Feb 14, 2020

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

PLOS One

Peter Kuhnert[1], Tobias Hidber[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

1 Works for me dx.doi.org/10.17504/protocols.io.bbh9ij96

Peter Kuhnert
Institute of Veterinary Bacteriology, Vetsuisse Faculty, Uni...

ABSTRACT

*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.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0229066

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

In vitro and ex vivo testing of alternative disinfectants to currently used more harmful substances in footbaths against Dichelobacter nodosus

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| TaqMan™ MGB Probe | 4316034 | Thermo Fisher |
| 7500 Real-Time PCR System, desktop | 4351105 | Thermo Fisher |
| TaqMan™ Fast Advanced Master Mix | 4444556 | Thermo Fisher |
| KingFisher™ Duo Prime Purification System | 5400110 | Thermo Fisher |
| VetMAX™ Xeno™ Internal Positive Control DNA | A29764 | Thermo Fisher |
| VetMAX™ Xeno™ Internal Positive Control - LIZ™ Assay | A29766 | Thermo Fisher |
| PMAxx™ Dye 20 mM in H2O | #40069 | Biotium |
| LED Floodlight 100 W 6000 K 8500 lm | FL5836 | Optonica |
| MagneSil®RED | A1641 | Promega |

## PMAxx treatment

1 place or dissolve your sample containing *Dichelobacter nodosus* in a total voulme of 50 µl 0.85% NaCl in a transparent 1.5 ml tube

2 in a darkened room add 100 µM (0.25 µl) of PMAxx™ to the tube

3 vortex and incubate for 3 min at room temperature in a metal box impervious to light

4 after incubation, place tube on ice and expose to LED light (white light, 100 W, 6000 K, 8500 lm) at a distance of 20 cm for 5 min

5 centrifuge tube at 15'000xg for 5 min in a microcentrifuge

6 to remove the supernatant, the tubes can be held in a horizontal position and twisted tissues used to absorb the liquid

7 resuspend pellet in 500 µl SV-lysis buffer (4 M guanidine thiocyanate, 0.01M Tris–HCl, 1% β-mercaptoethanol)

## DNA extraction

8 add 2 µl of VetMAX™ Xeno™ Internal Positive Control DNA to the sample in SV-lysis buffer (this is the extraction control and later also the internal PCR control)

9 add 30 µl MagneSil®RED and resuspend without vortexing

10 extract DNA using KingFisher™ Duo Prime Purification System (or an alternative magnetic bead based system) and elute the purified DNA in 60 µl $H_2O$

## real-time PCR

11 primers and probes as well as the real-time PCR are described in the publications **116. Dnodosus PCR.pdf** **140. Dnodosus infection SAT.pdf**

12 prepare 25 µl containing:
- 1x TaqMan™ Fast Advanced Master Mix
- 300 nM primer DnAprTM-L (CAATAGCCAAATTTCTTTAGATGGTGAT)
- 300 nM DnAprTM-R (CAAGAGCTGTCGCTTCTTTCTTT)
- 100 nM Probe DnAprTm-v (FAM-CGGTGGTTATCCTGAT-MGB)
- 250 nM Probe DnAprTM-b (VIC-TGGTCGTCCTGATC-MGB)
- pyrogen-free water
- 1 µl Xeno™ LIZ Primer Probe Mix

and 2.5 µl of DNA template

13 prepare at least duplicates for each sample and run on an appropriate instrument like 7500 Real-Time PCR-System instrument

14 run using cycles of 2 min at 50°C and 10 min at 95°C followed by 40 cycles with 15 s at 95°C and 1 min at 60°C

15 results are analyzed using the Sequence Detector 7500 software (v 2.3.) with the threshold set at 0.015

16 signals will only result from alive *D. nodosus* and no dead cell will be detected. For absolute quantification a standard curve using defined amounts of *D. nodosus* genome equivalents can be generaded in parallel

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited