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Evaluation of a PCR test for the diagnosis of Tritrichomonas foetus infection in bulls: effects of sample collection method, storage and transport medium on the test.

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Abstract

The objectives of this study were to determine the detection limit of a PCR test for Tritrichomonas foetus, to investigate the effect of sampling method, guanidinium thiocyanate (GuSCN), and sample storage, and to confirm the accuracy of the test on field samples. Serial 10-fold dilutions of culture material were used to determine the detection limit. For the sample handling trial, five positive bulls were sampled by sheath washing and scraping on six occasions over a period of 18 days (n=29 samples) and eight control bulls were sampled three, four or six times (n=28 samples). Samples were cultured, while portions with and without GuSCN were subjected to DNA extraction within 6h, after 30 h and after 5 days at 4 degrees C. PCR and agarose gel electrophoresis was performed. A two-tailed chi-square test was used to test for differences between treatments. The PCR assay showed a specificity of 98%. Its sensitivity declined with storage time, from 90% at 6h to 31% at 5 days. Sampling method and GuSCN had no effect on test sensitivity. The detection limit of the assay was 100 organisms. Parallel testing of 193 field samples gave complete agreement between culture and PCR results.