NEW: MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) – Automated Isolation of Bacterial DNA from Various Sample Materials

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Introduction

The efficient isolation of bacterial DNA from different sample materials is crucial for fast and sensitive detection of microorganisms by polymerase chain reaction (PCR). Automation of such procedures is of tremendous help in the microbiology lab [1-5]. We therefore developed the new MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) for the automated isolation of bacterial and fungal DNA from various types of research sample materials on the MagNA Pure LC Instrument. The standard protocol consists of very few manual steps (i.e., addition of a lysis buffer/proteinase cocktail and a heating step). All subsequent DNA isolation steps are automated on the MagNA Pure LC. The kit was tested with a variety of difficult sample materials like bronchoalveolar lavage (BAL), urine, sputum, stool, swabs, cerebrospinal fluid (CSF), tracheal secretion, blood and bacterial cultures. Samples were spiked with a known amount of certain Gram-positive and Gram-negative bacteria or with fungi, and the DNA was isolated and analyzed by species-specific PCR assays in the LightCycler (LC) Instrument [1-3].

Materials and Methods

Preparation of samples

The preparation method largely depends on the type of sample material. Samples like BAL, sputum, CSF, or stool vary to a great extent regarding their consistence, viscosity etc. All samples were processed within a safety cabinet until inactivation by boiling.

➔ Liquifying (optional):
For very viscous, mucous samples (e.g., some BALs, sputum), 1 volume of a freshly prepared 0.3 % DTT solution was added to a final concentration of 0.15 %. Samples were incubated at 37 °C until pipetteable (e.g., 30 minutes).

➔ Spiking with bacteria or fungi:
Samples were spiked with defined cell numbers of *Staphylococcus aureus*, *Escherichia coli* or *Helicobacter pylori* organisms. Other bacterial species were directly analyzed from culture suspensions. The number of bacteria was determined by the McFarland method. As for fungal organisms, *Aspergillus fumigatus* and *Candida albicans* were used for spiking.

➔ Centrifugation (optional):
Liquid samples with large volumes and low or unknown bacterial load (e.g., urine, CSF, BAL, aspirates) 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 pellet and some remaining supernatant (a volume of 50-100 µl) were processed.

➔ Addition of Bacteria Lysis Buffer/proteinase K:
130 µl of Bacteria Lysis Buffer and 20 µl of proteinase K solution from the MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) were added to the samples. After mixing, the lysates were incubated for 10 minutes at 65 °C. For very viscous or cell-rich samples, this step was extended 20-30 minutes.

➔ Inactivation by boiling:
To inactivate pathogenic organisms, all samples were incubated at 95 °C for 10 minutes. After boiling, the samples could be handled outside the safety cabinet and were allowed to cool down.

Special sample materials

➔ Stool:
Peanut-sized stool samples were suspended in about 5-10 volumes of PBS (final volume 500 µl). 100 µl of this suspension were used, Bacteria Lysis Buffer/proteinase K was added, and incubation/boiling was performed as described above.

➔ Swabs:
Swabs were submerged in Bacteria Lysis Buffer/proteinase K and incubated at 65 °C for 10 minutes. The liquid was squeezed out and boiled as described above.

➔ Blood cultures:
100 µl of blood culture medium were processed using the standard protocol described above. For Organon Teknika blood culture flasks, however, some carry-over of carbon particles was observed. Therefore, better PCR results were obtained in the LightCycler by a short centrifugation of the eluates prior to PCR to spin down the carbon particles.

DNA isolation on the MagNA Pure LC

The lysates were then transferred to the Sample Cartridge of the MagNA Pure LC, loaded onto the workstation together with the kit reagents, and the automated DNA isolation was started. The MagNA Pure LC automatically performs all isolation and purification steps such as addition of Lysis/Binding Buffer and magnetic glass particles (MGPs), binding of DNA to the MGPs, washing steps, elution of the pure DNA, and transfer to a cooled storage cartridge [6].

LightCycler PCR analysis

PCRs were performed on the LightCycler Instrument using species-specific primers and hybridization probes for *S. aureus*, *E. coli* and *H. pylori*. Only MagNA Pure LC eluates from cultured bacteria of other species were analyzed with "broad-range" eubacterial primers and hybridization probes. In general, 2 µl or 5 µl of MagNA Pure LC eluate (out of 100 µl) were used per PCR assay, together with primers, hybridization probes, and the LightCycler FastStart DNA Master Hybridization Probes (Roche Molecular Biochemicals), as described previously [1-3]. PCR results were analyzed with respect to LC Crossing Points (CP), indicative of the amount and quality of input DNA.

