPLING POINT 1: FLOOR OF ROOMS 110/310

Floor sample of a standard, not MRSA contaminated room.

Table 1: Count of different bacterial groups on the floor of rooms 110 and 310.

Bacterial group	1st floor	3rd floor
Total count	**$1{,}3 \times 10^4$**	**$1{,}4 \times 10^4$**
Coliform	**$3{,}7 \times 10^3$**	**$8{,}9 \times 10^2$**
S. aureus	**$7{,}8 \times 10^2$**	**$1{,}5 \times 10^3$**

CONCLUSION:

These samples were taken at day 1, just before the start of the PIP cleaning. Hence, these results do not yet provide any information on the effect of the PIP products. From the numbers in the above table it can be concluded that both floors show equal bacterial contamination and are suitable for this study.

II) SAMPLING POINT 2: FLOOR OF ROOMS 112/312

Floor sample of a standard, not MRSA contaminated room.

Table 2: Count of different bacterial groups on the floor of rooms 112 and 312.

Bacterial group	1st floor	3rd floor
Total count	$1{,}0 \times 10^4$	$3{,}0 \times 10^4$
Coliform	$1{,}2 \times 10^3$	$2{,}6 \times 10^3$
S. aureus	$2{,}0 \times 10^3$	$2{,}1 \times 10^3$

CONCLUSION:

Although total count numbers on the 3rd Floor are higher by about 0,3 log units, no significant effect on coliform bacteria and MRSA could be measured after 1 day of PIP cleaning.

III) SAMPLING POINT 3: FLOOR OF ROOMS 113/313

Floor sample of a standard, not MRSA contaminated room.

Table 3: Count of different bacterial groups on the floor of rooms 113 and 313.

Bacterial group	1st floor	3rd floor
Total count	**$1{,}4 \times 10^3$**	**$8{,}0 \times 10^3$**
Coliform	**$5{,}5 \times 10^2$**	**$5{,}5 \times 10^2$**
S. aureus	**$5{,}6 \times 10^2$**	**$3{,}3 \times 10^2$**

CONCLUSION:

The total count was about 0,5 log units higher on the 3rd Floor (PIP). Coliform numbers were identical between PIP-based and control cleaning. The MRSA count was 41 % lower in room 313 (PIP) compared to the MRSA count in room 113 (regular cleaning).

IV) SAMPLING POINT 4: FLOOR OF ROOMS 114/314

Floor sample of a standard, not MRSA contaminated room.

Table 4: Count of different bacterial groups on the floor of rooms 114 and 314.

Bacterial group	1st floor	3rd floor
Total count	**$1{,}0 \times 10^4$**	**$2{,}3 \times 10^4$**
Coliform	**$4{,}1 \times 10^3$**	**$2{,}5 \times 10^3$**
S. aureus	**$2{,}0 \times 10^3$**	**$1{,}1 \times 10^2$**

CONCLUSION:

After 3 days of cleaning with PIP products, the total count was about 0,2 log units higher in room 314. The number of coliform bacteria was about 0,2 log units lower and MRSA numbers dropped with 1,2 log units in the PIP-cleaned room, compared to the numbers in the control room (114).

V) SAMPLING POINT 5: FLOOR OF ROOMS 115/315

Floor sample of a standard, not MRSA contaminated room.

Table 5: Count of different bacterial groups on the floor of rooms 115 and 315.

Bacterial group	1st floor	3rd floor
Total count	**$3{,}0 \times 10^4$**	**$4{,}9 \times 10^4$**
Coliform	**$1{,}1 \times 10^4$**	**$1{,}9 \times 10^3$**
S. aureus	**$3{,}6 \times 10^3$**	**$1{,}1 \times 10^2$**

CONCLUSION:

The total count was about 0,2 log units higher in PIP-cleaned room 115. The coliform count was almost 1 log unit lower, and the MRSA numbers was 1,3 log units lower in room 315 (PIP) when compared to room 115 (control).

VI) SAMPLING POINT 6: FLOOR OF ROOMS 108/308

Floor sample of a standard, not MRSA contaminated room.

Table 6: Count of different bacterial groups on the floor of rooms 108 and 308.

Bacterial group	1st floor	3rd floor
Total count	**$2{,}9 \times 10^4$**	**$4{,}1 \times 10^4$**
Coliform	**$6{,}1 \times 10^3$**	**$1{,}6 \times 10^3$**
S. aureus	**$1{,}8 \times 10^3$**	**$6{,}7 \times 10^2$**

CONCLUSION:

The total amount of bacteria was about 0,1 log units higher in PIP-cleaned room 308, compared to the number in control room 108. Both the coliform number and MRSA count were approximately 0,5 log unit lower in room 308 (PIP) when compared to room 108 (control).

