



**Microbiological Validation of
PIP Hand Soap and PIP ProbioGel**

Study by Chrisal NV, Lommel, Belgium

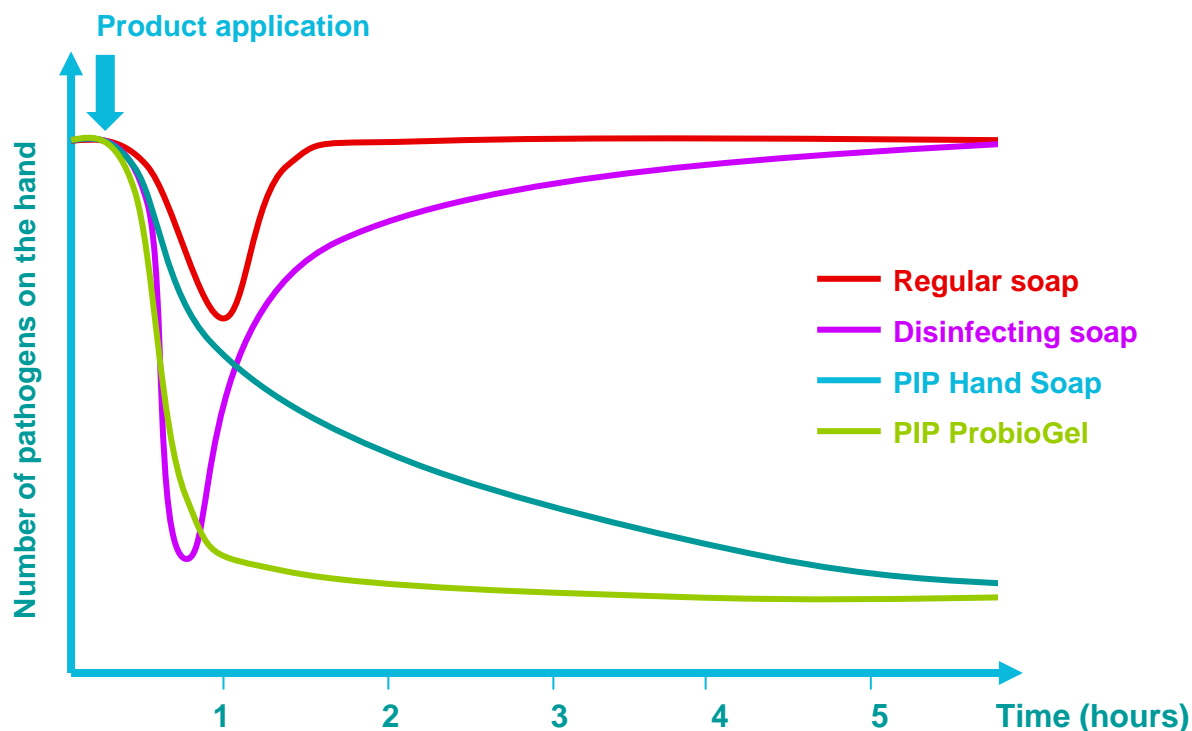


1. Introduction

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 probiotic hand soap (PIP Hand soap) and alcohol gel (PIP ProbioGel) based on the PIP technology in order to improve overall hand hygiene and sanitation.

- PIP Hand soap: is a creamy, probiotic skin cleanser creating a healthy and stable microflora on the hands.
- PIP ProbioGel: is a probiotic alcohol gel for hand sanitation. PIP ProbioGel has antibacterial and fungicidal effects and following disinfection, the probiotic bacteria in PIP ProbioGel create a healthy and protective skin microflora.

