

# Evaluation of the bactericidal activity of an antiseptic emollient wash formulation against Panton-Valentine Leukocidin producing *Staphylococcus aureus*

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## Introduction

Severe and recurrent skin and soft tissue infections (SSTIs) and, less commonly, necrotising pneumonia, are associated with rapidly emerging and highly pathogenic strains of *Staphylococcus aureus* that carry the virulence factor Panton-Valentine Leukocidin (PVL-SA). Some circulating strains are methicillin-resistant (PVL-MRSA), and multidrug resistant strains have rapidly spread across parts of North America and Australia, resulting in increasingly limited treatment options.

Dermol Wash (DW) is a hand wash product specially designed to confer skin protectant properties. The particular combination of antibacterial agents used, a mixture of benzalkonium chloride and chlorhexidine dihydrochloride, act synergistically, and therefore each agent is present at the low concentration of 0.1%. Cleansing action is achieved using a non-ionic soap substitute, cetomacrogol 1000, which is intended to avoid the irritancy problems that arise with ordinary anionic soaps and detergents. The wash also contains two emollient ingredients, liquid paraffin and isopropyl myristate, to form a physical barrier within the stratum corneum, thereby helping to maintain the skin's normal barrier function.

## Aim

The aim of this *in vitro* study was to test the bactericidal activity of DW against *S. aureus* strains with and without the PVL genes.

## Materials and Methods

- The suspension test protocol used was based on the European Standard for evaluating the bactericidal activity of chemical disinfectants and antiseptics (BS EN 1276:2009). This stringent standard requires a large reduction in microbial count (at least 5 log) within 5 minutes contact time with the test substance (1 minute for hand disinfectants) at 20°C in both clean or dirty conditions
- In total, 4 strains were tested: 3 clinical isolates of PVL-SA from patients with SSTIs (2 methicillin sensitive [MSSA] and 1 methicillin resistant [MRSA]); and a reference PVL-negative MSSA, (ATCC strain specified by BS EN 1276:2009)
- Neutralising solution consisted of tryptone, sodium chloride, lecithin, Tween 80, sodium thiosulfate, L-histidine and saponin
- Interfering substance was bovine serum albumin at 0.03% (clean conditions) and 0.30% w/v (dirty conditions)
- Samples were incubated at 20°C, and the product dilutions used in the methodology are such that DW was tested at 80% concentration

## Results

All control and validation data were within limits specified within BS EN 1276:2009. According to the criteria specified in the standard, DW exhibited satisfactory bactericidal activity (>5 log reduction) within 5, 10, 20 and 30 minutes contact time under both clean and dirty conditions against *S. aureus* ATCC 6538 reference strain (MSSA) as the test organism. Similarly, DW exhibited satisfactory bactericidal activity (>5 log reduction) within 5, 10, 20 and 30 minutes contact time under both clean and dirty conditions against each of the three PVL-positive clinical isolates of *S. aureus* irrespective of methicillin susceptibility status.

No appreciable difference was observed in the DW bactericidal activity between PVL-positive and PVL-negative *S. aureus* isolates. The PVL-MRSA strain showed higher survival rates than other isolates tested at 5 minutes contact time under simulated clean conditions but was nevertheless within required limits.

Table 1: Bactericidal activity of Dermol Wash

| Contact time (min) | ATCC 6538 (MSSA)      |                       | ARL-11-046 (PVL-MRSA) |                       | ARL-12-016 (PVL-MSSA) |                       | ARL-12-098 (PVL-MSSA) |                       |
|--------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                    | Log reduction (clean) | Log reduction (dirty) | Log reduction (clean) | Log reduction (dirty) | Log reduction (clean) | Log reduction (dirty) | Log reduction (clean) | Log reduction (dirty) |
| 5                  | >5.23                 | >5.23                 | 5.12                  | >5.36                 | >5.23                 | >5.23                 | >5.28                 | >5.28                 |
| 10                 | >5.23                 | >5.23                 | >5.36                 | >5.36                 | >5.23                 | >5.23                 | >5.28                 | >5.28                 |
| 20                 | >5.23                 | >5.23                 | >5.36                 | >5.36                 | >5.23                 | >5.23                 | >5.28                 | >5.28                 |
| 30                 | >5.23                 | >5.23                 | >5.36                 | >5.36                 | >5.23                 | >5.23                 | >5.28                 | >5.28                 |

## Conclusion

Dermol Wash can be described as bactericidal (as defined by BS EN 1276:2009) against PVL-SA. Within the limitations of the test protocol, the presence of the PVL genes in the MRSA and MSSA test strains did not affect sensitivity to the active substances in the antiseptic emollient wash.

## Bactericidal activity of Dermol Wash against PVL-*Staph aureus*

Severe and recurrent skin and soft tissue infections (SSTIs) are associated with rapidly emerging and highly pathogenic strains of *Staphylococcus aureus* that carry the virulence factor Panton-Valentine Leukocidin (PVL-SA). Some circulating strains are meticillin-resistant (PVL-MRSA), and multidrug resistant strains have rapidly spread across parts of North America and Australia, resulting in increasingly limited treatment options.

Dermol Wash is an antimicrobial emollient handwash designed to confer skin protectant properties. Two antiseptics, chlorhexidine dihydrochloride and benzalkonium chloride, act synergistically, and therefore each agent is present at the low concentration of 0.1%. Cleansing action is achieved using a non-ionic soap substitute, cetomacrogol 1000, that is intended to avoid the irritancy problems which can arise when ordinary anionic soaps and detergents are used.

The *in vitro* trial summarised overleaf shows that Dermol Wash is bactericidal against PVL-SA strains, both meticillin resistant (PVL-MRSA) and meticillin sensitive (PVL-MSSA).

### Summary of Poster Overleaf:

- The aim of this study was to test the bactericidal activity of Dermol Wash against *Staph aureus* strains with and without the PVL genes.
- Dermol Wash was tested (based on BS EN 1276:2009) against 4 strains of *Staph aureus*: 3 clinical isolates of PVL-SA from patients with SSTIs (2 of which were PVL-MSSA, 1 PVL-MRSA) and a reference PVL-negative MSSA.
- Bovine serum albumin was used as an interfering substance, at 0.03% w/v to simulate 'clean' conditions and at 0.30% w/v for 'dirty' conditions.
- Dermol Wash met the stringent test criteria of  $\geq 5$  log reduction within 5, 10, 20 and 30 minutes contact time under both 'clean' and 'dirty' conditions against the reference strain and all three PVL-positive clinical isolates of *Staph aureus* irrespective of meticillin susceptibility status.

### Conclusion:

*Dermol Wash can be described as bactericidal (as defined by BS EN 1276:2009) against PVL-SA. Within the limitations of the test protocol, the presence of the PVL genes in the MRSA and MSSA test strains did not affect sensitivity to the active substances in the antiseptic emollient wash.*