

## Rapid detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* by the direct thin-layer agar method

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### SUMMARY

We evaluated thin-layer agar (TLA) for the detection of resistance of *Mycobacterium tuberculosis* to rifampicin (RMP) and isoniazid (INH) as a direct method in patients at risk of multidrug-resistant tuberculosis (MDR-TB). Quadrant TLA plates contain 7H10 Middlebrook growth control, para-nitrobenzoic acid, INH and RMP. Detection of RMP and INH resistance by TLA was compared to that in indirect conventional drug susceptibility testing (DST) and conventional culture media. Median

time for growth was respectively 22, 10 and 7.6 days for Löwenstein-Jensen, TLA and the Mycobacterial Growth Indicator Tube. TLA sensitivity, specificity and predictive values for RMP and INH resistance were 100%. Time to resistance detection was respectively 11 and 11.5 days for RMP and INH. TLA showed a rapid turnaround time and performance comparable to conventional DST methods. **KEY WORDS:** MDR-TB; drug susceptibility testing; rapid DST

MULTIDRUG-RESISTANT tuberculosis (MDR-TB) has been reported worldwide. It is recommended to perform drug susceptibility testing (DST) in all new TB patients in settings where drug resistance is suspected.<sup>1,2</sup> Availability of DST results is necessary in providing appropriate treatment for MDR-TB, as treatment based on DST results has been associated with better clinical outcome and improved survival rates.<sup>3,4</sup>

Standard DST methods are slow in obtaining results.<sup>5</sup> Molecular tests, on the other hand, although rapid in yielding results, have not been adopted in routine practice due to their higher cost, especially in low-resource, high-burden countries.<sup>6</sup>

Alternative phenotypic DST, such as alamar blue, tetrazolium bromide, resazurin and bacteriophage-based assays,<sup>7</sup> have been described for DST of *Mycobacterium tuberculosis*. However, these need further development for application as direct tests. The microscopic-observation drug susceptibility assay (MODS) is a phenotypic test that has been evaluated for the detection of isoniazid (INH) and rifampicin (RMP) resistance and shows sensitivity and specificity close to reference methods.<sup>8</sup> Thin-layer agar (TLA) is a rapid method for the detection of *M. tuberculosis* from pulmonary and extra-pulmonary specimens<sup>9</sup> and for DST.<sup>10</sup>

This study evaluates a new format of TLA that uses

early detection of microcolonies by conventional microscopy applied not only for identification of *M. tuberculosis* but also for the simultaneous detection of resistance to RMP and INH directly from sputum samples.

### METHODS

Of 100 patients, 95 were included in the study. All patients, from Medellín, Colombia, had a diagnosis of pulmonary TB confirmed by sputum smear microscopy. Each had at least one epidemiological risk factor for MDR-TB.

All patients were asked to provide informed consent, previously approved by the Ethics Committee of the Corporación para Investigaciones Biológicas, Universidad Pontificia Bolivariana.

Sputum specimens were processed using the conventional sodium hydroxide-*N*-acetyl-L-cysteine method. A direct smear was prepared for Kinyoun staining to confirm acid-fast bacilli (AFB); then 0.1 ml of the digested sputum was inoculated into two tubes of Löwenstein-Jensen (LJ) media. *M. tuberculosis* was identified by standard biochemical tests and indirect DST was performed using the proportion method on 7H10 Middlebrook agar for first-line drugs. For growth detection, one mycobacterial growth indicator

tube (MGIT) was inoculated with 0.5 ml of digested sputum and incubated in MGIT 960 BACTEC instrument (BD Diagnostics, Sparks, MD, USA).

Quadrant petri plates were prepared with 5 ml of 7H11 Middlebrook agar per compartment containing the following: one as growth control with no additions; one with 0.5 mg/ml of para-nitrobenzoic acid (PNB), a specific inhibitor of *M. tuberculosis* complex; one with 0.2 µg/ml of INH; and one with 1 µg/ml of RMP. Before inoculation of 0.1 ml of digested sputum per compartment, dilutions were made according to AFB load on the smear as follows: >250 AFB/field, 10<sup>-3</sup> dilution; 25–250 AFB/field, 10<sup>-1</sup> dilution; <25 AFB/field, no dilution. The plates were sealed with tape and incubated at 37°C in a 5% CO<sub>2</sub> incubator. Plates were read every 2 days over 4 weeks, using a conventional microscope at 100× magnification. Inhibition of growth in the compartment containing PNB while growing in the control was considered positive for *M. tuberculosis*. Resistance was defined as growth in the compartments containing RMP and INH as compared to the growth control. All TLA plates were read blind to the results of reference standard methods.

