Automated Rapid Isolation of Bacterial DNA from Various Samples Using the MagNA Pure Compact System

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Introduction

The efficient isolation of microbial DNA from various kinds of sample materials is crucial for sensitive detection of microbial organisms by subsequent nucleic acid-based procedures. Automation of such physical and enzymatic purification steps offers considerable benefit for the laboratory workflow and facilitates standardized procedures for template DNA preparation [1, 2]. Most of the automated systems are designed for high throughput. However, many microbiology laboratories are also looking for automated devices which can process only a few samples in a versatile, effective and economic manner. The MagNA Pure Compact System meets these requirements: It is constructed for highly flexible and reliable DNA isolation, and allows processing of one to eight samples within a period of 20 to 50 minutes.

The MagNA Pure Compact System consists of a fully automated bench-top instrument and ready-to-use nucleic acid isolation kits with prefilled cartridges. One sealed cartridge is used per processed sample to prevent cross-contamination events and waste of reagents. The instrument has an integrated touchscreen computer and a barcode reader to avoid mixing-up of protocols or samples. It comprises full sample tracking and liquid, clot, and tip detection.

The standard protocol for bacteria consists of few manual steps including the addition of a lysis buffer/proteinase cocktail and a short heating step to inactivate pathogenic microorganisms potentially present in the investigated sample. All subsequent DNA isolation steps are automatically performed using the MagNA Pure Compact System, based on the well-established combination of chaotropic Lysis/Binding Buffer and Magnetic Glass Particles (MGPs) [2]. The DNA binds to the surface of the MGPs and is then purified in several washing steps. Finally, the total extracted DNA is recovered in a small volume of a special elution buffer and can be directly used as a template for subsequent analytical procedures.

We have developed protocols for the automated isolation of bacterial DNA from various types of research sample materials such as bronchoalveolar lavage (BAL), urine, sputum, stool, swabs, cerebrospinal fluid (CSF), tracheal secretions, blood cultures, and bacterial cultures.

Samples were spiked with a known amount of certain gram-positive and gram-negative bacteria. Total genomic DNA was subsequently isolated using the MagNA Pure Compact Nucleic Acid Isolation Kit I and analyzed by species-specific PCR assays with the LightCycler® System.

Materials and Methods

Preparation of samples

The preparation method depended largely on the type of sample material. Samples such as BAL, sputum, CSF, or stool may vary to a great extent, depending on consistency and viscosity. All samples were handled in a safety cabinet until inactivation by boiling. The processed sample volume was 200 µl. Although the MagNA Pure Compact Nucleic Acid Isolation Kit I is optimized for an input volume of up to 200 µl, even higher input volumes of liquid sample materials such as urine or CSF can be processed when a centrifugation step is applied (see below). In contrast, when handling very cell rich samples, it may be advisable to use less material to avoid overloading of subsequent PCRs.

Liquifying

In case of very viscous, mucous samples (BAL or sputum samples), one-fifth volume of a freshly prepared 0.75% Dithiothreitol (DTT) solution was added to obtain a final DTT concentration of 0.15%. Samples were then incubated at 37°C under occasional shaking until they were pipettable (10–30 minutes).

Spiking with bacterial organisms

Samples of various types were spiked with defined cell numbers of *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, or *Legionella gormanii*. Other

bacterial species were analyzed directly from culture suspensions. The amount of bacteria was determined by the McFarland method [7].

Optional: centrifugation ("enrichment step" for liquid samples)

In the case of liquid samples with large volumes (>200 µl) and low or unknown bacterial load (*e.g.*, urine, CSF, BAL, aspirates), samples were centrifuged for 10 minutes at 8000 x *g* to concentrate the bacterial cells in the pellet. Most of the supernatant was discarded, and the visible pellet, together with a portion of the remaining supernatant (total volume of about 200 µl), was processed. Thus, samples with an original volume of several milliliters could be processed in one isolation step to achieve maximum analytical sensitivity.

Optional: MagNA Lyser treatment

In order to check whether the lysis efficiency can be further increased by mechanical disruption, some samples spiked with the respective target organisms were transferred to MagNA Lyser Green Beads tubes and treated in the MagNA Lyser Instrument. To compensate for the dead volume between the beads, 250 µl sample and 250 µl of Bacterial Lysis Buffer/Proteinase K were used. Bead beating was performed at 6000 rpm for 30 seconds.

Optional: Enzyme cocktail digestion

To check whether the lysis efficiency could be further increased by enzymatical pre-treatment, some samples spiked with the respective target organisms were incubated with bacteriolytic enzymes before being processed on the MagNA Pure Compact Instrument. Two enzyme cocktails were tested. Enzyme cocktail I consisted of N-acetylmuramidase (0.625 mg/ml) and beta-1,3-glucanase (Zymolyase) (0.25 mg/ml), and enzyme cocktail II consisted of lysozyme (100 mg/ml) and lysostaphin (10 mg/ml) (all from Sigma). Ten microliters of the respective enzyme cocktails were added, and the mixture was incubated at 37°C for 30 minutes.

