



Medical Sciences Group

Report on the Microbiological Validation Trials of Chrisal StaBiotic Hand Soap And StaBiotic Hand Sanitizer Gel

(Part Of A Series On Probiotic Applications For Cleaning)

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OVERVIEW

The dangers of disinfecting soaps and gels, along with the problems of the over-use of antibiotics in creating resistant strains of pathogenic bacteria is now well understood and even penetrating to the general public.

However, there is still little understanding of the limited effectiveness of these disinfectant soaps and hand sanitizers and how long any beneficial action lasts.

The following study provides specific answers and also provides comparative data with an effective stabilized probiotic (StaBiotic) solution to the problems.

It is now being realized that to properly address the specific problems of nosocomial infections and the greater problem of reducing the spread of infections in general, requires a different methodology. A further study on how the use of disinfectants in general have been shown to actually be part generating infection cycles will be available under separate cover.

As can be seen from this any many other studies, that the core problem has been the attempt of the impossible task to eradicate all bacteria from specific areas from surfaces to wounds. There is the apocryphal report that on his deathbed, Louis Pasteur recanted and said "*Bernard was correct. I was wrong.*" The accuracy of that is in question and Pasteurization has been a huge benefit to Humans. However, Pasteur's core concept of declaring war on all bacteria has proved to be a disastrous mistake.

The key is to not fight nature's power, but instead use it. Each human is host to some ten times more bacteria on and in their bodies than the number of human cells. The 100 trillion cohabitants of your body are essential to your health. Therefore, it is surprising that this was not realized long ago, but it now can be seen that the use of beneficial bacteria is the only way to provide an extremely effective, easy to use and highly economical way to control the microscopic environment and prevent infections. And, to prevent the further rise of "superbugs". Which brings up the highly important additional factor that StaBiotic control of infections means that the same product can work with many different pathogens and continue to be effective as pathogens evolve, without the constant need for new medications.

There is also the significant additional benefit of reducing and eliminating many of the harsh chemicals now used that are creating so much environmental and direct damage to humans and all the other life forms on our planet. This series of reports address this situation.

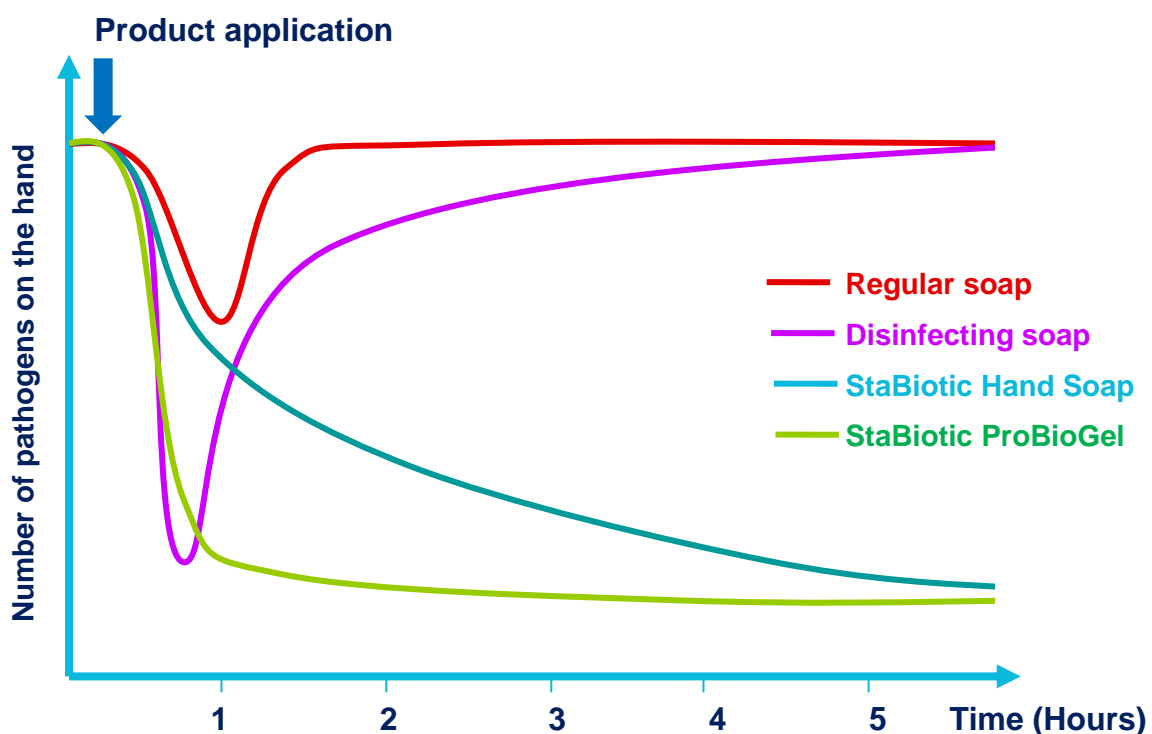
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1. Introduction

