Yeni İlaç Duyarlılık Testleri Çalışmaları

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Sunum içeriği I

- **Nitrat redüktaz testi (NRT)**
  - Direkt test;
  - İndirekt test;
    - LJ'de NRA
    - 7H9 sıvı besiyerinde tüpte NRA
    - 7H9 sıvı besiyerinde mikroplakta NRA
    - Kanlı agarda NRA
    - 7H11 agarda NRA

Sunum içeriği II

- **Kolorimetrik redoks indikatör metotları (KRİM)**
  - ***Alamar mavisi ile duyarlılık testi***
  - ***Rezasurin ile duyarlılık testi***
    - Mikrodilüsyon yöntemi ile
    - Tüpte kritik konsantrasyon kullanılarak
  - ***Tetrazolium tuzları ile duyarlılık testi***
    - MTT
    - XTT
  - ***Malaşit yeşili renksizleştirme testi (MYRT)***
  - ***Kristal viyole renksizleştirme testi (KVRT)***

Sunum içeriği III

- Mikroskobik izleme dayalı ilaç duyarlılık testi (MİDT/MODS)
- İnce tabaka agar testi (Thin Layer agar) (İTAT/TLA)
- Broth mikrodilüsyon metod (BMM)
- E test
  - 7H10 ve 11 agarda
  - Kanlı ve çukulatamsı agarda
- Kanlı agarda proporsiyon yöntemi ile duyarlılık testi
- Koyun serumlu kanlı agar temel besiyerinde proporsiyon yöntemi ile duyarlılık testi

Tüberküloz ile savaşta en etkili yol;

- ✓ Hastalarının erken saptanması
- ✓ Etkili tedavisi

ile bulaş zincirinin kırılmasıdır.

Etkin tedavi;

İDT sonuçlarına göre özgün tedaviler düzenlenmesi ile mümkündür.

Test edilecek ilacın seçimi

- ✓ Birinci seçenek ilaçlar
  - Her hastada öncelikle test edilmesi gereken ilaçlardır.
    - İzoniazid (INH)
    - Rifampisin (RIF)
    - Etambutol (EMB)
    - Pirazinamid (PZA) (Teknik zorluk nedeniyle ilk test edilecek ilaçlar arasına alınmayabilir)
    - Streptomisin (SM)

- ✓ İkinci seçenek ilaçlar
  - İzolat RIF'e dirençli ise test edilmelidir.

    (veya en az iki birinci seçenek ilaca dirençli ise)
    - Kanamisin (KAN)
    - Amikasin (AMİ)
    - Kapreomisin (CAP)
    - Ofloksasin (OFL)

İlaç direnci ile ilgili tanımlar

- ✓ **Primer direnç** (yeni olguda direnç)
  - Daha önce tedavi almamış veya bir aydan kısa süre tedavi almış olguda görülen direnç
- ✓ **Sekonder direnç** (tedavi görmüş olguda direnç)
  - En az bir ay kemoterapi almış olguda ortaya çıkan direnç

- ✓ **Çok ilaca dirençli tüberküloz (ÇİD-TB)**
  - En az RIF + INH direnci
- ✓ **Yaygın ilaç dirençli tüberküloz (YİD-TB)**
  - RIF + INH + florokinolon + bir enjektabl ikinci seçenek ilaç (kanamisin, kapreomisin, amikasin) direnci

İDT Yöntem Çeşitleri

- ✓ Kültüre Dayalı Yöntemler
  - **Proporsiyon Yöntemleri**
    - Agar Proporsiyon Yöntemi (MB 7H10/11)
    - LJ Proporsiyon Yöntemi
  - **Otomatize Sıvı Sistemler**
    - MGIT 960
    - VersaTREK

Kullanılan Besiyerinde Önerilen kritik konsantrasyonlar (µg/ml)

	LJ	7H10	7H11	MGIT 960	VersaTREK
İzoniazid (INH) *	0.2	0.2	0.2	0.1	0.1
Rifampisin (RIF)	40.0	1.0	1.0	1.0	1.0
Ethambutol (EMB)	2.0	5.0	7.5	5.0	5.0
Pirazinamid (PZA)	-	-	-	100.0	300.0
Streptomisin (SM)*	4.0	2.0	2.0	1.0	2.0

Kritik konsantrasyon;
ilaçla karşılaşmamış "wild type" *M. tuberculosis* suşlarının %95'ini inhibe eden en düşük ilaç konsantrasyonudur.

