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Evaluation of the Speed-oligo Direct *Mycobacterium tuberculosis* Assay for Molecular Detection of Mycobacteria in Clinical Respiratory Specimens

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We present the first evaluation of a novel molecular assay, the Speed-oligo Direct *Mycobacterium tuberculosis* (SO-DMT) assay, which is based on PCR combined with a dipstick for the detection of mycobacteria and the specific identification of *M. tuberculosis* complex (MTC) in respiratory specimens. A blind evaluation was carried out in two stages: first, under experimental conditions on convenience samples comprising 20 negative specimens, 44 smear- and culture-positive respiratory specimens, and 11 sputa inoculated with various mycobacterium-related organisms; and second, in the routine workflow of 566 fresh respiratory specimens (4.9% acid-fast bacillus [AFB] smear positives, 7.6% MTC positives, and 1.8% nontuberculous mycobacteria [NTM] culture positives) from two *Mycobacterium* laboratories. SO-DMT assay showed no reactivity in any of the mycobacterium-free specimens or in those with mycobacterium-related organisms. Compared to culture, the sensitivity in the selected smear-positive specimens was 0.91 (0.92 for MTC and 0.90 for NTM), and there was no molecular detection of NTM in a tuberculosis case or vice versa. With respect to culture and clinical data, the sensitivity, specificity, and positive and negative predictive values for the SO-DMT system in routine specimens were 0.76 (0.93 in smear positives [1.0 for MTC and 0.5 for NTM] and 0.56 in smear negatives [0.68 for MTC and 0.16 for NTM]), 0.99, 0.85 (1.00 in smear positives and 0.68 in smear negatives), and 0.97, respectively. Molecular misidentification of NTM cases occurred when testing 2 gastric aspirates from two children with clinically but not microbiologically confirmed lung tuberculosis. The SO-DMT assay appears to be a fast and easy alternative for detecting mycobacteria and differentiating MTC from NTM in smear-positive respiratory specimens.

Tuberculosis (TB) continues to be an important medical problem. Nontuberculous mycobacteria (NTM) causing clinical disease have become increasingly frequent and more varied, therefore the implementation of strategies for the rapid differentiation between NTM and *M. tuberculosis* complex (MTC) for early infection control and choice of antimicrobial therapy is now of primary importance (1–3).

Over the past 2 decades, the introduction of molecular sequence-based techniques for mycobacterial identification has enabled the recognition and reliable phylogenetic placement of more than 100 species (RIDOM [http://www.ridom-rdna.de/] and National Centre for Biotechnology Information databases [http://www.ncbi.nlm.nih.gov]). This has led to the widespread application by microbiology laboratories in industrialized countries of commercially available molecular assays for species identification and drug susceptibility testing of mycobacteria growing on culture media (4–9). They have also been used in clinical specimens with the suspected presence of *Mycobacterium* spp. (10–12). The utilization of these assays markedly reduces the time to diagnosis required by conventional phenotypic methods (13).

In this study, we evaluated the performance of a novel oligochromatographic assay (Speed-oligo Direct *Mycobacterium tuberculosis* [SO-DMT]; Vircell SL, Santa Fe, Granada, Spain) in the direct molecular detection of mycobacteria in respiratory specimens. This molecular assay is based on PCR technology combined with a dipstick to detect the presence of *Mycobacterium* and specifically to identify MTC in clinical respiratory specimens. It may represent a fast and easy alternative for differentiating between MTC and NTM in direct samples at laboratories with standard laboratory equipment (thermocycler and thermoblock). The applicability of the assay for routine mycobacterium laboratory testing is discussed.

(Part of this study was presented in poster form at the ECCMID meeting held in Milan, Italy, from 7 to 10 May 2011.)

MATERIALS AND METHODS

Study design. For the validation of the assay in a blind trial, it was evaluated in two stages: first, in a selection of acid-fast bacillus (AFB) smear-positive respiratory specimens (convenience samples) under experimental conditions, and second, in the routine workflow at two different laboratories (prospective evaluation).

Studies were also performed with aliquots from a pool of sputa from patients with no mycobacterial disease which were artificially inoculated with different mycobacterium-related organisms.

