

Evaluation of the GenoType MTBC assay for differentiating 120 clinical *Mycobacterium tuberculosis* complex isolates

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The majority of mycobacterial infections are caused by strains of the *Mycobacterium tuberculosis* complex (MTBC). MTBC includes the species *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti*, *M. canettii* and *M. pinnipedii*. The *M. bovis* species can be further divided into *M. bovis* subsp. *bovis*, *M. bovis* subsp. *caprae* and the *M. bovis*-derived BCG vaccine strain, whereas *M. africanum* includes two subtypes, I and II [1–5]. Identification of mycobacteria to the species level on the basis of growth rate, phenotypic characteristics and biochemical tests is laborious and extremely time-consuming. GenoType MTBC (Hain Lifescience, Nehren, Germany) is a recently developed commercial DNA-strip assay for differentiating MTBC strains isolated from cultured material. The procedure involves isolating DNA from cultured material, multiplex amplification with biotinylated primers and reverse hybridization of the single-stranded, biotin-labeled amplicons to membrane-bound probes. The resulting banding pattern indicates the species of the isolated mycobacterium. The assay permits genetic differentiation of the following species: *M. africanum* I, *M. bovis* BCG, *M. bovis* subsp. *bovis*, *M. bovis* subsp. *caprae*, *M. microti* and *M. tuberculosis*/*M. africanum* II. The aim of the current study was to evaluate the proficiency of this assay for identifying clinical MTBC isolates.

The study was performed using 120 consecutive clinical MTBC strains isolated from different patients at our hospital over a 5-year period (2000–2004). Specimens are routinely processed in accordance with international guidelines, and when necessary, an N-acetyl-L-cysteine-NAOH decontami-

nation procedure is used [6, 7]. Specimens are inoculated into BacTAlert 3D tubes (Organon Technika, Durham, NC, USA) and onto Löwenstein–Jensen agar (LJ; bioMérieux, Marcy l'Étoile, France) and incubated at 37°C for up to 6 and 8 weeks, respectively.

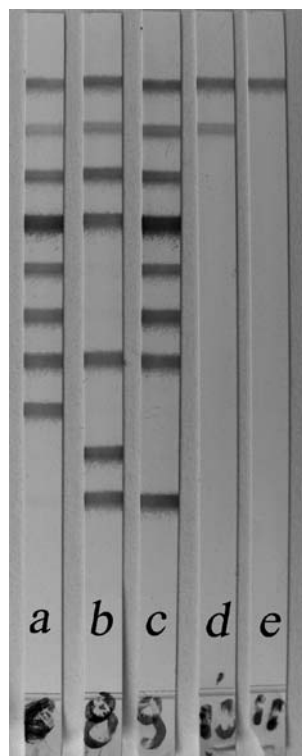
All 120 strains included in this study were identified as members of the MTBC using gene probes (AccuProbe; GenProbe, San Diego, CA, USA) and further differentiated to the species level by means of colony morphology and biochemical analysis [nitrate reduction (Nitrati Test; Liofilchem, Roseto, Italy), niacin accumulation (BBL Taxo TB Niacin Test Strips; Becton Dickinson, Sparks, MD, USA), and growth on LJ slants in the presence of 1 µg/ml thiophen-2-carboxylic acid hydrazide (TCH; bioMérieux)]. Of the 120 isolates, 119 (99.17%) were identified as *M. tuberculosis*; the remaining isolate was presumptively identified as *M. bovis* (negative niacin accumulation and nitrate reductase tests and no growth on TCH). Given this strain distribution, our results can only be applied to *M. tuberculosis*. Prior to use in this study, the LJ slants of all 120 isolates had been stored in a dark environment at room temperature and a loopful of culture material suspended in 1 ml distilled water had been preserved at –20°C.

Four control strains (two *M. africanum* and two *M. bovis*) obtained from Institute Pasteur (Paris, France) were used. In parallel, four non-MTBC microorganisms (one *M. kansasii*, one *Corynebacterium jeikeium*, one *Nocardia abscessus* and one *Escherichia coli*) were tested with GenoType for cross reactions.

The GenoType MTBC assay has formerly been evaluated in two German laboratories using control strains along with clinical isolates that were mostly obtained from positive liquid cultures [8, 9]. In the present study, the clinical isolates had been obtained exclusively from solid media. Moreover, these positive LJ slants had been stored for a prolonged period of time (ranging from weeks to years).

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Fig. 1 Examples of the results obtained with GenoType MTBC. Lane a, *M. tuberculosis*; lane b, *M. bovis* subsp. *bovis*; lane c, *M. africanum* subtype I; lane d, *Nocardia abscessus*; and lane e, *Escherichia coli*. In the case of *E. coli* only band 1 (conjugate control band) was seen, whereas in the case of *N. abscessus* the additional band 2 (universal control band for gram-positive bacteria with a high G+C content) was visible



This could prove valuable to numerous mycobacteriology laboratories worldwide that have unidentified mycobacteria stored in the form of positive LJ slants.

The GenoType assay was performed according to the manufacturer's instructions, using the reagents provided with the kit and Taq DNA polymerase (Qiagen, Hilden, Germany). The GenoType protocol consists of PCR amplification, hybridization of the PCR products to the strips, detection and interpretation of the results. The GenoType MTBC assay demonstrated 100% agreement with previous identification of the strains. In particular, all 119 *M. tuberculosis* isolates were again identified as *M. tuberculosis* by GenoType, whereas the one *M. bovis* isolate was identified as *M. bovis* subsp. *caprae*. The control strains were identified as *M. africanum* subtype I ($n=2$) and *M. bovis* subsp. *bovis* ($n=2$), whereas no cross reactions were noted for strains not related to MTBC (Fig. 1).

The GenoType assay performed well in almost all cases even though the LJ slants had been stored for a prolonged period. Nevertheless, in two cases it failed to give a result for culture material from the LJ slants. In these two cases the suspension preserved at -20°C was then used with good results.

In our study, the GenoType MTBC assay proved to be a rapid technique that is easy to perform and interpret and can easily be incorporated into the workflow of a routine diagnostic mycobacteriology laboratory. Since the GenoType assay includes an amplification procedure, it requires about three more hours to perform than AccuProbe. However,

since it provides immediate identification to the species level, the laborious and time-consuming step of biochemical analysis can be avoided. Another parameter that could restrict its use is its higher cost. We estimated it costed twice as much to identify each individual strain with the GenoType assay compared with the AccuProbe-biochemical analysis alternative. In this study, the GenoType MTBC assay proved to be reliable and the results were in full agreement with previous identification of the clinical MTBC isolates tested.

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