Application Note

Effective Mechanical Sample Lysis for Reliable Pathogen Identification

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In this application note, we describe the use of the TissueLyser LT, together with QIAamp® UCP Pathogen Kits, for effective mechanical disruption of microbial samples and successful isolation of high yields of genomic DNA for pathogen identification studies.

Introduction

Microbial DNA can be purified from various sample materials such as whole blood, swabs, cultures and body fluids. Since a high yield of microbial genomic DNA is crucial for reliable pathogen identification, thorough sample lysis prior to DNA extraction is of critical importance. Different sample pretreatment procedures may be required, depending on the type of microbe. For some pathogens, chemical lysis may be sufficient for sample pretreatment, while others, such as Gram-positive bacteria or yeast and other fungi require mechanical lysis for maximal lysis efficiency. Several different methods are available for the isolation of genomic DNA from microbes. For successful genomic DNA extraction, protocols must not only be adapted to meet the requirements of different sample types, but also to the morphology of the microorganism itself. One of the biggest obstacles in nucleic acid isolation from bacteria and fungi is the lysis step.

This study describes an optimized workflow for microbial genomic DNA isolation and highlights the importance of mechanical sample lysis for optimal results. The benefits of using the TissueLyser LT in combination with QIAamp UCP Pathogen Kits for sample disruption and microbial genomic DNA purification, respectively, are demonstrated.

Figure 1. The TissueLyser LT ensures simultaneous disruption of up to 12 samples.

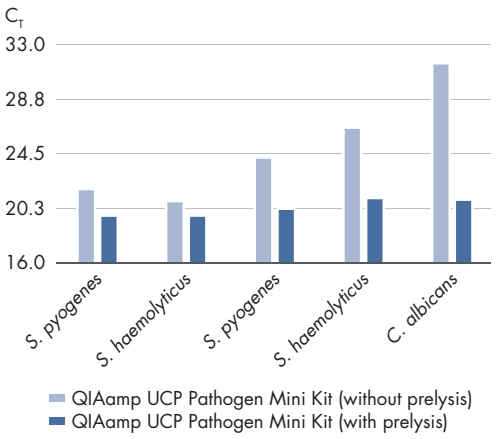

Figure 2. High yield of microbial DNA through efficient cell lysis. Cotton swabs or whole blood samples were spiked with a mixture of cells from the indicated microbes. DNA isolation was performed using the QIAamp UCP Pathogen Mini Kit with or without prelysis prior to DNA isolation. The QIAamp UCP Pathogen Mini Kit, when used in combination with Pathogen Lysis Tubes and the TissueLyser LT, resulted in efficient cell lysis, leading to high yields of microbial DNA, as demonstrated by the low C_T values in real-time PCR assays.

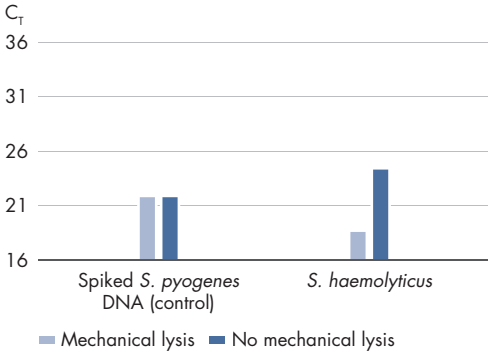

Figure 3. Efficient lysis of microbial cells and higher DNA yield due to mechanical disruption. Whole blood was spiked with purified DNA from Streptococcus pyogenes and with viable cells from Staphylococcus haemolyticus. Half the samples were mechanically lysed using Pathogen Lysis Tubes and the TissueLyser LT and the other half of the samples were not subjected to mechanical disruption. Microbial DNA from the spiked blood was then purified using the QIAamp UCP PurePathogen Blood Kit. Mechanical disruption of samples ensured efficient cell lysis, resulting in high yields of microbial DNA, as demonstrated by the low C_T values in real-time PCR assays.

Materials and methods

Sample disruption was achieved using Pathogen Lysis Tubes (Pathogen Lysis Tubes L for blood samples and Pathogen Lysis Tubes S for swabs) and the TissueLyser LT (Figure 1), according to the manufacturer's instructions. Microbial DNA was isolated using the QIAamp UCP Pathogen Mini Kit, the QIAamp UCP PurePathogen Blood Kit or the QIAamp DNA Mini Kit, according to the protocols described in the respective kit handbooks.

Pathogen DNA purification from swabs or 400 µl blood

Cotton swabs or whole blood samples were spiked with a mixture of *Streptococcus pyogenes*, *Staphylococcus haemolyticus*, and *Candida albicans* cells. Pathogen DNA was purified using either the QIAamp UCP Pathogen Mini Kit alone or in combination with Pathogen Lysis Tubes (S or L) and the TissueLyser LT. Purified DNA was quantified by performing species-specific, in-house real-time PCR assays.

Evaluating the effect of mechanical sample lysis on pathogen DNA recovery

Whole blood was spiked with purified DNA from *Streptococcus pyogenes* and with viable cells from *Staphylococcus haemolyticus*. While the first half of the samples were mechanically lysed using the Pathogen Lysis Tubes provided with the kit and the TissueLyser LT, the second half of the samples were not. Microbial DNA from 8 ml of the spiked blood was then purified in six replicates using the QIAamp UCP PurePathogen Blood Kit, according to the protocol described in the kit handbook. Eluates were analyzed by performing in-house real-time PCR assays specific for *S. pyogenes* and *S. haemolyticus*.