Various disinfecting hand soaps and gels exist on the market. Comparative microbiological tests demonstrate the inefficiency of any of these soaps and gels to prevent recontamination of the hands with pathogenic bacteria, yeasts and moulds. As a result, Chrisal has developed a StaBiotic™ (PIP-Probiotics-In-Progress) hand soap (StaBiotic Hand soap) and an Alcohol Gel Hand Sanitizer (StaBiotic Gel, aka: ProBioGell) based on Chrisal's StaBiotic (PIP-Probiotics-In-Progress) technology in order to improve overall hand hygiene and sanitation.

- The Chrisal StaBiotic™ Hand soap: is a creamy, probiotic skin cleanser creating a healthy and stable microflora on the hands and other skin areas.
- The Chrisal StaBiotic™ Gel Sanitizer: is a probiotic alcohol gel for hand sanitation. The Chrisal StaBiotic™ Gel has antibacterial and fungicidal effects and following disinfection, the probiotic bacteria in the StaBiotic Gel create a healthy and protective skin microflora that, importantly, provides ongoing protection for a long period of time compared to standard disinfectants.



The overall concept of StaBiotic™ hand hygiene is that immediately upon application the probiotic bacteria will colonize the hands and prevent pathogenic bacteria, yeasts and moulds from multiplying and spreading.



The main benefit of probiotic hand hygiene is the long lasting ongoing protection of a number of hours after application. This effect is demonstrated in the above theoretical graph.

2. Trial overview

The first round of testing covered the period from July 2008 through December 2008. This study was done by Dr. Robin Temmerman, Ph.D. and Guy Vossen, BG.Bc., Laboratory Director. A number of tests were performed in order to validate the efficiency of the Chrisal StaBiotic™ Probiotic Hand soap and the Probiotic Alcohol Gel) products. The tests comprised three phases:

- **Comparative test:** in a first test, the immediate pathogen removal efficiency of the StaBiotic Hand Soap was determined and compared to several other brands on the market
- **Efficacy test 1:** a first efficiency test with a number of test persons instead of inanimate surfaces in order to determine the protective effect of the probiotic bacteria in the hand soap and alcohol gel
- **Efficacy test 2:** extended comparative efficacy test with a number of additional test subjects to determine the efficiency of the probiotic hand soap and alcohol gel over time in real life situations as people used then in their normal daily routines.

2.1 Phase 1: Comparative test

The first phase determined the immediate pathogen removal efficiency of the StaBiotic Hand Soap compared to a number of commercial hand soaps and hand sanitizers, each with different active substances. The list of tested products and active components are shown in Table 1.

Table 1: Hand cleaning soaps and hand sanitizers used and compared in test phase 1.

Hand Hygiene Product	Supplier	Active Substance
StaBiotic Hand Soap	Chrisal	Bacillus sp.
Palmolive Liquid Hand Wash Hygiene & Protection –Plus	Colgate-Palmolive Company	2,4-Diethanolamine
GermControl	Degraen & Partners	Isopropylalcohol
IQ Quat Foam Soap	AERO	Quaternary ammonia
Antibacterial Premium Soap Septivon	TORK Omega Pharma	Triclosan Triclosan



Hand Hygiene Product	Supplier	Active Substance
Anti-bacterial Handwash	DETTOL	Parachlorometaxylenol
Alcogel 85	Dax	Ethanol

METHODOLOGY:

For 5 days, 4 times a day, hands of a test person were washed with one of the different hand cleaning soaps or sanitizers. Samples were taken every day before the hand washing, when the highest bacterial biota on the hands was expected, and after hand sanitization in order to determine the sanitizing efficacy of the different products, determined as the decrease in bacterial loading.