Results and Discussion

BAL samples

This type of sample material shows significant variation in terms of viscosity and cell content. However, even extremely viscous samples could be processed when using the pretreatment listed above. Six different BALs of varying viscosity were spiked with 10^5 colony forming units (CFU)/ml of *S. aureus,* as a typical organism found in such samples. After DNA isolation, all samples were clearly positive in the LC PCR (Figure 1). BALs with an extremely high viscosity or human cell load showed higher LightCycler crossing points indicating a suboptimal recovery. Therefore, the amount of input sample in such cases should be slightly reduced or the proteinase K step be extended to enable smooth processing, and to ensure maximum sensitivity. In turn, a BAL that was found positive for *S. aureus* by microbiological testing showed a significantly lower CP, indicating that the total number of *S. aureus* in this particular sample was much higher than the spiked amounts (Figure 1).

Urine samples

Nine different urines were spiked with cultured *S. aureus* organisms (approx. 10^5 CFU/ml) and the DNA was isolated as described above. 2 µl of eluate were analyzed in a specific LC PCR together with a dilution series of standard DNA. In all urine samples a clear PCR signal was obtained. Comparison of the LC CPs of the samples with those of the standards indicated a high recovery (Figure 2).

Stool samples

Three different stool samples were spiked with cultured *H. pylori* organisms (approx. 10^5 CFU/ml). The DNA was isolated with the MagNA Pure LC as described above and with a common filter tube method. 2 µl of each eluate was analyzed in a *H. pylori*-specific LC assay. For all samples, a clear PCR signal was obtained. The results of the MagNA Pure LC eluates were equal or better than those of the filter tube eluates (Figure 3).

Bacterial cultures and other sample materials

Cultures of *Enterococcus faecalis*, *Corynebacterium striatum*, *Legionella pneumophila*, *Streptococcus agalactiae*, *Bordetella pertussis*, *Chlamydia pneumoniae*, and a variety of other Gram-positive and Gram-negative bacterial species were used for DNA isolation. The DNA from

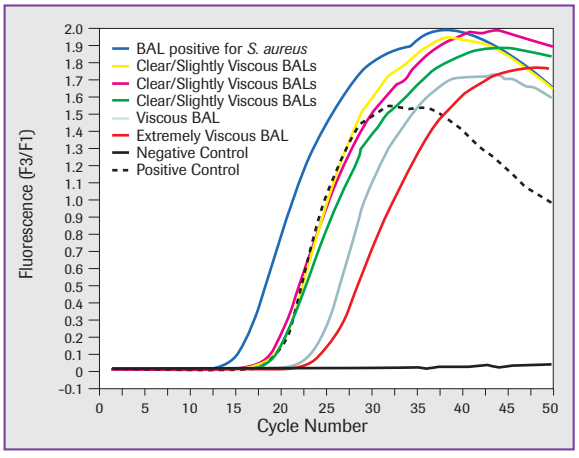

Figure 1: LC PCR of DNA from six different BAL samples spiked with *S. aureus* (approx. 10^5 CFU/ml)

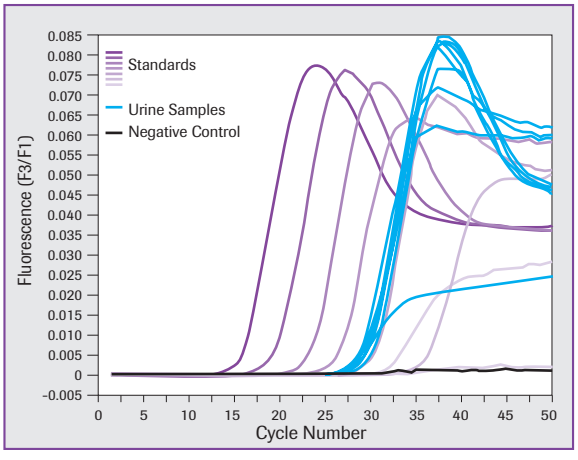

Figure 2: LC PCR of 9 different urine samples spiked with *S. aureus* (approx. 10^5 CFU/ml) and comparison to a dilution series of standard DNA

