USE OF CONTACT TESTS TO ASSESS THE IN-VITRO ACTIVITY OF TWO PRODUCTS CONTAINING CHLORHEXIDINE AGAINST *MALASSEZIA PACHYDERMATIS*

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Aim of the study. Chlorhexidine (CHX) is a biguanide antiseptic frequently used in topical therapy of dermatological diseases of dogs. It is contained in numerous marketed products at various concentrations and associated with different ingredients. The aim of this study was to assess the in vitro effectiveness against *Malassezia pachydermatis* of two commercial products containing CHX respectively at 0.45% (product A, Clorexyderm spot gel® - ICF) and 0.3% (product B, Clorexyderm spot gel + Tris EDTA® - ICF).

Materials and Methods. Antimicrobial activity was evaluated by contact tests according to guidelines of the European Standard EN 1275, which specifies a suspension test for establishing whether a chemical disinfectant or antiseptic (biocide) does or does not have a *basic* fungicidal activity. The activity is evaluated as the capability of a product to reduce the number of viable fungal cells under defined conditions. Five strains of *M.pachydermatis* were tested for each product, and tests were repeated twice. A sample of each product was added to a test suspension of the yeast. The mixture was maintained at 20 °C for 15 min (obligatory test conditions). At the end of this contact time, an aliquot was taken, and the fungicidal activity in this portion was immediately suppressed using a neutralizer solution (lecithin 3 g/l; polysorbate 80 30 g/l). The resulting suspension was then cultured on Sabouraud Dextrose Agar and the colony forming units counted after 72 h incubation. Products were considered effective (fungicidal) if a 4 decimal log (lg) reduction of the germ after a 15 minute contact time was observed (UNI EN 1275 - Quantitative suspension test for the evaluation of basic fungicidal or basic yeasticidal activity of chemical disinfectants and antiseptics).

Results and Conclusions. A 4 decimal log reduction of viable yeasts was obtained in all the experiments performed using five strains of the yeast. Both products can therefore be deemed effective against the target microorganism under in-vitro defined conditions, strictly fixed according to guidelines of European Standard UNI EN 1275. It's the authors' opinion that such an approach can represent an useful model able to take into account the several factors which are known to influence the activity of the active ingredients in biocides. Actually, the efficacy of antimicrobial products which are used topically depends on, and varies significantly with the period of contact and formulation effects, which can often enhance activity despite the presence of lower levels of the biocide. The mentioned factors are important in evaluating the modes of action of, and mechanisms of resistance to, biocides and are often regrettably overlooked (Russell and McDonnell 2000. J Hosp Infect 44: 1-3). This study confirms the effectiveness of CHX against *M.pachydermatis* and underlines the usefulness of contact tests when evaluating the antimicrobial effectiveness of topical therapy.