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Evaluation of GenoType Mycobacteria Direct Assay in Comparison with Gen-Probe *Mycobacterium tuberculosis* Amplified Direct Test and GenoType MTBDR*plus* for Direct Detection of *Mycobacterium tuberculosis* Complex in Clinical Samples[▽]

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Three molecular assays were evaluated for the direct detection of *Mycobacterium tuberculosis* complex bacteria in 125 respiratory and 22 nonrespiratory samples. The overall sensitivities obtained were as follows: GenoType MTBDR*plus*, 97.9%; GenoType Mycobacteria Direct, 93.7%; Gen-Probe *Mycobacterium tuberculosis* Amplified Direct Test, 89.6%. The specificity of the assays used was 100%.

Tuberculosis (TB) remains a significant public health challenge worldwide. Early diagnosis, adequate therapy, and measures to prevent further transmission are essential for TB control. Over the last few years, new commercial molecular assays for the direct detection of *Mycobacterium tuberculosis* in clinical samples have been introduced, leading to considerable improvement in the diagnostic rate (4). GenoType Mycobacteria Direct (GTDIR; Hain Lifescience, Nehren, Germany) is such a novel assay. In the present study, we comparatively evaluated GTDIR with two other commercial assays already in use by the majority of mycobacteriology laboratories worldwide, the Amplified *M. tuberculosis* Direct (AMTD) test (bioMérieux, Gen-Probe Inc., San Diego, CA) and GenoType MTBDR*plus* (GTPLUS; Hain Lifescience, Nehren, Germany).

GTDIR is based on nucleic acid sequence-based amplification (NASBA) applied to DNA strip technology. Three steps are involved in the use of the assay: isolation of 23S rRNA, amplification of RNA by the NASBA method, and reverse hybridization of the amplified products on membrane strips using an automated system. In addition to detecting *M. tuberculosis* complex (MTBC) organisms, this assay detects *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. malmonse*. The isolation of highly specific RNA is achieved by using the "magnetic bead capturing" method.

Both the AMTD and GTPLUS assays directly detect MTBC in clinical samples. In addition, GTPLUS offers the simultaneous detection of the most common resistance mutations in the *rpoB* (rifampin [rifampicin] resistance), *katG*, and *inhA* (isoniazid resistance) genes. A brief presentation of the three molecular assays is shown in Table 1.

In the present study, 147 samples from 132 patients who were strongly suspected of having TB were included. Samples were collected over a 3-year period (2006 to 2008) in our hospital. Respiratory samples ($n = 125$) included 94 sputa and 31 bronchial aspirates, while nonrespiratory samples ($n = 22$) included 6 tissue samples from lymph nodes, 4 urine samples, 1 fecal sample, and 11 fluid samples (4 pericardial, 3 pleural, 2 gastric, 1 abdominal, and 1 cerebrospinal).

Besides those from normally sterile sites, the samples were processed according to international guidelines by using the *N*-acetyl-L-cysteine–NAOH decontamination procedure and the Ziehl-Neelsen (ZN) technique for smear staining (3). Specimens were inoculated into BacTAlert 3D tubes (bioMérieux, Durham, NC) and onto Löwenstein-Jensen slants (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C for up to 6 and 8 weeks, respectively. Following inoculation of the culture medium, the remainder of each specimen was aliquoted into three parts and frozen at −20°C. The three aliquots of each sample were further used (after liquefaction at room temperature) to perform the three genetic assays. Isolates were identified to the species level by colony morphology, biochemical analysis, gene probes (AccuProbe; Gen-Probe, San Diego, CA), and/or use of the GenoType MTBC assay (Hain Lifescience, Nehren, Germany) for MTBC and GenoType CM and AS assays for nontuberculous mycobacteria (NTM). Procedures were performed according to standard guidelines (3) and the manufacturers' instructions. The three evaluated assays were performed according to the manufacturers' instructions by using an external *Taq* DNA polymerase (Qiagen) when needed.

Culture was considered the "gold standard" method. Of the 147 cultures, 96 were positive for *M. tuberculosis* (85 from respiratory and 11 from nonrespiratory samples). The 96 positive cultures were used as a reference for comparison with the

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TABLE 1. Commercial tests used for direct detection of mycobacteria in clinical samples

MTBC assay	Amplification technology	Target(s)	Detection principle	Turnaround time (h)	Equipment required	Cost (€)
AMTD	TMA[a]	rRNA	Chemiluminescence	3.5	Sonicator, heat block, luminometer	39
GTDIR[b]	NASBA	23S rRNA	Colorimetry	5.5	Magnetic separator, heat block, sonicator, thermocycler	50.5
GTPLUS[c]	PCR	23S rRNA, *rpoB*, *inhA*, *katG*	Colorimetry	5	Heat block, sonicator, thermocycler	56.8

[a] TMA, transcription-mediated amplification.
[b] Detects *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. malmonse*.
[c] Detects common mutations for rifampin and isoniazid resistance.

three molecular assays. Results of the evaluation are shown in Table 2. The statistical significance of the differences in sensitivity observed in the three molecular assays was determined by the Mann-Whitney U test. A probability (P) of 0.05 was regarded as statistically significant. Statistical analysis was performed with SPSS 11.5 software (SPSS, Chicago, IL).