The overall concept of probiotic 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 protection of several hours after application. This effect is visualised in the following theoretical graph:



2. Trial overview

From July till December 2008 a number of tests were performed in order to validate the efficiency of the PIP Hand soap and PIP ProbioGel products. The tests comprised three phases:

- **Comparative test:** in a first test, the immediate pathogen removal efficiency of the PIP Hand Soap was determined and compared to several other brands on the market
- **Efficacy test 1:** a first efficiency test with 2 test persons 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 5 test persons to determine the efficiency of the probiotic hand soap and alcohol gel over time in a real life situation

2.1 Phase 1: Comparative test

The first phase determined the immediate pathogen removal efficiency of the PIP 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
PIP 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
Anti-bacterial Handwash	DETTOL	Parachlorometaxilenol
Alcogel 85	Dax	Ethanol

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*).

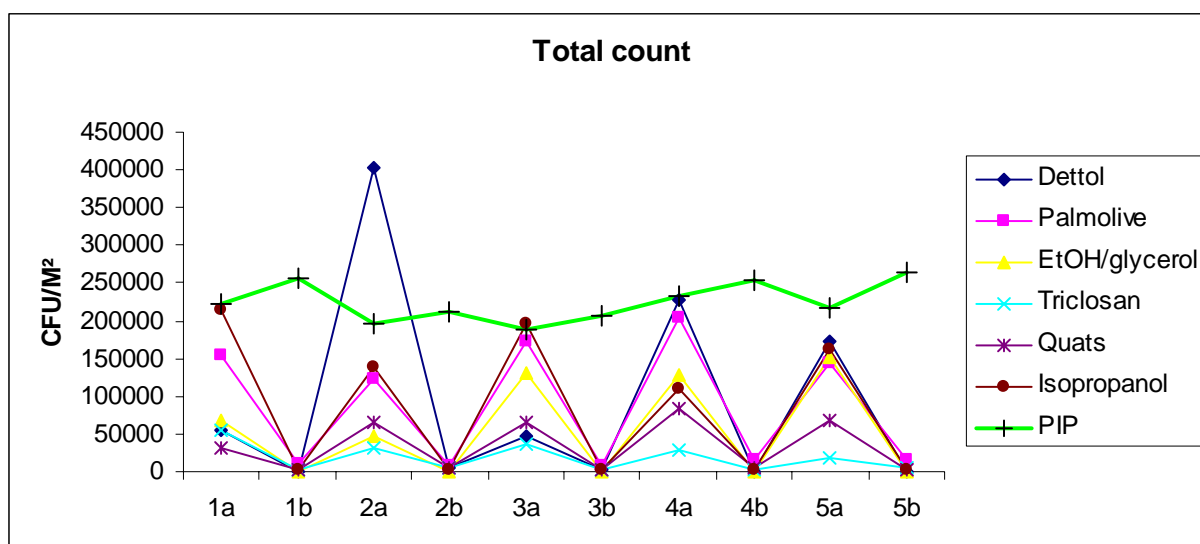


Figure 1: Total aerobic count before (a) and after (b) five distinct applications of various hand soaps and sanitizers

The above results demonstrate that the overall bacterial count is strongly reduced after applying all hand sanitizers and soaps (except PIP Hand soap) and that no significant distinction between the various brands can be made. The total count during application of the PIP Hand Soap remains constant, before and after application. This indicates that the probiotic bacteria are successfully transferred to the hands. However, specific pathogen counts are needed to demonstrate the efficiency of PIP Hand Soap in immediate reducing the number of pathogens (Figure 2).

