



**Shriners Hospitals**  
for Children®

**Shriners Hospital For Children  
Tampa Study Of Chrisal  
Probiotics As A Method For  
More Efficient Cleaning,  
Removal Of Biofilm And As A  
Method Of Decreasing  
Nosocomial Infections And  
Respiratory Reactions**

With observations on the relationship between  
ATP testing & microbial culture results

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## REASONS FOR THIS STUDY

The Shriners Hospitals for Children has always searched for ways to provide better infection control, reduce respiratory problems, increase safety, provide better cleaning, reduce material and labor costs and protect the environment.

The Shriners Hospital in Tampa has undertaken an urgent search for better and less toxic alternative solutions for cleaning and infection control as important additional factors affecting the growing problems with infection control and cleaning have become evident.

Major reasons for this accelerated search include the rise of serious problems of increasingly disinfectant and antibacterial agent resistant bacteria, and the growing awareness of the major impact biofilm has in the propagation of a wide variety of pathogens. Further, the recognition that existing cleaners and disinfectants oddly are proving to be part of the infection cycle itself and that they also exacerbate the growing respiratory problems with children, and engender allergic reactions in both the staff and the patients, are all part of the driving reasons for this study.

### NOSOCOMIAL INFECTIONS

Today's hospitals and care facilities have a number of important systemic problems to be overcome and needs to be addressed. Some of the current concerns would have been surprising 50 years ago. Hospitals have changed from being considered safe islands of healing into facilities to be avoided, except for the shortest possible stays, in order to evade nosocomial infections. In the USA these infections now cause as many deaths as from automobile accidents, AIDS and breast cancer combined, and cost hospitals well over \$30 billion annually.

Therefore, the number one pressing problem to be considered in this study is how to best address the requirement for environmental management to meet both the current and future needs for infection control.

### PROBLEMS WITH DISINFECTANTS

An important factor in this regard is the growing awareness of the negative effects triggered by disinfectants which, paradoxically, complicate the very problems they and other chemical solutions are designed to prevent.

An unexpected statement made at the first evaluation meeting for this project was that "*disinfectants cause infections*". The explanation for this statement proved most enlightening and became an important consideration in the investigation into a probiotic solution. This is covered in detail in the body of this study.

### RESPIRATORY PROBLEMS

Another major unsolved problem for our hospitals is that of respiratory distress. Ironically this problem is also triggered by the very disinfectants and other chemicals used in cleaning our facilities that are supposed to prevent such problems.

The negative effects of disinfectants on both patient populations and staff in medical facilities have not been effectively studied in the past. In recent evaluations users have determined that cleaning and sanitizing chemicals have far more negative impact on respiratory health than have been previously realized. Initially the concern was for the cleaning staffs that work directly with the chemicals. It was then observed that some members of the medical staff also had varying degrees of problematic reactions to the chemicals.

Furthermore, due to the growing incidence of asthma in children, and the reactions of some patients to cleaning chemicals, additional exposure avoidance steps have had to be implemented when using traditional cleaning and disinfection protocols that have varying degrees of toxic fumes.

### **EFFECTIVENESS & THE DEGREE OF CLEANLINESS**

The new probiotic technology has shown that the standards of what was traditionally considered “clean” are far outdated, and that the importance of biofilm elimination in the control of the microscopic environment has now been put under intense scrutiny. The removal of biofilm is now considered of prime importance in providing a far cleaner environment, with a significantly reduced risk of infection.

### **EFFICIENCY & THE LABOR FACTOR**

The reality of how training and management can make labor more effective also depends a great deal upon (a) the efficiency of the products used and (b) the degree of difficulty in using them (i.e. the use of caustic and toxic chemicals increases labor time and causes additional sick days). A part of this study is dedicated to quantifying the benefits of using probiotic products that have no negative effects on humans, animals, plants and the environment in general. It was also of interest that these Chrisal Probiotics-In-Progress (PIP) probiotic products can be applied using methods that allow areas normally unreachable to be cleaned more easily and on a regular basis thus mitigating mistakes and lapses of proper attention by staff.

### **COSTS**

Cost savings is always a high priority for all hospitals, even more so in the current distressed economy. Therefore measures that allow a reduction in cost and an increase in productivity (i.e. reduction in material costs and labor, less cleaning time and fewer employee sick days) while safer and adhering to a high level of standards and quality, are of a great interest. The subject products of this study became of interest, since under test, the Chrisal probiotics appear to meet these criteria and provide an effective and safe long term solution.

### **ENVIRONMENTAL IMPACT**

The environmental impact of the products we use are of great concern and a growing problem. This makes the use of green products more critical. In addition, the growing regulatory pressures mandating restrictions on what chemicals can be used in various applications, and the manner and cost of their disposal is of increasing concern.

Of major importance is that the components of the tested products are also environmentally benign. The probiotics that are the core of the tested products have been certified organic, and the chemicals used have received Ecolabel Certification. The Chrisal products have the added credibility of having been used successfully in many hospitals and medical facilities, as well as by a large number of other organizations in over 30 countries. Additionally, as an indication of safety and efficacy, according to the data provided, these Chrisal probiotic products have been utilized safely in applications on farms over the past seven years in the raising of all types of food animals and other life forms from dairy cows, poultry, pigs and fish to zoo animals, as well as horses and house pets.

## **STUDY GOAL SUMMARY**

This Shriners Hospital Study was designed as a comprehensive, in-depth evaluation of a new paradigm in cleaning that met the long term goals of providing a major improvement in efficacy, infection control, cost effectiveness, safety, ease of use, reduced environmental impact, and reduced material and labor costs.

The study was also designed to go much further than to simply consider only a substitution of products. It is part of an in-depth strategic effort to consider new methodologies that would solve a number of today's concerns and meet the requirements in the future for all of our facilities nation-wide.

## **PROJECT INTRODUCTION & EVALUATION**

This study was initiated as a result of the problems experienced with existing cleaning and disinfection solutions and the ramifications posed by their varying degrees of toxicity. The problem is that there are so many new products claiming a large range of benefits that require investigation and evaluation that the Shriners had to determine what products were most likely to meet the criteria before even committing to testing. When one of the Shriners' respected support entities suggested our organization look at a new probiotic solution for preventing nosocomial infections and providing more effective and economical cleaning, our staff was willing to review the data to see if the solution merited further study.

A number of factors were considered. Due to the source of the recommendation and the initial studies provided from universities and other hospitals, our staff looked into the Chrisal solutions. A large amount of additional medical and scientific data was presented. These factors and initial informal tests of the products yielded impressive results which lead us to proceed with the full study of these probiotic solutions.

### **MICROBIOLOGICAL MECHANISM:**

The most important questions to be answered by the Infection Control Department revolved around understanding the mechanism upon which the products are based. It was determined that if the products worked as stated, then they were far ahead of other existing solutions because they provided a controlled domination of the microscopic environment (CDME).

### **THE BIOFILM FACTOR:**

It should be noted that it is impossible to control the microscopic environment of any surface without being able to remove the existing



biofilm and prevent its regrowth. The general public is typically unaware of the existence of the biofilm that microscopically coats almost all surfaces, which is produced by pathogens and other bacteria for their own protection. It is important to understand that biofilm is not just the plaque that bacteria build up on teeth and that darkens the grout between tiles. Biofilm also exists on all surfaces, including skin – and is estimated to be the key factor in the generation of over 80% of all infections.

However, despite the advances in understanding of the much larger role played by the microbiome in daily life, many professionals are still unaware of the mechanisms and processes required to actually control infection without causing complications or mutation in pathogens.

Due to significant advances in the bio-sciences over the last few years, a major consideration in any study of both infection control and general cleaning, is that medical researchers and scientist have determined how much more abundant and versatile microscopic life forms are in both the general environment and on and in our bodies. The result has been the development of a new and superior approach to cleaning and infection control.

Lately, the general public has also been exposed to an increasing number of articles and news stories about the fact that the ratio of bacterial cells to human cells in the human body is ten to one; that the average adult human body is made up of 10 trillion cells, but hosts ten times that amount, 100 trillion bacteria, making humans in actuality a composite life form comprised of 90% bacteria and 10% “Human” by count.

Understanding how rampant and hardy the biofilm that bacteria produce to house and protect themselves is, provides insight into to why this particular method of using good bacteria to combat bad bacteria, without harming the environment in any way, has been found to be an ideal solution for removing the threat posed by biofilm. For those in infection control, the removal of biofilm is a major factor in the prevention of infection and re-infection that is, in part, caused by the use of disinfectants.

This knowledge is now leading to an understanding that the only sensible choice is between controlling a healthy balance of environmental bacteria for protection or allowing an unhealthy balance with its risks of infection and that the attempted total destruction of all bacteria with disinfectants is counterproductive.

The capability of the tested probiotic products to work at the microscopic level and to deconstruct biofilm was of primary interest because: (a) no other products have been shown to be able to eliminate biofilm in a simple and easy to use manner and (b) that the only waste product that occurs in the conversion (deconstruction) of the biofilm by the Chrisal products is harmless CO<sub>2</sub>. The Chrisal products were the only products able to eliminate biofilm in a way that was safe, practical and environmentally responsible. The fact that the products worked by using the normal functioning of natural biological processes and were safe and easy to use, were the keys to the decision to formally test the products. Other considerations included operational needs and cost effectiveness.

#### **REQUIREMENTS OF USE:**

The Hospital prefers not to use any products or solutions that require special equipment, handling or training. In this regard, the PIP probiotic cleaning products were extremely simple to use and required no special conditions, training or equipment. The only conditions in using probiotic products was that they not be mixed or used with other cleaning or disinfection products, and that that they should not be stored where they would freeze. The probiotics were to be used in

specific areas during the trials and were not to be mixed with any other products used in the same locations. The undiluted products have a shelf life of years. Once the concentrated products are mixed with water for use, they should be used within a week. The instructions for use were to simply substitute the Chrisal PIP Probiotic cleaners for the currently used products – and proceed with cleaning as the staff would normally operate with any other products.

Prior to the decision to start this study, some of our staff tested and used the products on an individual basis and provided management with highly positive reviews. At the same time additional product usage and test data, from universities and hospitals, was received and submitted to the Study Management Team. A meeting of all the parties was held at the Tampa Children’s Hospital and the decision to proceed with a full scale study was proposed by the Management Team. This was agreed to by all parties, and the basic procedures were laid out and further refined at additional meetings over the month subsequent to the first evaluation meeting.

## **TEST AREAS**

25 locations in the Hospital were selected. These included:

- Public areas: lobby, the Self-Check-in Desk computer keyboard and mouse used by the patient’s parents
- The screens and hand controllers used by the children waiting in the lobby
- Men’s and woman’s rest rooms, with special attention to the handicap stalls, sink handles, soap dispenser and baby changers
- Patient room area
- Patient exam rooms, exam tables, sink, counter and floor
- Cast rooms
- Nurses stations
- Nurses locker room and bathroom
- Hallway hand sanitizer dispenser
- Hallway mounted staff computer keyboard and mouse
- Floor areas
- Kitchen areas
- Plus additional areas for visual changes such as public water fountains, etc.



## **TESTING PROCEDURES**

The list of the final testing locations were approved by a team from the Tampa Shriners Children’s Hospital including Sheryl Chewning, RN, CIC, CPHO, LHRM, Director of Performance Improvement, Risk Management, and Infection Control, Carol Ann Jenkins, Administrative Director of Support Services, Roberta Hardy, Director of Environmental Services and several other staff members.

## **BASELINE TESTING PRIOR TO THE USE, TESTING AND EVALUATION OF PROBIOTICS UTILIZING CULTURES & ATP METERS**

In order to provide a base-line reference prior to the study, each area to be tested was first cleaned with the hospital’s normal cleaning products.

At each location:

1. First, ATP (Adenosine Triphosphate) testing was performed using Hygeina ATP test swabs for the sampling and processed using a Hygeina ATP Meter.
2. Then, cultures were taken from the same area using 3-M culture swabs which then were taken to the lab and poured onto 3-M culture media and incubated for 24 hours before being checked and then frozen for photographs.