VII) SAMPLING POINT 7: MRSA CONTAMINATED ROOMS (115/314)

At sampling day 7, on both the 1st and 3rd floor, an MRSA infected room had to be thoroughly cleaned after departure of the patients. At 7 am, these rooms were cleaned following the AZ Lokeren prescribed protocols in case of MRSA contaminated rooms (including a universal disinfection of the room). **By means of experiment, for once, no disinfection step proceeded the PIP cleaning on the 3rd floor; on the 1st floor disinfection was performed as prescribed.** At 8.30 am, samples were taken on the floor, table and lavatory in these rooms. Hence, the following results are those of 1 hour after cleaning, comparing disinfection of an MRSA contaminated room with PIP cleaning of an MRSA contaminated room, without prior disinfection (note: disinfection of room 314 did happen during the stay of the MRSA contaminated patient).

Table 7: Count of different bacterial groups on the floor of rooms 115 and 314, 1 hour after cleaning, respectively with regular cleaning products (incl. disinfection) and with PIP-based cleaning products (excl. disinfection).

Bacterial group	1st floor	3rd floor
Total count	**$2,4 \times 10^4$**	**$1,7 \times 10^4$**
Coliform	**$7,4 \times 10^3$**	**$4,4 \times 10^2$**
S. aureus	**$8,0 \times 10^3$**	**$1,1 \times 10^2$**

Also the **service tray** (on which food is placed) of these rooms were sampled.

Table 8: Count of different bacterial groups on the service tray in MRSA contaminated rooms (115/314) on the 1st and 3rd floor.

Bacterial group	1st floor	3rd floor
Total count	**$4,4 \times 10^3$**	**$3,3 \times 10^4$**
Coliform	**$1,8 \times 10^3$**	**$3,3 \times 10^2$**
S. aureus	**$5,5 \times 10^2$**	**0**

CONCLUSIONS:

On this sampling day, an interesting situation occurred due to the availability of an MRSA infected room on both Floors. The hospital cleaning procedures require a thorough disinfection and subsequent cleaning before a new patient may enter the room. By means of experiment, room 314 was only cleaned using the PIP products, without disinfection. Compared to room 115 (disinfected), the following results were found:

- **Total count was equal between both rooms**
- **Coliform bacteria were approx. 80% lower in room 314**
- **MRSA count was 99% lower in room 314**

Although both rooms can not be considered 100% identical, these results may indicate that disinfection efficiency in room 115 was rather low (8,0 x 10^3 CFU/m^2 remaining), especially when compared to the results from a previous sampling in that room (see Table 5), where the MRSA count in room 115 was at that time 3,6 x 10^3 CFU/m^2. Because of the lack of any direct biocidal activity of the PIP products (see further), it is suggested that spreading of MRSA in the room cleaned with PIP may have been hampered by the probiotic bacteria that colonized the surfaces since 1 week in a pre-emptive way.

Also the service trays of these rooms were compared. On the 1st Floor, this tray was also disinfected, whereas the tray on the 3rd floor had only been cleaned using the following PIP product: PIP Universal Cleaner. Results were similar as those of the samples taken on the floor, with MRSA counts in the PIP room being zero (= below detection limit).

The results obtained in the MRSA contaminated rooms indicate that a daily PIP treatment prevents the build-up and spread of pathogenic bacteria, such as coliform and MRSA. As already demonstrated during a previous study, disinfection produces unreliable results, which may be due to resistance of the pathogenic bacteria to these agents.

VIII) SAMPLING POINT 8: SEATING OF LEATHER CHAIR

At the end of the central hall, a seating corner is located. This corner contains four leather chairs and one table. Samples were taken from the seating of one of these chairs.

Table 9: Count of different bacterial groups on the seating of a leather chair at the end of the central hall, on 1st and 3rd floor.

Bacterial group	1st floor	3rd floor
Total count	**$1{,}4 \times 10^4$**	**$1{,}8 \times 10^2$**
Coliform	**$1{,}1 \times 10^3$**	**$1{,}1 \times 10^2$**
S. aureus	**$2{,}2 \times 10^2$**	**$2{,}2 \times 10^2$**

CONCLUSION:

In general, total count and coliform count on the chair was lower on the 3rd Floor, cleaned with PIP, as compared to the 1st Floor, cleaned with regular products. No difference in MRSA level was observed.

IX) SAMPLING POINT 9: MATTRESS OF ROOMS 109/309

Sample of a mattress, cleaned with PIP Allergy Free, of a not MRSA contaminated room.