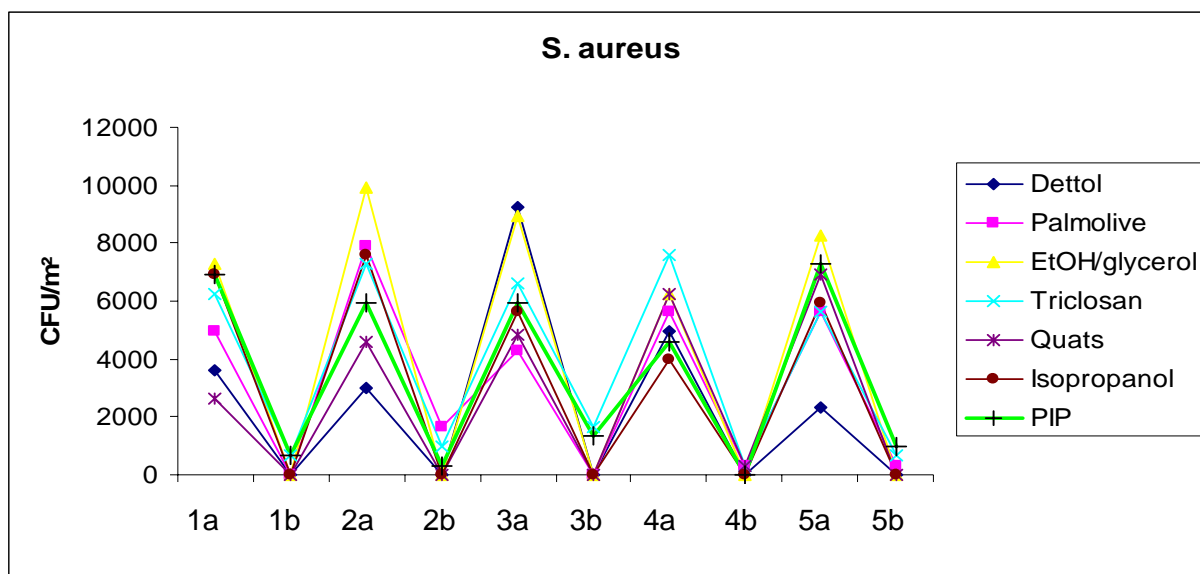


Figure 2: Specific *Staphylococcus aureus* (MRSA bacteria) count before (a) and after (b) five distinct applications of various hand soaps and sanitizers.

Figure 2 clearly demonstrates that, using *S. aureus* as indicator organism, the PIP 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 PIP 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 PIP bacteria in the PIP 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 PIP hand soap and PIP 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 PIP Hand Soap and PIP ProbioGel 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 PIP Hand Soap and PIP ProbioGel application.

	Total Count (before)	MRSA (before)	Total Count (5' after)	MRSA (5' after)	Total count (2h after)	MRSA (2h after)
Person 1 (PIP Hand Soap)	> 200 diverse	45 CFU	>500 Bacillus	0 CFU	>500 Bacillus	2 CFU
Person 2 (PIP Probiogel)	> 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 PIP Hand Soap and ProbioGel 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 PIP Hand Soap and PIP ProbioGel 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 PIP Hand Soap and PIP ProbioGel (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.

	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%
	All (start)	TC (2 min)	COL (2 min)	Y/M (2 min)	TC (2h)	COL (2h)	Y/M (2h)
ProbioGel – PIP bacteria	100	- 99%	- 94%	- 100%	- 95%	+ 2%	- 82%
ProbioGel + 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 PIP 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 PIP 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 PIP Hand Soap.

- **ProbioGel conclusions:**

- i. The immediate reduction (values after 2 minutes) in microbial count is best with the probiotic version of PIP ProbioGel, 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 PIP ProbioGel.
- ii. The protective effect (values after 2 hours) is again much better for the probiotic version of PIP ProbioGel. 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 PIP ProbioGel.

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 PIP Hand Soap and probiotic PIP ProbioGel.

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 PIP Hand Soap (2 test persons) or the PIP ProbioGel (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 PIP Hand Soap and PIP ProbioGel 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 PIP Hand Soap or PIP ProbioGel; 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 probiotic PIP Hand Soap and PIP ProbioGel 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; PIP Hand Soap and PIP ProbioGel. The following main conclusions can be made:

- Phase 1: Compared to its competitors, PIP Hand Soap performs equally well in reducing microbial counts; efficiently creating a probiotic hand microbiota.
- Phase 2: Both PIP 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 PIP 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 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.



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