*Kritik konsantrasyonunda direnç görülürse , ilacın daha yüksek bir konsantrasyonu için duyarlılık testi yapılması önerilmektedir.

Dezavantajları

- Proporsiyon yöntemi;
  - 3-4 hafta gerekli
  - 7H10 ve 11 agar ya da LJ'de uygulanmakta
  - LJ hazırlanması zor
- MGIT 960;
  - Otomatize ve hızlı
  - Pahalı
  - Ekipman gerekli

NRT

- Testin temeli;
  - Bakteri nitrat redüktaz enzimine sahip
    - Nitrat redüktaz enzimi olmayan bakteri oranı <%1
  - Duyarlılık testi yapılacak olan besiyerine KNO_3 ekleniyor (1000 µg/ml)
  - Eğer bakteri dirençli ise ortamda çoğalmaya başlıyor.
  - Ortamdaki canlı bakteri enzimiyle nitratı nitrite indirgiyor
  - Canlı bakteri kolonileri görünür hale gelmese de
  - Canlı bakteri varlığına bağlı oluşan nitrit Griess ayracı ile görünür hale getiriliyor

NRT

- Direkt test;
- İndirekt test;
  - LJ'de NRA
  - 7H9 sıvı besiyerinde tüpte NRA
  - 7H9 sıvı besiyerinde mikroplakta NRA
  - Kanlı agarda NRA
  - 7H11 agarda NRA