Set A. Set A was comprised of 20 respiratory specimens, specifically bronchoalveolar and nasal lavage fluids from pediatric patients with no mycobacterial disease.

Set B. Set B was comprised of 44 selected respiratory smear-positive specimens with different AFB loads (AFB/field, ×200 magnification),

Received 16 July 2012 Returned for modification 21 August 2012
Accepted 10 October 2012
Published ahead of print 24 October 2012
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doi:10.1128/JCM.00533-12

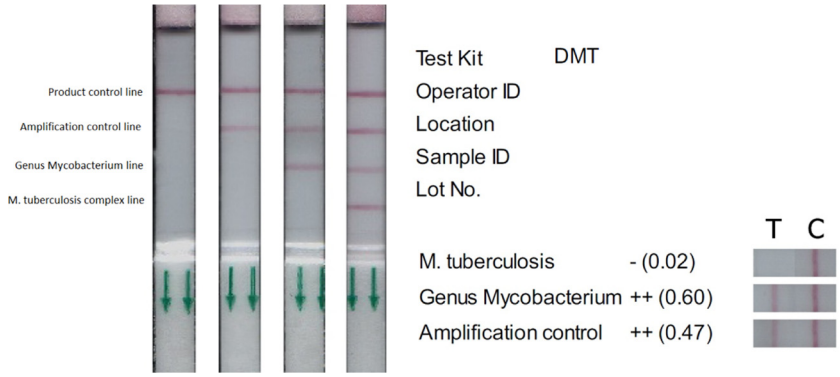


FIG 1 Automatic reading and interpretation of dipstick results after 5 min of incubation. T, test line; C, control line.

scored as 1+ (less than 1:13), 2+ (1:10 to 10:10), 3+ (10:8 to 100:8), and 4+ (more than 100:13), from 29 patients with pulmonary *Mycobacterium* disease under treatment at Torrecárdenas Hospital (Almería, Spain).

Twenty-four specimens were from 17 TB patients, 21 MTC culture-positive and 3 culture-negative specimens were collected from patients under therapy, and 20 NTM culture-positive specimens were from 12 patients fulfilling the criteria for NTM lung disease (14).

Set C. To assess the specificity of the assay, set C was comprised of 11 aliquots from a pool of smear- and culture-negative sputa that were artificially inoculated with 11 different mycobacterium-related organisms supplied by the Instituto Valenciano de Microbiología (IVAMI): *Corynebacterium amycolatum* IVAMI 4023656, *C. xerosis* NCTC 7243, *Streptomyces ambofaciens* IVAMI 4021542, *S. gardneri* IVAMI 4022939, *S. sampsonii* IVAMI 4023161, *Nocardia brasiliensis* NCTC 10300, *N. asteroides* IVAMI 4022938, *N. farcinica* IVAMI 4016593, *N. nova*, *Rhodococcus equi* CECT 555, and *Propionibacterium acnes* IVAMI 4023683.

Routine specimens. SO-DMT and culture were used to prospectively assay 566 fresh respiratory specimens from 460 patients suspected of having mycobacterial disease and included the following: 495 expectorated sputa, 38 bronchial aspirates, 9 bronchoalveolar lavages, and 21 gastric aspirates. This sample comprises specimens processed at mycobacterium reference laboratories (MRLs) in two different hospitals: Carlos Haya University Hospital (Malaga, Spain), which tested 317 specimens from 261 patients between March and June 2011, and Virgen de las Nieves University Hospital (Granada, Spain), which tested 249 specimens from 199 patients between April and June 2011.

These centers are localized in the Mediterranean coastal area of southeast Andalusia, opposite the Morocco coast. The mean annual rate of respiratory TB in Andalusia was 8.49 per 100,000 inhabitants in 2010 (http://revistas.isciii.es/bes/public/journals/1/pdf_69.pdf), and a high percentage of the cases involved individuals from north and sub-Saharan Africa (15). However, the true incidence of other mycobacterioses is unknown because NTM is not a reportable disease in Spain.