Addition of Bacteria Lysis Buffer/proteinase K

180 µl of Bacteria Lysis Buffer and 20 µl of proteinase K solution from the MagNA Pure Compact Nucleic Acid Isolation Kit I were added to the samples (screw-capped reaction tubes were used). To minimize manual pipetting, the two components were first mixed and then added in one step. After brief vortexing, the lysates were incubated for 10 minutes at 65°C. For very viscous or cell-rich samples, the incubation time was extended to up to 30 minutes, depending on the degree of liquefaction (visual inspection). Alternatively, the samples were incubated overnight.

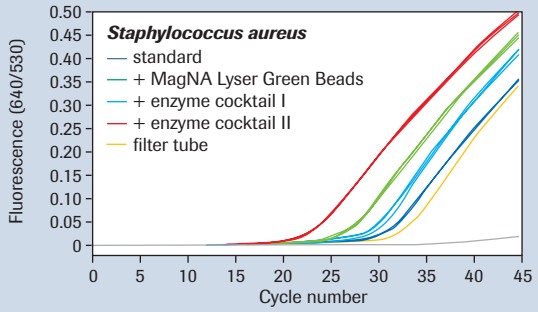

Figure 1: Comparison of different *Staphylococcus aureus* isolation protocols. LightCycler® PCR was performed in four replicates using template DNA extracted from urine samples spiked with *S. aureus* (approximately 10^4 colony forming units CFU/ml).

Inactivation by boiling

To inactivate, or at least to diminish, the infectiousness of potentially pathogenic microorganisms, samples were subjected to a further incubation step at 95°C for 10 minutes. Then the digested and/or liquified sample material could be handled outside the safety cabinet. After the samples had been cooled down to room temperature, they were directly transferred to the MagNA Pure Compact Instrument.

DNA isolation with the MagNA Pure Compact System

The lysates were transferred to the MagNA Pure Compact Sample Tubes and loaded onto the instrument together with the necessary disposables (Elution Tubes, Tip Trays) and Reagent Cartridges from the MagNA Pure Compact Nucleic Acid Isolation Kit I. The automated DNA isolation was started ("Bacteria Protocol"). The MagNA Pure Compact System automatically performs all isolation and purification steps. For comparison, a common filter tube method was run in parallel.

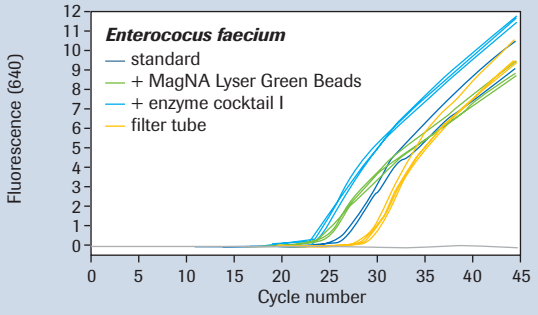

Figure 2: Comparison of different *Enterococus faecium* isolation protocols. LightCycler® PCR was performed in four replicates using template DNA extracted from urine samples spiked with *E. faecium* (approximately 10^4 CFU/ml).

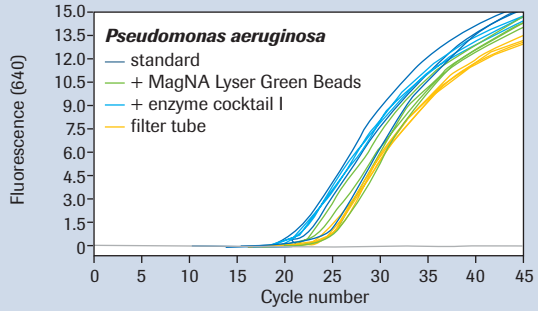

Figure 3: Comparison of different *Pseudomonas aeruginosa* isolation protocols. LightCycler® PCR was performed in four replicates with template DNA extracted from urine samples spiked with *P. aeruginosa* (approximately 10^4 CFU/ml).

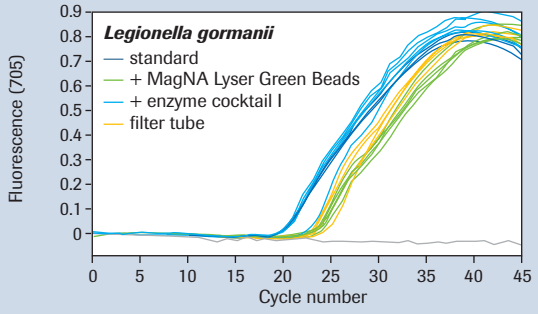

Figure 4: Comparison of different *Legionella gormanii* isolation protocols. LightCycler® PCR (*Legionella spp.*-specific protocol) was performed in four replicates on template DNA extracted from urine samples spiked with *L. gormanii* (approximtely 10^4 CFU/ml).