The applied soaping time was 20 seconds, unless otherwise mentioned by the product supplier. Afterwards, the hands were dried with a sterile paper towel, to avoid cross-contamination, unless otherwise mentioned by the manufacturer. Samples were taken from the (dried) hands with sterile, moist sampling paper, which was consequently printed on a Trypticase Soy Agar plate (total aerobic count) and Baird Parker Agar plate (*Staphylococcus aureus*).

After proper incubation of the agar plates, the total aerobic count value is an indication for the overall bacterial removal and/or the efficiency of probiotic inoculation of the hands. Results are shown in figure 1 (total aerobic count) and figure 2 (*Staphylococcus aureus*).

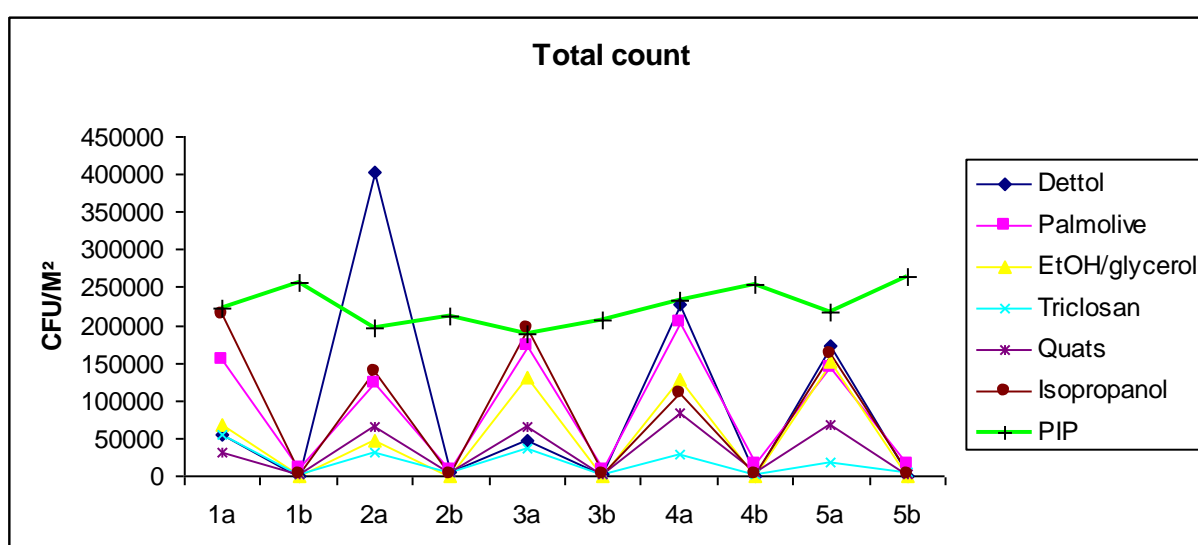


Figure 1: Total aerobic count before (a) and after (b) five distinct applications of various hand soaps and sanitizers



The above results demonstrate that the overall bacterial count is strongly reduced after applying all hand sanitizers and soaps (except StaBiotic Hand soap) and that no significant distinction between the various brands can be made. The total count during application of the StaBiotic Hand Soap remains constant, before and after application.

This indicates that the StaBiotic (probiotic) bacteria are successfully transferred to the hands. However, specific pathogen counts are needed to demonstrate the efficiency of StaBiotic Hand Soap in immediately reducing the number of pathogens (Figure 2).