Specimen processing, microscopy, culture, and storage conditions. Respiratory specimens were decontaminated, directly examined (with auramine O), and cultured by following standard protocols (16, 17). Mycobacteria were identified by the GenoType mycobacterium CM/AS assay (Hain Lifescience, Nehren, Germany) or AccuProbe hybridization probes (Gen-Probe Inc., San Diego, CA). At least one aliquot of all decontaminated specimens was maintained for a maximum of 7 days at 4°C until assayed with SO-DMT; if the assay procedure was delayed, samples were frozen at −80°C until their analysis.

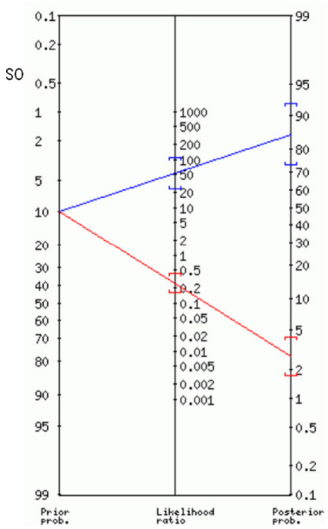
SO-DMT assay. The SO-DMT assay was carried out by previously trained technicians in two different centers. Positive and negative controls were always included in each test run. The SO-DMT comprises four steps: DNA extraction, amplification, PCR product detection, and result reading, as detailed below.

DNA is extracted from a previously concentrated decontaminated sample using the reagents included in the kit according to the manufacturer's instructions. Invalid assay results (absence of the human amplification control line) are usually obtained from specimens with a volume of <200 μl. Sample preparation of a set of 12 specimens takes approximately 45 min.

DNA amplification is performed with 10 μl of disrupted sample supernatant and 15 μl of the ready-to-use PCR mix. The amplification process is a multiplex PCR in which a triple target is amplified. DNA extraction is controlled by amplification of the human gene *RNase P*, which is also used to control for the presence of PCR inhibitors. The presence of *Mycobacterium* is assayed by amplifying a fragment of the 16S rRNA sequence and the presence of MTC members by amplifying a fragment of the insertion sequence IS*6110*. DNA amplification was performed using a standard thermocycler and took 1 h to complete.

A dipstick with probes bound to colloidal gold and to the membrane is used for PCR product detection. The PCR product is denatured for 2 min and added to a preheated running solution in a thermoblock. After introduction of the dipstick and a 5-min incubation period, the result is obtained from the presence of visible lines by using an automatic reading system. In our study, the results interpretation was performed automatically by the software according to the intensity of the line, but a visual interpretation is also possible based on the presence or absence of the red color on control and test lines (Fig. 1). The dipstick has four lines: a product control line, a PCR amplification control line, a test line for the whole *Mycobacterium* genus, whose presence in the absence of the *M. tuberculosis* complex line indicates the presence of NTM genetic material, and a test line for MTC members. In our present experience with this assay, the turnaround time was about 2 h (45 min for DNA extraction, 60 min for DNA amplification, and 10 min for PCR detection).

Data analysis and resolution of discrepancies. Smear microscopy was used for the initial assessment of the *Mycobacterium* load on specimens. Conventional culture was selected as the gold standard for initial assessment of the test performance because of the lower sensitivity of AFB smear examination (18, 19). When discrepancies were observed between the results of direct molecular and microbiological methods, the SO-DMT assay was repeated, and all available clinical, radiological, and smear data were taken into account to classify the patient as clinically positive or clinically negative for TB or NTM (14). A patient was considered clinically positive when (i) clinical symptoms (based on X-ray or TB-specific therapy) were recorded in the medical record or (ii) a positive mycobacterial culture result was available for the same episode. A patient was considered clinically negative when the lung symptoms were compatible but there was no history of *Mycobacterium* infection or recent positive TB or NTM culture findings (20). To minimize false-negative microscopic examinations, smear-negative results of culture-positive specimens were reanalyzed using a new auramine-stained smear of the stored specimens for examination by a second specially trained technician.



