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Detection of viable *Dichelobacter nodosus* by real-time PCR using PMAxx™

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Protocol Information

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Summary: *Dichelobacter nodosus* is a gram-negative fastidious anaerobic bacterium and the causative agent of ovine footrot. The disease has a global presence and is endemic in many countries. Clinical symptoms range from mild interdigital dermatitis in benign footrot to severe underrunning and separation of the hoof horn from the underlying tissue in virulent footrot. Clinical symptoms start as early as 2 weeks after first contact and the disease leads to pain, lameness, decreased meat and wool production as well as animal welfare issues. Diagnosis has improved significantly by the development of a real-time PCR to detect and discriminate virulent (aprV2-positive) and benign (aprB2-positive) *D. nodosus* strains. However, the real-time PCR also detects dead cells making its use for testing e.g. disinfecting agents limited. A PMA (propidium monoazid) real-time PCR using the improved dye PMAxx™ was therefore developed for virulent *D. nodosus* that allows discrimination of viable and dead bacteria. The distinction between viable and non-viable cells is possible, based on membrane integrity. For that purpose, the samples containing *D. nodosus* are treated with the improved nucleic acid intercalating PMA dye PMAxx™ that selectively enters cells with compromised cell membranes, whereas the intact cell membrane presents a natural barrier for this molecule. After exposure to strong light, it covalently binds to the DNA, preventing DNA from being amplified by PCR, thereby enabling differentiation of viable from non-viable cells. The PMA-qPCR proved to be a valid method for comparison of antimicrobial efficiency in ex vivo experiments.

Associated Publications: Hidber T, Pauli U, Steiner A, Kuhnert P (2020) *In vitro* and ex vivo testing of alternative disinfectants to currently used more harmful substances in footbaths against *Dichelobacter nodosus*. PLoS ONE 15(2): e0229066. doi: [10.1371/journal.pone.0229066](https://doi.org/10.1371/journal.pone.0229066)

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