The overall sensitivity of each assay for the detection of *M. tuberculosis* was as follows: AMTD, 89.6% (86/96); GTDIR, 93.7% (90/96); GTPLUS, 97.9% (94/96). These results are in accordance with previous results for AMTD (5, 6, 7), GTDIR (1), and GTPLUS (2, 8). In the three assays used, the sensitivity for respiratory samples was higher than that observed for nonrespiratory samples, while the sensitivity of GTPLUS was higher than that of the other assays for both respiratory and nonrespiratory samples. The difference in sensitivity between GTPLUS and AMTD was statistically significant for the respiratory samples ($P = 0.017$) and overall performance ($P = 0.017$). No statistically significant difference was found in the sensitivities of the three assays for the nonrespiratory samples. Since all positive signals corresponded to *M. tuberculosis*, assay specificity was found to be 100%.

It should be noted that in 81 respiratory samples with positive GTDIR results, this assay detected the presence of NTM in addition to the *M. tuberculosis* complex organism where two cases of *M. intracellulare* and one case of *M. avium* were detected. On the other hand, in eight respiratory samples with a negative GTDIR result, this assay detected the presence of *M. avium* in three cases and *M. intracellulare* in one case. The presence of NTM was verified by the culture and GenoType CM and AS assays.

Among the 48 ZN-positive samples, the sensitivity of AMTD was 93.7% (45/48), that of GTDIR was 97.9% (47/48), and that of GTPLUS was 100% (48/48). These results suggest that ZN remains useful and should be used concomitantly with the molecular assays. Among the 48 ZN-negative but culture-positive samples, the sensitivity of AMTD was 85.4% (41/48), that of GTDIR was 89.6% (43/48), and that of GTPLUS was 95.8% (46/48).

TABLE 2. Sensitivities of the genetic assays tested for MTBC detection in samples culture positive for *M. tuberculosis*

Assay	% Sensitivity[a]		
	Respiratory samples ($n = 85$)	Nonrespiratory samples ($n = 11$)	Overall ($n = 96$)
ZN	55.3 (47)	9.1 (1)	50.0 (48)
AMTD	90.6 (77)	81.8 (9)	89.6 (86)
GTDIR	95.3 (81)	81.8 (9)	93.7 (90)
GTPLUS	98.8 (84)	90.9 (10)	97.9 (94)

[a] In parentheses is the total number of samples in each category.

The sensitivity of ZN for nonrespiratory samples was very low (9.1%). In contrast, the molecular assays showed sensitivities of >80%, underlining their usefulness in the early detection of *M. tuberculosis* in nonrespiratory samples.

In the 51 culture-negative samples (40 respiratory and 11 nonrespiratory), there was 1 with positive ZN and GTPLUS results and 1 with a positive GTPLUS result only, indicating the presence of *M. tuberculosis* DNA in the samples. The two patients were undergoing anti-TB therapy when the samples were collected. Finally, in the 51 samples culture negative for *M. tuberculosis*, there was 1 nonrespiratory sample (feces) that gave a positive result for *M. avium* with GTDIR. This result was verified by a positive culture for *M. avium*.

The highest sensitivities were obtained with GTPLUS in all categories. All assays performed well, especially with respiratory samples, where sensitivities were >90% in all cases. Thus, our experience showed that the three commercial assays can readily be incorporated into the work flow of a mycobacteriology laboratory and be completed within the same working day. With the exception of the magnetic bead capturing method of GTDIR, which is laborious and intense, all of the other steps involved in the three assays can be performed easily. Interpretation of the results of the three assays is also rapid and straightforward. Of note is that the AMTD assay is easier to perform, its turnaround time is shorter, and it is less expensive than GTDIR and GTPLUS.

GTDIR and GTPLUS showed high sensitivities. The advantage of these assays is that in each run additional data are obtained. The advantage of the ability of GTPLUS to simultaneously detect resistance to isoniazid and/or rifampin suggests that this assay be used in areas with a high incidence of TB and resistance to anti-TB agents. In contrast, GTDIR should be used in areas with a low incidence of TB and a high incidence of infections by NTM, due to its ability to simultaneously detect various species of mycobacterial pathogens in one assay and in the same sample. Similarly, in everyday hospital practice, when a patient is strongly suspected of having TB because of the clinical or radiological picture, GTPLUS should be selected, whereas when clinical or radiological data indicate an NTM infection, GTDIR should be used.

A notable disadvantage of these assays is that they are not very cost-effective and therefore should only be used selectively. A proposed algorithm is for a molecular test to be performed for all smear-positive samples, as well as samples

from patients who are strongly suspected of having TB when the specimen is smear negative.

In conclusion, the aforementioned assays performed well, with GTPLUS exhibiting the highest sensitivity. The assays evaluated in this study can therefore easily be incorporated into the work flow of a mycobacteriology laboratory and should be used selectively on samples from patients who are strongly suspected of having TB.

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