	All specimens N: 538 Estimate (n/N) (95% CI)	AFB+ specimens n: 28 Estimate (n/N) (95% CI)	AFB- specimens n: 510 Estimate (n/N) (95% CI)	MTC & FN-MTC specimens n: 43 Estimate (n/N) (95% CI)	NTM & FN-NTM specimens n: 10 Estimate (n/N) (95% CI)
Sensitivity	**0.75** (40/53) (0.61-0.85)	**0.93** (26/28) (0.75-0.98)	**0.56** (14/25) (0.35-0.75)	**0.86** (37/43) (0.71-0.94)	**0.30** (3/10) (0.08-0.64)
Specificity	**0.99** (478/485) (0.97-0.99)	-	**0.99** (478/485) (0.97-0.99)	-	-
PPV	**0.85** (40/47) (0.71-0.93)	**1.00** (26/26) (0.84-1.00)	**0.67** (14/21) (0.43-0.85)	**1.00** (37/37) (0.88-1.00)	**1.00** (3/3) (0.31-1.00)
NPV	**0.97** (478/491) (0.95-0.99)	-	**0.98** (478/485) (0.96-0.99)	-	-
LR+	**52** - (25-111)	-	**39** - (17-88)	-	-
LR-	**0.25** - (0.16-0.40)	-	**0.45** - (0.29-0.69)	-	-

FIG 2 Sensitivity, specificity, predictive values, likelihood ratios, and pre- and posttest probabilities of mycobacterial disease for the SO-DMT system in routine samples. SO-DMT assay results for all specimens were the following: prior probability (odds), 10% (0.1); positive likelihood ratio (LR+) of SO-DMT positive test, 52 (95% CI, 25 to 111); posterior probability (odds), 85% (5.7) (95% CI, 73 to 92%); negative likelihood ratio (LR−) for the SO-DMT negative test, 0.25 (95% CI, 0.16 to 0.40); posterior probability (odds), 3% (0.0) (95% CI, 2 to 4%).

Statistical analysis. The concordance correlation coefficient kappa was used to evaluate the degree of agreement. When evaluating the cumulative results of the SO-DMT assay in the specimens received from each patient, correlation was considered when molecular assay and culture results matched for all specimens from the patient or when an initial discrepancy was resolved by reviewing the patient's clinical records. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratios (LR+ and LR−), and positive and negative posttest probabilities of the molecular assay were calculated with a 95% confidence interval (CI) using the Diagnostic Test Calculator (http://www.medcalc.org/calc/diagnostic_test.php) and VassarStats Calculator1 (http://faculty.vassar.edu/lowry/vsclin.html) for the data analyses.

RESULTS

Convenience samples. (i) SO-DMT assay of AFB- and culture-negative specimens (set A). The SO-DMT showed no reactivity in any of the 20 *Mycobacterium*-free specimens (absence of MTC and *Mycobacterium* genus test lines and presence of amplification control line).

(ii) SO-DMT assay of AFB- and culture-positive specimens (set B). Compared to culture, the overall sensitivities of the selected smear-positive specimens were 0.91 (95% CI, 0.77 to 0.97) (22/24 MTC and 18/20 NTM) and 0.97 (95% CI, 0.81 to 1.00) for strong positives, 0.77 (95% CI, 0.46 to 0.94) for weak positives, 0.92 (95% CI, 0.72 to 0.99) for MTC, and 0.90 (95% CI, 0.69 to 0.98) for NTM. There was no molecular detection of NTM in a TB case or vice versa.

(iii) SO-DMT assay of specimens with *Mycobacterium*-related organisms (set C). The SO-DMT showed no reactivity with any of the *Mycobacterium*-related strains tested.

Routine sample. (i) AFB examination and culture results. The results of the two laboratories were grouped together for the assay evaluation.

Out of the 566 specimens, 28 (4.9%) were AFB smear positive; 13 (2.3%) were strongly positive and 15 (2.7%) were weakly positive.

According to the culture results, 46 (8.1%) were positive (37 [6.5%] for MTC and 9 [1.6%] for NTM), 492 (87.0%) were negative, and 28 (5.2%) became contaminated with saprophytic flora. Culture cross-contamination was not detected.

