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Diagnostic utility of the molecular assay GenoType MTBC (HAIN Lifescience, Germany) for identification of tuberculous mycobacteria

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Abstract

Introduction: The GenoType system (HAIN Lifescience, Germany) offers new perspectives of detecting the tuberculous and non-tuberculous mycobacteria at the molecular level. The system compromises five independent tests that could be performed either on direct specimens or isolated strains, to identify the strains and test the resistance against rifampin and isoniazid. Up to now, non GenoType test was applied in Poland. The aim of the study was an evaluation the accuracy of GenoType MTBC test in speciation of the clinical isolates, previously classified as *M. tuberculosis* complex by HPLC analyze of mycolic acids.

Material and methods: 161 clinical isolates, derived from the TB patients hospitalized in the Warsaw Medical University Hospital between 1999 and 2007 were assayed.

Results: On the basis of the hybridization patterns, all 161 studied strains were identified as *M. tuberculosis*/*M. canettii*.

Conclusions:

1. The GenoType MTBC test (HAIN Lifescience, Germany) precisely recognizes *M. tuberculosis* complex. The 100% accordance in speciation of *M. tuberculosis* by the GenoType MTBC test as compared to HPLC method was demonstrated. The GenoType MTBC test can replace HPLC in detection of tuberculous mycobacteria in clinical isolates.
2. As the GenoType MTBC test performs well, the other tests of GenoType system may be considered to be verified in diagnostic procedure of mycobacterial infection.

Key words: hybridization, multiplex PCR, *Mycobacterium tuberculosis* complex

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Introduction

Nowadays, molecular biology techniques are intensively used in the laboratory diagnostics of tuberculosis, both in direct examination of specimens, as well as in order to identify the species of mycobacteria isolates. In studies of direct specimens, commercial diagnostic kits, such as MTD (GenProbe Inc. USA) or Amplicor MTB system (Roche Diagnostics, USA), which apply nucleic acid amplification reactions, are used to detect the genetic material of *Mycobacterium tuberculosis* complex. For typing isolates, highly specific nucleic acid hybridization reaction is applied. The most commonly accepted commercial system applying this method is AccuProbe (GenProbe Inc. USA), which offers specially designed DNA probes for *M. avium*, *M. gordonae*, *M. intracellulare*, *M. kansasii* and *M. tuberculosis* complex.

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Set of five probes of AccuProbe genetic system usually allows for an effective diagnosis in the routine procedure. However, it is not possible for this molecular system to replace the high-performance liquid chromatography (HPLC) in typing the mycobacteria finally. The HPLC, which is based on an analysis of mycolic acids, allows the typing of more than 50 mycobacteria species, depending on the range of reference strains in the laboratory [1]. The only serious limitation of this method is a very high cost of equipment and disposal of organic solvents. In addition, some closely related species, having a similar set of mycolic acids, are indistinguishable by HPLC, for example within the *M. tuberculosis* complex (except the *M. bovis* BCG).

For the comprehensive diagnosis of various mycobacteria infections, a new molecular system, GenoType Mycobacterium (HAIN Lifecience, Germany) has been developed, which may replace the chromatography in routine diagnostic procedure. The GenoType Mycobacterium comprises five independent tests, but there is no need to perform all of them. The advantage of the system is such that the assays can be applied either separately or in combination, depending on the demand.

- GenoType Mycobacteria Direct test, directly applied to the clinical specimens, identifies five species of mycobacteria: *M. avium*, *M. intracellulare*, *M. kansasii*, *M. malmoense* and *M. tuberculosis* complex [2].
- The tests GenoType Mycobacterium CM for common mycobacteria, and GenoType Mycobacterium AS for additional species, which can be performed together, enable to identify 30 types of mycobacterial strains [3].
- GenoType MTBC test is intended for the differentiation of members of the *M. tuberculosis* complex and particularly the identification of *M. bovis*, which is not possible using HPLC [4].
- GenoType MTBDRplus, which has been developed to detect drug resistance to rifampicin and isoniazid, can be applied not only to the mycobacterial strains, but also to the direct clinical specimens, provided they are AFB positive [5]. Implementation the GenoType MTBDRplus into the routine laboratory work up would greatly accelerate the detection of multidrug resistant strains.

Before the implementation of the GenoType Mycobacterium system into the routine laboratory diagnostic procedure of mycobacterial infection, the accuracy of the individual tests needs to be verified, both in terms of typing precision, as well as in respect to the complexity execution. According to the authors' knowledge, none of the tests of GenoType system has been used so far in the diagnostics of tuberculosis in Poland.

First, we have chosen the GenoType MTBC, which provides a rapid and accurate identification of the presence of various members of the *M. tuberculosis* complex (*M. africanum, M. bovis* BCG, *M. bovis ssp. bovis, M. bovis ssp. caprae, M. microti, M. tuberculosis*/ *M. canetti*) when it is used with growth-positive cultures. The assay is based on the detection of single nucleotide polymorphism of the gyrase B gene [6], and the Region of Difference 1 (RD1) deletion of *M. bovis* BCG [7]. Specific oligonucleotides targeting these sequences are immobilized on membrane strips. Amplicons derived from a multiplex PCR hybridize to these probes. Species can be identified according to the interpretation chart provided by the manufacturer. Although the assay does not provide tools for the differentiation *M. tuberculosis* from *M. canetti*, there is a poor chance to isolate *M. canetti* in Poland as this novel pathogenic taxon of the MTBC has been described recently, and rare cases have been reported in patients living in Africa [8].

