Evaluation of the GenoCard as a tool for transport and storage of samples for tuberculosis molecular drug susceptibility testing

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SUMMARY

Early identification and monitoring of the spread of resistant *M. tuberculosis* strains is essential to control tuberculosis. The paper-based transport device GenoCard enables the safe shipment of inactivated biological material and strains to be used for molecular detection of drug resistance.

KEY WORDS: Genocard, MDR-TB, Molecular DST

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The increasing number of multi-drug resistant (MDR) and the onset of extensive-drug resistant (XDR) *M. tuberculosis* strains require fast collection of data for monitoring resistance (Coker R.J., 2004, Cohen J., 2006).

Identification of drug resistance can be performed either by conventional methods (culture and drug susceptibility testing - DST) or, for selected drugs (Riska P.F., *et al.,* 2000), by nucleic acids amplification techniques (NATs) targeting selected genes. NATs allow fast identification of mutations responsible for the resistant phenotype (Cheng V.C.C., *et al.*, 2005). In many countries the low number of tuberculosis (TB) laboratories performing cultures, or their localization only in the capital, is the limiting factor preventing collection of reliable nationwide data on MDR/XDR strains and their clustering. In addition, the shipment of infectious materials must follow very strict international rules making it extremely time-consuming and expensive.

The aim of the present study is to illustrate the value of the GenoCard (Hain Lifescience, Nehren, Germany), an easy- and rapid-to-use solid support enabling collection and storage of biological materials to be used on molecular assays such as direct testing performed for identification of MDR-TB and molecular typing for epidemiological purposes.

18 respiratory and 2 non-respiratory samples, scored from negative to 2+ on smear microscopy, collected from patients with a high clinical suspicion of active TB, and 28 *M. tuberculosis* strains isolated on cultures in high incidence areas (with known sensitivity pattern), were selected to evaluate the use of the GenoCard for transport and medium-term storage of samples containing mycobacterial DNA. 100 µL of homogenized clinical samples or 100 µL of TB culture suspension were dispensed on the GenoCard paper, dried at room temperature for 2 hours and inactivated by incubation at 110°C for 15' and transported to the laboratory in Milan, Italy.

A spot (ø 1mm) of the GenoCard, withdrawn with a washable punch and a provided punching mat,

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was used directly as DNA template in the amplification reaction.
Figure 1 schematically describes the collection of the samples and the use of spotted support in amplification reaction. The spotted GenoCard were stored at room temperature up to six months. In order to evaluate the inactivation of the samples, we cultured several spots in liquid medium and, after incubation at 37°C for up to 60 days, no growth was observed. Samples were stable after spotting and the GenoCard could be used up to 6 months without any damage to the DNA or anomalies on the results.
We evaluated the performance of the card spotted with clinical samples and strains in assays aimed at rapid detection of resistance associated genes and typing by Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats (MIRU-VNTR). We used the commercial kit Genotype MTBDR (GT-MTBDR, Hain Lifescience) for rifampin and isoniazid resistance detection (MDR) and we sequenced the Quinolones Resistance Determining Regions (QRDRs) of *gyrA* and *gyrB* genes and the *rrs* gene for identification of selected mutation associated to resistance to fluoroquinolones and aminoglycosides, respectively (Ramaswamy S., *et al.*, 1998; Maus C.E., *et al.,* 2005). The GT-MTBDR test was performed as described elsewhere using 2U of HotStar*Taq* DNA-Polymerase (Qiagen) in the amplification protocol (Miotto P., *et al.*, 2006). The genotyping of samples by MIRU-VNTR was performed as described by Supply *et al.* using 2.5U of HotStar*Taq* DNA Polymerase (Qiagen) (Supply P., *et al.*, 2001).
Characteristics of the samples and amplification results for MIRU-VNTR and GT- MTBDR assays are reported in Table 1. Microscopy scoring was done according to the WHO standard (World Health Organization). Positive amplification results marked (*) were obtained after heat extraction of the DNA from 5 spots of GenoCard in 75 µL of water. This protocol was used in smear negative or "scanty" samples in order to avoid false negative reactions due to the uneven distribution of mycobacteria in the sample.
The DNA extracted from GenoCard spotted with the strains allowed to correctly perform the GT-MTBDR for all samples. Three samples were classified as non-tubercular mycobacteria (NTM) by