LJ'de NRT

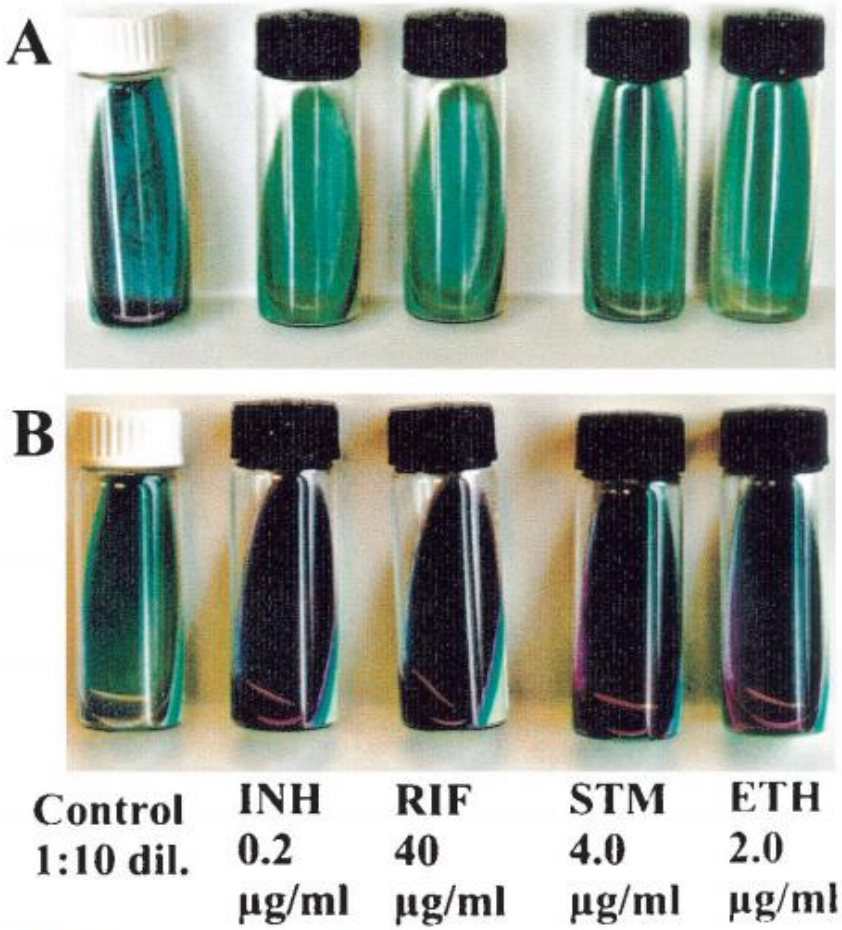
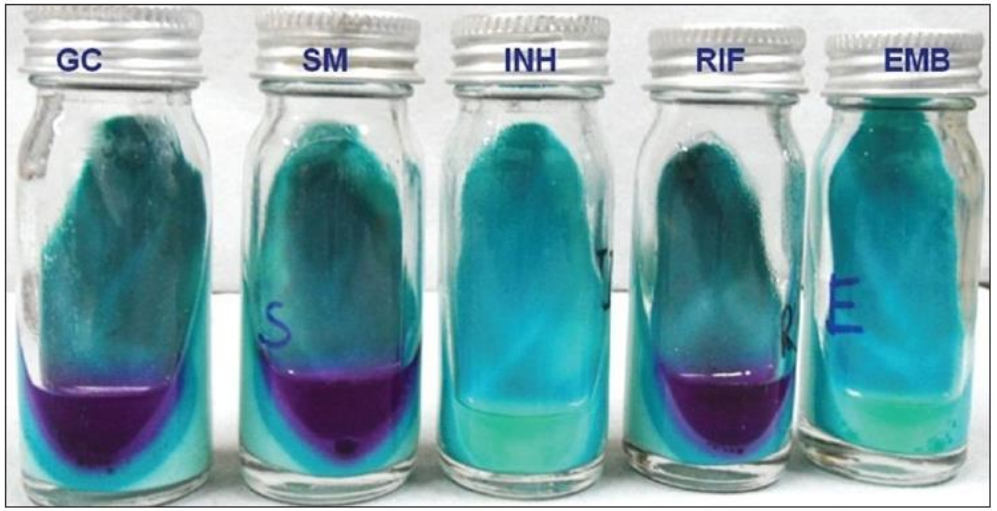
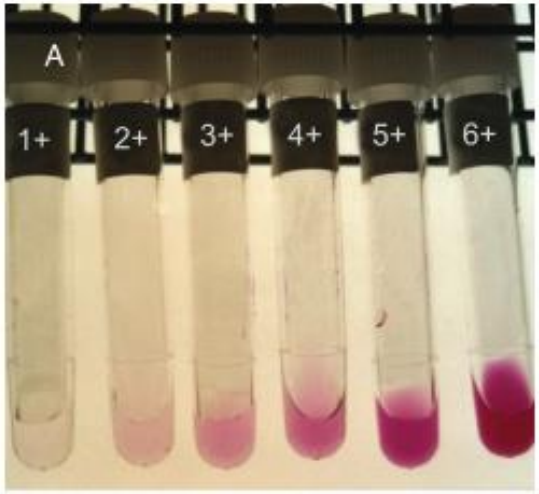


FIG. 1. Examples of NRA results. A strain is considered resistant to a certain drug if there is a color change in the drug-containing tube greater than that in the 1:10-diluted (1:10 dil.) control tube. (A) Fully susceptible strain; (B) strain resistant to all four tested drugs.



Demonstration of nitrate reductase assay results: resistant to two drugs. GC: growth control tube without any drug, S streptomycin, INH: growth sensitive to isoniazid, RIF: growth resistant to rifampicin, EMB: growth sensitive to etha

Tüpte NRT



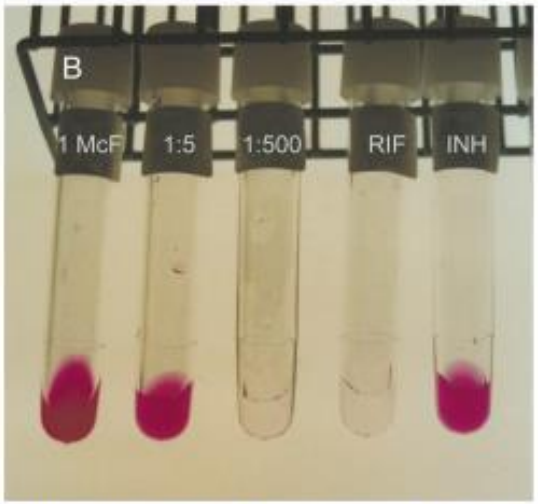


FIG. 1. (A) Serial twofold dilutions of a standard stock solution of sodium nitrite ($NaNO_2$) resulting in an increasing color intensity graded from 1+ through 6+. (B) Example of a strain examined by the CONRAS test. The color intensity of the 1:500 dilution of the bacterial suspension was compared with the color intensity in the standard tubes (panel A). A strain was considered to be resistant if the color intensity

