



Rapid Extraction and Detection of DNA from *Candida* Species in Research Samples by MagNA Pure LC and the LightCycler System

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Here we describe a fully automated method for the extraction of DNA from different *Candida* species in research samples using the MagNA Pure LC technology and a real-time PCR format based on the LightCycler. Combining these two methods, we developed an extremely rapid, sensitive and reliable assay for automated DNA preparation and quantitative PCR analysis of many different yeast species.

Introduction

DNA extraction from different fungal species such as yeast, moulds, dimorphic fungi and dermatophytes has been described to be rather complicated. Because of the structure and composition of the fungal cell wall, mainly consisting of mannan, mannoproteins, β -glucans and chitin [1], the release of fungal DNA usually requires additional lysis steps, such as mechanical disruption and enzymatic digestion or use of toxic chemicals.

Furthermore, most established methods for extraction and amplification of *Candida* DNA are laborious and time-consuming, requiring six to eight hours for DNA extraction followed by at least nine hours for amplification and post-PCR analysis [2].

The aim of this study was to reduce the time necessary for fungal DNA extraction significantly and to simplify the working process. Here we present an assay using the MagNA Pure LC Instrument for fully automated extraction of *Candida* DNA, achieving high purity and speed.

Materials and Methods

Fungal cultures

Cultures of the following fungi were obtained from the German Collection of Microorganisms (DSMZ), Braunschweig/Germany: *Candida albicans* (DSMZ 1665), *Candida krusei* (DSMZ 70065), *Candida glabrata* (DSMZ 70614), *Candida inconspicua* (DSMZ 70631), *Candida lusitanae* (DSMZ 70102) and *Aspergillus fumigatus*

(DSMZ 790). The sample of *Candida dublinensis* was obtained from the Institute of Medical Microbiology, University of Tübingen.

After subculturing for 48 hours at 30°C on Sabouraud-Glucose-Agar, cell suspensions from all fungi were prepared using sterile 0.9% NaCl solution.

Blood samples

For evaluation of the detection limit, EDTA anti-coagulated whole-blood samples from healthy volunteers were spiked with serial dilutions of *C. albicans* cells (10^5 – 10^0 per ml blood). DNA was extracted and subsequently analyzed according to the protocol described below and compared to a method routinely used in our laboratory [3, 4].

Manual steps

Blood specimens were initially incubated with a hypotonic red-cell lysis buffer to remove PCR-inhibiting agents such as hemoglobin. The erythrocyte-free pellet was subsequently treated in the same way as the samples from fungal dilution series.

Using serial dilutions of fungal cultures, 200 μ l of each sample were transferred into a vial containing glass-beads (180 μ m, Sigma, Deisenhofen/Germany). The vials were vortexed thoroughly and the supernatant was pipetted into the wells of the MagNA Pure LC sample cartridge.

DNA of all samples was extracted concurrently according to our in-house method using recombinant lyticase (Sigma, Deisenhofen/Germany) and a spin column system as published previously [3].

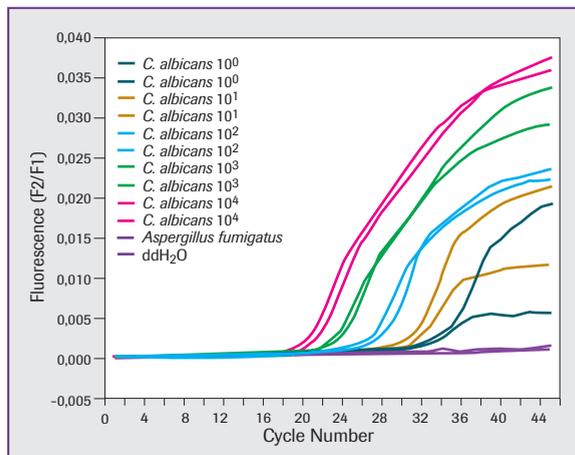


Figure 1: Sensitivity and reproducibility of the MagNA Pure LC assay. Serially diluted *Candida albicans* cells (10^4 – 10^0 CFU) were extracted in duplicate. DNA extracted from *Aspergillus fumigatus* (10^3 CFU) and ddH₂O remained negative

MagNA Pure LC isolation

The MagNA Pure LC Total Nucleic Acid Isolation Kit was used for all preparations. The setting and preparation of the instrument were performed according to the instruction manual.

LC amplification and detection

10 μ l of the eluted DNA were automatically pipetted into pre-cooled LightCycler capillaries, together with the same volume of Mastermix, using the Post-Elution module of the MagNA Pure LC Instrument.

The Hot Start LightCycler PCR was performed for 45 cycles. Primers (5'-ATT GGA GGG CAA GTC TGG TG, 5'-CCG ATC CCT AGT CGG CAT AG, Roth, Karlsruhe/Germany) bind to conserved regions of the fungal 18S rRNA gene as described before [4]. The LightCycler FastStart DNA Master Hybridization Probes Kit was used as recommended by the manufacturers. The probes hybridize to an internal species-specific sequence of the 18S rRNA gene of various *Candida* species. One probe was labeled at the 5'-end with the LightCycler-Red 640 fluorophore (5'-TGG CGA ACC AGG ACT TTT ACT TTG A), (Tibmolbiol, Berlin/Germany), the other at the 3'-end with fluorescein (5'-AGC CTT TCC TTC TGG GTA GCC ATT) (Tibmolbiol, Berlin/Germany).

Gel electrophoresis

Gel electrophoresis was performed according to a standard protocol using a TAE-2% agarose gel and Gel Star (BME, Rockland/USA) for staining of the amplicons.