Table 10: Count of different bacterial groups on the mattresses in rooms 109 and 309, on 1st and 3rd floor.

Bacterial group	1st floor	3rd floor
Total count	**$3{,}1 \times 10^3$**	**$2{,}1 \times 10^4$**
Coliform	**$7{,}7 \times 10^2$**	**$2{,}2 \times 10^2$**
S. aureus	**$7{,}7 \times 10^2$**	**$2{,}2 \times 10^2$**

CONCLUSION:

Total count on a mattress in room 309 was generally higher than on a mattress in room 109. However, numbers of coliforms and MRSA were approx. 0,5 log units lower on mattress '309' when compared to mattress '109'. **PIP cleaning of the room's bed and furniture may result directly in a safer microbial situation to the patient.**

X) SAMPLING POINT 10: BED PUSHING BAR OF ROOMS 116/316

A potential source of microbial spread throughout the hospital is the patient's bed. Each bed is equipped with a pushing bar for the hospital staff. Samples from these chrome bars were taken.

Table 11: Count of different bacterial groups on the bed pushing bars of rooms 116 and 316.

Bacterial group	1st floor	3rd floor
Total count	**$2{,}6 \times 10^3$**	**$1{,}6 \times 10^3$**
Coliform	**$3{,}3 \times 10^2$**	**0**
S. aureus	**$2{,}5 \times 10^2$**	**$1{,}1 \times 10^2$**

CONCLUSION:

Although no significant results was noted concerning total count and MRSA count, PIP cleaning resulted in a strong decrease of coliform numbers on the patient's bed pushing bars: this count decreased to a level below the detection limit in PIP-cleaned room 316.

IX) SAMPLING POINT 11: LAVATORY SINK OF ROOMS 116/316

In order to specifically evaluate the potential of the sanitary cleaner, samples were taken from the sink in the lavatory of rooms 116 and 316.

Table 12: Count of different bacterial groups on the lavatory sink in rooms 116 and 316.

Bacterial group	1st floor	3rd floor
Total count	**$7{,}7 \times 10^3$**	**$2{,}9 \times 10^4$**
Coliform	**$8{,}8 \times 10^2$**	**$3{,}3 \times 10^2$**
S. aureus	**0**	**0**

CONCLUSION

Although total bacterial count was higher due to PIP treatment with beneficial bacteria, coliform levels were 60% lower in the lavatory sink on room 316 compared to room 116. No MRSA bacteria were found in both sinks, probably due to the higher rate of washout.

PART 3

STUDY PROJECT

RESULTS – PHASE 2

SECTION

1. INTRODUCTION

This part of the report presents the results obtained during PHASE 2 of the study.

Following PHASE 1 of this study, a buffer period of 1 month was inserted, during which regular cleaning of the entire hospital was carried out. This was intended to bring the microbial community of the 3rd Floor back to the same level as the other Floors. The results presented in this part of the report cover those of PHASE 2 of the study with PIP cleaning starting on the 11th of April 2007.

All presented results are those from **fixed sampling points**, with the first value corresponding to regular cleaning in the buffer period, followed by a number of sampling dates during PIP cleaning.

The following sampling points were chosen:

- **Emergency**
- **Maternity**
- **1st Floor**
- **2nd Floor**
- **3rd Floor (= control Floor; regular cleaning)**
- **4th Floor**
- **5th Floor**

All samples were taken **on the floor** in the middle of the central halls of these divisions.

Results of all sampling points are combined into one graph for each of the organism types monitored (total count, coliform, *S. aureus* and *C. difficile*) and are presented as line plots over time. Each graph contains the number of colony forming units per square meter of surface (= **CFU/m²**) for both the control Floor (black line, Regular cleaning) and the average of all PIP cleaned Floors (green line). All **results are the average values of triplicate sampling and analysis**. These threefold analyses facilitated the statistical valorisation of the obtained quantifications.

2. MICROBIAL ANALYSES

The following part presents the results of PHASE 2 by means of four graphs:

1. **Total count**
2. **Coliform bacteria**
3. ***Staphylococcus aureus***
4. ***Clostridium difficile*.**

Each graph comprises the results of all fixed sampling points (3rd Floor = control) and is followed by a brief discussion and conclusion of the results.

A. TOTAL COUNT

Figure 13 presents the combined results of total count values measured during the first 2 weeks of phase 2. The third Floor served as control with regular cleaning (black line) whereas the PIP cleaned sampling points are averaged and presented by the green line. The first values (10th April 2007) were taken the day before actual start of the PIP cleaning protocol.