Out of the 492 culture-negative specimens, 7 were considered false negatives after a review of the patients' clinical records and other mycobacterial cultures, including 3 AFB smear-negative specimens from TB patients with other MTC-positive specimens from the same episode, 3 AFB smear-positive specimens collected from patients under TB therapy, and 1 smear-positive specimen from a patient with other NTM-positive cultures during the same episode.

All 7 false negatives were considered true-positive cultures (6 for MTC and 1 for NTM) in the SO-DMT assay evaluation, giving a total of 53 culture-positive specimens (43 [7.6%] MTC and 10 [1.8%] NTM culture positives) (Fig. 2 and Table 1).

TABLE 1 Direct examination and *Mycobacterium* culture results for the routine workflow specimens

Culture type	AFB result: Negative	Weak positive	Strong positive	Total
MTC	19	12	12	43
NTM	6	3	1	10
NEG[c]	485[a]	0[b]	0	485
Contaminated	28	0	0	28
Total	538	15	13	566

[a] Including three AFB and *Mycobacterium* culture-negative specimens from TB patient with other MTC-positive specimens in the same episode.
[b] Three of the AFB-positive and culture-negative specimens were obtained from TB patients under therapy, and one was from a patient with other NTM-positive cultures in the same episode. Culture-negative results of 7 specimens were considered false negatives (6 MTC, 1 NTM) for the SO-DMT assay evaluation because they were obtained from clinically positive patients.
[c] NEG, absence of mycobacterial growth.

TABLE 2 Comparison of SO-DMT assay results to smear and culture results

AFB smear test result	SO-DMT positive ($n = 47$)			SO-DMT negative ($n = 491$)		
	No. MTC assay and culture positive	No. NTM assay and culture positive	No. culture negative	No. with MTC positive culture	No. with NTM positive culture	No. with negative culture
Negative	13	1	7	6	5	478
Weak positive	12	1	0	0	2	0
Strong positive	12	1	0	0	0	0
Total	37	3	7[a]	6	7	478

[a] SO-DMT was positive for MTC in 1 specimen and for NTM in 6 specimens.

Out of the 460 patients in the study, 18 (3.9%) were AFB smear positive and 34 (7.4%) had one or more positive mycobacterium cultures (28 [6.1%] MTC positive and 6 [1.3%] NTM positive). The NTM species isolated in these six patients were *M. avium* ($n = 2$), *M. fortuitum* ($n = 2$), *M. kansasii* ($n = 1$), and *M. abscessus* ($n = 1$). One (0.2%) TB patient under therapy had a single AFB smear-positive and culture-negative specimen.

Specimens from 406 (88.3%) patients were all AFB smear and culture negative (one cultured specimen from some of these patients was contaminated). All specimens from the remaining 19 (4.1%) patients were AFB smear negative, and the cultures were contaminated with saprophytic flora.

(ii) SO-DMT assay results for specimens in routine workflow. In the direct comparison between culture and SO-DMT results, 28 specimens from clinically negative patients were excluded because the culture was contaminated and specimens had always produced a negative SO-DMT result. Among the remaining 538 specimens, the SO-DMT results were negative in 491 specimens, positive for MTC in 38 specimens, and positive for NTM in 9 specimens (Table 2).

In the detection of *Mycobacterium* in the 538 evaluated specimens, the SO-DMT assay showed a sensitivity of 0.75 (40/53) (95% CI, 0.61 to 0.85), specificity of 0.99 (478/485) (0.97 to 0.99), PPV of 0.85 (40/47) (0.71 to 0.93), NPV of 0.97 (478/491) (0.95 to 0.99), prior probability (odds) of 10% (0.1), LR+ of 52 (25 to 111), positive posterior probability (odds) of 85% (5.7), LR− of 0.25 (0.16 to 0.40), and negative posterior probability (odds) of 3% (0.0) (Fig. 2).

A total of 28 specimens were AFB smear positive. In this group, the sensitivity of the SO-DMT was 0.93 (26/28) (CI, 0.75 to 0.98), and the PPV was 1.00 (26/26) (0.84 to 1.00) (Fig. 2). The sensitivity was 1.00 (13/13) (0.72 to 1.00) and the PPV was 1.00 (13/13) (0.72 to 1.00) in the AFB strongly positive subgroup, while the sensitivity was 0.86 (13/15) (0.56 to 0.97) and PPV 1.00 (13/13) (0.70 to 1.00) in the AFB weakly positive subgroup.

