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# Use of commercial cards for freeing DNA from mycobacterial strains

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**SUMMARY**

A new commercial system for rapid extraction of DNA, consisting of a card on which a drop of bacterial suspension is spotted, was evaluated with 43 mycobacterial strains. Once dried, a small disk of the seeded area was directly transferred in the amplification mixture. All the samples produced good quality amplification products which were satisfactorily sequenced.

*KEY WORDS:* DNA extraction, Genetic sequencing, Mycobacteria, PCR

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Because of the steadily increasing number of mycobacterial species the accurate identification of such organisms is nowadays available only by using molecular methods.

Several all-inclusive kits are commercially available but their use is possible only in laboratories which isolate suitable numbers of mycobacteria. In addition such methods can identify only a limited number of the around 130 species officially recognized at present.

For the strains escaping the identification with the methods above the sequencing of highly conserved genetic regions is unanimously considered the reference method. Small laboratories and the ones which do not have access to an automatic sequencer usually rely on reference centers to which they send their strains. As the mailing of living organisms is becoming more and more cumbersome and expensive, the shipment of extracted DNA is increasingly used. We evaluated the reliability of a commercial card-system which seems particularly suited for the shipping of DNA with regular mail and which, without any operation, provides free, directly amplifiable and stable, DNA.

The GenoCard (Hain Lifescience, Nehren, Germany) is a cardboard layer on which a small drop of bacterial suspension can be pipetted and, once dried, stored at room temperature for at least three months.

The amplification of the DNA is made by detaching a small disk from the seeded area of the card, using a special punch, and by directly transferring it in the amplification mix. Extraction or purification steps are not needed.

Forty-three mycobacterial strains (Table 1) grown in solid or liquid culture were used for the test. From solid media 1 µL loopful was suspended in 200 µL of distilled water, while 20 µL, of liquid cultures were diluted with water to the same final volume.

From each suspension 15 µL were dropped in one round sample-field of GenoCard.

As negative control one sample field was dropped with 15 µL of distilled water. The cards were left to dry for 15 min within the flow cabinet they had been seeded in and then stored, with the lid

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TABLE 1 - *Mycobacterial strains used in the experiment*

| *Species* | *Strains* |
|---|---|
| *M. abscessus* | 3 |
| *M. arupense* | 2 |
| *M. avium* complex | 1 |
| *M. chelonae* | 2 |
| *M. florentinum* | 1 |
| *M. gastri* | 1 |
| *M. genavense* | 1 |
| *M. gordonae* | 4 |
| *"M. insubricum"* | 2 |
| *M. kansasii* | 3 |
| *M. kumamotonense* | 1 |
| *M. mucogenicum* | 1 |
| *M. nonchromogenicum* | 2 |
| *M. parascrofulaceum* | 1 |
| *M. simiae* | 3 |
| *M. szulgai* | 2 |
| *M. terrae* | 4 |
| *M. triplex* | 2 |
| *M. xenopi* | 5 |
| M. spp. N1740C | 2 |

flipped closed, at room temperature. The amplification was carried out by transferring a fragment of the plotted matrix directly to the amplification mix containing the primers specific for a trait, of approximately 500 bp, within the first third of the 16S rDNA, and following standard procedure (Kirschner *et al.*, 1993).

The amplification products were checked, by means of agarose gel electrophoresis, for the presence of 500 bp long DNA and than sequenced using the BigDye-terminator chemistry on an AB3730 automated instrumentation (Applied Biosystems); the strains were identified by comparison of forward and reverse strands with the GenBank database.

In order to ensure the safety of the GenoCard system, five cards were inactivated for 15 min at 115°C after use. They were then punched again twice, one disk was submitted to amplification and sequencing and the other was seeded in liquid medium for mycobacteria (MGIT, Becton Dickinson). To ascertain whether an accurate standardization of the suspension was required several twofold dilutions were used to spot different cards.

Forty of 43 mycobacterial strains revealed an approximately 500 bp long amplification product by gel electrophoresis, while the presence of amplified DNA was not clearly evident in the remaining three and was absent in the negative control. All the strains, including the latter three, but not the negative control, produced good quality electropherograms and were correctly identified. Equally satisfactory results were obtained from inactivated cards. Amplifications products obtained from cards inoculated with different dilutions of the bacterial suspension did not reveal significant differences such suggesting a high detection limit of the method as well. No growth was obtained in MGIT tube inoculated with inactivated disks.

Freeing of nucleic acids is the first step in different molecular techniques. Several approaches exist for the extraction of DNA from bacterial cells. The reference method, which is based on the use of detergents and proteolytic enzymes followed by phenol-chloroform extraction (Blin and Stafford, 1973), is cumbersome and time-consuming.

For diagnostic bacteriology purpose several rough methods have been developed. Probably the most simplified one is the method relying on boiling and centrifugation, with the DNA being detectable in the supernatant. Although this method works quite well in most circumstances, unsatisfactory extracts are not rare, in particular with organisms characterized by a highly resistant cell wall.

A direct comparison of the yield of extractions achieved with GenoCard and with the latter method is not possible here as the strains were not processed in parallel with the two approaches. An indirect comparison with the extractions

obtained in a 3-month period by boiling revealed a clearly better performance of the GenoCard method (100% versus 83% of successful DNA amplification) whose extracts also produced, on average, better quality electropherograms.
GenoCard, which keeps handling to a minimum, with the freeing of DNA requiring only the blotting of a drop of bacterial suspension, revealed an excellent yield and a 100% effectiveness on different species of mycobacteria. Each round sample-field is large enough to allow the detachment of at least eight fragments, thereby allowing the possible repetition of the test and/or the amplification of multiple genetic regions from the same extract.
The format of the system and the possibility of heat inactivating samples without damaging the DNA are added values that allow the safe and inexpnsive mailing of stable DNA samples. The cards, which can hold up to four DNA samples, fit into any small-size envelope.

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## ERRATA CORRIGE

The authors' names of the article "Distribution of HSV-1 IgG antibodies by two methods comparing in Turkish atopic children" published in New Microbiol. 2007 Apr; 30 (2): 109-112

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