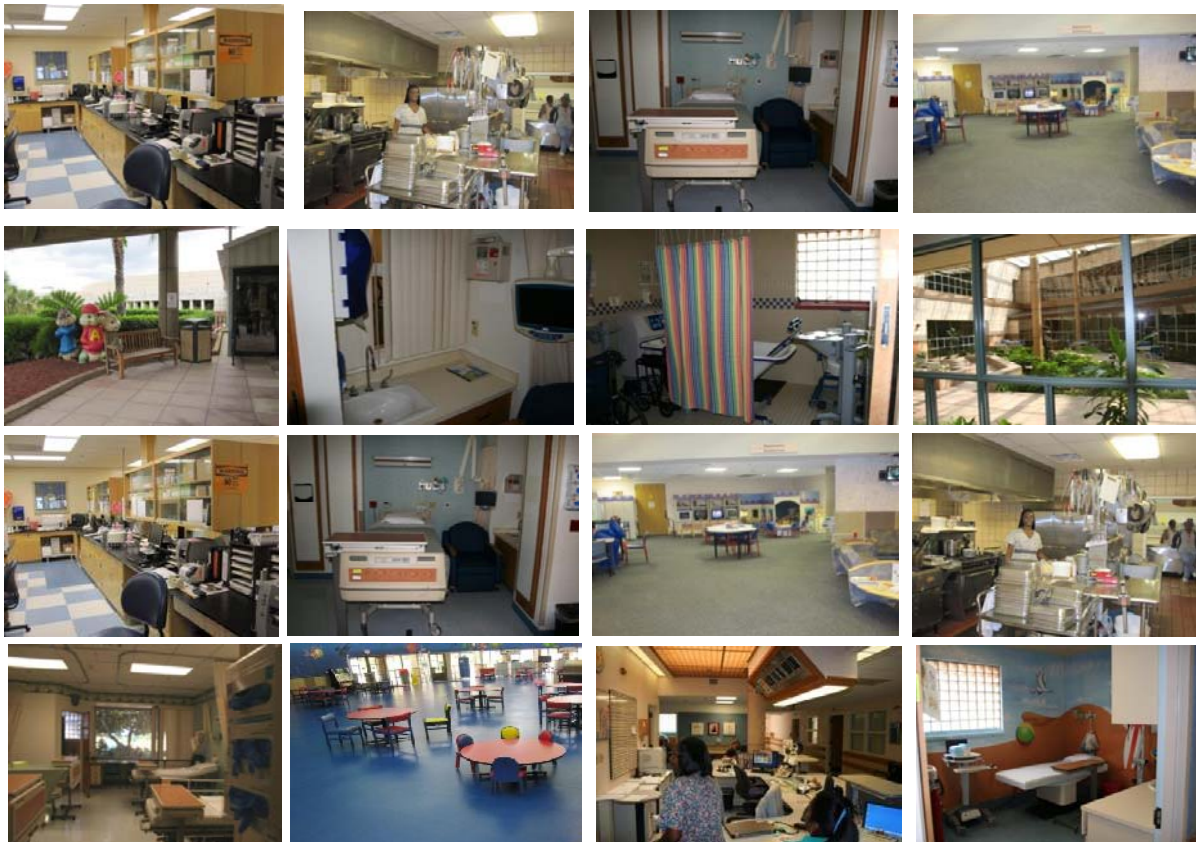
**ONGOING PROCEDURES & TESTING DURING THE STUDY**

After the initial base line testing was completed, the trial was started by taking the following steps:

- Each test area was listed and assigned a specific number;
- Signs were posted in each room or area noting that only Chrisal products were to be used for that area by the Hospital staff;
- A special “Chrisal Only” cart was designated and labeled to carry only the Chrisal cleaning products and the mops and other cleaning items used;
- One of the sinks in the janitor’s closet in each study area was set up to be used for the Chrisal PDU – Automatic Dilution Units.
- The Hospital’s day and the night cleaning staff were instructed to use only the Chrisal Probiotic PIP Products in each of the designated areas from that point on.

Thereafter, on Thursday of each week over the next month, both ATP testing and cultures were taken at each site where the Chrisal PIP Probiotic Products were used for cleaning. In addition, visual inspection notes were taken as well as reactions to the use of the products by the cleaning staff, the medical staff and the patients.

**The Shriners Hospitals for Children in Tampa and various test areas**



## **TRAINING**

A week before the testing started, and under the supervision of Roberta Hardy, Director of Environmental Services, a day of training on the use of the probiotic products for cleaning was provided by Chrisal. The Day Cleaning Crew trained on the products were: McNeil, Blackman, Lancaster, Miller and Sneed. Later the same day additional training was provided to the Night Cleaning Crew consisting of Crew Supervisor Fernandez, Munoz and Ray.

In addition, automatic dilution units were installed in the cleaning closets, and hooked directly to water outlets. It was clearly stated that the Chrisal probiotic products are highly concentrated and are diluted by 99 parts water to 1 part product.

It has been found nationally in medical facilities over the years that staff should not be depended upon to dilute concentrated products, because it often results in over use. This wastage is significant and is derived from the mentality that, *“if a little bit is good – then more must be better”*. This fallacy is negated and the proper dilutions provided through a simple calibrated dispensing system.

The Chrisal products, are extremely effective at full dilution, (100:1), and increasing the concentration is not required to improve efficacy. Therefore, process automation has been implemented to remove any required judgment by local staff. Furthermore, the products have been color coded and numbered in addition to having instructions in Spanish as part of the labels.

The vendor provided support personnel to work with the Hospital staff as requested during the test period. Various observers from interested local groups were invited and visited the test sites to become familiar with what was considered a new paradigm in controlling the microscopic environment.

## **NOTE ON INITIAL BASE LINE RESULTS**

Several external observers noted that the initial testing to establish a base line showed extremely low readings compared to the measurements normally found in hundreds of other facilities from other hospitals and universities, and food processing plants. These readings were exceptionally lower than anticipated ATP readings and corroborating cultures showed the same results in almost all the areas tested at the Shriners Hospital. The group of Hospital staff and external people observing the initial testing noted and discussed the reasons for the unexpectedly low contamination levels found in this particular hospital facility, which were noted as being exceptionally good.

The Hospital staff was understandably proud of these results, especially when the professionals observing the tests commented that in the pre-testing, that the Shriners Hospital for Children in Tampa had the lowest readings of any of the other facilities seen tested over four years.

In discussions as to the reasons the Shriners facilities had such low ATP readings, bias due to advanced knowledge of the testing was dismissed as the staff were not informed, nor were the locations selected until the morning of the commencement of the study. It was noted that an important factor might have been that the staff was working with Children, and were therefore more diligent than they might otherwise be. Outside observers noted that they had not seen comparable levels of dedication shown by the cleaning staff in comparison to staff efforts at other facilities. This may be no small factor. It is rare to find anyone who is not affected when working with children who have health issues. In evaluating the staff from this perspective, it was



noted that the feeling of dedication appears not to dissipate over time by staff that stays in service with the hospital, which might be a possible interesting study in itself.

### **TESTING WITH CULTURE MEDIA VS. ATP TEST RESULTS:**

In addition to testing with cultures, the Hospital also performed ATP testing. This was to evaluate the effectiveness and convenience of ATP testing which was of interest due to the short time required to obtain the test results, and the ease with which the staff was able to perform the ATP tests.



*The photos above show ATP testing of several of the Hospital's sites that are part of this study*

**NOTES ON THE RELATIONSHIP BETWEEN CULTURES AND ATP TESTING:** Cultures can be extremely specific, depending on the type of media being used and what will grow on that media. Cultures are commonly used to detect the presence of and quantifying the amount of infectious bacteria.

It should be noted that ATP testing reflects not only the presence of total organic load but also other sources of ATP, hence there is not always a direct correlation between the two, however, ATP testing is a good indicator of the relative cleanliness and presence of contamination. ATP testing measures adenosine triphosphate (ATP) which is the universal energy molecule found in all animal, plant, bacteria, yeast and mold cells. Organic residues, particularly food, contain large amounts of ATP, and when left on a surface can harbor and grow bacteria, cause cross-contamination, and develop biofilm. Microbial contamination also contains ATP except in rare cases including the probiotic solutions developed by Chrisal.



An ATP reading does not specify the specific organisms or sources that produce the detected ATP, and so, though an excellent indicator of cleanliness, ATP meters cannot directly determine the presence of dangerous pathogens.

ATP testing is normally an extremely good indication of cleanliness and is sufficient to indicate the relative level of safe contact on surfaces.

It must be noted however, that even small amounts of pathogens that fall within the accepted range of ATP testing numbers, can still culture into substantial growth over a relatively short period of time. This is a normal occurrence and has been seen in this and all testing.

In spite of fact that pathogens falling below an acceptable range of an ATP meter may still be hazardous, ATP testing has proven to be of great value, most importantly, due to the speed of testing since an ATP meter requires only 15 seconds for a determination whereas cultures require 24 hours for a determination results, depending on the type of testing performed. Due to

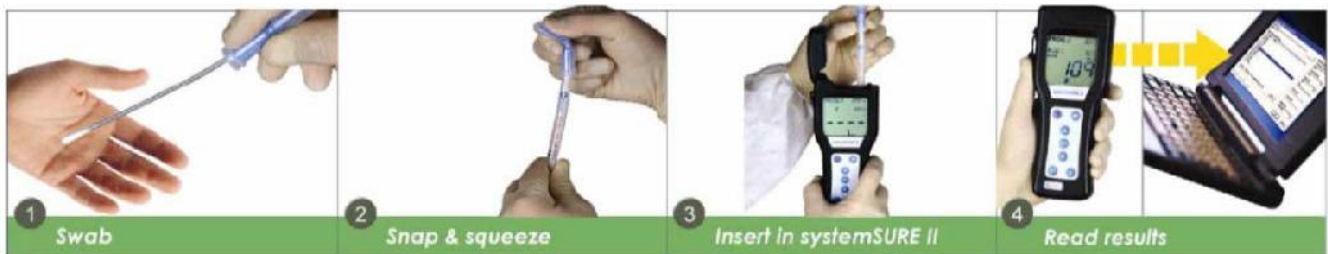
the possible discrepancy, periodic cultures should also be added to the routine testing to ensure that pathogen control is maintained.

**CORRELATION:** A base line correlation between ATP readings and cultures, is possible where each numerical reading of, “1” would equal approximately 1,000 bacteria. It should be noted that a reading of 10 would be considered low, but in a culture starting off with only 10,000 the bacteria count can rapidly grow in to billions in 24 hours. Therefore the comparison of ATP and 24 hour cultures may diverge over a period of hours. ATP readings are primarily useful and accurate as an instantaneous indication.

**TEST STEPS:** ATP Testing is performed by simply rubbing the swab on any surface to be tested, from skin or a kitchen counter to an operating room table. The swab is then reinserted in the tube, the bulb is bent to break the seal holding the reagent, the liquid drops into the swab compartment, and the entire assembly is shaken and then inserted into the meter. The OK button is depressed and an exact digital read out of the number value is obtained in 15 seconds. The system also numbers each test and is compatible with, and plugs in to any computer to provide permanent electronic records.

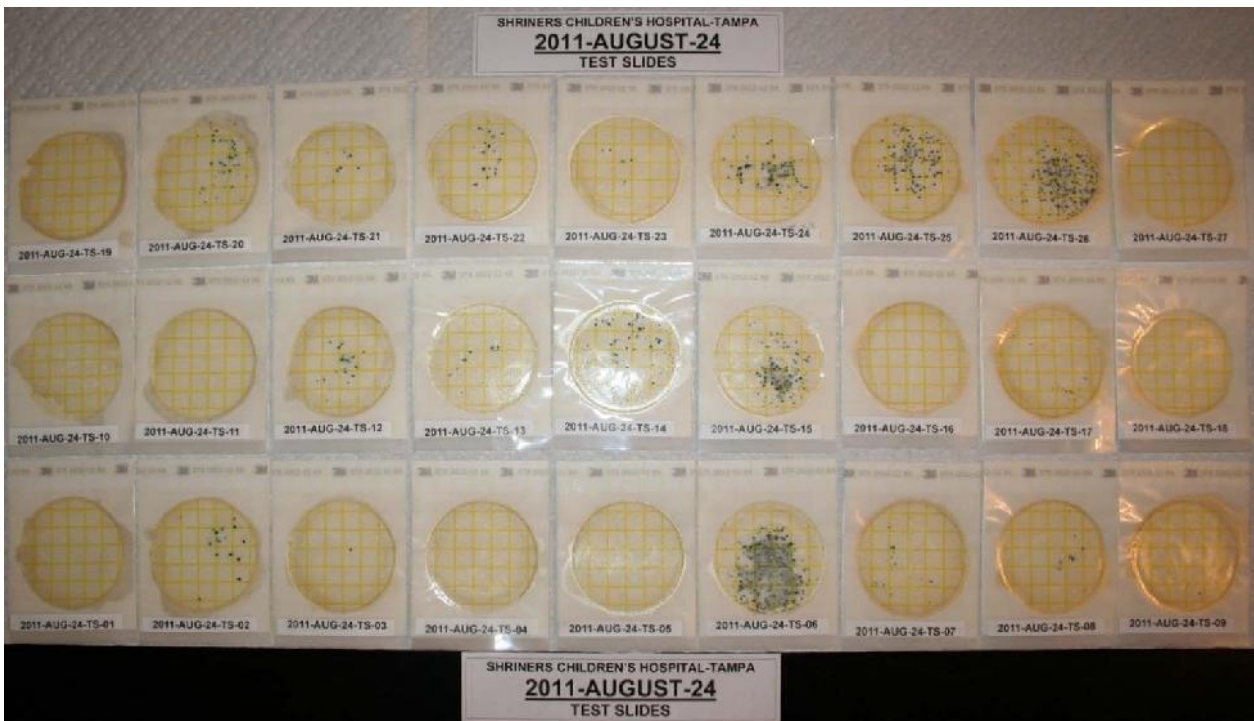
Note that any ATP reading of less than 30 is considered safe and over 30 is considered to have bacterial and/or other contamination.