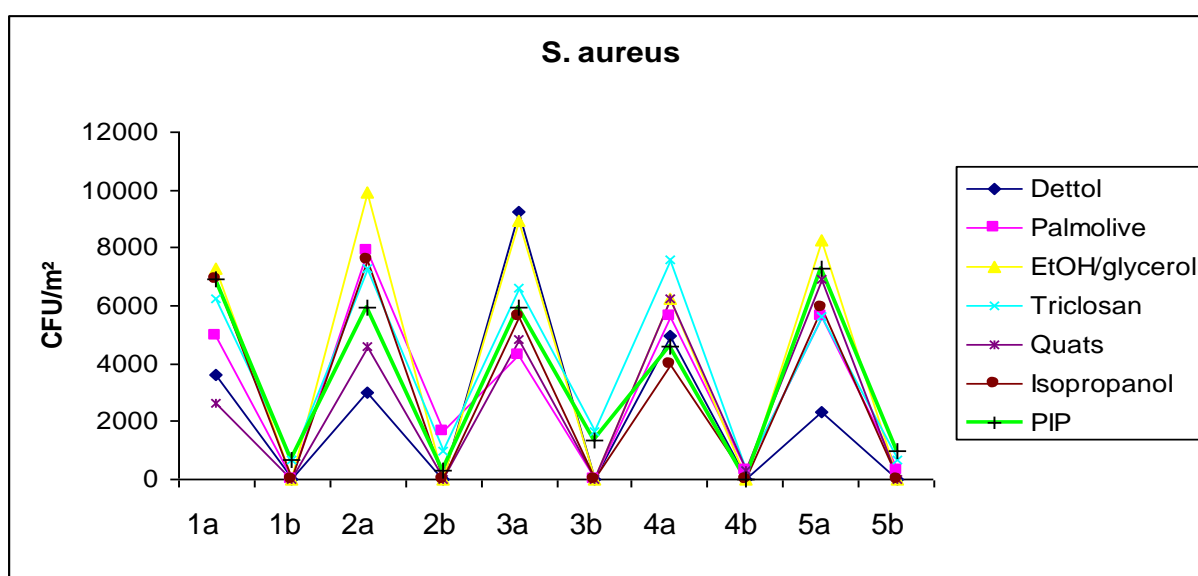


Figure 2: Specific *Staphylococcus aureus* (MRSA bacteria) count before (a) and after (b) five distinct applications of various hand soaps and sanitizers.

Figure 2 clearly demonstrates that, using *S. aureus* as indicator organism, the StaBiotic Hand Soap has an equal efficiency for the immediate reduction in pathogen count compared to other commercial hand soaps and sanitizers.

Conclusion phase 1:

The tests performed in phase 1 show that the formulation of the STABIOTIC Hand Soap has equal efficiency in the immediate removal of pathogenic organisms compared to other hand soaps and sanitizers. In addition, it was proven that the probiotic bacteria in the StaBiotic Hand Soap are successfully transferred to the hand skin during washing.

These probiotic bacteria will create a healthy microbiota on the hand and protect it from new pathogenic colonization and spread. The latter aspect was further assessed in phase 2 and 3 of this validation trial.



2.2 Phase 2: Efficacy test 1

During phase 2 a first efficiency test with 2 test persons was conducted in order to determine the protective effect of the StaBiotic hand soap and StaBiotic alcohol gel. Due to the presence of the probiotic bacteria after application of the products, pathogenic organisms arriving on the skin will no longer be able to multiply and recontaminate the hands. This is the long lasting protective effect that conventional cleaners and sanitizers cannot provide.

In a first small test, two test persons were sampled after applying StaBiotic Hand Soap and StaBiotic Gel respectively. Total count and MRSA samples, following the protocol of phase 1, were taken before, 5 minutes and 2 hours after application of the respective products. Results are presented in Table 2:

Table 2: Microbiological results before and after StaBiotic Hand Soap and StaBiotic Sanitary Gel application.

	Total Count (before)	MRSA (before)	Total Count (5' after)	MRSA (5' after)	Total count (2h after)	MRSA (2h after)
Person 1 (StaBiotic Hand Soap)	> 200 diverse	45 CFU	>500 Bacillus	0 CFU	>500 Bacillus	2 CFU
Person 2 (StaBiotic Gel)	> 200 diverse	86 CFU	>500 Bacillus	0 CFU	>500 Bacillus	1 CFU

The above results show that the hands of the test persons had a diverse hand microbiota (over 200 CFU with various colony morphologies) with a significant presence of pathogenic bacteria (*S. aureus* as indicator organisms). Both StaBiotic Hand Soap and Sanitary Gel are capable of immediately reducing the number of pathogens on the hand upon application and colonizing it with probiotic *Bacillus* bacteria.

The measurements 2 hours after product application still demonstrate a probiotic hand microbiota with almost no pathogenic bacteria.

In the following experiment it was verified, by means of test persons, whether the probiotic version of the StaBiotic Hand Soap and Sanitary Gel performed better than the same product formulation without the probiotic bacteria added.