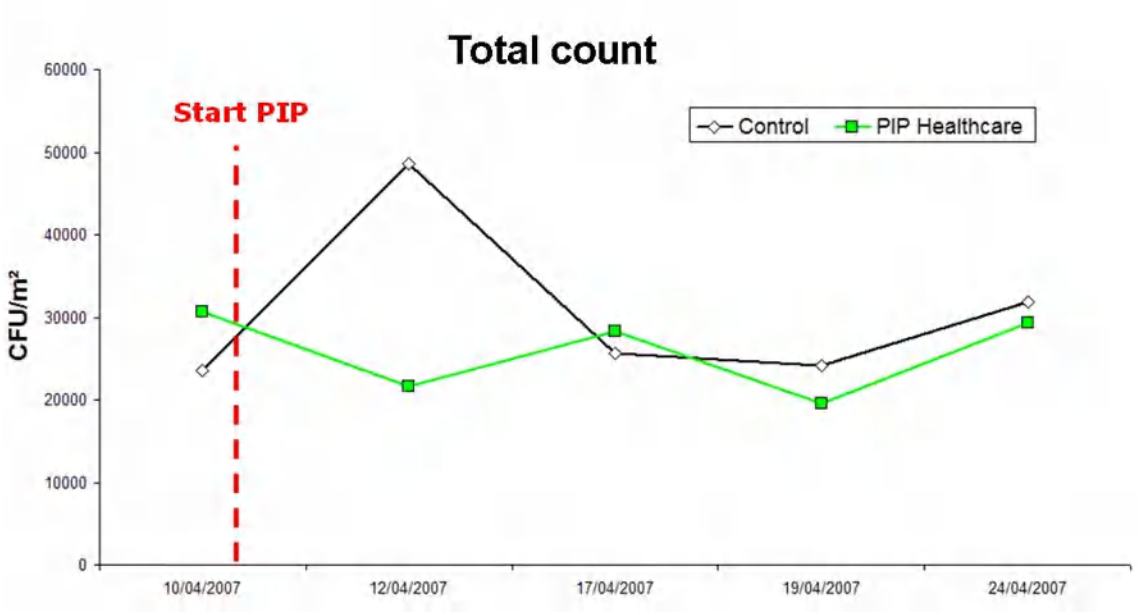


Fig. 13: Total count values during PHASE 2 of the study.

CONCLUSION:

As also observed during PHASE 1 of this study, total count on average did not statistically increase on the PIP cleaned surfaces. This indicates that the applied concentrations and dilution of the various PIP products are well-calculated in order to obtain total bacterial counts of equal quantities compared to regular cleaning.

B. COLIFORM BACTERIA

Figure 14 presents the combined results of total coliform values measured during the first 2 weeks of phase 2. The third Floor served as control with regular cleaning (black line) whereas the PIP cleaned sampling points are averaged and presented by the green line. The first values (10th April 2007) were taken the day before actual start of the PIP cleaning protocol.

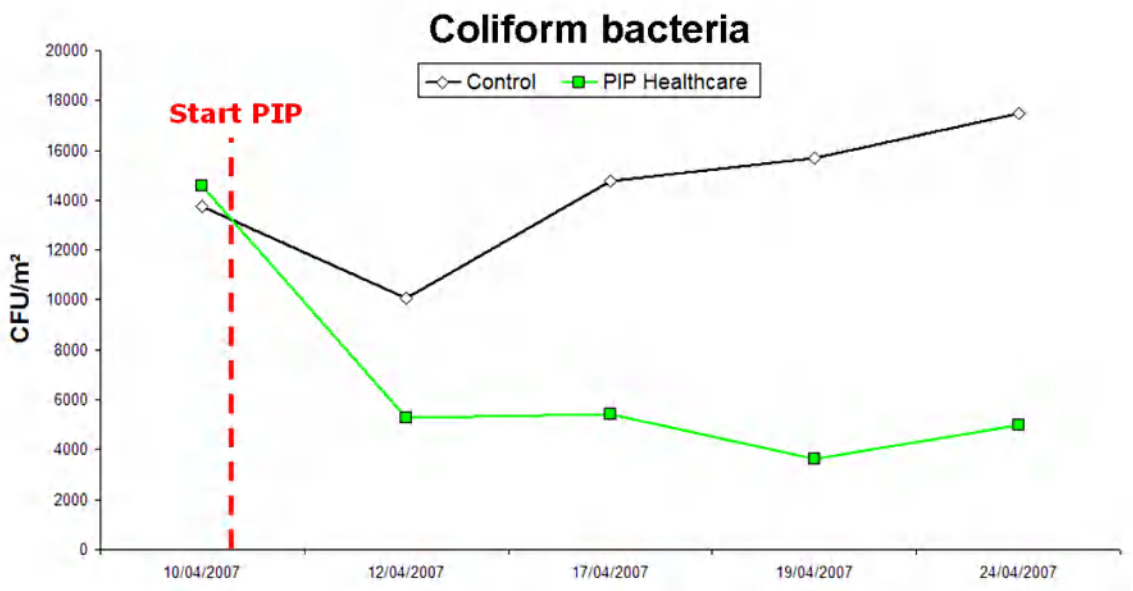


Fig. 14: Coliform count during PHASE 2 of the study.