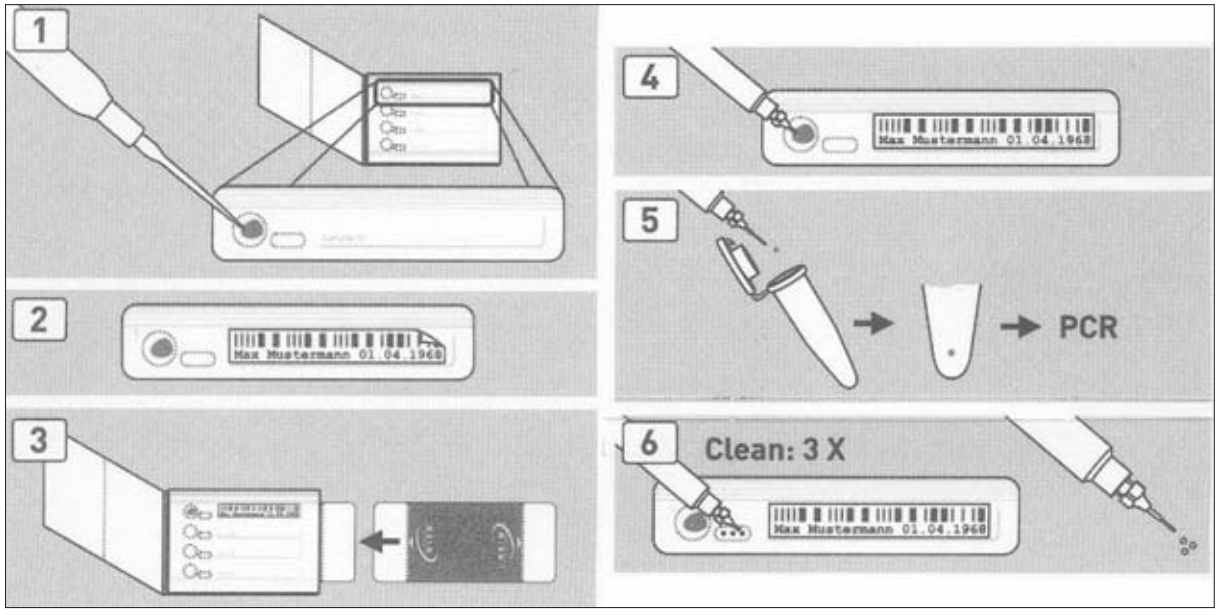

FIGURE 1 - *Schematic representation of the use of the GenoCard. The figure schematically describes the collection of the samples and the use of spotted support in amplification reaction. Each card can be used for four different samples and it is provided with an area for sample spotting and a different area for cleaning the punching device. 100 L of sample are spotted on the labelled GenoCard paper (steps 1-2), after inactivation and transport; the plastic punching mat is placed under the GenoCard and a spot is collected with the washable punch (steps 3-4-5). The puncher is cleaned 3-4 times between samples (step 6).*

TABLE 1 - *Characteristics of the samples and amplification results for MIRU-VNTR genotyping, the GT-MTBDR assay.*

Sample		*Assay*		*N (%)*
AFB	*Material*	*MIRU-VNTR*	*GT-MTBDR*	
NEG	Pus	+*	+*	1 (5)
	Pus	-	-	1 (5)
	Sputum	+	+*	1 (5)
	Sputum	-	-	1 (5)
	Sputum	+*	+*	2 (10)
	Bronchial Aspirate	+*	+*	1 (5)
+	Bronchial Aspirate	+*	+*	1 (5)
	Bronchial Aspirate	+	+	2 (10)
	Sputum	+	+	8 (40)
++	Sputum	+	+	2 (10)
Total				**20**

Microscopy scoring was done according to the WHO standard: negative, no acid-fast bacilli (AFB) observed; scanty, 1 to 9 AFB in 100 fields; +, 10 to 99 AFB in 100 fields; ++, 1 to 10 AFB per field in at least 10 fields; +++, >10 AFB per field in at least 10 fields. *Positive results obtained after heat extraction of the DNA from 5 spots of GenoCard in 75 µL of water.

the lack of hybridization of the MTB-Complex control probe. All the other isolates resulted MDR by DST. The results obtained with the molecular assay were all consistent with those obtained with DST results.

For 23 out of 25 MTB-positive samples we obtained a complete MIRU-VNTR genotyping profile and sequence results for QRDR regions of *gyrA* and *gyrB* genes, and the *rrs* gene. Two samples carried the Ala90Val substitution in the QRDR of the *gyrA* gene and 2 other samples carried the nucleotidic substitution a1401g in the *rrs* gene, predicting cross-resistance among streptomycin, kanamicin, amikacin and capreomycin (Maus C.E., *et al.*, 2005). No XDR sample was detected. For 2 samples we were unable to obtain any amplification result.

The use of the GenoCard as DNA template source yielded reliable data on resistance to rifampin and isoniazid on genotyping and sequencing data. From 19 clinical specimens out of 20 we obtained successful hybridization results using the commercial line probe assay GT-MTBDR and complete genotyping analysis by MIRU-VNTR. Specificity and sensitivity of the GT-MTBDR were consistent with those previously obtained (Miotto P., *et al.*, 2006).

Notably, molecular DST were obtained within 3 days. The GenoCard could be considered a useful tool for collection and transport of clinical specimens and/or clinical isolates to reference laboratories for a quick monitoring of drug-resistant TB in high incidence Countries.

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