Sample preparation methods for high-quality nucleic acid isolation from a variety of veterinary samples

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Introduction

Effective and reproducible isolation and purification of nucleic acids is one of the key success factors for amplification and detection of pathogenic nucleic acids using molecular methods. Diagnostic tests using PCR (e.g., for Johne's disease in cattle) are replacing traditional ELISA or culture-based methods due to higher sensitivity and speed.

For reliable results in molecular assays, nucleic acid purification systems need to remove potential PCR inhibitors and other contaminants while providing maximum recovery of DNA and RNA. Furthermore, molecular assays must enable specific amplification and sensitive detection of target sequences. In addition, the entire procedure — from sample to result — needs to fit into the workflow of routine laboratories, and provide minimal hands-on time and increased user safety when handling potentially infectious samples.

An easy and standardized method for nucleic acid isolation that gives high-quality nucleic acids is required. The major challenge when working with such diverse material is to develop optimized pretreatments for all sample types. Depending on sample type, content of inhibitors, and content of nucleic acids, the samples demand different pretreatment conditions, such as mechanical disruption, enzymatic digestion, and extended incubation.

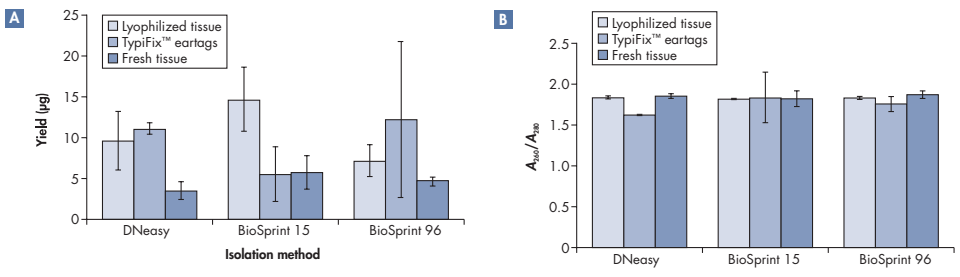
The presentation shows data from various animal organ tissues, fresh and dried pig ears, horse hair, and ticks using manual and automated sample preparation.

Materials and methods

Manual preparation was carried out manually using the DNeasy® Blood & Tissue Kit. For automated sample preparation, the low-throughput QIAGEN® QIAcube® or BioSprint® 15 workstation, and the high-throughput BioSprint® 96 workstation were used.

Quality of the isolated DNA is shown by gel electrophoresis, enzymatic digestion, and standard as well as real-time PCR as downstream applications.

Results — DNA from various animal samples I



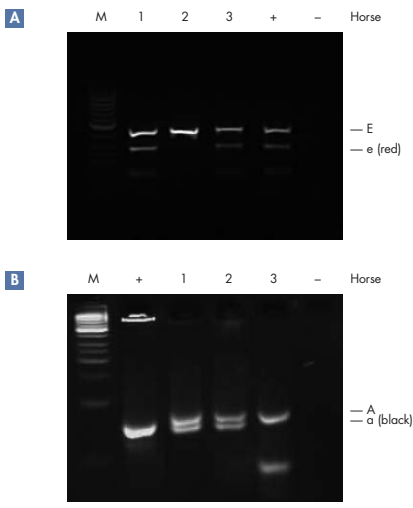
Comparison of DNA isolation methods. DNA was isolated from different types of eartags with either the DNeasy Blood & Tissue Kit (**DNeasy**), the BioSprint 15 DNA Tissue protocol (**BioSprint 15**) or the BioSprint 96 DNA Tissue protocol (**BioSprint 96**). A DNA yield and B purity were measured by spectrophotometric scans and the purity was calculated by the A_{260}/A_{280} ratio.

Complete removal of inhibitors

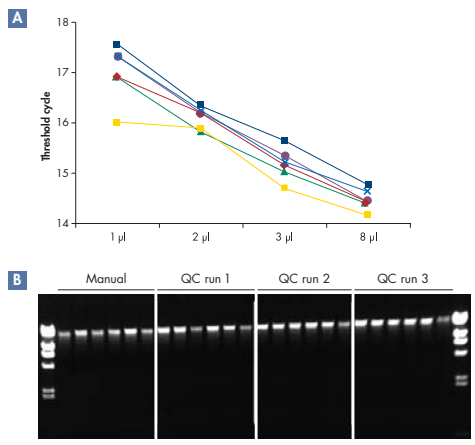
Sample	2 µl	0.2 µl	0.02 µl	delta 1	delta 2
Lung	14.7861	17.5067	21.1123	–2.7206	–3.6056
	14.3551	16.2466	19.6384	–1.8915	–3.3917
	13.9544	15.6540	19.3342	–1.6997	–3.6801
Heart	14.4435	16.7119	20.5708	–2.2685	–3.8588
	14.6298	17.3352	21.2359	–2.7054	–3.9007
	14.4397	16.9923	20.1397	–2.5526	–3.1474
Kidney	13.8868	15.2995	18.4825	–1.4127	–3.1830
	13.8136	15.5126	18.4145	–1.6990	–2.9019
	13.8871	15.4679	18.6948	–1.5808	–3.2269