CONCLUSION:

Although most Floors demonstrated higher coliform counts compared to the control Floor at the start of PHASE 2, PIP cleaning efficiently reduced these counts to values below that of the control. During the first 2 weeks of PIP cleaning, all PIP Floors had lower coliform counts than the control Floor. On average, the reduction of coliform counts by means of PIP cleaning was 60%, slightly higher compared to the reduction obtained during PHASE 1 of this study. This might indicate that expansion of PIP cleaning results in lower cross-contamination and more efficient pathogen control.

C. STAPHYLOCOCCUS AUREUS

Figure 15 presents the combined results of *Staphylococcus aureus* values measured during the first 2 weeks of phase 2. This bacterial species is the source of MRSA in hospitals. The third Floor served as control with regular cleaning (black line) whereas the PIP cleaned sampling points are averaged and presented by the green line. The first values (10th April 2007) were taken the day before actual start of the PIP cleaning protocol.

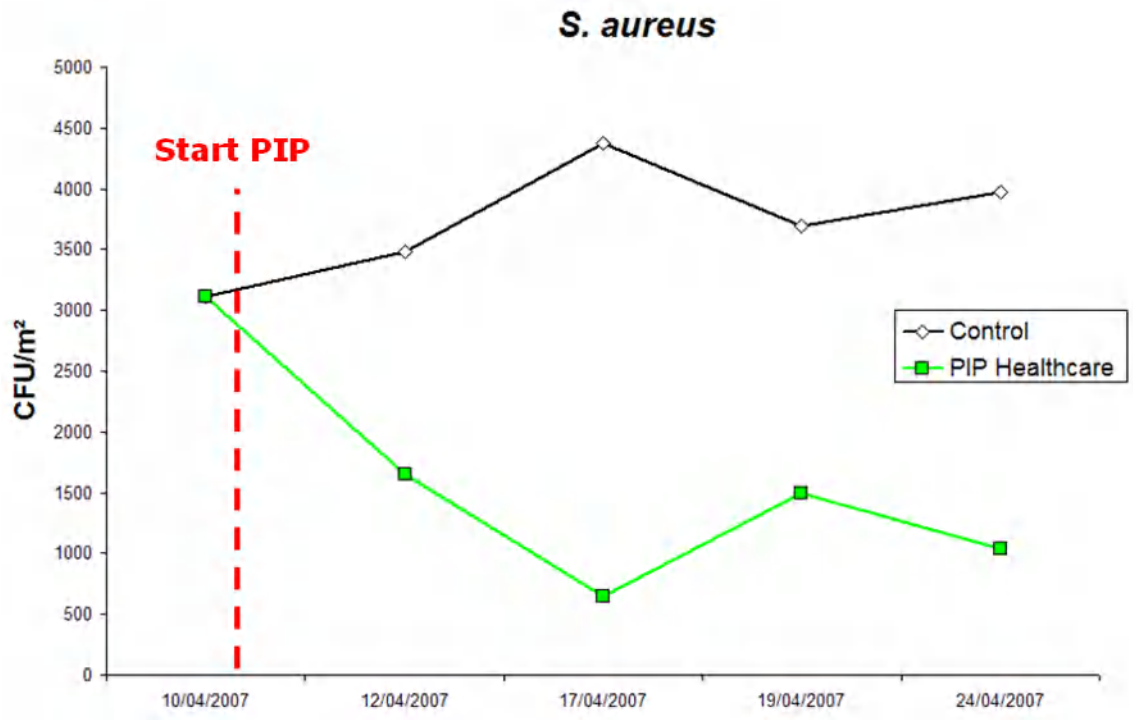


Fig. 15: *S. aureus* counts during PHASE 2 of the study.

CONCLUSION:

Although most Floors demonstrated higher *S. aureus* counts compared to the control Floor at the start of PHASE 2, PIP cleaning efficiently reduced these counts to values below that of the control. During the first 2 weeks of PIP cleaning, all PIP Floors had lower *S. aureus* counts than the control floor. On average, the reduction of *S. aureus* counts by means of PIP cleaning was 74%.