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Rapid Colorimetric Method for Testing Susceptibility of *Mycobacterium tuberculosis* to Isoniazid and Rifampin in Liquid Cultures

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We have developed a rapid colorimetric method for testing the susceptibility of *M. tuberculosis* to isoniazid (INH) and rifampin (RIF) based on incorporation of nitrate in broth cultures containing growth supplements. The performance of this colorimetric nitrate reductase-based antibiotic susceptibility (CONRAS) test was compared with that of the radiometric BACTEC 460TB system in determining the susceptibilities of 74 *M. tuberculosis* strains to INH and RIF. By using the BACTEC 460TB system as the "gold standard," the sensitivity (i.e., the ability to detect true drug resistance) and specificity (i.e., the ability to detect true drug susceptibility) of the CONRAS test were 100 and 95% for INH and 94 and 100% for RIF, respectively. The repeatability of the CONRAS test was excellent (for INH, kappa = 1 and $P < 0.001$; for RIF, kappa = 0.88 and $P < 0.001$). For the majority of strains, results were obtained within 5 days. The CONRAS test is rapid, accurate, and inexpensive and is an adequate alternative, particularly for resource-poor countries.

Mikroplakta NRT

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Comparative evaluation of the microplate nitrate reductase assay and the rezasurin microtitre assay for the rapid detection of multidrug resistant *Mycobacterium tuberculosis* clinical isolates

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The microplate nitrate reductase assay (MNRA) and the rezasurin microtitre assay (REMA) were used for the susceptibility testing of 73 clinical isolates and the results were compared with those that were obtained using the Bactec 460 TB and Bactec MGIT 960 systems. The REMA and the MNRA were performed in 96-well plates. For the REMA, the concentrations of isoniazid (INH) and rifampicin (RIF) ranged from 1.0-0.01 µg/mL and 2.0-0.03 µg/mL, respectively. For the MNRA, the INH concentration was between 1.0-0.03 µg/mL and the RIF concentration was between 2.0-0.06 µg/mL. For the MNRA, the sensitivity, specificity, positive predictive value, negative predictive value and INH/RIF agreement were 100/95.6, 97.6/100, 96.8/100, 100/98 and 98.6/98.6, respectively, and for the REMA, they were 100/91.3, 90.4/100, 88.5/100, 100/96.1 and 94.5/97.2, respectively. Our data suggest that these two rapid, low-cost methods may be inexpensive, alternative assays for the rapid detection of multidrug resistant tuberculosis in low-income countries.

Key words: *Mycobacterium tuberculosis* - MIC determination - microplate nitrate reductase assay - rezasurin microtitre assay