Genomic DNA was isolated from 25 mg of pork lung, heart, and kidney samples using the DNeasy Blood & Tissue Kit on the QIAcube. Genomic DNA was eluted in 200 µl of Buffer AE, and different amounts of the eluate were analyzed in quantitative PCR (total reaction volume 25 µl) using the QuantiFast™ SYBR® Green PCR Kit to detect the 18S rRNA gene.

Results — DNA from various animal samples II

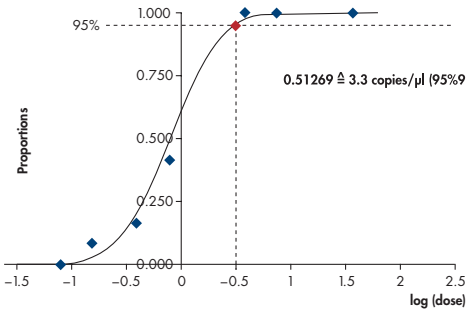


Genotyping with DNA from horse hairs. DNA was purified from horse hair of 3 brown horses and analyzed for coat-color genotypes using the MSH and ASIP PCR systems. MSH PCR products were digested with *Taq*I. Horses 1 and 3 were identified as Ee heterozygotes, carrying the recessive e allele for red coat color. Analysis of ASIP PCR products identify horses 1 and 2 as Aa heterozygotes, carrying the recessive a allele for black coat color. +: Positive Ee aa control DNA from a black horse. –: Negative PCR control. **M**: markers.

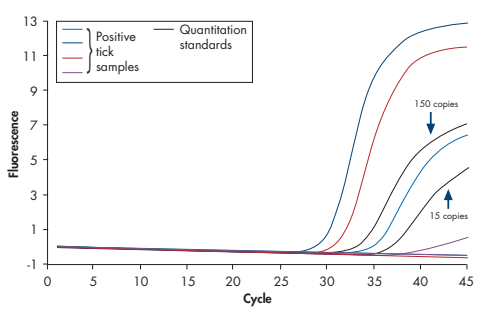


Genomic DNA isolation from goat blood. Genomic DNA was isolated from different samples of goat blood using the DNeasy Blood & Tissue Kit manually or on the QIAcube. Genomic DNA was eluted in 200 µl of Buffer AE, and different volumes of the eluate were analyzed. A Analysis by quantitative PCR (total reaction volume 25 µl) using the QuantiFast SYBR Green PCR Kit to detect the 18S rRNA gene. B 15 µl of the eluate was subjected to agarose gel electrophoresis.

Results — Detection of *Borrelia* DNA and *M. paratuberculosis*



Analytical sensitivity of the artus M. paratuberculosis PCR Kit on the LightCycler® 1.1/1.2/1.5 Instrument. Probit analysis was carried out using a standard dilution series. Testing was carried out on three different days on eight replicates.



Real-time PCR detection of *Borrelia* DNA in ticks. *Borrelia* sp. is a pathogen carried by ticks that causes Lyme disease in humans and other mammals. DNA was purified from ticks using the DNeasy Blood & Tissue Kit. *Borrelia* DNA was detected in real-time PCR using the *artus*® Borrelia LC PCR Kit on the LightCycler® system. The lowest positive curve corresponds to 5 copies per PCR.

Conclusions

- Manual and automated DNA isolation from a variety of different samples and different animal species was successful, and a range of typical DNA yields are shown, depending on the starting material.
- All types of pig ear samples, including frozen, lyophilized, and dried tissue, gave good yields and were successfully used in PCR analysis.
- Detection of *Borrelia* DNA with the *artus* Borrelia LC PCR Kit subsequent to DNA isolation from ticks demonstated high sensitivity: the lowest positive curve in the real-time PCR corresponded to 5 copies.

DNeasy Blood & Tissue Kit, BioSprint 15, BioSprint 96, QIAcube, *artus* Borrelia LC PCR Kit, *artus* M. paratuberculosis LC PCR Kit, QuantiFast SYBR Green PCR Kit: For Reasearch Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

The *artus* Borrelia LC PCR Kit and *artus* M. paratuberculosis LC PCR Kit are not USDA approved in the USA and not approved by the Friedrich-Loeffler-Institute in Germany for veterinary diagnostic use.

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