*The four steps in ATP testing*



**TEST RESULTS – 2011 AUG-24 – PRE-CHRISAL TESTING:**

The following test results are from areas prior to the initial application of probiotic cleaning



materials. Cultures were taken shortly after cleaning by staff using standard hospital cleaning materials. Seven of the cultures showed no activity due to the cleanliness of the facility. Though pathogen growth is to be expected in public facilities, especially in hospitals, other than in some locations, the readings were well under what is normally prevalent in most hospitals.

**TEST RESULTS – 2011-SEP-01 – START OF THE CHRISAL USE TESTING PERIOD:**

Below are the test results after the first week of cleaning with the Chrisal PIP Probiotic Products



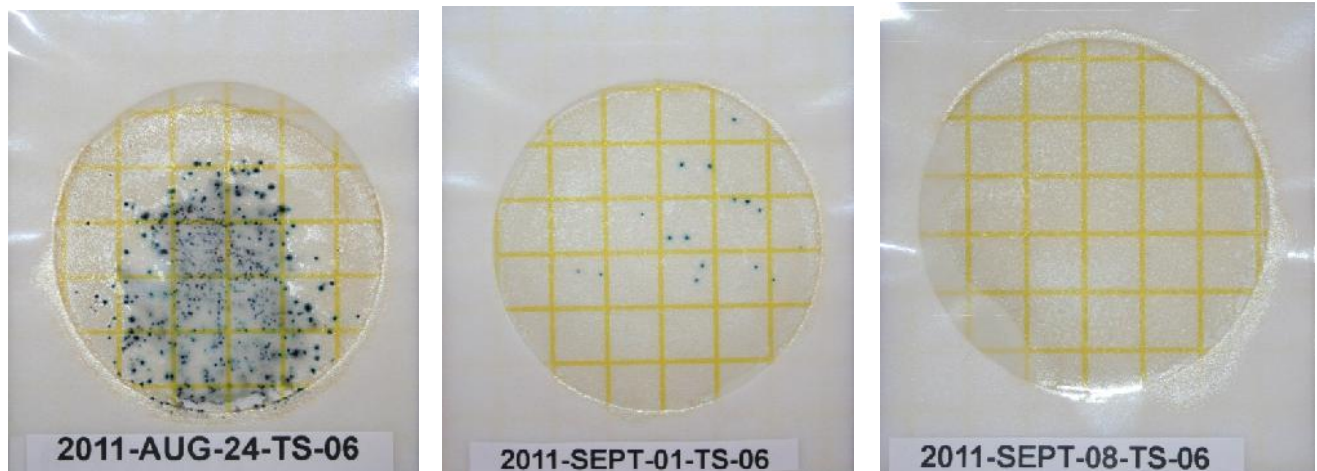
**TEST RESULTS – 2011-SEP-08 – CHRISAL USE TESTING PERIOD:**

These weekly results, when grouped together, show the progression and advantage of cleaning with probiotic. One of the most important aspects and advantages provided by the Chrisal PIP probiotic cleaning products is the control and deconstruction of biofilm.



Since it is now recognized that an important aspect of cleaning and infection control is to deconstruct biofilm, an important advantage of these probiotic products is that they work down to the microscopic level, deconstructing the protective biofilm that bacteria create and reside within.

In older facilities a great number of biofilm layers can build up, in which case it takes from a few days to two or more weeks to rid all surfaces of the biofilm. That is why the products are called PIP, for Probiotics-In-Progress. Once the probiotics deconstruct the biofilm, from that point on, the probiotic products provide an effective ongoing control of the microscopic environment. (Chrisal calls this “**CDME**”, for Controlled Domination of the Microscopic Environment.)

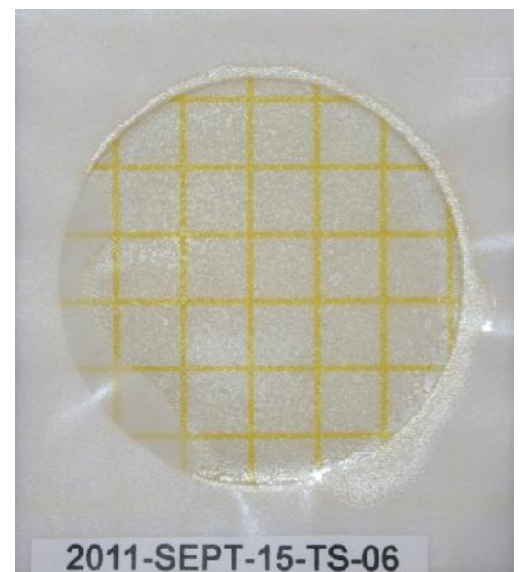


The 3 slides above represent the normal progression of the probiotic steps in controlling biofilm.

It can be seen from the fourth culture (*the slide to the right* →) of this series that the biofilm has been deconstructed, the probiotics that cover the surfaces, applied during the original cleaning, continue to work and protect all the surfaces cleaned.

The key point and objective, is that once all the biofilm has been removed, there is nothing left on the surfaces to protect the pathogens, other bacteria and contaminants, including viruses and dirt, so that the surfaces stay clean and safe for year after year as long as the regimen is followed.

**ONGOING PROTECTION IS PROVIDED BY THE VERY NATURE OF PROBIOTICS:** Unlike chemical cleaners that stop working as soon as they are dry, probiotics are a living solution that normally keep working at full strength for up to three (3) days.



During the cleaning process using the Chrisal PIP probiotic products, it was noted that the probiotics deposited as part of the cleaning process, will continue to function for days, providing ongoing cleaning and protection for all surfaces.

Subsequent testing of the probiotic cleaners indicated progressively lower (cleaner) ATP readings taken hours after use - and then again the next day, without any additional cleaning.

The standard non-probiotic, chemical cleaners resulted in re-contamination within hours.

**CONFIRMATION TESTING METHOD:**

Testing was performed using an ATP Meter. Readings were taken from the selected areas and then additional culture samples were taken directly adjacent to the ATP test sites to then be cultured for 24 hours.

The surfaces were tested both prior and subsequent to cleaning.

An optimal testing regimen is to section off a larger surface on a selected area, tape off and number the separate sections. Each test site should be directly adjacent to the other. Uniform testing of each area after cleaning with different cleaners should be performed to make a valid comparison. The tested cleaners included the range of standard hospital cleaners currently in use, disinfectants, other cleaners on hand, regular bleach and the Chrisal probiotics.

Subsequent to cleaning the surfaces, additional tests were performed. In the vast majority of instances the probiotic product had had the lowest ATP reading and has proven to be the best cleaner.

In performing additional tests after a few hours and again the next day, invariably in all cases, the areas cleaned with traditional toxic cleaners were contaminated again within a short period of time, while the segments cleaned using Chrisal probiotic products actually resulted in far lower ATP (cleaner) readings, and progressively continued to reduce contamination over time.

**TEST RESULTS – 2011-SEP-15 – CHRISAL USE TESTING PERIOD:**



As noted in all of the testing to date, here in the Shriners Children’s Hospital in Tampa, and corroborated by test results from other hospital and university studies, by the end of the third week, all probiotic treated surfaces have become biofilm free, and as long as the surfaces continue to be cleaned with the probiotics once every three days, they tend to stay free of pathogens, contaminants and allergens. It should be noted that if fresh contamination is placed

on top of the surfaces cleaned with probiotics, and swabbing performed, there may be short period of time for which a microbial spike will occur. However, once the probiotics have had the time to take hold and control an area, the contamination does not spread and becomes inactive due to the competitive advantage held by the probiotic solutions.

### **SCHEDULE RESTRICTIONS WHEN USING TRADITIONAL CLEANING SOLUTIONS:**

Different areas within the facility have different cleaning schedules and restrictions. Public access areas are cleaned twice a day, by the day and evening shift personnel, with special cleaning for any spills or problems. However, there are a number of patient areas that cannot be cleaned with regular schedules, such as some of the out-patient rooms which are dependent upon staff and patient use and scheduling.

In addition to issues revolving around scheduling, there are special considerations when using traditional toxic chemicals in proximity to patients and hospital personnel. Special consideration must be made when using normal chemical cleaners in areas where patients with respiratory conditions, such as asthma are in residence. In cases where allergic reactions ranging from slight to extreme present, toxic chemical use must be carefully controlled. One of the nurses that works within the area under study reacted so violently to any of the conventional cleaning solutions that she was not able to be in close proximity to any area cleaned for a period of time after the application of any materials with toxic or chemical fumes.

Once the Chrisal probiotic PIP products were used to clean the nurse's desk area, her phone, computer and the floors around the nurse's station and the entire wing, that the nurse experienced no adverse reactions and had no idea that the area was being cleaned with anything other than water.

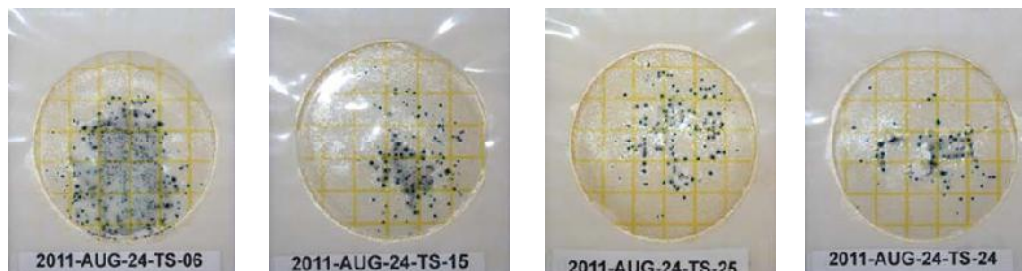
Another observed benefit was that asthmatic patients in the area under study had no adverse reactions to the probiotic cleaning solution.

### **PATTERN OF SAMPLING RESULTS & NOTES ON ANY VARIATIONS:**

In the pre-probiotic sampling, 7 out of 27 cultures showed a zero count.



5 of the 27 locations had high counts. These included areas such as on the baby changing station, the game controllers used by the children in the lobby section and the patient log-in area.

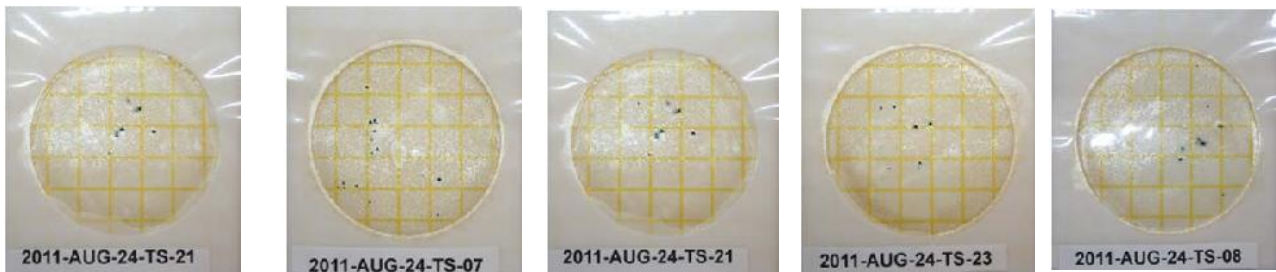


The medium count plates were from areas like the sink handles and counter tops, computer keyboards and mouse, the push bar for the hand sanitizer, the handicap railing in the bathrooms.

The light count plates included exam room floors, and a mix of the same type of areas that showed both medium counts and zero counts.

