INT J TUBERC LUNG DIS 20(6):753–756

http://dx.doi.org/10.5588/ijtld.15.0528

Capilia™ TB-Neo assay: a new tool for rapid distinction between tuberculous and non-tuberculous mycobacteria

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SUMMARY

SETTING: The ability to rapidly distinguish between *Mycobacterium tuberculosis* complex (MTC) and non-tuberculous mycobacteria (NTM) is critical in clinical practice.

OBJECTIVE: To evaluate the usefulness of an immunochromatographic (IC) assay to distinguish between MTC and NTM.

DESIGN: We analysed a panel of 145 cultures from 128 patients. The routine molecular identification approaches, such as the AccuProbe™ *Mycobacterium tuberculosis* complex culture identification test and GenoType® *Mycobacterium* assays, were used as reference methods.

RESULTS: Of the 101 positive cultures, 98 were correctly identified using the Capilia™ TB-Neo Assay. Of the three discordant isolates, one was identified as *M. bovis* bacille Calmette-Guérin (BCG) and two as *M. tuberculosis*. Although we have not performed the sequencing of these strains, some false-negative results have been described due to mutations in the *mpb*64 gene or with some *M. bovis* BCG strains. We did not observe false-positive results or any cross-reaction with 22 NTM strains, 12 non-mycobacterial micro-organisms and 10 negative cultures.

CONCLUSION: We report good overall performance (sensitivity 97%, specificity 100%, positive predictive value 100% and negative predictive value 96%) of this rapid assay that is easy to perform and interpret and does not require sample preparation, trained technicians or expensive equipment.

KEY WORDS: mycobacteriology; tuberculosis; immunochromatographic assay; diagnosis

DESPITE PROGRESS in antimicrobial chemotherapy over the last 50 years, tuberculosis (TB) remains one of the leading causes of death due to infection worldwide. In 2013, 9 million persons developed TB and 1.5 million died of the disease.[1] According to the World Health Organization's (WHO's) 2013 estimates, TB incidence in Portugal was 26 per 100 000 population.[1] Improving laboratory diagnostic capacity is a crucial component of TB control. Moreover, there is both an increasing number of clinical isolates of non-tuberculous mycobacteria (NTM) in many countries and growing awareness of their ability to cause disease.[2] NTM are capable of causing a wide range of infections in humans, with pulmonary NTM disease being the most common, especially in patients with pre-existing pulmonary disease.[2] NTM are mainly opportunistic pathogens that can occasionally cause severe disseminated disease, especially in patients with systemic impairment of immunity.

The differentiation between *Mycobacterium tuberculosis* complex (MTC) and NTM is critical for TB control as well as for clinical practice, as the management and treatment of patients with TB differs from that of patients infected with other mycobacteria. Rapid confirmatory molecular tests are available for the identification of MTC from culture. However, these methods are comparatively expensive and generally require technical expertise and sophisticated equipment.

A number of immunochromatographic (IC) assays, such as the Capilia™ TB-Neo assay (TAUNS Laboratories, Numazu, Japan), based on the detection of the MPB64 antigen, have been developed. MPB64 has been found in unheated culture media of *M. tuberculosis*, *M. bovis* and some, but not all, substrains of *M. bovis* bacille Calmette-Guérin (BCG). MPB64 is a protein that is highly specific to the MTC. NTM do not produce MPB64.[3–5] Other tests, such as the BD MGIT™ TBc Identification Test (BD, Franklin Lakes, NJ, USA), have been developed based on MPB64. Tests based on the MPT64 antigen, such as the SD TB Ag MPT 64 Rapid (Standard Diagnostics, Seoul, South Korea) have also been developed. The Capilia TB-Neo assay is an improved version of Capilia TB and, although this second

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Article submitted 22 June 2015. Final version accepted 8 January 2016.

generation Capilia test has been validated in some laboratories, as with the first generation test, false-negative results due to mutations in the *mpb*64 gene have been reported.[6–9]

In the present study, we evaluated the performance of the Capilia TB-Neo assay at the Clinical Pathology Department of the São João Hospital Centre, Porto, Portugal, in identifying MTC by comparing it with our routine molecular identification methods.