Efficiency was verified towards both immediate pathogen removal and protective effect. Instead of *S. aureus*, this time the group of coliform bacteria were used as an indicator of



overall hygiene and risk of pathogenic presence. Also, yeasts and moulds were measured. Measurements were done by means of 3M Petrifilms for the StaBiotic Hand Soap and Sanitary Gel (with and without probiotic bacteria) before and 2 min/2 hours after application. Results are presented in Table 3 as percentage of reduction or increase of a certain microbiological group compared to the value before product application (set at 100% value).

Table 3: Percentage of increase or reduction of Total Count (TC), Coliforms (COL) or Yeasts and Moulds (Y/M), 2 min and 2 hours after application of the products, compared to the initial value [set at 100% for all organisms (All)] before product application.

<u>StaBiotic Hand Soap</u>	All (start)	TC (2 min)	COL (2 min)	Y/M (2 min)	TC (2h)	COL (2h)	Y/M (2h)
Hand Soap - PIP bacteria	100	- 86%	- 89%	-100%	- 20%	- 3%	- 78%
Hand Soap + PIP bacteria	100	+ 135%	- 78%	-100%	+ 230%	- 53%	- 100%
<u>StaBiotic Sanitary Gel</u>	All (start)	TC (2 min)	COL (2 min)	Y/M (2 min)	TC (2h)	COL (2h)	Y/M (2h)
StaBioticGel– PIP bacteria	100	- 99%	- 94%	- 100%	- 95%	+ 2%	- 82%
StaBioticGel– PIP bacteria	100	- 3%	- 100%	- 100%	- 4%	- 100%	- 100%

- **Hand soap conclusions:**

- i. The **immediate reduction** (values after 2 minutes) in microbial count is slightly better for the non-probiotic version of the StaBiotic Hand Soap, although the reduction in pathogenic count is almost equal for both versions. The rise in Total Count for the probiotic version is due to the probiotic bacteria being transferred on the hands.
- ii. The **protective effect** (values after 2 hours) is much better for the probiotic version of StaBiotic Hand Soap with 50% less coliforms and still no yeasts and moulds. The amount of coliforms in case of the non-probiotic version has almost returned to the initial value before product application. Also, yeasts and moulds are growing again 2 hours after applying the non-probiotic version, whereas still completely absent 2 hours after applying the probiotic StaBiotic Hand Soap.



- **ProbioGel conclusions:**

- i. The **immediate reduction** (values after **2 minutes**) in microbial count is best with the probiotic version of the Chrisal StaBiotic Sanitizing Gel, with the exception of total count remaining almost the same because of the probiotic bacteria being transferred on the hands. Both coliforms and yeasts and moulds were removed completely with the probiotic StaBiotic Gel.
- ii. The **protective effect** (values after **2 hours**) is again much better for the probiotic version of the StaBiotic Gel. In case of the non-probiotic version coliform count after 2 hours of product application is already 2% higher compared to the initial value. Also, the yeasts and moulds have started to multiply again with the non-probiotic product. Both coliforms and yeasts and moulds are still completely absent 2 hours after applying the probiotic StaBiotic Sanitary Gel.

Conclusion phase 2:

The tests performed in phase 2 show that the addition of probiotic bacteria to a hand soap and alcohol gel formulation indeed results in an improvement of the products performance. Total count increases due to the transfer of the probiotic bacteria, but all other micro-organisms (coliforms, *S. aureus* and yeasts/moulds) are efficiently reduced and suppressed for a long period of time after application of the probiotic StaBiotic Hand Soap and Sanitary Gel.

2.3 Phase 3: Efficacy test 2

Tests performed so far were short term performance tests with a limited number of test persons in lab conditions. The final phase 3 tests will demonstrate the products efficacy in a real life situation with 4 test persons ('consumers') using either the StaBiotic Hand Soap (2 test persons) or the StaBiotic Gel (2 test persons) in their everyday life.

The overall test period lasted for 4 weeks:

- week 1 and 2: the 4 test persons applied the non-probiotic hand soap or alcohol gel
- week 3 and 4: the 4 test persons applied the probiotic hand soap or probiotic alcohol gel



Twice every week microbiological prints of the hands from each test person were taken by means of the 3M Petrifilm technology. As the test simulates a real life situation, each test person carried on with its normal daily activity, being a mixture of office and lab activities. The test persons applied the products as their normal hand hygiene measures with no imposed frequencies of application. Samples were taken 1 hour after application of one of the products by means of the 3M Petrifilm technology.