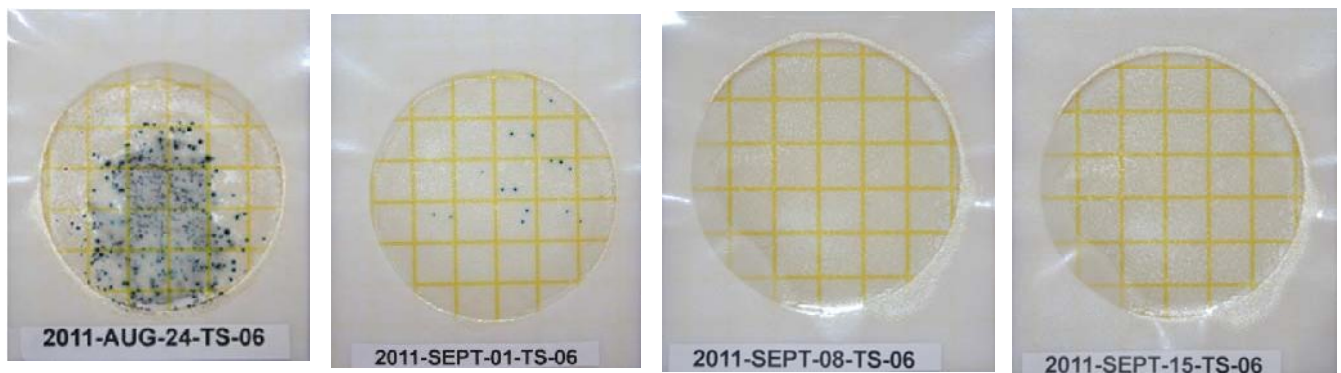
This indicated that for similar type locations and items tested, the variables depended upon what users had access to the area, and how the cleaning schedules were arranged.

Therefore, it was expected that the use of a probiotic cleaner would be able to help alleviate the variance by removing the biofilm that supported a great deal of contamination and pathogen activity.



### **PROGRESSION OVER THE TEST PERIOD**

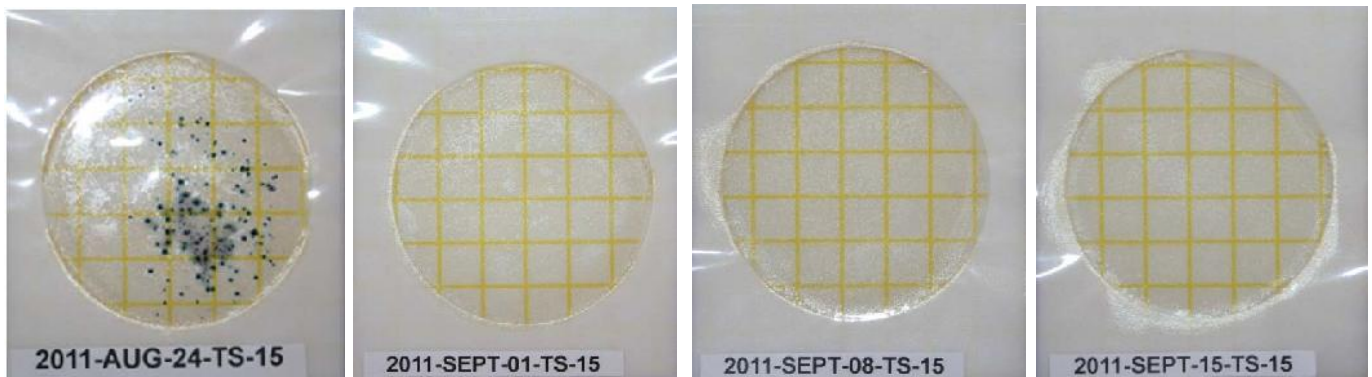
**Results Test Site-06):** Taking the high count plates as a good example of the progression of probiotic cleaning, the following depict the progress of each area tested, starting with test site-06



The above cultures show the progression of the results in test location-06, with the first culture on the left being the results of testing the area cleaned with the hospital's normal cleaning and disinfecting products.

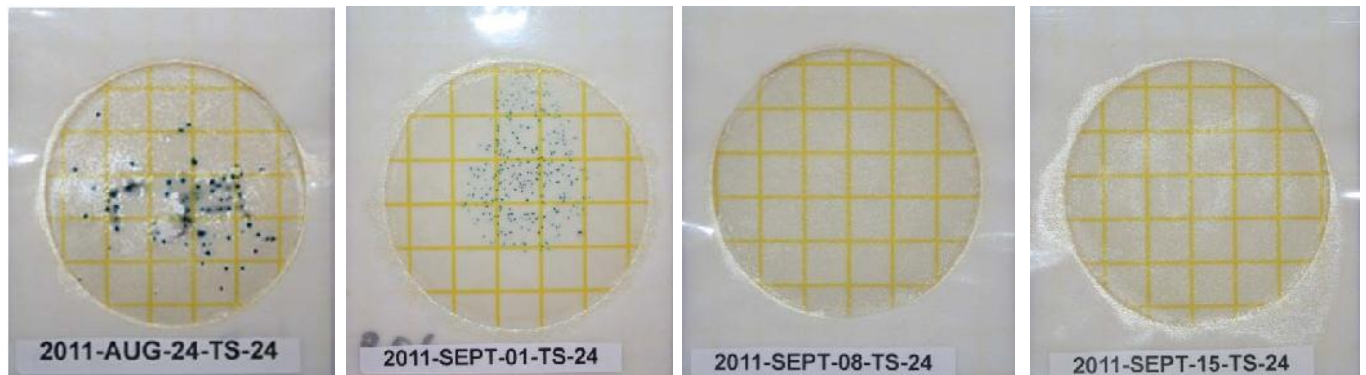
The second culture is the week after application of the PIP Probiotic cleaners. Each subsequent slide thereafter indicates the weekly test results from the PIP Probiotic cleaned area.

**Results Test Site-15:**



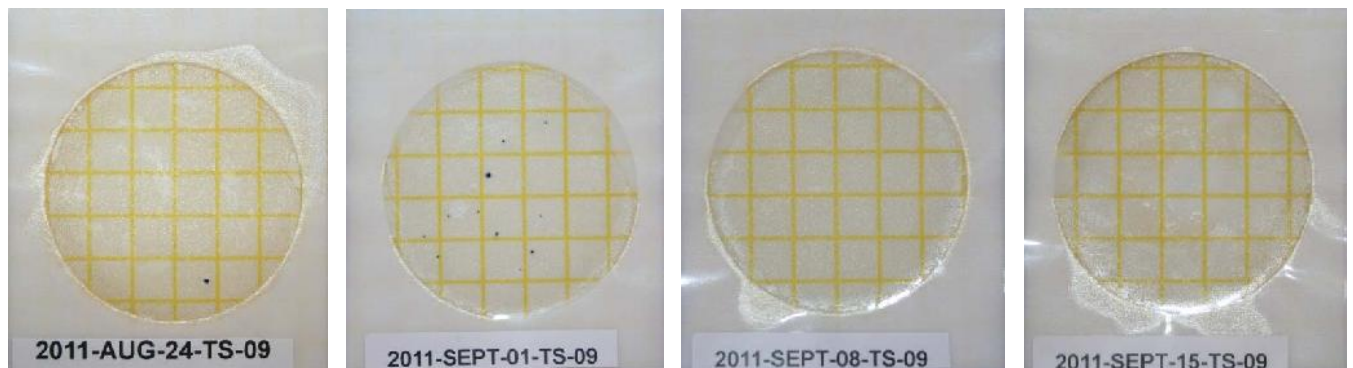
The above slides indicate the progressive reduction in pathogens when the probiotic solutions are used. In all cases the results match those found in dozens of other studies we examined.

**Results Test Site-24:**



Site-24 is another normal progression of probiotic cleaning.

**Results Test Site-15 – Note The Variations:**



The anomaly in, Site-09 demonstrates that, until the biofilm is totally removed, there remain areas that afford protection for pathogens and other contaminants. As a result, because there were resources remaining for pathogen growth in the first week, until the second week when the probiotic took full control, the pathogens were able to continue to survive.

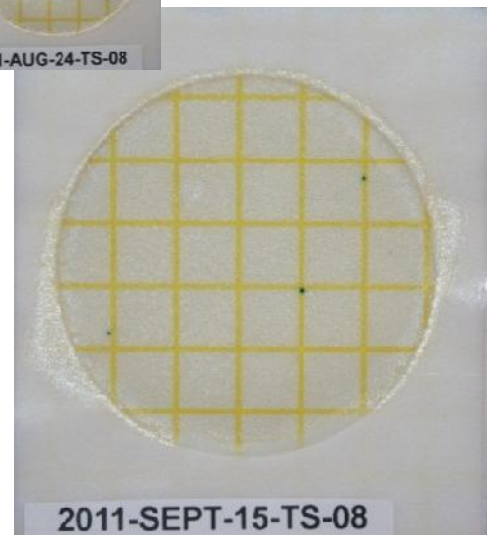
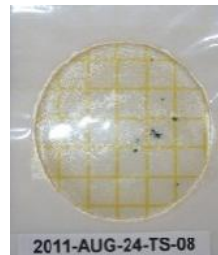
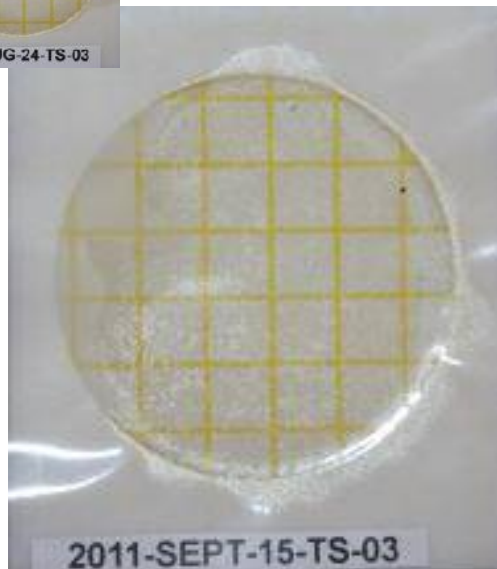
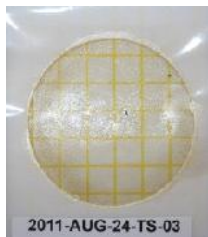


**Notes on Additional Variations:**

Below are the cultures from two sites that showed minimal counts during the third week of testing. It was noted at the time of testing that these two cultures were from test sites in front of urinals.

The other test was performed on the grout in front of a toilet where at the time of the tests, photos were taken of a large splatter of fresh urine and footprints in front of the urinal that had not been cleaned. There was also recent soiling around the toilet.

The interesting thing about both of these situations is that test swabbing of the soiled matter, spiked the microbial load, but the bacteria did not gain a foothold and did not continue to grow on the surfaces treated with the Chrisal PIP Probiotics.