Results

Sensitivity and reproducibility

The in vitro sensitivity obtained by MagNA Pure LC extraction was 1 CFU (colony forming units, in case of yeast corresponding to fungal cells) per LC assay (Figure 1). This corresponded to the sensitivity observed with manual DNA extraction by using spin columns.

Figure 1 demonstrates the reproducibility of the assay, as two samples of each dilution step (extractions were performed in parallel and on two following days) showed almost identical graphs and crossing points. Figure 2 indicates the linear range of the assay from 10^4 to 10^0 yeast cells.

Specificity

As the test system is based on a panfungal PCR, and primers are binding to conserved regions of the fungal 18S rRNA gene, DNA from *C. albicans*, non-*albicans Candida* species and from filamentous fungi was extracted by using the MagNA Pure LC Instrument.

As shown by gel electrophoresis (Figure 3), DNA from the following species could be extracted successfully by MagNA Pure: *C. albicans*, *C. krusei*, *C. glabrata*, *C. inconspicua*, *C. lusitaniae*, *C. dublinensis* and *A. fumigatus*.

Using hybridization probes designed to detect a variety of *Candida* species, DNA from the following fungi could be detected by LightCycler: *C. albicans*, *C. krusei*, *C. glabrata*, *C. inconspicua* and *C. dublinensis*. Melting curve analysis showed the probe melting temperature for DNA from *C. albicans* cells to be 68°C, whereas the melting temperature for *C. dublinensis* was 66°C, for *C. inconspicua* 58°C, for *C. krusei* 57°C and for *C. glabrata* 56°C.

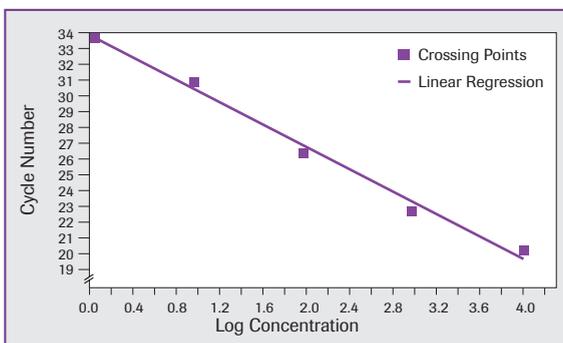


Figure 2: DNA extraction of serially diluted *Candida albicans* cells (10^4 – 10^0 CFU) by MagNA Pure LC and amplification by LightCycler. The standard curve report shown indicates the linear range of the assay

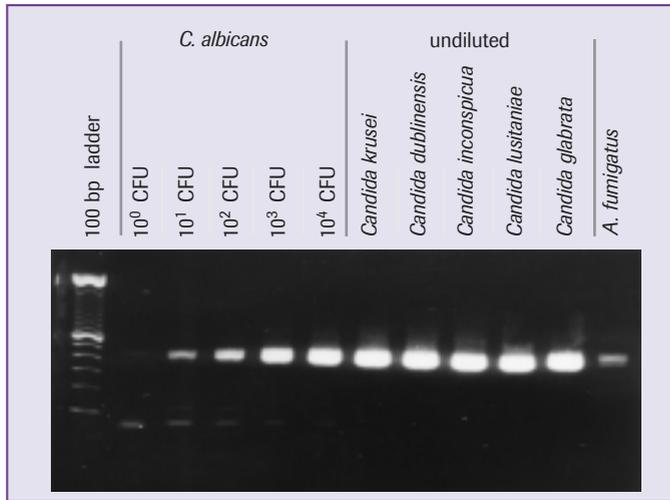


Figure 3: MagNA Pure based extraction of DNA from *Candida albicans* in different dilutions (10^0 - 10^4 CFU), other *Candida* species and *Aspergillus fumigatus* and amplification by LightCycler. Amplicon analysis by 2 % agarose gel electrophoresis

All negative controls, consisting of ddH₂O or DNA extracted from cytomegalovirus or blood from healthy volunteers, remained negative in LightCycler runs as well as in the gel electrophoresis.

Discussion

The method described was found to allow rapid and precise DNA extraction and detection from culture material as well as from blood of healthy volunteers, spiked with serial dilutions of *C. albicans*.

In contrast to routinely used manual DNA extraction protocols [3], the amount of hands-on time is reduced to less than 2 hours including extraction, amplification and data analysis (from 8 hours for the standard protocol). Automated DNA extraction allows to perform several tests on the same day. The total time requirement of the assay is only 4 hours compared to at least 16 hours according to manual protocols from initial DNA extraction to quantitative results.

Due to the overall presence of fungal spores in the environment, drastic precautions to prevent contamination, including the concurrent extraction and amplification

of negative controls are mandatory. As filtration, centrifugation or any other manual steps are completely eliminated when fungal DNA is extracted by MagNA Pure LC, the risk of contamination occurring from environmental sources or cross-contamination between the samples is reduced to an absolute minimum.

In conclusion, combining two extremely fast methods using MagNA Pure LC and the LightCycler technique, we developed an extremely rapid, sensitive and reliable assay for automated preparation and amplification of DNA from various *Candida* species. The results obtained indicate that the method is a valuable tool for DNA extraction from a wide range of fungal species.

References

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<http://biochem.roche.com/magnapure>



Product	Pack Size	Cat. No.
MagNA Pure LC Instrument	1 instrument plus accessories	2 236 931
MagNA Pure LC Total Nucleic Acid Isolation Kit	1 kit (192 reactions)	3 038 505
LightCycler Instrument	1 instrument	2 011 468
LightCycler - FastStart DNA Master Hybridization Probes	1 kit (96 reactions)	3 003 248
	1 kit (480 reactions)	2 239 272