In the 510 smear-negative specimens, the SO-DMT showed a sensitivity of 0.56 (14/25) (0.35 to 0.75), specificity of 0.99 (478/485) (0.97 to 0.99), PPV of 0.67 (14/21) (0.43 to 0.85), and NPV of 0.98 (478/485) (0.96 to 0.99) (Fig. 2).

In the 43 specimens with the presence of MTC species, the assay showed a sensitivity for MTC identification of 0.86 (37/43) (0.71 to 0.94) and PPV of 1.00 (37/37) (0.88 to 1.00) (Fig. 2).

In the 10 specimens (9 NTM positive and 1 false-negative NTM) showing the presence of *Mycobacterium* species, the assay showed a sensitivity for NTM detection of 0.30 (3/10) (CI, 0.08 to 0.64) and a PPV of 1.00 (3/3) (0.31 to 1.00) (Fig. 2).

(iii) SO-DMT assay results in routine workflow specimens. Considering cumulative SO-DMT results in the specimens from each patient, the concordance between SO-DMT assay results and culture-confirmed and/or clinically diagnosed mycobacterial lung disease was 97.8, with a Cohen's kappa index of 0.85 (standard error, 0.0475), indicating excellent concordance. The correlation (% concordance) between SO-DMT assay results and clinically and/or microbiologically confirmed findings for mycobacterial disease was 100% (18/18) in the smear-positive patients (17 TB and 1 clinical NTM lung disease cases), 65% (13/20) in the smear-negative patients (80% [12/15] in TB cases and 20% [1/5] in NTM culture-confirmed and/or clinically diagnosed cases), and 92.2% (400/403) in the patients without NTM or MTC. In four patients, three with TB and another one fulfilling the criteria for NTM lung disease, the SO-DMT assay failed to detect any mycobacterium in some of the culture-positive specimens tested, but these were considered concordant cases for this analysis.

(iv) Discrepancies between SO-DMT assay results and clinically and/or microbiologically confirmed TB or mycobacterial lung disease. There were 14 specimens with initially divergent results. In four of these cases, the discrepancy was resolved by retesting and identifying technical errors in the SO-DMT assay performance ($n = 2$), by finding positive mycobacterial cultures in another specimen(s) from the same episode not included in this study, and/or by reclassifying the clinical diagnosis after a review of the patient's medical records ($n = 2$). In the remaining 10 specimens from 10 patients, the SO-DMT assay result differed from the clinical and/or microbiological findings on the presence of mycobacterial disease. Discrepancies were the following: (i) SO-DMT negative both in 1 patient with TB and in 4 NTM culture-positive patients with or without confirmed lung disease; (ii) in 3 cases the molecular test was positive for *Mycobacterium* spp. in culture-negative and clinically negative patients; and (iii) two cases were positive for *Mycobacterium* spp. by SO-DMT of TB patients. The latter discrepancies occurred when testing two gastric aspirates from two children with clinically but not microbiologically confirmed lung TB; both resulted from domestic contacts with different confirmed lung TB cases.

DISCUSSION

In a convenience sample of preselected specimens with different AFB loads, the molecular assay was able to efficiently detect and classify MTC and NTM specimens. Three out of the four false negatives had a low bacterial load (1 to 10 AFB/10 fields), and the two false negatives were for specimens with NTM. *M. gordonae* was present in one of these, but the smear microscopy study revealed it to be a paucibacillary sample, probably below the assay

detection limit. *M. intracellulare* was present in the other false-negative specimen that produced a test line signal level below the automatic reading cutoff limit recommended by the manufacturer, although a faint color was visible on the *Mycobacterium* genus test line.