SHRINERS HOSPITALS FOR CHILDREN-TAMPA - TEST SITES & RESULTS FOR CHRISAL													
2017 Test Dates:		PRE-CHRISAL TESTING-AUG-24			POST-CHRISAL TESTING SEPT-01			POST-CHRISAL TESTING SEPT-08			POST-CHRISAL TESTING SEPT-15		
Site #	Fac. Site Location	ATP #	Culture Results	Notes	ATP #	Culture Results	Notes	ATP #	Culture Results	Notes	ATP #	Culture Results	Notes
<b>GUEST QUARTERS</b> Bathrooms Pharmacy Hallway				planned 25 used 25	Numbers??			Numbers??			# of 101-128		
<b>Men's Bathroom</b>													
1	Baby Changing	70	0	L19-T1/T27 (T21?)	1	0		0	4		1	0	
2	Handicap Stall Handrail	176	17	L19-02/T21	3	29	L19-02??	0	0		0	0	
3	Floor in front of Light-umbral	153	2	L17-12/T124	5	2	L17-12??	18	0		5	4	
<b>Woman's Bathroom</b>													
4	Handicap Stall Rails	77	0	L4-T2	3	0	L16-T2	0	0		7	7	
5	Handicap Push button	76	0	L3-T2	3	0	L20-T2	1	0		0	0	
6	Diaper Changer	598	N/C	L18-12/T122	1	18	L18-12??	4	0		0	0	
	Roller in middle stall	43		L18-11/T125	3								
7	Soap Dispenser Top	70	23	L15-T2/T33		0	L15-T2??	6	0		3	0	
<b>BEACHCOMBERS InPatient Unit</b>													
<b>Nurse's Locker Room</b>													
8	Floor in front of toilet (group)	143	11	L9-T2/T36	47	9	L9-T2??	35	1		35	4	
9	Sink Handles	6			3	11		2	0		2	0	
	Wall Visual												
	Floor Visual												
<b>Patient Room #20</b>													
10	TV by sink	2	0	T23 Not occupied		0		0	1		22	0	
11	Stocker Chair by Sink	7	0	L10-T1 recently		0		4	0		3	0	
<b>Patient Exam Room</b>													
12	Sink Handle & counter top by blue cabinet top dispenser	7	23	L8-T1 L13-T2		0	L13-12??	0	1		8	0	
13	Patient bedside table	22	9	L7-T1 L14-T2	2	0	L14-12??	2	0		6	0	
<b>Nurse's Desk</b>													
14	Computer keyboard B	28	33	L6-T1 L11-T2		2	L11-T2??	4	2		4	0	
15	Telephone-Locker in cabinet	103	168	N/C	8	0		1	0		8	0	
<b>OUTPATIENT DEPARTMENT</b>													
<b>Exam Room 17</b>													
16	Exam Table Mattress	3	0	L13-T1		0						0	
17	Counter Top & Sink Handles	7	18			2		2	0			0	
<b>Exam Room 18</b>													
18	Exam Table Mattress	3	0	L15-T1		0		0	0		7	0	
19	Floor in front of Exam Table	4	7	L14-T1		0		7	0		20	0	
20	Computer keyboard mouse	26	43	L7-T1		6		6	0		0	0	
21	Floor @ table head next to trash marked can	122	11			0		7	2		4	0	
<b>OUTPATIENT HALLWAY</b>													
22	Computer Keyboard/Hallway betw rooms 17+18	103	28	L7-T1		0		3	1		7	0	
23	Hand sanitizer between rooms 4+6	59	9	L16-T1		3		10	0			0	
<b>MAIN LOBBY &amp; KIDS PLAY AREA</b>													
24	Patient Login Computer & Mouse	75	73?	L4-T1	17	N/C			0			0	
25	Topo Screen game for kids	37	13?	L5-T1		9			0			0	
26	Kids game controller - left side	104	N/C	L12-T1		10						1?	
	Lobby Water Fountains & Wall												
	Lobby Feet Rests Mens												
<b>MENS BATH ROOM</b>													
				Just cleaned	23		See urine splash in photo						
27	Handicap stall	9	10?	L1-10-T0? Just cleaned									
	Diaper changer	no	2	L1-T1 Just cleaned		0							
	Handicap stalls	30	L5-T2	Just cleaned									
	Floor in front of stall	1	X	Just cleaned									
<b>KITCHEN</b>													
	Floor in front of fryer	30		L3-T1	60								
	Refrigerator/water cooler	74	L1-T1	Washed after each use									
	Floor in front of rear rest sink	86	L2-T1		18								
24-Aug-2017 notes													
Note-01: Had just been cleaned minutes before testing.													
Note-02: Had just been cleaned minutes before testing.													
Note-03: Had just been cleaned minutes before testing.													
Note-04: Had just been cleaned minutes before testing.													
Note-05: Patient room had not been occupied recently since disinfecting.													
Note-06: Patient room had not been occupied recently since disinfecting.													
Note-07: Mattress was cleaned with disinfectant recently after patient was discharged from room.													
Note-08: Sink and counter tops were cleaned with disinfectant recently after patient was discharged from room.													
Note-09: Mattress was cleaned with disinfectant recently after patient was discharged from room.													
Note-10: Very odd AT-11 testing on floor below where patient's is what it may be due to being wiped with disinfectant if anything dripped on floor.													

## **STUDY CONCLUSIONS**

The study undertaken at the Shriners Hospital in Tampa started with a list of key questions and goals. The questions about the viability of probiotic products for applications in our facilities were formulated in a logical order to cover all items of concern and of interest utilizing a step by step protocol.

The results of the study were well received by all parties, and the answers to the questions and conclusions from those results are as follows:

- 1) **DO THE CHRISAL PIP PROBIOTIC CLEAN AS WELL AS THE CURRENT HOSPITAL CLEANING PRODUCTS?** The answer is, yes, they do. In every single case, using the PIP probiotic products yielded better results in all aspects of cleaning and infection control when compared to the existing products used in the hospital.
- 2) **HAVE THE CHRISAL PIP PROBIOTIC PRODUCTS PROVEN TO BE SUPERIOR TO THE CURRENT HOSPITAL CLEANING PRODUCTS – AND IF SO, HOW?** All of the probiotic products tested proved to provide far superior results in a number of ways.
  - a. **CLEANING ABILITY:** in just the first few days of the study it was noted that the Chrisal Probiotic Products produced visibly better cleaning than conventional products. An ATP meter was not required to see the substantial positive difference in cleanliness of the surfaces. In the Nurses Locker Room with only one cleaning, a badly soiled and dull blue tile wall became so much cleaner that a number of the staff commented on the visible improvement in its appearance.

In the lobby area, the water fountains and the splash guard on the wall behind them that were previously cleaned every day with the Hospital's standard products were cleaned by the PIP products. After use of Chrisal, both the fountains and the wall behind and adjacent to the fountains looked like new. These types of improvements were evident on all surfaces tested over the course of the study
  - b. **FLOORS:** An interesting effect that we noted is that in normal mopping, the water starts turning cloudy and slightly gray as the staff mops the floors. However, with the Chrisal Probiotic Products, as they started to break up the biofilm, the water actually started to turn black from all the extra dirt removed by the PIP Floor & Carpet Cleaner. It was also determined that by simply fogging over carpeted areas with the Chrisal Probiotic Environmental Control product, dirt and soil on carpets become unbound by the probiotic action as it breaks up the biofilm, so that simple vacuuming becomes far more effective. The fact that the dirt was more easily removed from the carpet with vacuuming alone means that shampooing of carpet areas can actually be performed less frequently providing additional cost and labor savings, as well as extending the life of the carpet. An added benefit of the Chrisal probiotic action is that by ridding the carpet of biofilm, the contaminants and the microbial load are reduced thus providing safer surfaces. This is of special benefit where children are playing on carpets.
- 3) **HOW SAFE ARE THE CHRISAL PIP PROBIOTIC PRODUCTS COMPARED TO THE STANDARD HOSPITAL PRODUCTS USED?** This is one of the most important aspects of the probiotic cleaners from Chrisal. We noted:
  - a. **NO DANGEROUS CHEMICALS - OR FUMES.** The cleaning crew and management greatly appreciated the elimination of the toxic cleaners and fumes. Even though the

Chrisal Probiotic products proved to be extremely effective cleaners, there are no dangerous chemicals in the products so they can be used without protective equipment or gloves. Of course in a hospital setting gloves should always be used where there is a possibility of infectious materials from patients being present. We noted however that chemical irritation and many associated problems that are common with chemical use, disappear when the staff used Chrisal products. Therefore, even when spills or accidents occur, there is no risk of injury as a result of Chrisal use instead of the normal high risk of sustaining permanent and irreparable damage from standard cleaning chemicals.

**The greatest risk to the general and patient population using conventional cleaners is the release of toxic fumes generated by traditional chemicals.** This is a well-recognized problem of major concern. The change to Chrisal probiotics has eliminated the risks and impact posed by these toxic chemicals on patients, staff, management and the public visiting the facility.

- b. **SAFETY NOTE:** Because the Chrisal Probiotics are safe to use, it was noted that these non-toxic products would be of benefit for use in facilities treating patients that present physically aggressive behaviors as well as mental and dementia patients as these products cannot be used as weapons to inflict harm intentionally or unintentionally.
- 4) **EASE OF USE CONSIDERATIONS?** The Chrisal Probiotic Products have proven to be simple to use, even without instructions. In most situations it is a case of simply spraying on the product and wiping it off, or mopping on and mopping off. The unique ability to fog and spray with the Probiotic Environmental Control products adds a totally new dimension to cleaning and labor saving procedures. The probiotics are shelf stable for years and once diluted for use, should be used within a week. They retain full efficacy when stored above freezing and below 122 degrees F.
- 5) **DILUTION:** Each of the hospital cleaning sinks have a PDU (automatic self contained Portable Dilution Unit) attached so that the probiotic products are properly metered automatically for use and the staff is not responsible for determining or measuring for accurate dilution. The product containers simply thread into any standard sink with a hose. Activating the unit using a button, automatically delivers exactly the correctly diluted product dilution into spray bottles – or into buckets to wash the floors. The unit hooks on the side of any wash-bucket and has a lock on the trigger for filling large volumes.

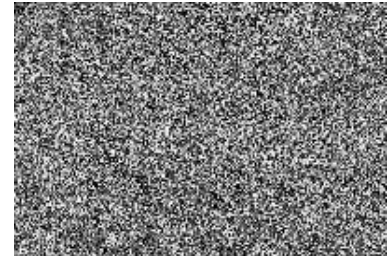
## **INFECTION CONTROL**

This was the crux of the study. Even the best run hospital will be contaminated to some degree by bacteria constantly brought in from the outside, transported in from public areas by patients, staff and visitors, resulting in microbes managing to gain a foothold in areas that are difficult to clean on a consistent basis.

The new scientific data generated over the last few years pinpoints the degree of culpability of biofilm in the propagation of infection. As stated in previous sections, the recent information of how much greater and more pivotal the role of the microscopic environment is on health is being taken into consideration more widely. Compounding the problem is the rapidly growing resistance of pathogens to antibiotics and the realization that the problems are being caused by the very chemicals created as a means to combat them.

Chrisal's probiotic products were chosen for this study because no other products claiming to have probiotic activity have been found to meet all of the criteria required and to have sustainable non-toxic probiotic constituents and prolonged shelf life. In addition, there is a large body of scientific and medical testing that has been conducted over the past five years on the Chrisal products that are extremely positive. Therefore the following factors of prime importance were met:

- 6) **THE REDUCTION OR ELIMINATION OF BIOFILM:** The need to focus on cleaning and reducing or eliminating biofilm as a way of reducing bacteria load rather than trying to just kill bacteria with disinfectants was clearly demonstrated.



**Biofilm is produced by bacteria as a “protective housing”** – it is an aggregate of microorganisms in which cells adhere to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS), which is also referred to as slime. Biofilm will form on both organic and on non-organic surfaces and is prevalent in natural, industrial, commercial and hospital settings, as well as homes and all other surfaces.

- 7) **UNDERSTANDING THE DANGERS OF BIOFILM:** Biofilms have been found to be involved in a wide variety of microbial infections in the body, by some estimates as much as **80% of all infections**. Infectious processes in which biofilms have been implicated include common problems such as:

- urinary tract infections**
- middle-ear infections**
- gingivitis**
- infections in cystic fibrosis**
- and less common but more lethal processes such as endocarditis**
- and infections of permanent indwelling devices such as joint prostheses and heart valves**
- catheter infections**
- formation of dental plaque**
- coating contact lenses**

**More recently it has been noted that bacterial biofilms may also:**

- impair cutaneous wound healing**
- reduce topical antibacterial efficiency in treating infected skin wounds**

- a) **“DISINFECTANTS CAUSE INFECTIONS”:** This counter intuitive statement is the quandary posed by disinfectants and an extremely important factor that has only recently come to be recognized about the disinfectant process. The key to understanding the reasons that the disinfecting process causes re-infections is simple. Many of the organisms killed by disinfectants cannot be mechanically removed. The normal method and mechanics of wiping with paper towels or cloth to pick up the microbial bodies remaining (the proteins and carbohydrates), even on the smoothest of surfaces is ineffective and fails to remove all of the microbial carcasses. This is a major problem in that many of these microbial bodies are physically unreachable in cracks, holes, pits, depressions and other geographic features of the surfaces of most objects on a microscopic level. Hence it remains on and in surfaces as a food source for subsequent generations of bacteria.

- b) **Add the serious complication of BioFilm.** There are normally many complex layers of biofilm in all of the fissures on surfaces as well as on the surfaces themselves (i.e. the plaque on teeth and stubborn darkening of the grout between tiles are excellent

examples). The problem is that disinfectants normally never properly penetrate biofilm – and so all the bacteria, viruses and other contaminants protected by the biofilm are not affected by even the strongest hospital grade disinfectants.

- c) Another major factor in infection control is that all **disinfectants and cleaners stop working as soon as they are dry or shortly thereafter**. The Chriscal products normally continue to keep working and clean for up to 72 hours after each application, even when dry.
- d) **Why Conventional cleaning and disinfectants cause increase risk of infection:** As stated above, after being disinfected, the treated surfaces retain billions of dead organisms that are a food source available to the first opportunistic organisms that land on that surface or migrate upward from the biofilm below. This surface then provides pathogens, which appear more aggressive than beneficial bacteria, with a massive source of food, the protein and carbohydrates of the carcasses, while eliminating the competing benign bacteria that normally keep dangerous organisms in check.

Recognizing this problem was an important step toward understanding the dangers of the overuse of antibiotics which leads to producing resistance in bacteria. The over-use of antibiotics and disinfectants are now creating serious problems everywhere from hospitals to gyms and from schools to homes.

