Application Note

Isolation of Viral RNA, Viral DNA and Bacterial DNA from Animal Samples

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Successful pathogen identification employing real-time PCR or RT-PCR is dependent on a multitude of parameters of which the isolation of high-quality nucleic acid is paramount. However, increasing the overall efficiency, and thus success rate, of veterinary pathogen detection can also be achieved by parallel and automated processing of different sample types. In this article, we describe how this can be achieved. Utilizing a combination of the QIAamp® *cador*® Pathogen Mini Kit, the QIAgility® for automated PCR setup, and universal real-time PCR cycling protocols with the QuantiFast® Pathogen PCR and RT-PCR +IC Kits, on the Rotor-Gene® Q, permits multi-pathogen identification from multiple sample types.

Introduction

Pathogen identification by real-time PCR and RT-PCR has become a standard method and is replacing classical culture-based methods. However, isolation of high-quality nucleic acid can prove problematic due to complex and variable matrix characteristics of animal-derived sample material. Specialized procedures for nucleic acid isolation for many animal sample types are available. However, employing various protocols for each different sample type is laborious and increases the risk of errors. This can be overcome by simultaneously processing different sample types, in regard to sample material and pathogen type, with a single universal protocol. The QIAamp *cador* Pathogen Mini Kit uses proven silica membrane-based spin-column technology and enables co-purification of pathogen RNA and DNA from a broad range of veterinary sample types using a single universal protocol. Furthermore, by automating sample preparation and PCR setup, and using universal real-time PCR cycling protocols with QuantiFast Pathogen PCR and RT-PCR +IC Kits on the Rotor-Gene Q, the efficiency and reliability of veterinary pathogen detection can be further improved.

A further bottleneck can be introduced by the localization of pathogens and/or workflow demands which require the use of animal whole blood as a sample source. Silica membrane spin columns provided with current sample preparation kits can become clogged due to the composition of animal blood. This may result in lower purification efficiency and carryover of inhibitory substances ▷

into the eluate, leading to impaired downstream performance. The QIAamp *cador* Pathogen Mini Kit also allows the processing of up to 200 µl of animal whole blood with greatly reduced risk of silica membrane clogging.

Here, we demonstrate how the QIAamp *cador* Pathogen Mini Kit can be employed for reliable processing of animal whole blood samples, parallel processing of various sample types and for demanding sample materials such as feces. We also describe an integrated workflow for efficient nucleic acid isolation and detection from multiple animal sample types and multiple pathogens.

Materials and methods

Robust isolation of viral RNA from animal whole blood samples

Whole blood samples of various species were spiked with a fixed concentration of bovine viral diarrhea virus (BVDV) particles from cell culture supernatant and then frozen at –20°C. Three replicates for each sample were processed with the QIAamp *cador* Pathogen Mini Kit (manual procedure), and the procedure was repeated twice on different days. Viral RNA was detected using the QuantiFast Pathogen RT-PCR +IC Kit according to handbook recommendations, using BVDV-specific primers and probe.

Parallel and automated setup of nucleic acid isolation and pathogen detection from various sample types

Sample types and the pathogen content within each sample are shown in Table 1. Pathogen content was due to either natural infection or following the spiking of virus particles or bacteria from cell/bacterial culture. Where required, a pretreatment was applied according to the instructions provided in the *QIAamp cador Pathogen Mini Kit Handbook*. The QIAcube®, in combination with the QIAamp *cador* Pathogen Mini Kit, was utilized to automatically co-isolate viral RNA, viral DNA or bacterial DNA from each sample dependent on the pathogenic infection associated with each specific sample (Table 1). The run was repeated on different days resulting in a total of three extraction replicates for each sample type. All samples (36 samples) were simultaneously analyzed using PCR or RT-PCR.

PCR and RT-PCR setup was automated on the QIAgility. Four different reaction mixtures were prepared in one setup run using primers and probes specific for BVDV, bovine herpesvirus (BHV), *E. coli* and *Staphylococcus haemolyticus*. The 72-well rotor on the Rotor-Gene Q was utilized. Each well contained reaction mixes and sample eluates and was loaded using the QIAgility. Each of the four pathogens was co-detected with the internal control (IC; added to the master mix as an amplification control) in duplex RT-PCR or PCR using the appropriate QuantiFast Pathogen +IC protocol, according to handbook instructions, without optimization of the duplex setup.

Table 1. Sample types and pathogen infection

Sample ID	Sample material	Pathogen in sample	Pathogen type	Pathogen origin
1	Bovine blood	BVDV	RNA virus	Natural infection
2	Bovine blood	BVDV	RNA virus	Natural infection
3	Bovine blood	*S. haemolyticus*	Gram-positive bacterium	Artificially spiked
4	Bovine serum	BVDV, BHV	RNA, DNA virus	Artificially spiked
5	Bovine serum	BVDV, BHV	RNA, DNA virus	Artificially spiked
6	Bovine milk	BVDV, *E. coli*	RNA virus, Gram-negative bacterium	Artificially spiked
7	Bovine serum	*E. coli*	Gram-negative bacterium	Artificially spiked
8	Swab in UTM	BHV, *S. haemolyticus*	DNA virus, Gram-positive bacterium	Artificially spiked
9	Kidney tissue	BVDV	RNA virus	Natural infection
10	Spleen tissue	BVDV	RNA virus	Natural infection
11	Bovine serum	*E. coli*	Gram-negative bacterium	Natural occurrence
12	Bovine feces	*E. coli*	Gram-negative bacterium	Artificially spiked

UTM: Universal transport medium.