The palm of the hand was pressed on the Petri films for one minute after which the films were closed and incubated at proper temperature and time. After 24h or 96h the number of CFU/cm² was determined.

During each sampling 4 measurements were performed:

- Total aerobic count (CFU count after 24h at 28°C)
- *Enterobacteriaceae* (CFU count after 24h at 37°C)
- *Staphylococcus aureus* (CFU count after 24h at 37°C)
- Yeasts and moulds (CFU count after 96h at 28°C)

Results are presented in Table 4. Microbial count values for the non-probiotic version are set as reference value = 100%. The effect of the probiotic version of StaBiotic Hand Soap and StaBiotic Gel can as such be seen as a percentage of increase or decrease of microbial counts.

Table 4: Percentage of increase or reduction of Total Count, *Enterobacteriaceae*, Yeasts/Moulds after 2 weeks of using either StaBiotic Hand Soap or Sanitary Gel; compared to the initial value [set at 100% for all organisms (All)] when using the non-probiotic version of the respective products.

	All (start)	P1 Soap	P2 Soap	P3 Gel	P4 Gel
Total Count	100	+ 44%	+ 470%	- 5%	N/A ¹
Enterobacteriaceae	100	- 96%	N/A ²	- 34%	- 75%
S. aureus	100	- 91%	- 93%	- 91%	- 93%
Yeasts/moulds	100	+ 38%	- 50%	- 42%	- 52%

¹ = Percentage not available due to overgrowth on petrifilms

² = No reliable *Enterobacteriaceae* quantification possible



Conclusion phase 3:

Although less pronounced and reproducible than the results of the lab tests in phase 2, the obtained results show that the use of both the StaBiotic Hand Soap and Sanitary Gel in every day life provides a long lasting microbial protection of the hands. Except for one case with yeasts/moulds, the probiotic hand soap and alcohol gel performed much better than the conventional products.

3. General conclusion

A three-phase validation trial was performed in order to verify the efficacy of the newly developed probiotic hand hygiene products from Chrisal; StaBiotic Hand Soap and StaBiotic Gel. The following main conclusions can be made:

- **Phase 1: Compared to its competitors, the StaBiotic Hand Soap performs equally well in reducing microbial counts; efficiently creating a probiotic hand microbiota.**
- **Phase 2: Both StaBiotic products result in a good immediate reduction in pathogenic count on the hands of test persons and also provide an efficient long lasting protection against recolonisation of the hands with pathogenic microorganisms.**
- **Phase 3: Everyday use of the StaBiotic hand hygiene products result in an improved hand hygiene with a lower number of pathogenic microorganisms and a stable probiotic hand microbiota**

Although occasional bacterial contaminations by hand contact with highly contaminated substances or surfaces can never be prevented, the use of probiotic based StaBiotic hand hygiene products certainly reduces the risk of further development of pathogenic microorganisms on the hand.

In turn, this lowers the risk of cross-contamination between persons and helps as such in the restraint of pathogenic development and spread.

The above covers the first round of testing performed by Dr. R. Temmerman and G. Vossen.

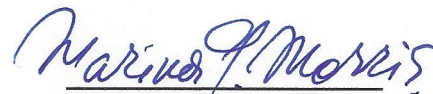


SUMMARY

After the above initial testing was completed, the same testing procedures have been rerun a number of times over the years, plus ATP Testing has been used on hands and skin areas in a wide range of ongoing testing that, in each case, demonstrated the ability of the StaBiotic products to provide lasting protection for many hours compared to the large range of standard non-Stabiotic products, such as Purell, Germ-X and many others tested.

It should be noted that the Chrisal StaBiotic products provide ongoing protection that can be measured in days on static surfaces, such as counters, floors, etc. However, since skin areas on hands are a dynamic environment and are constantly being rubbed, washed and exposed to many changing factors, the protection time is reduced compared to surfaces and is influenced by a number of external factors.

When taking these factors into consideration, the Chrisal StaBiotic Soap and Gel still always provide a number of hours of extra protection in comparison to all the regular disinfecting gels and soaps tested. It is also of interest that since the StaBiotic products deconstruct biofilm, that the longer they are used on any surface, the more effective they become.


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