STUDY POPULATION AND METHODS

Study population and samples

The study was conducted at the Microbiology Laboratory of the Clinical Pathology Department of São João Hospital Centre, the largest hospital in the northern region of Portugal. The laboratory receives specimens from an academic hospital and various district hospitals. Clinical performance was determined using 145 samples from 128 patients: 101 MTC-positive cultures, 22 NTM-positive cultures, 12 other non-mycobacterial micro-organism isolates and 10 negative cultures (growth units: 0 in BACTEC™ MGIT™ 960 [BD] after 42 days of incubation). Clinical samples were mostly pulmonary specimens (70 sputum, 23 bronchial lavage, 7 gastric lavage, 5 pleural fluid, 5 bronchoalveolar lavage, 2 pleural biopsy and 1 bronchial brush), but also included extra-pulmonary specimens (12 urine, 3 stool, 3 pericardial fluid, 1 peritoneal ganglion, 1 pus from breast abscess, 1 pus from sternum abscess, 1 pus from bone abscess and 2 blood samples). Eight specimens of unknown identification were also included.

The institutional review board of São João Hospital Centre, Porto, Portugal, approved the study. As this was a retrospective study, the need for informed consent was waived.

Mycobacterial culture

The samples were decontaminated using the *N*-acetyl-L-cysteine-sodium hydroxide routine method.[10] The digested, decontaminated and concentrated samples were inoculated into BACTEC™ MGIT™ 960 medium (BD), supplemented with BBL™ MGIT oleic albumin dextrose catalase (BD) enrichment and BBL MGIT PANTA™ antibiotic mixture (BD) or in modified Middlebrook 7H9 broth with carbon dioxide (CO2) BACTEC MYCO/F-Sputa (BD) and incubated for 42 days at 37°C. The MGIT 960 medium and BACTEC MYCO/F-Sputa tubes were incubated in the BACTEC MGIT 960 and BACTEC 9000 MB (BD) systems, respectively. Four samples were also inoculated in Löwenstein-Jensen medium (Bio-Rad Laboratories, Hercules, CA, USA) and incubated for 60 days at 37°C.

Mycobacterial molecular identification

Cultures that were positive on BACTEC MGIT 960 (manufacturer-set threshold: 75 growth units) were examined using smear microscopy by Kinyoun's staining to examine acid-fast bacilli (AFB). To determine the species of the isolates, one or two of the following routine molecular approaches were used as a reference identification method: the AccuProbe™ *M. tuberculosis* complex culture identification test (Hologic® Inc, San Diego, CA, USA), which relies on isothermal amplification of nucleic acids and on oligonucleotide probes complementary to 16S rRNA, and the GenoType® *Mycobacterium* CM and AS kits (Hain Lifescience, Nehren, Germany), based on polymerase chain reaction (PCR) and the DNA-STRIP technology.

The two molecular methods are based on the ability of complementary nucleic acid strands to associate to form stable double-stranded molecules. In the first method, a single-stranded DNA probe with a chemiluminescent label complementary to the rRNA of the target micro-organism is used. After RNA extraction and amplification, the selection reagent allows discrimination of non-hybridised probe and DNA:RNA hybrids. The hybrids are measured in a GenProbe luminometer. In the second method, a multiplex PCR with biotinylated primers is performed, followed by reverse hybridisation and addition of a streptavidin/alkaline phosphatase conjugate. The staining reaction is mediated by an alkaline phosphatase. Three controls (conjugate, universal, and genus) are included in each strip.

Capilia TB-Neo assay

The Capilia TB-Neo assay was performed according to the manufacturer's instructions. This IC assay uses a nitrocellulose membrane with specific anti-MPB64 mouse monoclonal antibody immobilised on it. Briefly, 100 μl of the positive broth culture was applied directly into the Capilia well without any manipulation. In positive cultures in solid media, a few colonies from the medium were scraped using a loop (1 μl) and suspended in 0.2 ml of extraction buffer (TAUNS). The suspension was vortexed and approximately 100 μl of this suspension was added to the sample well. The results are available 15 min after inoculation of the test cartridge. Results were read as positive for MTC by observing the presence of purple-red lines on the MTC area as well as on the test area of the internal quality control.