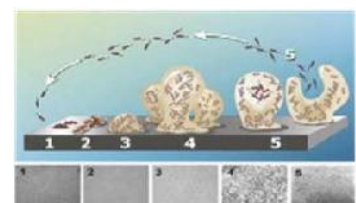
Therefore, the overwhelming concern about overuse of disinfectants and a search for solutions to these quandaries provided a major incentive for staff to be open to reviewing the benefits of the probiotic paradigm over the conventional “kill 99.9% method”. It became obvious in a short period of time to the Hospital study team, that the evaluation of the probiotic solutions provided by Chriscal clearly demonstrated the ability of the probiotics to control the microscopic environment, instead of laying waste to it.

Traditional disinfectants constantly create an environmental wasteland where competition has been removed, and the carcasses of the killed bacteria become a ready food source, giving pathogens a cyclic advantage each time a surface is disinfected. Consequently since pathogens tend to be hardy and prevalent organisms, even though they normally are only an extremely small percent of the total microscopic population, pathogens seem to be the ones prevalent in rapid growth spikes and so greatly increase the risk of infections.

Therefore, by destroying **all** of the bacteria, including the vast balance of “good” bacteria that help provide protection from, and compete with, the harmful bacteria, **disinfectants have actually created a breeding ground that leads to an upward cyclic spiral of increasing pathogens after a short period of relative safety after each treatment**.

The Chriscal products did not promote the cyclic growth of pathogens because they did not provide additional food with every cleaning. Instead the probiotic solution changed the paradigm to that of removing the resources and the biofilm protection that pathogens require to live, thus causing their elimination from the cleaned environment.

- 8) **INFECTION AND THE BIOFILM FACTOR:** Again, It is impossible to control the microscopic environment of any surface without being able to remove the existing biofilm and prevent its re-growth. Once biofilm has been eliminated with the Chriscal Probiotic Products, it has been found that as long as the



probiotics are used at least once every three days, biofilm is not regenerated. In other studies it was noted that on surfaces where biofilm has been removed using Chrisal probiotics, the risk of MRSA and other infections dropped by a considerable factor.

**DECONSTRUCTING BIOFILM:** The results of our testing indicate that the efficacy of the Chrisal products was well evident from the initial application from the first day of the study. The concept of PIP (probiotics in progress) proved also to be accurate; not only do the Chrisal products perform as well or better in keeping surfaces visually free of contamination as conventional products, but they provided the added benefit of totally deconstructing and removing biofilm down to the bare surface over a short period. This is something no other traditional cleaner or disinfectant is able to do in a practical way. As result, as each layer of biofilm is eaten away, the surface becomes cleaner and safer.

**PIP – THE TIME FACTOR IN THE REMOVAL OF BIOFILM:** We noted that probiotics require from two days to several weeks to deconstruct biofilm on most surfaces. (again, this is the reason for the “PIP” designation). For locations with extremely well entrenched biofilm, it may take up to three or four weeks, but this seem to occur only in rare situations and typically substantial and measurable improvement can be seen in the first weeks.

- 9) **SOLUTION CRITICAL AREAS - THE COMBINATION OF CHRISAL AND DISINFECTANTS:** Recognizing the downside of disinfectants only raises the additional issue of the regulatory requirement to disinfect to 99.9%. Because it is currently impossible for Hospital and medical facilities to stop using disinfectants for specific areas for the foreseeable future (as such changes require overcoming conventional thinking and acceptance of a new paradigm), we have noted that there is a way to gain the critical advantage that the new probiotic methodology provides, while adhering to the regulatory requirements for certain locations. It is to utilize the “best of both worlds” method as noted below.

**FORMULATING A “ONE-TWO PUNCH” COMBINATION:** When it is understood that one of Chrisal’s key functions is to totally eliminate contaminants and harmful biological matter from any surface, it becomes obvious that, for high-risk areas, Chrisal and a disinfectant provides a synergistic benefit. In another study we noted that at the Lokeren Hospital, Chrisal is being applied in every area of the hospital ongoing and for the last five years. In order to adhere to the regulations requiring disinfection in critical areas such as in operating rooms, the hospital utilizes Chrisal on a normal basis for cleaning, but disinfects prior to procedures.

Using probiotics between disinfections can vastly reduce the risk of infection since the probiotics remove and then prevent the growth of biofilm plus control the microscopic environment against contamination until the next disinfection procedure. Since changing the hospital’s cleaning supplies to Chrisal, the Hospital rose to being rated as one of the top 10 hospitals in the country for having the least amount of nosocomial infections.

Therefore, the Chrisal probiotics not only make disinfectants far more effective by eliminating the biofilm that protect pathogens from disinfectants, but the probiotics then also eliminate the organic matter resulting from the disinfection process. This is a vast improvement in infection control and meets or exceeds all of the criteria of our study.

- 10) **ADDITIONAL PROTECTION OF INTEREST:** It should be noted that in addition to all the studies to date, provided to the team, showing that the use of the Chrisal Probiotic Products tend to lower infection rates by large percentages in hospitals, there are also studies

showing how effective the Chrisal probiotics can be against spore based organisms. This includes C-Diff, which is now a serious problem. (we referenced the report from the University of Liverpool report on C-Diff and Chrisal). Of additional interest to our study are the reports indicating the elimination of MRSA by Chrisal, including one from the University of Ulster and another from the Burn Center in Kiev. The study relevant to the control of MRSA is covered in-depth in the study from the University of Gent, the Lokeren Hospital and in the University of Ferrara and St. Anne Hospital Study. We have reviewed the findings in these studies and subsequent to comparing the results of our studies concur with their findings.

- 11) **WHAT IS THE WASTE PRODUCT?** In the case of the Chrisal Probiotic solutions, the waste product from the deconstruction of the biofilm and digestion of dead organisms is CO<sub>2</sub>. Therefore these probiotics exhibit a highly effective modality that has a number of beneficial attributes while having no negative properties or processes.
- 12) **NEGATIVE FACTORS IN USING CHRISAL.**  
We did not experience any negative factors in using the Chrisal products
- 13) **DID THE CHRISAL PRODUCTS VALIDATE THE ABILITY OF THE NEW PARADIGM TO PROVIDE BETTER CLEANING AND INFECTION CONTROL:** This study provided clear and consistent results indicating that the application of the PIP probiotic products from Chrisal radically reduced the risk of pathogenic bacteria levels in any sites where they were used. This is demonstrated by the series of culture plates collected before, during, and after the study. ATP testing further corroborated these findings.

In addition, all areas cleaned with Chrisal demonstrated visibly cleaner and brighter surfaces and most importantly, became odor free. It should be noted that the Chrisal products used during this test did not cover up odors, but actually eliminated all of them.

It was determined that the ability of the Chrisal products to effectively remove odors was due to the fact that Chrisal PIP worked down to the microscopic level, actually eliminating all odor causing contamination and bacteria, so that there was nothing left on the surfaces to produce odors.

Another facet of interest is that the surfaces cleaned with Chrisal tended to feel smoother to the touch. Because this was subjective measure, a number of professional personnel were asked to test for this characteristic along with confirming the visual perception of improved cleanliness. Ultimately there was a general consensus that the surfaces actually did feel smoother after cleaning with Chrisal. This appears to be as a direct result of removing the biofilm which is holds contaminants which increase surface friction.

## **IMPORTANT RESPIRATORY FACTORS**

**RESPIRATORY DYNAMICS:** Another major consideration that has been solved by utilizing Chrisal Probiotic Products is the elimination of the respiratory problems triggered by the disinfectants and chemical cleaners currently in use.

Hospitals are treating more and more asthmatic patients and, sadly, we are noting a rise in asthma in child populations as well. In addition there also seems to be a rise in the incidence of other respiratory problems. Our study has shown that Chrisal Probiotics do not trigger any respiratory problems, distress or negative responses in patients. This is contrary to most



other conventional products. Chrisal is also effective in removing allergens and irritants from the environment which provides additional benefits to both patients and staff. During the test period, no negative comments were noted from the cleaning, medical staff, management or patients.

Comments about the odors and fumes from standard cleaning materials are not uncommon. In comparison, when using Chrisal, the comments received from the staff were that the probiotic products were pleasant to use. We are acutely aware that employee compensation claims due to chemical fumes and even burns are a regular occurrence. The study clearly allowed us to conclude that this problem would be alleviated by using the Chrisal probiotic products.

- 14) **COST FACTORS:** In addition to being more effective, it has been estimated that using the Chrisal Probiotic products should lower the direct costs of cleaning materials by up to 50%. In addition, Chrisal products have been shown to reduce labor costs by a significant factor and a report by one group in neighboring Clearwater showed up to 50 percent reduction in labor in their facilities.

**THE COST OF INFECTIONS:** There is an extremely large cost benefit to the reduction of infections caused by hospital stays. Preventing just one hospital contracted infection per year yields a cost savings in excess of the yearly cost of using the probiotic products. In essence it is easy to determine that changing to Chrisal products effectively reduces the costs of cleaning to the hospital to zero when considering cost avoidance.

- 15) **REDUCTION IN NUMBERS OF DIFFERENT PRODUCTS REQUIRED:** Normally a large number of different cleaning products are used to accomplish the required results in a hospital facility. The Chrisal PIP product line appears able to replace most of these traditional chemical cleaners with only two to four probiotic products that have greater efficacy, are safer and more cost effective. Using these products also reduces overhead and space costs as well as lowering labor demands on the cleaning staff.

- 16) **ENVIRONMENTAL IMPACT:** The environmental impact of the disinfectants and chemical products as well as the growing local, state and federal regulatory pressures in how these products are to be used, stored and in the methods required for disposal are a growing problem. Chrisal eliminates these problems as the core ingredients in the products are organic, have been FDA registered as a dietary supplement and are non-toxic and benign. It should be noted that the products are beneficial when used in facilities requiring septic tanks.

- 17) **REACTIONS AND EVALUATIONS FROM STAFF & USERS:** Every staff member using the Chrisal products have evaluated them indicating that they are better than the traditional products used to date. They rated Chrisal products ranging from “very good” to enthusiastically, “We love the products and never want to go back”. Almost all staff members noted that they can now breathe without worry about respiratory allergic responses or topical allergic reactions. In each case, individually and as a group, the staff endorsed the concept of probiotic cleaning and wanted to change from traditional cleaners to the Chrisal products.

- 18) **PRE-DETERMINATION USER REPORTS:** A study of the products was not initially considered when the Hospital was first presented with information about the Chrisal Probiotic Products. They were simply presented as a solution that might be of interest. Samples were provided to some of the management and staff to try off-site in a home setting. At the time, it was proposed that if the users saw positive results, perhaps the hospital would like to

test the products in auxiliary building areas like the trash room and other non-medical areas and then over time the use of the products might expand to more areas.

The test results proved so positive that it was recognized that the probiotic products might prove to be of great use to the Hospital. The fact that a great deal of test data in a number of other hospitals and universities supported and were consistent with our initial findings expedited our conclusion that a full onsite study was in order. Examples of the off-site reports from users included examples of:

- (a) Far better cleaning and reduction of the risk of infection**
- (b) Complete odor removal, including areas of strong urine odors**
- (c) And, of the elimination of allergens and other factors that relieve respiratory distress.**

In other tests, staff members reported that their children with asthma had significantly reduced breathing problems in just a matter of days after starting to use the probiotic products. This of course is of significant interest to a children's hospital.

- 19) **TESTING USER REPORTS:** Once the study was initiated at the Hospital, the staff was constantly polled for their opinions.

Some withheld judgment, but within a week, as stated above, all of the staff responses about the products were enthusiastically positive.

An example of employee response is the report from one of the night staff<sup>(1)</sup> that cleaned the lab area. This particular employee has problems with the fumes from the standard chemical cleaners. The employee reported that using the Chrisal products, not only cleaned better, but was like "a breath of fresh air." Another strong indication of acceptability was that a number of staff members asked how they could purchase the products for their own use as they felt enthusiastic and safe using them in their own homes once they saw the results.

The final indications from all of the cleaning staff, including the director and both the day supervisor and the night crew supervisor, was that they all highly endorsed the Chrisal products and all thought working with these probiotic solutions was a major plus for the hospital's cleaning efforts.

They felt that that the Chrisal products afforded the hospital its staff and patients, a safer, chemical and odor free environment, while improving the convenience and cleanliness of the facilities over the performance of existing products.

No negatives were reported by any of the cleaning crews or any of the associated staff members, and there were a number of comments referencing reluctance to return to using the old products.