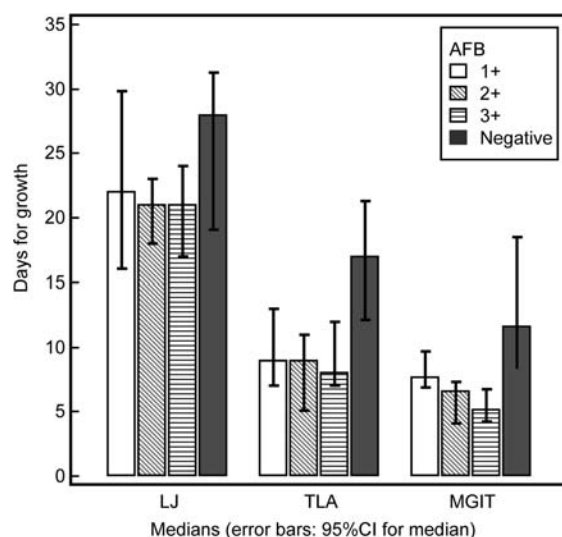
The Wilcoxon rank test for paired samples was performed to compare times to detection of growth in TLA, MGIT and LJ. A *P* of <0.05 was considered significant. Sensitivity, specificity and predictive values were calculated for the detection of resistance using TLA including 95% confidence intervals (CIs), as compared to the proportion method. All analyses were performed using MedCalc version 9.2.1.0 (MedCalc software, Mariakerke, Belgium).

## RESULTS AND DISCUSSION

More *M. tuberculosis*-positive cultures were detected using TLA (91.3%) and MGIT (96.7%) than with LJ (84.7%). TLA and MGIT had a shorter detection time for *M. tuberculosis* growth (median 10 and 7.1 days respectively) as compared to LJ (median 22 days) and according to specimen bacillary load (Figure). All isolates obtained on TLA were confirmed by standard tests as *M. tuberculosis*. The rate of contamination was higher for LJ (10.5%) than for TLA and MGIT, respectively 4.1% and 2.2%.

The prevalence of RMP and INH resistance was respectively 10.8% and 14.3%. Compared to the proportion method in agar, TLA showed 100% sensitivity for detection of resistance to both RMP (95%CI 93.7–100) and INH (95%CI 95.8–100). Specificity was 100% for both RMP (95%CI 99.3–100) and INH (95%CI 99.3–100). Positive and negative predictive values were also 100% for both drugs. The mean time to detection of resistance on TLA was 11.1 days (95%CI 6.7–15.4) for RMP and 11.25 days (95%CI 9.2–13.2) for INH, compared to the standard 21 days for the proportion method.

These results show that a patient could be diag-



**Figure** Days to detection of growth using three culture methods by bacillary load in specimens from tuberculosis patients at high risk for drug resistance. AFB = acid-fast bacilli; LJ = Löwenstein-Jensen; TLA = thin-layer agar; MGIT = Mycobacterial Growth Indicator Tube; CI = confidence interval.

nosed with *M. tuberculosis* and the presence of resistance to RMP or INH within a median of 11 days. MODS, a comparable method, detects the formation of cords characteristic of *M. tuberculosis* growth in liquid medium.<sup>8</sup> Higher contamination rates have been reported for MODS than for the TLA method described here. A major disadvantage of MODS is the need for an inverted microscope and the labour time involved. In contrast, TLA uses standard microscopy with additional advantages such as the identification of grown colonies as *M. tuberculosis* by both PNB inhibition and characteristic microcolony morphology; in addition, the manipulation of a solid media plate format is easier.

In conclusion, these results show that the TLA method is rapid and simple for the diagnosis of *M. tuberculosis* and simultaneous detection of resistance to both RMP and INH. The simplicity of the one-plate format favours its implementation and applicability in mycobacterial diagnostic laboratories without additional expenses in equipment. The rapid and reliable results makes this TLA method a powerful tool for establishing appropriate treatment and reducing the impact of MDR-TB.

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## RÉSUMÉ

Nous avons évalué l'agar en couche fine (TLA) pour la détection de *Mycobacterium tuberculosis* et la résistance à rifampicine (RMP) et à l'isoniazide (INH) comme méthode directe chez les patients à risque de tuberculose multirésistante (TB-MDR). Les plaques de TLA contiennent du 7H10, un produit de contrôle de la croissance, de l'acide paranitrobenzoïque, de l'INH et de la RMP. La détection de la résistance à l'INH et à la RMP dans les TLA a été comparée aux méthodes conventionnelles de détermination indirectes de la résistance et les milieux de

culture. La durée médiane avant détection est respectivement de 22, 10 et 7,6 jours pour Löwenstein-Jensen, TLA et MGIT. La sensibilité et la spécificité de TLA, et sa valeur prédictive pour une résistance à l'INH et à la RMP ont été à 100%. La durée moyenne avant l'obtention du résultat de la résistance a été de 11 et 11,5 jours pour RMP et INH. Grâce à la TLA, la durée globale est plus courte et la performance est comparable à celle des méthodes conventionnelles de détermination de la résistance.

## RESUMEN

Se evaluó el agar de capa delgada (ACD) para detección de resistencia a rifampicina (RMP) e isoniácida (INH) utilizando un método directo de siembra de muestras clínicas de pacientes con riesgo de tuberculosis multirresistente (TB-MDR). Se utilizaron cajas de ACD divididas en cuadrantes que contenían 7H10 como control de crecimiento, ácido paranitrobenzoico y concentraciones de RMP y INH. La resistencia para RMP e INH se comparó con el método de proporciones indirecto y los medios de

cultivo convencionales. El tiempo medio de detección 22, 10 and 7,6 días para Löwenstein-Jensen, ACD y MGIT. Para la detección de resistencia a RMP e INH, el ACD mostró sensibilidad, especificidad y valores predictivos de 100% ; la media en días para la detección de resistencia fue 11 and 11,5 días. ACD demostró resultados más rápidos y desempeño equiparable a los métodos convencionales de detección de resistencia.