Assessment of discordant results

Discordant results were assessed as follows: isolates that were positive for MTC on Accuprobe or GenoType CM but negative on Capilia TB-Neo were tested using the GenoType MTBC assay (Hain Lifescience), based on PCR and the DNA-STRIP

Table 1 Performance of the Capilia TB-Neo assay

Strain	Total (cultures)	Positive Capilia assay	Negative Capilia assay
MTC*	101	98	3
NTM*	22	0	22
Mycobacterium spp ($n = 2$)			
M. intracelullare ($n = 4$)			
M. avium ($n = 1$)			
M. kansasii ($n = 2$)			
M. fortuitum ($n = 5$)			
M. gordonae ($n = 3$)			
M. chelonae ($n = 3$)			
M. peregrinum ($n = 1$)			
Other micro-organisms	12	0	12
Nocardia spp. ($n = 3$)			
Klebsiella pneumoniae ($n = 1$)			
Pseudomonas aeruginosa ($n = 3$)			
Escherichia coli ($n = 1$)			
Staphylococcus aureus ($n = 1$)			
Candida albicans ($n = 2$)			
Proteus mirabilis ($n = 1$)			
Negative cultures†	10	0	10

* Routine molecular identification methods were used as reference method.
† MGIT broth cultures that were negative in BACTEC MGIT 960 system after 42 days of incubation.
MTC = *M. tuberculosis* complex; NTM = non-tuberculous mycobacteria; MGIT = Mycobacteria Growth Indicator Tube.

technology according to the manufacturer's instructions. The protocol for the GenoType *Mycobacterium* CM and AS kits is the same as described above.

Statistical analysis

The sensitivity, specificity and positive (PPV) and negative predictive values (NPV) of the Capilia TB-Neo assay were determined using the routine molecular identification approaches as reference method.

RESULTS

Of the 101 positive cultures (97 broth and 4 solid cultures) with MTC molecular identification, 98 were correctly identified using the Capilia TB-Neo assay. The test failed to detect three cultures (Tables 1 and 2). The discordant results were assessed using the GenoType MTBC test. Of the three discordant isolates, one was identified as *M. bovis* BCG and two, isolated from the same patient in different samples, were identified as *M. tuberculosis*. We did not observe any cross-reaction with any of the 22 NTM and 12 other micro-organisms, such as *Nocardia spp*., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Proteus mirabilis*. No colour reaction was observed in the MTC reading area in the 10 negative cultures also tested (MGIT broth cultures that were negative on MGIT 960 after 42 days of incubation). These results indicate a sensitivity of 97%, a specificity of 100%, a PPV of 100% and an NPV of 96% of the Capilia TB-Neo assay for the identification of MTC.

Table 2 Evaluation of the Capilia TB-Neo assay

	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
Capilia TB-Neo	97	100	100	96

DISCUSSION

In this study, we evaluated the performance of an IC assay for the identification of MTC and its differentiation from NTM. We found the assay useful, with a performance similar to that of molecular methods. In our hospital, 19 629 samples from 10 595 patients were analysed for mycobacteria between January 2010 and September 2014. Of the 19 629 samples, a positive result was found in 1421 (7.2%), corresponding to 650 (6.1%) patients, of whom 455 (70%) were males and 195 (30%) females, with an average age of respectively 53 and 55 years. An NTM strain was isolated in 253 (38.9%) of the positive patients (153 males, 100 females). In hospitals with high NTM prevalence, it is especially important to rapidly differentiate between these two groups of mycobacteria.

Molecular assays such as the AccuProbe MTC culture identification test and GenoType *Mycobacterium* kits are available for the identification of MTC and NTM from cultures. However, these methods are laborious and expensive. Various IC assays have been developed based on the MPB64 antigen detection. The MPB64 protein is highly specific to MTC, including *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti*, *M. pinnipedii*, and some, although not all, substrains of *M. bovis* BCG.[3–5]

Consistent with previous reports, the Capilia TB-Neo assay demonstrated a specificity of 100%.[7,11] We observed a sensitivity of 97%, higher than reported by Gomanthi et al. but similar to that reported by Muyoyeta et al. and lower than those reported by Pokam et al. and Chikamatsu et al.[6,7,11,12] Reactivity with NTM such as *M. avium*, *M. kansasii*, *M. fortuitum*, *M. gordonae*, *M. chelonae*, *M. peregrinum* and other micro-organisms, such as *Nocardia spp*., *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *S. aureus*, *C. albicans* and *P. mirabilis,* was checked and a cross-reaction was confirmed as absent for all of these as well as for the negative cultures tested. Although we detected no false-positive results, we did detect three false-negative results. The existence of false-negative results on Capilia TB-Neo was not further evaluated, but it may have been due to one of the following reasons: either the *mpb*64 gene was deleted, as previously reported for some

BCG strains, such as substrains of the Pauster and Glaxo strains,[7,13] or the *mpb*64 gene was mutated, leading to the production of an incomplete protein.[6,7]

Despite the false-negative results, we found the sensitivity of the Capilia TB-Neo to be similar to that observed for AccuProbe (96.8%).[14] Unlike the molecular method, the IC test is a rapid one-step test (15 min after positivity of cultures), that is less expensive and does not require sample preparation, trained technicians or sophisticated equipment. These features can simplify the identification procedure and reduce the turnaround time for MTC identification.