In this retrospective work, we evaluated the GenoType MTBC assay to test a large number of clinically significant isolates, using HPLC-based mycolic acids analysis as a reference method. The strains were derived from patients of The Central Clinical Hospital of The Medical University of Warsaw between 1999 and 2007.

Material and methods

Strains analyzed

A set of 161 *M. tuberculosis* complex strains, isolated from pulmonary or extrapulmonary specimens (70 sputum, 52 bronchial washings, 14 bronchioalveolar lavage fluids, 13 pleural effusions, 6 pleural tissue samples and 6 others) were analyzed. Only one specimen per patient was used in the present analysis. The specimens were initially processed according to national guidelines [9] using an *N*-acetyl-L-cysteine-NaOH decontamination procedure, inoculated onto solid slant media (Löwenstein-Jensen), and incubated at 37°C for up to 8 weeks. The clinical strains were typed with HPLC based mycolic acids fingerprinting according to the procedure being routinely performed in our laboratory, as described previously [10]. Only specimens with growing tuberculosis bacteria were included in the study.

GenoType MTBC assay

For an isolation of DNA, 1 loopful of bacteria was suspended in distilled water (500 μl) and sub-

jected to isolation procedure performed with AMPLICOR Respiratory Specimen Preparation Kit, (Roche Diagnostics, USA), according to the manufacture's instruction. The GenoType MTBC assay was performed as recommended by the manufacturer. Briefly, for an amplification, 35 μl of a primer nucleotide mixture (provided with the kit), amplification buffer containing 1.5 mM $MgCl_2$ and 1 U of Platinum Taq polymerase (Invitrogen, USA) (not provided with the kit), and 10 μl of DNA in a final volume of 55 μl were used. The amplification protocol consisted of 300 s of denaturation at 95°C, followed by 10 cycles comprising 30 s at 95°C and 120 s at 58°C, an additional 20 cycles comprising 25 s at 95°C, 40 s at 53°C, and 40 s at 70°C, and a final extension at 70°C for 480 s.

Hybridization and detection were carried out in a TwinCubator washing and shaking device (HAIN Lifescience, Germany). The GenoType MTBC assay was performed as recommended by the manufacturer, and the species were identified according to the interpretation chart provided with the kit. Only those strips, that developed both control bands, UC (Universal Control) and CC (Conjugate Control), were regarded as interpretable. The GC (Genus Control) reaction zone of the membrane strip, which hybridizes with amplicons generated from all members of the *M. tuberculosis* complex, should be positive for the whole set of studied strains.

Results

Amplification and hybridization controls (UC and CC, respectively) verified the test procedures: All GenoType MTBC assays gave unequivocal results and none of the amplification reactions were inhibited. All of the 161 specimens investigated showed the pattern *M. tuberculosis*/*M. canettii*. The identification results for all specimens were in complete accordance with the previous differentiation results obtained by HPLC.

The GenoType MTBC assay fits easily into the work flow of a routine laboratory, provided the basic equipment for molecular biology assays is accessible and can be completed within 5 hours for 12 specimens.

Discussion and conclusion

The GenoType MTBC assay has been CE marked for the use in Europe for several years and some authors have already evaluated the accuracy of the test in clinical practice [4, 11–13]. The authors generally emphasize that the GenoType MTBC assay is an easy-to-use and reliable method for the routine identification of members of the *M. tuberculosis* complex. The assay could be implemented in any mycobacteriology laboratory in order to speed up the mycobacterial diagnostics. Richter et al. [11] compared the GenoType MTBC assay with the AccuProbe test. The sensitivity was slightly higher for the GenoType MTBC test than for the AccuProbe assay (100% and 97.4%, respectively). Somoskovi et al. [13] noticed the high sensitivity (93.2%) and specificity (100%) of the GenoType MTBC assay when used directly on a set of 79 smear-positive clinical specimens.

The goal of the present study was to evaluate the GenoType MTBC assay in practice, in our local laboratory. We have chosen this test to be verified as the first one from the GenoType system (HAIN Lifescience, Germany) for the tuberculosis sake and on grounds of positive opinions cited above. A set of 161 clinical isolates of *M. tuberculosis* complex was analyzed using HPLC-based mycolic acids analysis as a reference method. Yielded results were absolutely in concordance.

From a practical point of view, the GenoType MTBC assay has several advantages. First, as it is a molecular biology based technique, the total cost of species differentiating is much lower then the cost of using a HPLC method, while the expense of sophisticated equipment is very high and the utilization of organic solvents is even much more higher. The GenoType system demands basic equipment, easily accessible at a routine laboratory, and the price of the assay is comparable to other molecular tests used in Poland. Second, it takes 5 hours to complete the assay for a set of 12 specimens. On the contrary, the HPLC analysis is much more time consuming. Third, thanks to control bands put on each hybridization strip, there is no need to perform the whole set of 12 assays at the time. In consequence, each specimen could be assayed on demand, which does not influence the price of the test.

Considering the laboratory diagnosis of tuberculosis, the HPLC technique, which has been used for detecting *M. tuberculosis* complex isolates up to date, could be substituted with the GenoType MTBC assay without difficulty. However, the assay does not address the nontuberculous mycobacteria differentiation. Other tests of the GenoType system could easily be applied to eliminate this disadvantage without spending extra money for new equipment [3]. In this situation, some new benefits would be observed: The AFB positive clinical specimens could be examined directly along with the drug susceptibility testing to rifampicin and isoniazid [2, 5].

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