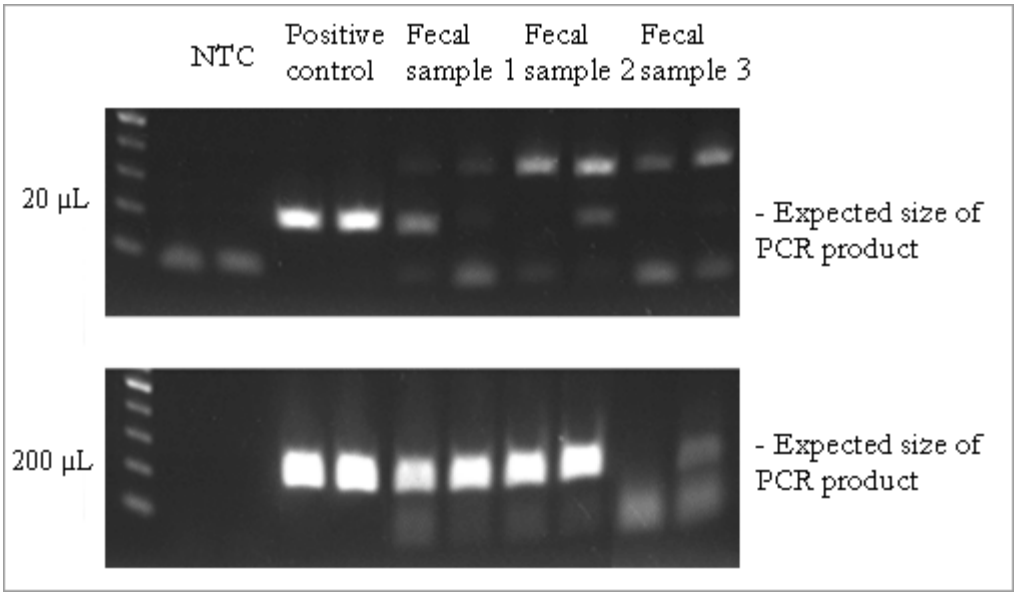
Overcoming PCR inhibition

SuperConvection improves the detection sensitivity since it enables rapid and sensitive PCR in larger reaction volumes (see AlphaHelix' definition of detection sensitivity). AlphaHelix' technology allows the use of more of the sample than any other platform by allowing rapid and efficient PCR reactions in volumes up to 200 µl. This is advantageous for samples with complex matrices and inhibiting substances in combination with a low number of target molecules, since a large volume PCR is much less inhibited by contaminating factors (see figure below).

The results presented below show that DNA amplification as well as probe binding is inhibited in bovine fecal samples positive for Mycobacterium avium subspecies paratuberculosis.

When analyzed in 20 µl PCR reactions when analyzed in 20 µl PCR reactions and that amplification and detection is rescued by re-analyzing the samples (using the same sample amount) in 200 µl PCR reactions.

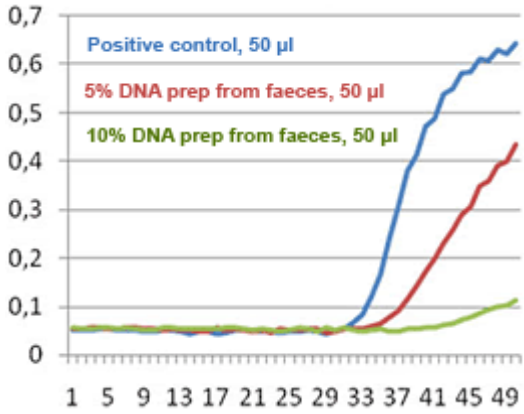


Increasing reaction volumes dilute the effect of PCR inhibitors present in DNA preparations from bovine feces, thereby rescuing DNA amplification. Agarose gel electrophoresis (above) highlight differences in amplification efficacy between the same samples run in 20 and 200 µl PCR reaction volumes, respectively.

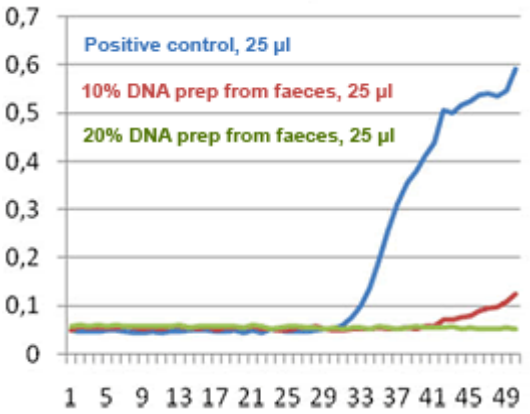
Proof-of-principle study

Use of large-volume superconvective real-time PCR to overcome inhibitor induced amplification failure in bovine faeces samples.

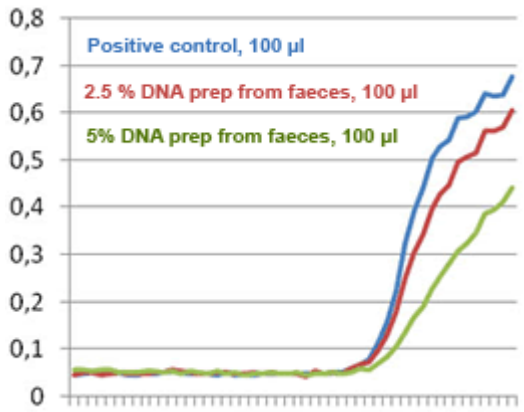
25 µl



50 µl



100 µl



200 µl