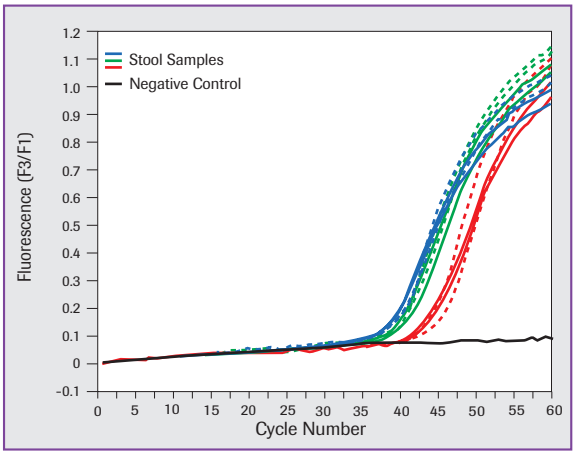

Figure 3: LC PCR of DNA obtained from 3 different stool samples (blue, green, red) spiked with cultured *H. pylori* organisms (approx. 10^5 CFU/ml), and isolated with the MagNA Pure LC (full lines) and a filter-tube method (dashed lines)

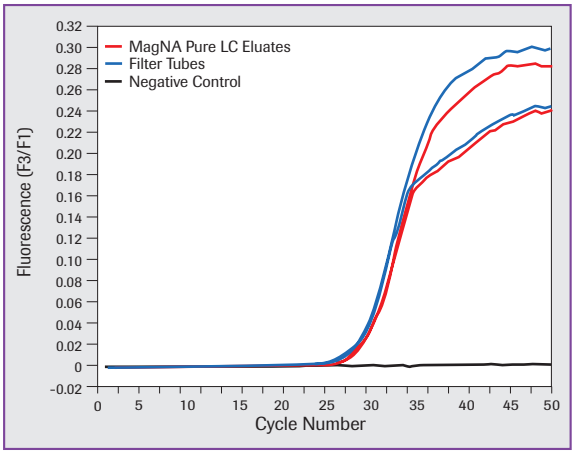

Figure 4: LC PCR of DNA from BAL spiked with *A. fumigatus* organisms (10^5 CFU/ml). The MagNA Pure LC eluates showed PCR results equal to the eluates from the filter tubes

MagNA Pure LC could be detected with a sensitivity similar to or higher than the DNA from filter-tube methods (data not shown). Furthermore, other materials such as swabs, sputum, CSF and certain blood culture media could be processed with the MagNA Pure LC and the eluates could be directly analyzed by LC PCR (data not shown).

Detection of fungi

DNA from both *A. fumigatus* and *C. albicans* could be efficiently isolated from spiked BALs. PCR results from MagNA Pure LC eluates were equal to those from parallel DNA isolations with filter tubes (Figure 4).

Conclusion

The MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) can be used for a broad spectrum of research sample materials. The procedure is largely automated with few manual preparation steps and allows the processing of up to 32 samples at a time. The automated part takes about 60-90 minutes, depending on the number of samples. The overall time strongly depends on sample type and pre-treatment. A boiling step is recommended to inactivate pathogenic organisms 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, blood and bacterial cultures). The isolated DNA is of high quality and shows no inhibition of PCR. The sensitivities obtained in the LC were similar to those achieved with other isolation methods.

Overall, more than 20 different bacterial species were investigated and could easily be detected (except *Mycobacterium* or *Nocardia*, which were not tested since those are known to require special lysis procedures, for example alkaline lysis [7]). All other analyzed bacteria, both Gram-positive and Gram-negative species, as well as fungi could be lysed efficiently, and the isolated DNA could be detected by real-time PCR with a high sensitivity.

Therefore, the new MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) allows many new applications of the MagNA Pure LC/LightCycler System in the microbiology lab and significantly reduces the workload for nucleic acid isolation and analysis.

References

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http://biochem.roche.com/magnapure

Order INFO

Product	Pack Size	Cat. No.
MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)	1 kit (192 isolations)	3 264 785
MagNA Pure LC Instrument	1 instrument plus accessories	2 236 931