CONCLUSIONS

Capilia TB-Neo is a good screening method for rapidly identifying MTC and distinguishing between MTC and NTM in AFB-positive cultures. As a reduction in the laboratory turnaround time could reduce transmission, a rapid IC confirmation test may have important implications in TB control.

Acknowledgements

The Capilia TB-Neo assay kits were supplied by Quilaban-Quimica Laboratorial Analitica, Sintra, Portugal.

Conflicts of interest: none declared.

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RESUME

CONTEXTE : La capacité de distinguer rapidement le complexe de *Mycobacterium tuberculosis* (MTC) et les mycobactéries non-tuberculeuses (NTM) est cruciale en termes de pratique clinique.

OBJECTIF : Évaluer l'utilité d'un test immunochromatographique afin de distinguer les MTC des NTM.

SCHÉMA : Nous avons analysé un panel de 145 cultures provenant de 128 patients. Les approches d'identification moléculaire de routine, comme l'AccuProbe™, le test d'identification par culture du MTC et le test GenoType® *Mycobacterium*, ont été utilisées comme méthode de référence.

RÉSULTATS : Sur 101 cultures positives, 98 ont été correctement identifiées par le test Capilia™ TB-Neo. Parmi les trois isolats discordants, l'un a été identifié comme *M. bovis* du bacille Calmette-Guérin (BCG) et deux autres comme *M. tuberculosis*. Bien que nous n'ayons pas réalisé le séquençage de ces souches, quelques résultats faux négatifs ont été décrits à cause de mutations du gène *mpb*64 ou avec quelques souches de *M. bovis* du BCG. De plus, nous n'avons pas observé de faux positifs ni de réaction croisée avec les 22 NTM, les 12 bactéries non-mycobactériennes et les 10 cultures négatives.

CONCLUSION : Nous faisons état d'une bonne performance d'ensemble (sensibilité 97%, spécificité 100%, valeur prédictive positive 100% et valeur prédictive négative 96%) de ce test rapide, facile à réaliser et à interpréter, qui ne demande pas de préparation des échantillons, de techniciens formés ou d'équipement coûteux.

RESUMEN

MARCO DE REFERENCIA: La capacidad de diferenciar el complejo *Mycobacterium tuberculosis* (MTC) y las micobacterias no tuberculosas (NTM) es primordial en la práctica clínica.

OBJETIVO: Evaluar la utilidad de una prueba inmunocromatográfica en la diferenciación del MTC y las NTM.

MÉTODO: Se analizó un conjunto de 145 cultivos provenientes de 128 pacientes. Se utilizaron como métodos de referencia pruebas corrientes de identificación molecular como la prueba AccuProbe™ de identificación del cultivo del MTC y la prueba GenoType® *Mycobacterium*.

RESULTADOS: En los 101 cultivos positivos, la prueba Capilia™ TB-Neo detectó correctamente 98 muestras. De los tres aislados discordantes, uno se identificó como *M. bovis* bacille Calmette-Guérin (BCG) y dos como *M. tuberculosis*. No se practicó la secuenciación de estas cepas, pero se han descrito algunos casos de resultados negativos falsos debido a mutaciones en el gen *mpb*64 o con algunas cepas de *M. bovis* BCG. Además, no se observaron resultados positivos falsos ni reacciones cruzadas con las 22 cepas de NTM ni con las otras 12 bacterias diferentes ni hubo reacción en los 10 cultivos negativos.

CONCLUSIÓN: Los resultados del presente estudio ponen de manifiesto un buen rendimiento diagnóstico global (sensibilidad 97%, especificidad 100%, valor diagnóstico de un resultado positivo 100% y valor diagnóstico de un resultado negativo 96%) de esta prueba rápida, de realización e interpretación sencillas, que no exige preparación de la muestra, personal técnico capacitado ni equipos costosos.