Isolation of bacterial DNA from fecal samples

Fecal samples were provided by GD B.V. Animal Health Service Ltd. (Netherlands) during the 2012 International Proficiency Testing Scheme (PTS) for *Brachyspira hyodysenteriae* DNA detection in porcine feces. Bacterial DNA was isolated with the QIAamp *cador* Pathogen Mini Kit with a modified pretreatment for feces. Briefly, the sample was centrifuged after lysis and the supernatant was transferred into a new tube before addition of the binding buffer. *B. hyodysenteriae* DNA was identified using the *cador* B. hyodysenteriae PCR Reagent* on the Rotor-Gene Q.

Results

Robust isolation of viral RNA from animal whole blood samples

Utilizing the QIAamp *cador* Pathogen Mini Kit permits the processing of animal whole blood samples without clogging of the spin column. BVDV RNA was isolated from whole blood samples from various species. Figure 1 illustrates the high intra- and inter-assay reproducibility with minimal prep-to-prep variation in C_T values. This is confirmed by the low intra-and inter-assay coefficient of variation (CV). Using the C_T values for all samples the intra-assay CV was 0.30–1.49% (mean 0.82%) and the inter-assay CV was 0.18–1.45% (mean 0.67%).

* Not available in Germany and Canada.

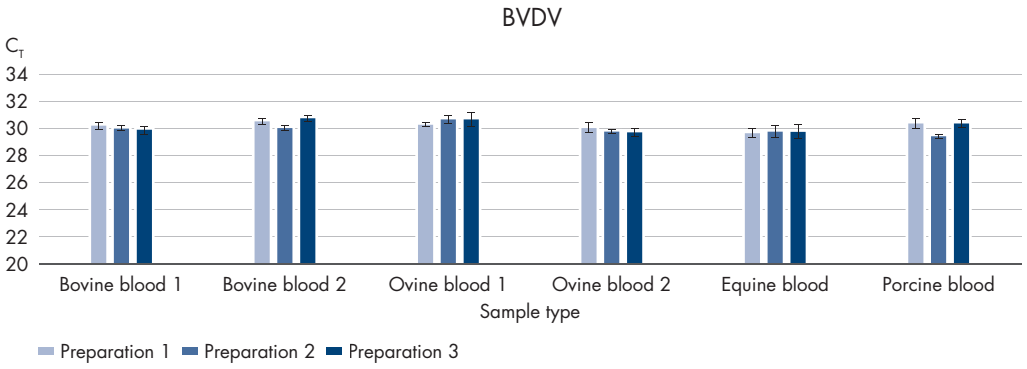

Figure 1. High precision and reproducibility for isolation of BVDV RNA from whole blood samples. Blood samples were spiked with BVDV particles and RNA was isolated in three manual runs with the QIAamp *cador* Pathogen Mini Kit. Isolated RNA was subsequently amplified using BVDV-specific primers and probe and the QuantiFast Pathogen RT-PCR +IC Kit. The bar chart shows the C_T values obtained. Error bars represent ±1 SD of three purification replicates.

Nucleic acid isolation and pathogen detection from various sample types in a parallel and automated setup

Viral RNA, viral DNA and bacterial DNA of four different pathogens were reliably isolated from various sample materials using one universal protocol. Two different pathogen types were co-isolated from some of the samples (see Table 1). For all four pathogens targets, parallel PCR setup was fully automated in one single QIAgility run, keeping hands-on time to a minimum. All pathogens were identified correctly. The low inter-assay variability (CV values of 1.27 ± 1.16%), following automated sequential isolation of nucleic acids on three different days, illustrates the high reproducibility of the sample preparation procedure (Figure 2).