When looking at the course of *S. aureus* numbers on the 3rd Floor during the two phases of this study (Fig 16), it can be seen that upon finishing PIP cleaning at the end of PHASE 1, *S. aureus* counts again increased to approximately 3200 CFU/m² at the start of PHASE 2. During the second phase of the study, the 3rd Floor served as a control, with regular cleaning. During this period, *S. aureus* numbers remained at the same level.

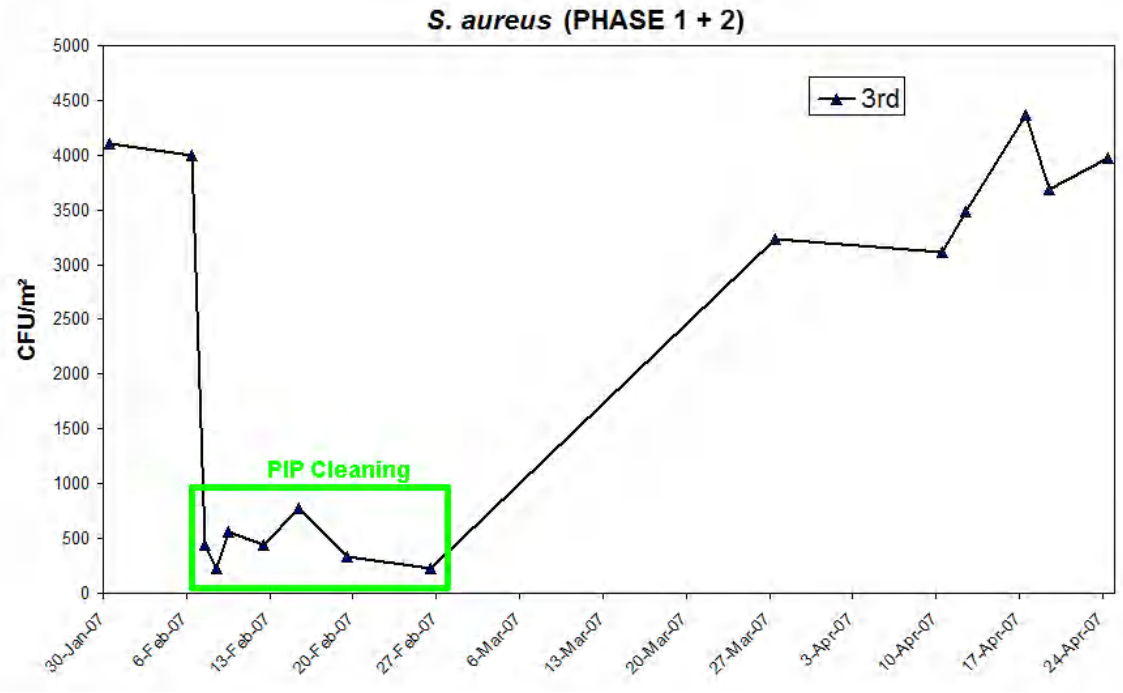


Fig 16: *S. aureus* count on the 3rd Floor during both PHASES of the study. Only during PHASE 1, this Floor was subjected to PIP cleaning, demonstrating clearly lower *S. aureus* counts.

CONCLUSION:

Fig 16 clearly shows that PIP cleaning decreases the number of *S. aureus*. From the moment PIP cleaning stops, the number of *S. aureus* increases again to values within the same range as those prior to PIP cleaning. These observations demonstrate that the observed reduction in *S. aureus* is indeed the result of PIP cleaning.

D. CLOSTRIDIUM DIFFICILE

Figure 17 presents the combined results of *Clostridium difficile* values measured during the first 2 weeks of phase 2. Because of its sporulation capability, this bacterial species is very hard to remove with currently existing cleaning/disinfection procedures and causes severe diarrhoea in hospitals. The third Floor served as control with regular cleaning (black line) whereas the PIP cleaned sampling points are averaged and presented by the green line. The first values (10th April 2007) were taken the day before actual start of the PIP cleaning protocol.