In the routine specimens, a good correlation was found between smear microscopy and SO-DMT results. The sensitivity of the assay was very satisfactory in AFB smear-positive specimens and correctly classified samples as MTC or NTM in all cases. The sensitivity was lower (56.6%) in AFB smear-negative samples within the range previously reported for smear-negative samples (21, 22). The overall specificity was very high. The sensitivity for specimens with NTM species was low (3/10), probably because there is only one copy number of the amplified target. In fact, the overall sensitivity of the *Mycobacterium* genus line was relatively weak in the routine specimens, likely because this sample contained six smear-negative NTM samples but only one that was strongly smear positive. A weakness of the present study was the low prevalence of NTM-positive specimens in the routine workflow series. Four of the false negatives were obtained from a single patient with *M. avium* infection for whom no other positive cultures were available to identify any genetic variant that might have interfered with the amplification or detection processes of the assay, and no other molecular method was available for the direct detection of *M. avium*. In contrast, the assay detected 18 out of 20 smear-positive NTM samples in the convenience sample. Assay results and clinical data showed a concordance of 100% in smear-positive cases, 65% in smear-negative cases, and 99.2% in clinically negative cases.

No cross-reactions were detected in assays of different mycobacterium-related organisms (convenience sample), and the specificity of the assay in all study samples was very high (99%), demonstrating that the test is useful to correctly differentiate between MTC and NTM in smear microcopy-positive samples and that there is a very low likelihood of a false-positive result with this system. However, five discrepancies between the assay and clinical data could not be resolved. Out of these, three samples were reproducibly positive for the *Mycobacterium* genus test line but the *Mycobacterium* cultures were negative. One explanation is the presence of contaminant *Mycobacterium* DNA that could not be recovered by standard culture methods due to the impaired viability of the organisms (23). Additionally, a negative assay result in a culture-positive specimen may have various explanations: the presence of a low number of bacteria or strains of MTC (notably *M. bovis*) having a low number of copies of IS*6110*, the unequal distribution of mycobacteria in the specimen, or deficient lysis of the mycobacterial wall. According to the kit manufacturer, the analytical sensitivity of the assay was determined with serial dilutions of *M. tuberculosis* H37Rv purified DNA, demonstrating an ability to detect up to 2 genome copies per reaction on the *M. tuberculosis* complex test line and 20 genome copies per reaction on the *Mycobacterium* genus test line.

Several commercially available nucleic acid amplification tests have been extensively evaluated for the detection of MTC species in respiratory samples, showing high specificity and good positive predictive values for smear-positive samples and variable sensitivity for smear-negative and extrapulmonary TB samples (22–25). However, most of these tests require the use of real-time PCR or precision instruments that are not affordable in many countries with a high TB burden, or they involve technologies that require extensive hands-on time. In contrast, the SO-DMT assay combines a PCR amplification method with a simple detection procedure that does not require long postamplification preparation or exclusive equipment, reducing manipulation to only two pipetting steps. As limitations, assay automation is not possible and separate laboratory areas should be used for specimen preparation, target amplification, and amplicon detection. In conclusion, the SO-DMT assay requires only 30 min of hands-on time and offers a total turnaround time of 2 h from sample reception. It directly detects *Mycobacterium* species in a clinical specimen and simultaneously differentiates them from MTC species. Results obtained in a convenience sample of AFB smear-positive specimens suggest that utilization of this assay for MTC/NTM detection and differentiation can avoid the need to wait for culture results from smear-positive samples. This is an important benefit for case management and contact investigation, making the assay especially valuable for clinicians in countries with a high incidence of NTM.

ACKNOWLEDGMENTS

We are grateful to Juan García de Lomas for providing mycobacterium-related organisms from the Instituto Valenciano de Microbiología (IVAMI) and to the personnel in the clinical mycobacteriology laboratories of H. Carlos Haya, H. Virgen de las Nieves, and C. H. Torrecárdenas and to the research group of tuberculosis at Almería CTS626 (IndalTB) for their collaboration.

SO-DMT kits used in this study were a gift of Vircell SL, Granada, Spain, of which P. Mendoza-Lopez is an employee.

All authors voluntarily publish this article and have no personal interest in this study other than publishing the scientific findings that they have been involved in via planning, setting up, assaying, and analyzing the results. M.J.M.-L. designed the study and was responsible for the first draft of the manuscript, which was then substantially revised by all authors.

The study was partially financed by Junta de Andalucía (PI-0444/2008 and PI-0306-2009) and SEPAR (763-09).

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