LightCycler® PCR analysis

DNA of the respective target organisms was detected by LightCycler® PCR using pathogen-specific LightCycler® kits, or well-evaluated home-brew protocols for *S. aureus*, *E. faecium*, *P. aeruginosa*, and *Legionella spp.* [3, 4]. In general, 5 µl of MagNA Pure Compact eluate (out of 100 µl) was used per 20 µl LightCycler® assay, together with the corresponding primers, hybridization probes, and the LightCycler® FastStart DNA Master Hybridization Probes, as previously described [1, 4]. PCR results were analyzed with respect to LightCycler® Crossing Points (CP) that are indicative of the amount and quality of input DNA.

Results and Discussion

Several variants of isolation protocols were compared to identify the most efficient DNA extraction and purification procedure. Incubation with DTT proved to be very efficient in liquefying mucous respiratory samples such as sputum or BAL. All evaluated protocols included a proteinase K digestion step that further improves the consistency of viscous samples and removes impurities. This step is followed by a short boiling step to inactivate infectious organisms.

The cell-wall structure of some gram-positive bacteria is known to be resistant to a number of common lysis methods. Therefore, additional sample-pretreatment strategies were systematically evaluated, including digestion with bacteriolytic enzymes, freeze/boil cycles, or bead beating with the MagNA Lyser System. To obtain comparable results, DNA was also manually extracted using a filter-tube method. The corresponding extraction efficiencies were assessed by quantitative real-time PCR assays on the LightCycler® Instrument.

The results differed significantly depending on the bacterial species as shown in Figures 1-4. The application of several freeze/boil cycles did not have any significant effects on DNA extraction efficiency for the bacterial species investigated (data not shown). For *S. aureus*, the enzyme cocktail II dramatically increased the lysis efficiency (about 500-fold), whereas bead-beating pretreatment improved the sensitivity about 100-fold, and enzyme cocktail I by about five-fold compared with the standard MagNA Pure Compact extraction procedure.

For *E. faecium*, both enzyme cocktail I and the bead-beating procedure resulted in a tenfold increase in the analytical sensitivity. For gram-negative species, such as *P. aeroginosa* or *L. gormanii*, additional pre-treatments did not further increase the analytical sensitivity.

Conclusion

The MagNA Pure Compact System has proven to be a useful and versatile tool for efficient, automated isolation of bacterial DNA from various sample types. All DNA isolation steps are performed automatically by the MagNA Pure Compact Instrument within less than 30 minutes. All buffers and reagents needed for the automated extraction and purification of individual samples are ready to use in a sealed cartridge. The “one cartridge per sample” concept offers great flexibility and avoids both cross-contamination events and waste of reagents and consumables. A boiling step is recommended to inactivate pathogenic organisms that may be present in the sample.

It was demonstrated that most of the relevant types of sample materials can be processed, (e.g., BAL, urine, stool, sputum, swabs, CSF, tracheal secretion, or cultures). The resulting DNA preparation is of high quality and shows no inhibition in subsequent PCR analyses. From spiked samples of identical composition, identical or sometimes better analytical sensitivities were observed in the Light-

Cycler® experiments when using MagNA Pure Compact extracts compared with template DNA obtained by other isolation methods.

Especially for gram-positive bacterial species, a significant increase in lysis efficiency and, as a consequence, in the analytical sensitivity of subsequent nucleic acid-based assays can be achieved by implementing additional steps to the MagNA Pure Compact extraction workflow (*i.e.*, incubation with a cocktail of bacteriolytic enzymes or physical pre-treatment with the MagNA Lyser System). For gram-negative bacterial species, additional enzymatic or physical pre-treatment steps did not show a significant benefit with respect to extraction efficiency.

However, even when extra steps are applied, the entire isolation procedure can be performed within 45–75 minutes. In combination with rapid cycle real-time PCR on the LightCycler® Instrument [1], the complete analytical workup from receipt of sample material to the reporting of the PCR result can be performed in less than two hours.■

References

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MagNA Pure Compact Nucleic Acid Isolation Kit I	1 kit (32 isolations)	03 730 972 001
MagNA Pure Bacteria Lysis Buffer	20 ml	04 659 180 001
Proteinase K, rec. PCR grade, solution	5 ml	03 115 828 001
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MagNA Lyser Instrument	1 instrument (220 V)	03 358 976 001
MagNA Lyser Green Beads	100 tubes	03 358 941 001

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