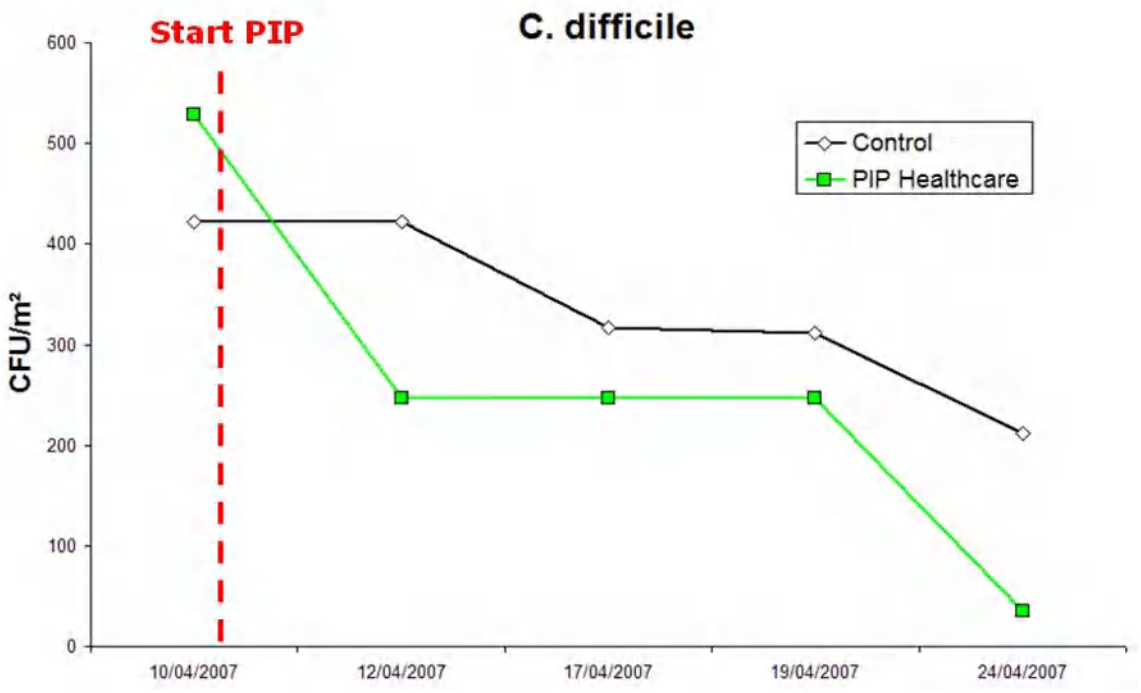


Fig. 17: *C. difficile* count during PHASE 2 of the study.

CONCLUSION:

Determination of *Clostridium difficile* was only performed during PHASE 2 of this study. In general, due to the anaerobic nature of this organism, counts are never high, but the resilient spores may induce severe illness when the appropriate conditions occur. The *C. difficile* count at the control Floor was too low to serve as a statistically significant control, but the obtained quantifications clearly demonstrate that *C. difficile* can be reduced strongly by means of PIP cleaning. The average reduction of *C. difficile* over 2 weeks was approximately 90%. Most likely, the property of the PIP bacteria to sporulated, together with their aerobic metabolism, is a unique and efficient way to control *C. difficile*.

3. BIOCIDAL ACTIVITY

In order to verify whether the PIP bacteria exhibit a direct biocidal effect towards other bacteria, an *in vitro* experiment was conducted, using live/dead staining on flow cytometric analysis. It was determined whether the filtrate of a 48h old bacterial suspension of the PIP product was able to kill *Staphylococcus aureus* and/or *Streptococcus faecalis*.

Table 13: Viability counts on *Streptococcus faecalis* and *Staphylococcus aureas* to determine a possible biocidal action of the PIP product.

Control: *Streptococcus faecalis*

Live	Dead	Total	Live (%)	Dead (%)
9317	18	9335	99,81	0,19
9342	25	9367	99,73	0,27
9311	12	9323	99,87	0,13

***Streptococcus faecalis* + bacterial filtrate**

Live	Dead	Total	Live (%)	Dead (%)
9338	95	9433	98,99	1,01
9365	43	9408	99,54	0,46
9349	45	9394	99,52	0,48

Control*: Straphylococcus aureus*

Live	Dead	Total	Live (%)	Dead (%)
8925	7	8932	99,92	0,08
8846	13	8859	99,85	0,15
8868	12	8880	99,86	0,14

***Straphylococcus aureus*+ bacterial filtrate**

Live	Dead	Total	Live (%)	Dead (%)
9677	40	9717	99,59	0,41
9635	23	9658	99,76	0,24
9629	28	9657	99,71	0,29

CONCLUSION:

As claimed by Chrisal, no direct biocidal activity of the PIP product towards other bacteria was witnessed.

4. INFECTION DATA

Although the time frame of this study was too short to be able to formulate solid conclusions whether PIP cleaning also resulted in a lower incidence of nosocomial infections, a remarkable observation was done during this study. From the moment PIP cleaning started, THE NUMBER OF INFECTIONS ORIGINATING IN THE HOSPITAL, DROPPED WITH APPROXIMATELY 60%.

No claims will be attached to this observation at this time, but it is a strong motivation to further track infection data during the following 6 months of PIP cleaning in the AZ Lokeren hospital.