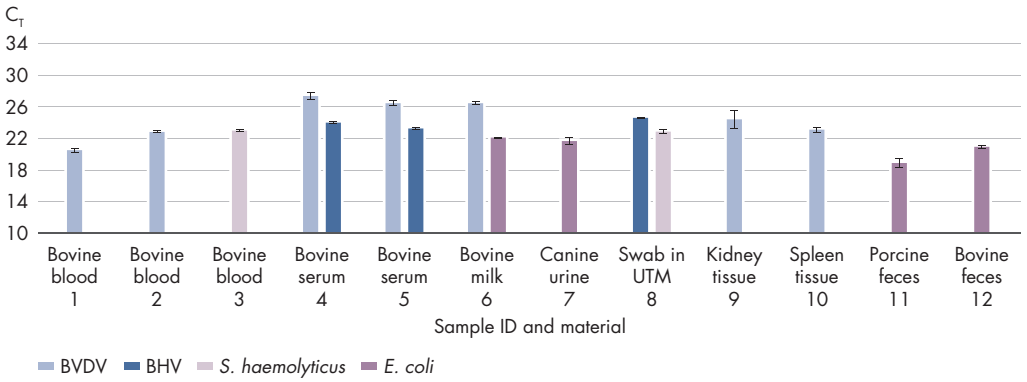

Figure 2. Automated co-isolation of viral RNA, viral DNA and bacterial DNA. Utilizing the QIAcube, in combination with the QIAamp *cador* Pathogen Mini Kit, viral RNA, viral DNA and bacterial DNA were co-isolated from various sample materials. Three replicates of the samples were processed in three sequential runs. All isolated pathogen nucleic acids were detected in one single Rotor-Gene Q run, following automated PCR setup on the QIAgility using PCR systems as described above. Error bars represent ±1 SD of three purification replicates from sequential QIAcube runs.

Superior detection of *B. hyodysenteriae* DNA from fecal samples

QIAGEN participated in the 2012 PTS for *B. hyodysenteriae* DNA detection in porcine feces, which was organized by GD B.V. Animal Health Service Ltd. (Netherlands). All samples were correctly identified with QIAGEN® assays delivering comparatively lower C_T values than other participating labs (Figure 3).

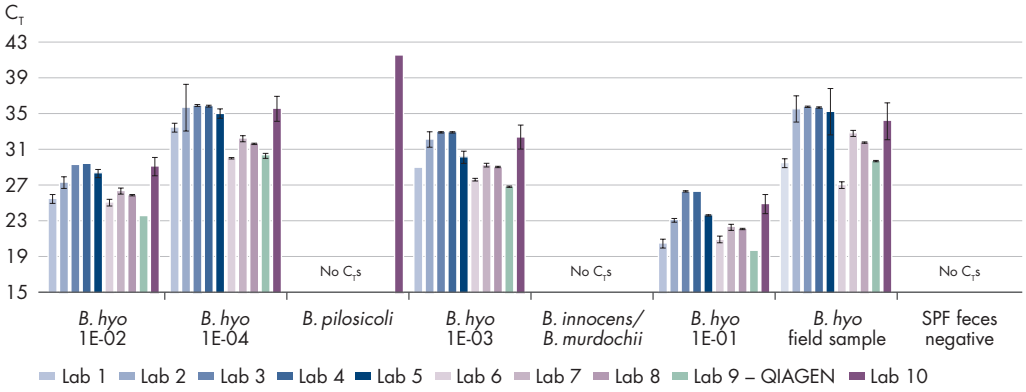

Figure 3. Lab-to-lab comparison of *B. hyodysenteriae* DNA detection. Bacterial DNA was isolated using the QIAamp *cador* Pathogen Mini Kit. *B. hyodysenteriae* DNA was identified using the *cador* B. hyodysenteriae PCR Reagent* on the Rotor-Gene Q. Mean values from two C_T values reported by each participant are shown. For the QIAGEN data (green bars), the reported C_Ts are from two purification replicates. Error bars represent ± 1 SD.

Conclusions

- The QIAamp *cador* Pathogen Mini Kit enables parallel processing of a broad range of sample types using one universal protocol.
- Pathogen nucleic acids are reliably and efficiently co-purified — even from demanding sample types, such as animal whole blood and feces.
- Up to 200 µl undiluted animal whole blood can be processed with a much reduced risk of silica membrane clogging.
- Pathogen identification can be streamlined by parallel processing of various combinations of sample material and pathogen type.
- Efficiency and reliability of pathogen identification can be further increased by automation of nucleic acid isolation and PCR setup.

* Not available in Germany and Canada.

Ordering Information

Product	Contents	Cat. no.
QIAamp *cador* Pathogen Mini Kit (50)	For 50 RNA/DNA preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers	54104
QIAamp *cador* Pathogen Mini Kit (250)	For 250 RNA/DNA preps: 250 QIAamp Mini Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers	54106
QuantiFast Pathogen PCR +IC Kit (100)	For 100 x 25 µl reactions: Master Mix, lyophilized Internal Control Assay, lyophilized Internal Control DNA, ROX™ Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE	211352
QuantiFast Pathogen RT-PCR +IC Kit (100)	For 100 x 25 µl reactions: Master Mix, RT Mix, lyophilized Internal Control Assay, lyophilized Internal Control RNA, ROX Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE	211452
cador B. hyodysenteriae PCR Reagent*	For 96 x 25 µl reactions: Master Mix, lyophilized Internal Control Assay, lyophilized Internal Control DNA, *B. hyodysenteriae* Primer/Probes, *B. hyodysenteriae* Control DNA, ROX Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE	285215

* Not available in Germany and Canada.

The QIAamp *cador* Pathogen Mini Kit is intended for laboratory use. Not for use in veterinary diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Discover more at **www.qiagen.com/QIAampcadorPathogenMiniKit**!