#### **ADDITIONAL PROTECTION OF INTEREST:**

It should be noted that the use of the Chrisal Probiotic Products tend to lower infection rates by a large number against such problems as Staphylococcus aureus, and according to other studies, and spore based organisms, including Clostridium difficile (C-Diff), which is a serious problem (we referenced the report from the University of Liverpool report on C-Diff and Chrisal).

The reports indicating the control of MRSA and other pathogens have been covered in this study (see the University of Ulster, the Burn Center in Kiev, the University of Gent, the Lokeren Hospital and in the University of Ferrara and St. Anne's Hospital studies).

### **STUDY CONCLUSION**

**SUMMATION: The final result of the Shriners Hospital study demonstrates that in all categories the Chrisal probiotic products exceed the criterion set forth in our goal to find new methods and solutions that:**

- 1) improves cleaning,**
- 2) reduces the risk of infection,**
- 3) is green, safe to use directly on skin without gloves or protective gear,**
- 4) eliminates adverse respiratory and allergic response in staff and patients,**
- 5) reduces both material and labor costs,**
- 6) and reduces our impact on the environment.**

**Therefore, the results clearly demonstrate the need to change from conventional cleaners to these probiotic solutions, and to continue to use the products in our Hospital and to introduce Chrisal to the rest of the hospitals in our organization as a solution of choice for the benefit of our patients, staff and management.**

# ADDENDUM

## STUDY ENTITIES

### THE SHRINERS HOSPITALS FOR CHILDREN.

The Shriners Hospitals for Children is a pediatric health care system of 22 hospitals dedicated to improving the lives of children by providing specialty care, innovative research and outstanding teaching programs.

Children up to age 18 with orthopedic conditions, burns, spinal cord injuries, and cleft lip and palate are eligible for care. They receive the services in a family-centered environment without financial obligation to patients or their families. Shriners Hospitals for Children rely on the generosity of donors to deliver this mission every day. Locations are listed below and for more information, visit [www.ShrinersHospitals.org](http://www.ShrinersHospitals.org).

#### Boston, MA

51 Blossom St.  
Boston, MA02114  
617-722-3000

#### Canada

1529 Cedar Ave.  
Montreal, Quebec, CanadaH3G 1A6  
514-842-4464

#### Chicago, IL

2211 North Oak Park Ave.  
Chicago, IL60707  
773-622-5400

#### Cincinnati, OH

3229 Burnet Ave.  
Cincinnati, OH45229-3095  
800-875-8580

#### Erie, PA

1645 West 8th St.  
Erie, PA16505  
814-875-8700

#### Galveston, TX

815 Market St.  
Galveston, TX77550-2725  
888-215-3109

#### Greenville, SC

950 West Faris Rd.  
Greenville, SC29605  
864-271-3444

#### Honolulu, HI

1310 Punahou St.  
Honolulu, HI96826-1099  
808-941-4466

#### Houston, TX

6977 Main St.  
Houston, TX77030  
713.797.1616

#### Lexington, KY

1900 Richmond Rd.  
Lexington, KY40502-1204  
859.266.2101

#### Los Angeles, CA

3160 Geneva Street  
Los Angeles, CA90020-1199  
213-388-3151

#### Mexico

Mexico City, MX  
Av. del Iman No. 257, Col. Pedregal de  
Santa Ursula, Deleg. Coyoacan, 04600,  
Mexico, D.F.  
011-52-555-424-7850

#### Northern California

2425 Stockton Blvd.  
Sacramento, CA95817  
916-453-2000

#### Philadelphia, PA

3551 N. Broad St.  
Philadelphia, PA19140-4131  
215-430-4000

#### Portland, OR

3101 S.W. Sam Jackson Park Rd.  
Portland, OR97239-3009  
503-241-5090

#### Salt Lake City, UT

Fairfax Road at Virginia St.  
Salt Lake City, UT84103  
801-536-3500

#### Shreveport, LA

3100 Samford Ave.  
Shreveport, LA71103  
318-222-5704

#### Spokane, WA

911 W. 5th Ave.  
Spokane, WA99204  
509-455-7844

#### Springfield, MA

516 Carew St.  
Springfield, MA01104-2396  
413-787-2000

#### St Louis, MO

2001 S. Lindbergh Blvd.  
St. Louis, MO63131-3597  
314-432-3600

#### Tampa, FL

12502 USF Pine Dr.  
Tampa, FL33612-9411  
813-972-2250

#### Twin Cities

2025 East River Pkwy.  
Minneapolis, MN55414  
612-596-6100

## **THE STUDY TEST SITE - THE SHRINERS HOSPITALS FOR CHILDREN - TAMPA**

This study was performed at the Tampa Shriners Hospital for Children located on the Campus of The University of South Florida at 12502 USF Pine Dr, Tampa, FL 33612-9411.

The hospital includes 179,000 square feet of treatment and support areas and provides a large range of services to children of all ages.

When the Shriners first became aware of the availability of a probiotic cleaning solution, the Tampa hospital near the Shriners' international headquarters was picked as the test site.

A multidisciplinary team was assembled to manage this study since there were a significant number of factors to be considered in addition to the direct testing. These included managing the possible changes in procedures and evaluating the associated potential health benefits. The members of the team involved in this study include:



Gene Bracewell,  
Chairman Emeritus  
Board of Directors (and Board of Trustees)  
Shriners Hospitals for Children

Sheryl Chewning, RN, CIC, CPHO, LHRM  
Director of Performance Improvement, Risk Management, and Infection Control  
Pediatric Specialty Care, Tampa

Carol Ann Jenkins  
Administrative Director of Support Services, Tampa

Jim Gamez  
International Headquarters  
Pediatric Specialty Care  
Supply Chain Sourcing Specialist, Supply Chain Management Department, Tampa

Roberta 'Bert' Hardy  
Director of Environmental Services, Tampa

Patty Veasey, Tampa

### **Tampa Hospital DAY Cleaning Crew Trained On Products:**

Mcneil  
Blackman  
Lancaster  
Miller  
Sneed

Hospital NIGHT Cleaning Crew Trained On Products:

Fernandez

Munoz

Ray

Other Support Entities:

Dr. Marina G. Morris, M.D., Medical Sciences Group

Kim Metzler-Rice, Hygiene USA

Robert W. Yates, 3M Microbiology

Lino G. Morris, CEO, Chrisal Group

Howard Zalkin, Training Director, Chrisal USA

**SEGREGATING THE TEST AREAS:**







Note that each room and area where the Chrisal PIP Probiotic Products were used had signage placed conspicuously restricting use of normal disinfectants and other chemical cleaning materials in the area under test.

The staff was instructed to not cross-contaminate any of the areas with different products.

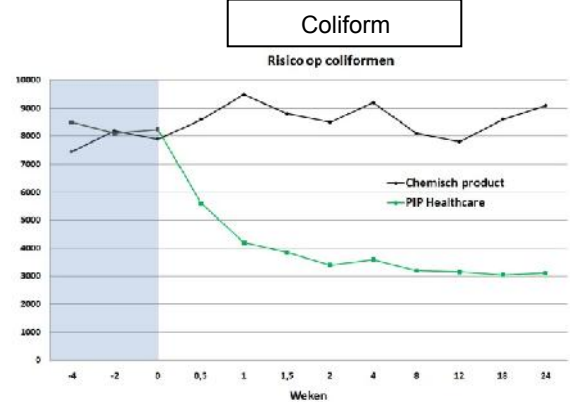
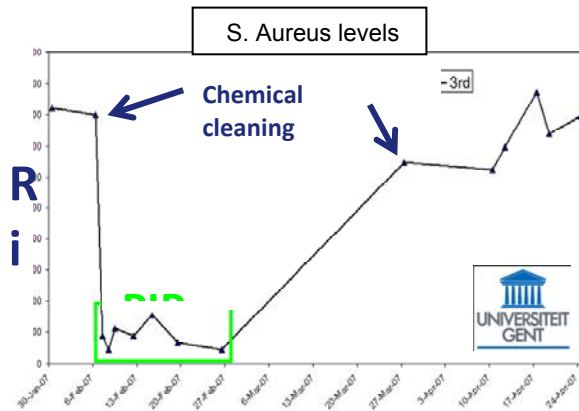
In some of the areas, of course, disinfectant pump bottles were on treatment counters, however these were restricted in use, for the most part, to patient clean up and for instruments.



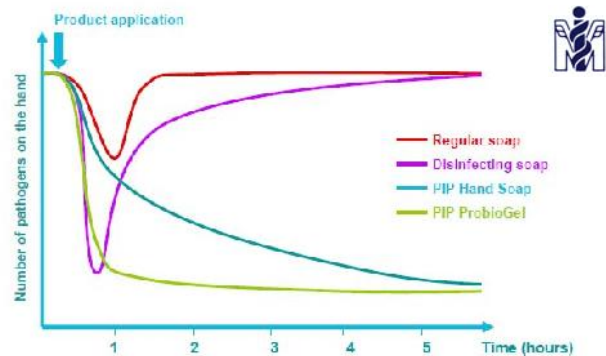
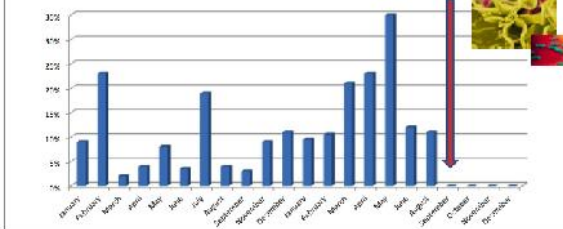
## REFERENCES

- 01) **THE UNIVERSITY OF GHENT & THE LOKEREN HOSPITAL - 18 MONTH INDEPENDENT STUDY** The University of Ghent in conjunction with the A. Z. Lokeren Hospital, with study reviewed by Avecom, conducted 18 months of testing of the Chrisal PIP probiotic products and their effectiveness covered by over 10,000 individual laboratory tests. The hospital has continued to purchase and use the Chrisal probiotics for the last five years. 
- 02) **740 BED MIAMI JEWISH HOSPITAL & HOME FOR THE AGED MEDICAL COMPLEX - INDEPENDENT STUDY** The Miami Jewish Home & Hospital for the Aged is a 740 bed hospital and nursing home facility on 28 acres and 8 buildings in Miami, Florida. 
- 03) **UNIVERSITY OF LIVERPOOL – ENGLAND STUDY REPORT** Testing the effect of PIP cleaning products on viability of Staphylococcus Aureus and Clostridium Difficile. 
- 04) **UNIVERSITY OF ULSTER – IRELAND - HEALTH CARE WORKER STUDY ON THE USE OF CHRISAL FOR THE CONTROL OF MRSA INFECTIONS** 
- 05) **UNIVERSITY OF FERRARA & THE ST. ANNE HOSPITAL STUDY** A major independent one year study of the effects of cleaning with the Chrisal Probiotic products with thousands of individual laboratory culture tests. 
- 06) **POST STUDY UPDATE LETTER FROM THE MIAMI JEWISH HOSPITAL**
- 07) **LOKEREN GENERAL HOSPITAL (AZL) POST STUDY UP-DATE LETTER FOR THE UNIVERSITY STUDY**
- 08) **MIDLAND MEDICAL CLINIC - FT. LAUDERDALE, FL, SPECIALIZING IN AIDS CARE** 
- 09) **INSTITUTE OF OSTRAVA - INDEPENDENT EFFECTIVENESS TRIAL OF PROBIOTIC CLEANING PRODUCTS FOR THE MEDICAL FACILITIES**
- 10) **PIP HEALTHCARE CLEANING AT KIEV CITY MEDICAL BURN CENTRE**
- 11) **KFC AND TACO BELL TESTING YEAR REPORT (YUM Brands) (request access times)**
- 12) **IMPROVEMENT OF ROOM HYGIENE WITH PROBIOTIC PRODUCTS (Hendrik van de Sand, LWZ Haus Riswick)** In the context of the special training course for milk producers in January 2008 at the agriculture center Haus Riswick, Dr. van Eecke presented the improvement of space hygiene by means of PIP
- 13) **LABMET – EFFECT CHRISAL AHS CLEANING PRODUCTS ON CAMPYLOBACTER JEJUNI**
- 14) **AVECOM – REPORT ON HOSPITAL TESTS**
- 15) **TEST REPORT – PIP ALLERGY FREE MATTRESS CLEANING**
- 16) **HOSPITAL & HEALTHCARE REFERENCES LISTING FOR CHRISAL PIP USER SITES**
- 17) **NON-PROFIT CHURCH ORGANIZATION REPORT**
- 18) **FITNESS, ASSOCIATED FACILITIES & FOOD FACILITIES STUDIES**
- 19) **ATP METER TESTING REPORTS USED FOR TESTING CHRISAL PROBIOTICS AGAINST OTHER PRODUCTS IN SCHOOLS, FOOD FACILITIES, PUBLIC TRANSPORTATION SYSTEMS & OFFICES**
- 20) **SUBWAYS RESTAURANTS** (Tests: Hollywood, Miami, Palm Beach at Sterling Road, Jackson Hospital and PGA Blvd).
- 21) **QUARTERDECK RESTAURANT (P.B. AREA)** re floor test results.
- 22) **DUST MITES – MICROBIAL TESTS TOWARDS DUST MITE REPORT BY METLAB**
- 23) **LABMET – EFFECT TOPIDOL STABILIZER PRODUCT ON LISTERIA MONOCYTOGENES**
- 24) **CLOSTRIDIUM PERFRINGENS-EFFECT BY PIP AHS CLEANING PRODUCTS LABMET REPORT**
- 25) **LISTING OF EU FARMS USING CHRISAL PRODUCTS - ALL POSITIVE RESULTS**
- 26) **KLEANTEK INDUSTRIES REFERENCE LETTER**
- 27) **HOTEL ENVIRONMENTS – MICROBIOLOGICAL VALIDATION**