5. GENERAL CLEANING REMARKS

During the course of the study, the cleaning staff applying the PIP products was asked to comment on the overall cleaning characteristics of these products and provide information on potential deviations of the cleaning protocol.

No complaints or negative effects were communicated concerning the PIP products.

The following remarks were made:

- All products showed good cleaning power and nice smell.
- The PIP Sanitary Cleaner did not bother any of the users or cause breathing problems, in contrast to the regular products, which did cause problems in staff.
- One person requested a stronger degreasing power of the PIP Sanitary cleaner.
- Without the use of chlorine tablets, the inside of the toilets were not sufficiently clean.
- The PIP Allergy Free spray cans were experienced as very handy products to apply in hard to reach places.

Overall, the cleaning staff was very satisfied to work with the PIP products and experienced them as much more healthy to work with, compared to the regular (chemical) cleaning products. Also, the added value of the PIP products was experienced by the cleaning staff as an additional motivation to actively aid in the overall hygiene of the hospital.

PART 3

STUDY PROJECT

GENERAL

CONCLUSIONS

SECTION

This report provides information demonstrating the problem of the prevalence of several harmful bacterial groups in hospital environments and the report results clearly demonstrates the high efficiency of the Chrisal's PIP Healthcare products to manage the harmful pathogenic hospital bacteria.

This study validated the efficiency of **PIP Healthcare®** cleaning products (from Chrisal NV) in a clinical environment. The effect on **total count, coliform, *Staphylococcus aureus* (MRSA) and *Clostridium difficile* count** was monitored and assessed, in comparison with regular cleaning products.

THE FOLLOWING GENERAL CONCLUSIONS CAN BE MADE FROM THIS STUDY:

1. **A significantly lower pathogen count was measured on all hospital floors, sanitary, furniture and equipment on various hospital divisions, when daily PIP-based cleaning was applied. The following average count evolutions exist during PIP Healthcare® cleaning:**
    - **Total count: + 10%**
    - **Coliform count: - 50%**
    - **S. aureus count: - 80%**
    - **Clostridium count: - 90%**
2. **The obtained reduction in pathogen count remained stable during the full course of the PIP cleaning, indicating the effective stabilization of the microbiota. Note that only prolonged termination of PIP cleaning resulted in a deterioration of pathogen counts.**
3. **Despite the lack of a direct biocidal activity, PIP cleaning results in a microbial community of equal size, but with a much lower percentage of pathogens.**

This study clearly demonstrates that the use of probiotic cleaning products results in lower levels of pathogenic bacteria.

The PIP probiotic bacteria colonize the treated surfaces and prevent (potential) pathogenic bacteria from colonizing these surfaces after cleaning. Based on the total count numbers, which are only slightly higher during PIP cleaning, it can be concluded that the probiotic bacteria of the PIP products gradually take over the microbial "hospital ecosystem" and replace the pathogenic organisms. These results directly lead to a reduced risk of cross-contamination between patients, personnel and visitors.

Although PIP cleaning almost never resulted in a complete removal of pathogenic bacteria when measured after 24 hours, the most important aspect of PIP cleaning is the stability of the obtained results, preventing any pathogen from peaking at certain times.

The study at the AZ Lokeren hospital demonstrates that daily cleaning with Chrisal's PIP Healthcare® products effectively reduces the level of pathogens in the hospital, leading to a more healthy and stable microbial environment to all patients, personnel and visitors.

Prof. Dr. ir. Willy Verstraete
Ghent University

Dr. ir. Wim Dewindt
Avecom NV

CONTACT INFORMATION

Ghent University
Laboratory of Microbial Ecology and Technology
Coupure Links 653
B9000 Gent
+32-9-264.59.76

Prof. Willy Verstraete Willy.Verstraete@ugent.be 09/264.59.76

AZ Lokeren
Lepelstraat 4
B9160 Lokeren
+32-9-340.86.11

Koen Van Landeghem Koen.van.landeghem@azlokeren.be 09/340.83.86

Avecom
Industrieweg 122P
B9032 Wondelgem

Dr. ir. Wim De Windt R&D Manager Wim.Dewindt@avecom.be 0473/61.46.36

The tested company:

Chrisal N.V.
Priester Daensstraat 9
B3920 Lommel
+32-11-54.80.00

Corrie Gielen	General manager	Corrie@chrisal.be	0497/58.91.06
Dr. Robin Temmerman	R&D Manager	Robin@chrisal.be	0496/27.41.10

To contact the tested company directly in North & Central America:

CHRISAL LTD.
PO Box 61-0400
North Miami, Florida

Lino G. Morris, CEO **Lino@CHRISAL.net** **305-940-8000**