Purification of bacterial DNA from culture

DNA from *Escherichia coli*, *Bacillus subtilis* and *Streptococcus pyogenes* was purified from various volumes (0.1 ml, 0.4 ml and 1.6 ml) of overnight cultures using either the QIAamp DNA Mini Kit or the QIAamp UCP Pathogen Mini Kit, alone, or in combination with Pathogen Lysis Tubes and the TissueLyser LT. For analysis, 10 µl of the purified DNA was run on a 1% TAE agarose gel.

Results

The results of this study demonstrate the importance of mechanical disruption of samples for effective lysis prior to microbial DNA purification (Figures 2–4).

Efficient sample lysis leads to high yields of DNA – crucial for reliable pathogen identification

Pathogen identification studies require high yields of microbial DNA for successful analysis. The yields of microbial DNA achieved are influenced by the efficiency of sample disruption and cell lysis prior to DNA extraction. Our results demonstrate the effectiveness of using the TissueLyser LT in combination with QIAamp UCP Pathogen Kits for optimal microbial DNA purification and reliable pathogen identification (Figures 2–4). Together, these products provide a complete solution for efficient lysis of bacteria, yeast or other fungi, and for successful purification of nucleic acids from whole blood or other sample types – enabling reliable pathogen identification.

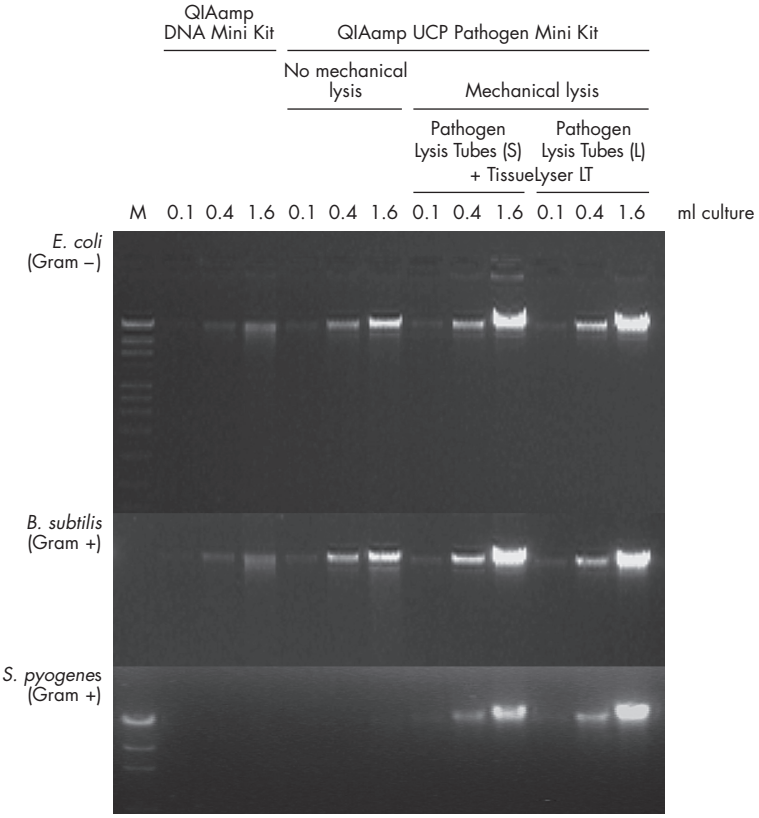

Figure 4. Mechanical lysis ensures high yields of microbial DNA. Bacterial DNA from the indicated volumes of bacterial overnight cultures was purified using either the QIAamp DNA Mini Kit or the QIAamp UCP Pathogen Mini Kit, alone, or in combination with Pathogen Lysis Tubes and the TissueLyser LT. High yields were achieved for samples that were subjected to mechanical disruption. The highest yield of microbial DNA was achieved when using the QIAamp UCP Pathogen Mini Kit in combination with Pathogen Lysis Tubes and the TissueLyser LT. **M**: marker.

Conclusions

- High yields of microbial genomic DNA are required for reliable pathogen identification.
- Thorough lysis of samples prior to DNA extraction is of critical importance to ensure successful recovery of high yields of microbial DNA.
- Effective sample disruption and homogenization is achieved using the TissueLyser LT.
- The QIAamp UCP PurePathogen Blood Kit and QIAamp UCP Pathogen Mini Kit simplify purification of microbial DNA, delivering high yields and consistent quality.
- Use of QIAamp UCP Pathogen Kits in combination with the TissueLyser LT provided higher yields of DNA (due to effective cell lysis prior to DNA purification), enabling sensitive pathogen identification.

Ordering Information

Product	Contents	Cat. no.
TissueLyser LT*	Bead mill; requires TissueLyser LT Adapter, 12-tube	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Pathogen Lysis Tubes S	50 Pathogen Lysis Tubes (with small beads) and 1 vial Reagent DX	19091
Pathogen Lysis Tubes L	50 Pathogen Lysis Tubes (with large beads) and 1 vial Reagent DX	19092
QIAamp UCP PurePathogen Blood Kit	For 10 preps: QIAamp UCP Mini Columns, Pathogen Lysis Tubes L, Tube Extenders (20 ml), Proteinase K, Buffers, VacConnectors, and Collection Tubes (1.5 ml and 2 ml)	50112
QIAamp UCP Pathogen Mini Kit (50)†	For 50 preps: 50 QIAamp UCP Mini Columns, Collection Tubes, Tube Extenders, Elution Tubes, and Buffers	50214

* The TissueLyser LT must be used in combination with the TissueLyser LT Adapter, 12-Tube.

† QIAamp UCP Pathogen Mini Kit does not include the Pathogen Lysis Tubes (L and S). Pathogen Lysis Tubes are sold separately.

The QIAamp UCP PurePathogen Blood Kit is not for use in detecting fungal nucleic acid by PCR and hybridization.

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.