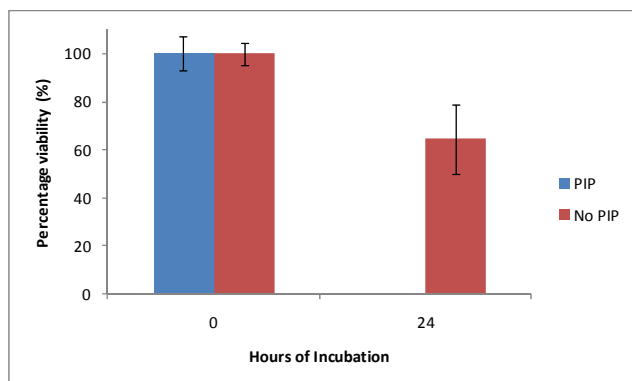
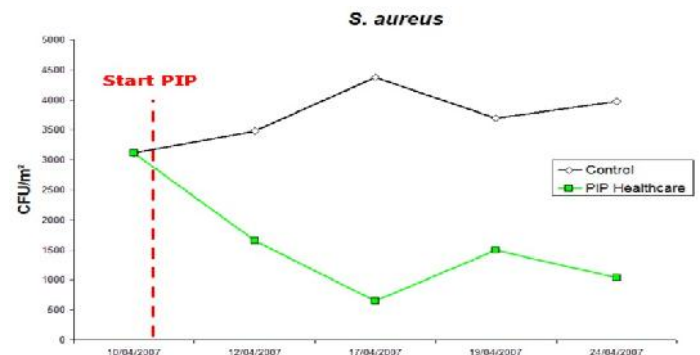
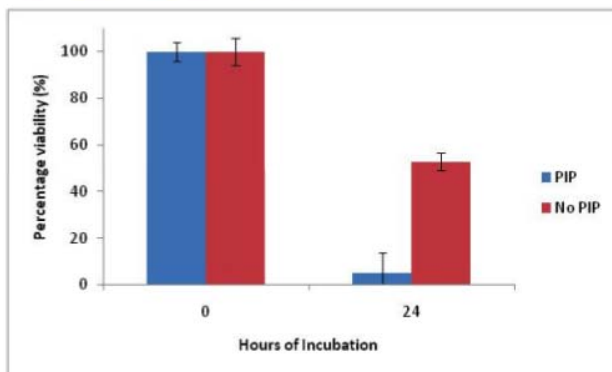
**REFERENCE-II: Example graphics from universities, hospitals & other studies of the Chrisal probiotic solutions since 2005 that provided additional data for the decision by the Hospital to pursue a study of the probiotic products in-house.**



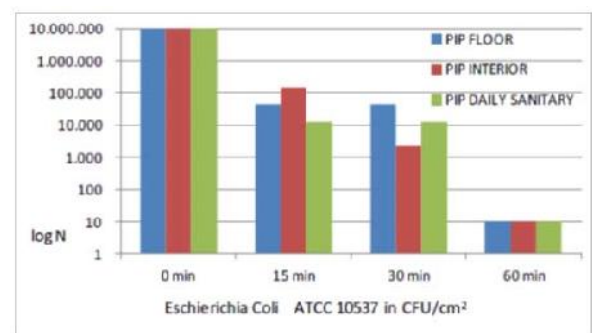
Two year history of Percentage of Salmonella contamination in a large poultry facility – then note the results of starting to use Chrisal PEC in September of the second year:



UNIVERSITY OF LIVERPOOL  
**Clotidium Difficile (C-Diff)**



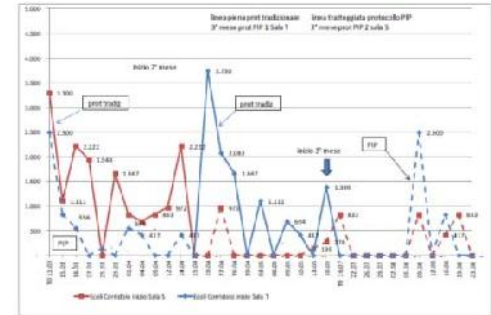
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**TABLE 7 - PERCENTAGE OF TOTAL REDUCTION OF PATHOGEN PROCEDURES WITH RESPECT TO THE PROCEDURES PIP VS TRADITIONAL EXPRESSED IN ABSOLUTE PERCENTAGES**

Sampling Points	Pathogen	General Medicine Phase 1 & 2	Outpatient Ward Phase 1&2	Mean value of General Medicine Ward Phase 3 (*)
Entry and end of corridor	<i>Staphylococcus aureus</i>	29,56%	36,64%	81,03%
	<i>Coliforms spp</i>	72,38%	46,62%	79,72%
	<i>Pseudomonas spp</i>	93,09%	64,49%	88,44%
	<i>Candida spp.</i>	68,88%	56,21%	68,47%
	<i>Acinetobacter</i>		44,74%	
Toilet floor	<i>Staphylococcus aureus</i>	58,75%	51,33%	85,88%
	<i>Coliforms spp</i>	89,15%	78,13%	78,31%
	<i>Pseudomonas spp</i>	55,28%	75,94%	78,57%
	<i>Candida spp.</i>	82,90%	67,80%	71,78%
	<i>Acinetobacter spp.</i>	74,25%		
Toilet sinks	<i>Staphylococcus aureus</i>	55,74%	52,50%	95,59%
	<i>Coliforms spp</i>	81,56%	75,83%	85,12%
	<i>Pseudomonas spp</i>	67,53%	50,41%	95,16%
	<i>Candida spp.</i>	50,38%	27,93%	94,86%
	<i>Acinetobacter</i>	16,39%	31,25%	75,99%


**Figure 8 – Reductions of Escherichia coli in patient areas for S and T ; PIP 1 month -76.67% PIP 2 months - 87.5%, PIP 3 months - 79.72%**

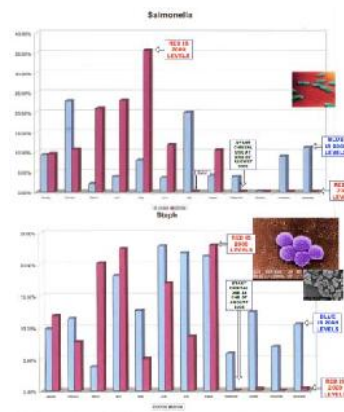
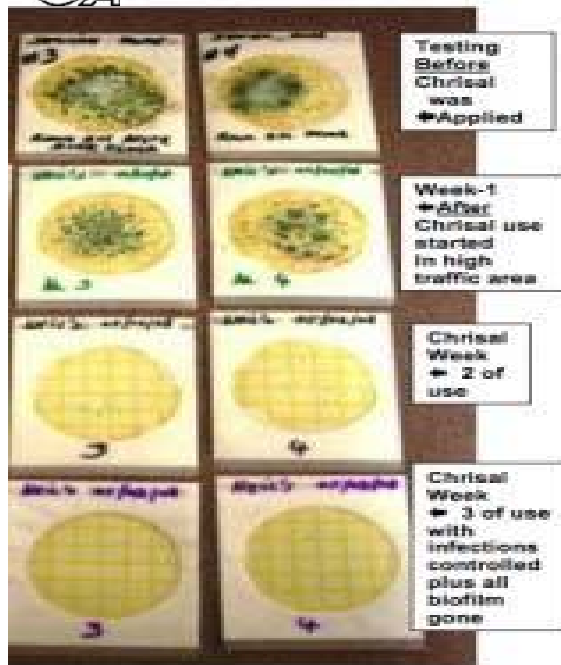
It was found that after two months of use, the prolonged action of the PIP probiotics caused a substantial decrease in the pathogenic microbial load compared to the areas that were treated with traditional products. In many cases the populations of the microorganisms of interest were reduced by close to 90%. One example was from the sink in the toilets, which is a critical surface for patients.

### UNIFE & ST. ANNE HOSPITAL STUDY – CONCLUSIONS

“The mean overall percentage of reduction of pathogens by using the Chrisal PIP Probiotic Products protocol compared to the use of traditional disinfectants is more than a 70 - 80% reduction in pathogens.

Therefore, these results are statistically significant because they have been obtained from the results of the testing of more than 12,000 microbiological samples.

Further, that these samples were taken in many different areas of the hospital, and were subject to everyday recontamination.”



## ADDITIONAL RELEVANT DATA:

In addition to the previous studies and research materials on the probiotic products in the References Section above, are articles of interest related to respiratory and other problems caused by standard cleaning materials:

Bello A, Quinn MM, Perry MJ, Milton DK [2009]. Characterization of occupational exposures to cleaning products used for common cleaning tasks—a pilot study of hospital cleaners. *Environ Health Mar 27(8):11*

Green behind the scenes (2008). *Lodging Hospitality 64(4):52*.  
[http://lhonline.com/green/housekeeping/green\\_behind\\_scenes\\_0315/](http://lhonline.com/green/housekeeping/green_behind_scenes_0315/)

Hansen KS [1983]. Occupational dermatoses in hospital cleaning women. *Contact Dermatitis 9(5):343–351*

Henneberger PK (NIOSH) [2005]. How "clean" is the cleaning profession? *Occup Environ Med. 2005 Sep;62(9):586-7* Comment on: *Occup Environ Med. 2005 Sep;62(9):598-606*

Mei-Lien C, Wan-Ping L, Hsin-Yi C, Bey-Rong G, I-Fang M (2005). Biomonitoring of alkylphenols exposure for textile and housekeeping workers. *Int J Environ Anal Chem 85(4/5):335-347*

<http://www.cdph.ca.gov/programs/ohsep/Documents/cleaningproducts.pdf>

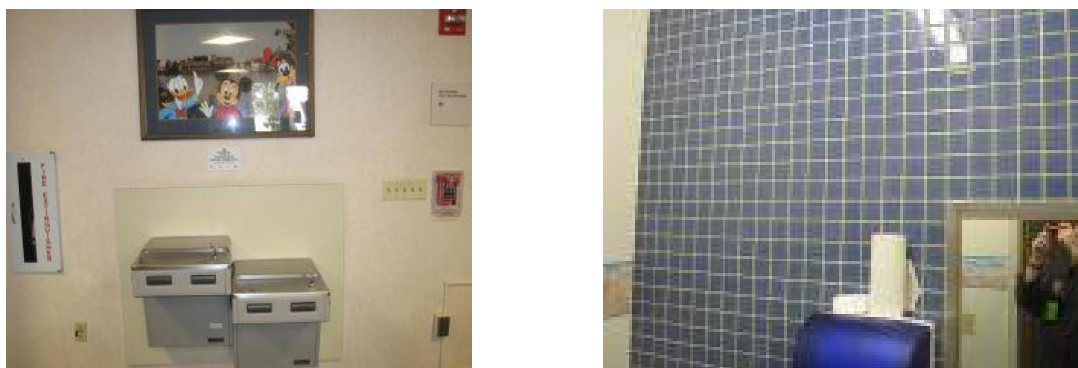
<http://www.arbeitsinspektion.gv.at/NR/rdonlyres/12868056-2A96-4FA3-A189-38509BF412EF/0/Cleanersanddangeroussubstances.pdf>

## Photo Group-2 of Training and Testing Locations

*The photos above show ATP testing of several of the Hospital sites that are part of this study*



*Below is some of the cleaning staff during training on Chrisal and while using the products:*



*Above left are the water fountains cleaned with the tested probiotic products in the visitor's lobby, plus above right is the tile wall in the Nurse's Locker that all looked refurbished by using the products.*