Mikroplakta NRT

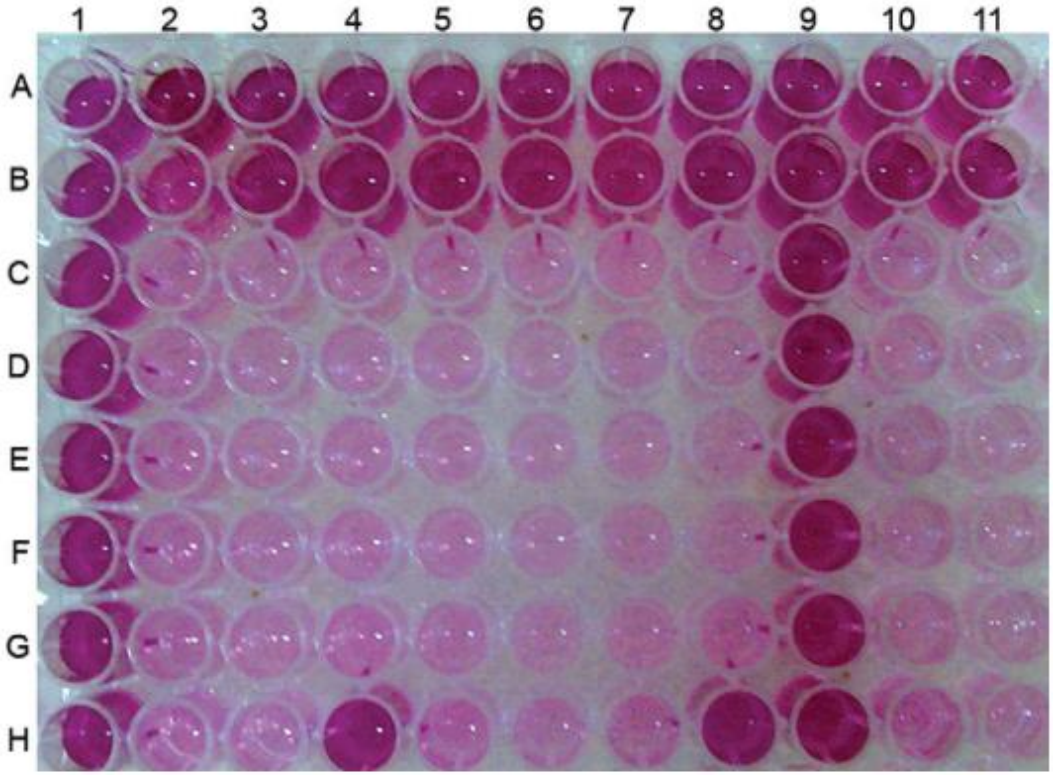

Fig. 2: assessment of microplate nitrate reductase assay (MNRA) results. A1-11: positive control wells; B1-11: positive control wells; C1-11: 1.0 µg/mL for isoniazid (INH) and 2.0 µg/mL for rifampicin (RIF); D1-11: 0.5 µg/mL for INH and 1 µg/mL for RIF; E1-11: 0.25 µg/mL for INH and 0.5 µg/mL for RIF; F1-11: 0.125 µg/mL for INH and 0.25 µg/mL for RIF; G1-11: 0.06 µg/mL for INH and 0.125 µg/mL for RIF; H1-11: 0.03 µg/mL for INH and 0.06 µg/mL for RIF.

Kanlı agarda NRT

A rapid detection of multidrug-resistant *Mycobacterium tuberculosis* by a nitrate reductase assay on blood agar

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The susceptibility of 49 Mycobacterium tuberculosis *clinical isolates to isoniazid (INH) and rifampisin (RIF) (28 multi-drug resistant-tuberculosis samples) was determined by a nitrate reductase assay (NRA) on blood agar. Agreement between the NRA and other testing methods was found to be 93.8% for both INH and RIF. The sensitivity, specificity, positive predictive value and negative predictive value for INH were 92.8%, 94.2%, 86.6% and 97%, respectively. The sensitivity, specificity, positive predictive value and negative predictive value for RIF were 90.4%, 96.4%, 95% and 93.1%. In conclusion, we show here that blood agar can be used effectively for the NRA test.*

Key words: *Mycobacterium tuberculosis* - nitrate reductase assay - blood agar

Tuberculosis remains an important public health problem and has recently been increasing in incidence (Affolabi et al. 2007). The early detection of antimicrobial susceptibility is especially important for creating an appropriate treatment protocol for patients with tuberculosis. Multi-drug resistant (MDR) tuberculosis isolates present a major challenge for tuberculosis control programs (Shikama et al. 2009). Therefore, microbial susceptibility to at least isoniazid (INH) and rifampisin (RIF) should be ascertained to diagnose MDR tuberculosis. Conventional methods [proportion methods in Löwenstein-Jensen (LJ) and Middlebrook 7H10 agar] for tuberculosis drug susceptibility testing have many disadvantages, including being labour intensive and time

susceptibility for determining MDR tuberculosis using an NRA test on blood agar, a method which has recently been developed to test the antibiotic susceptibility of tuberculosis bacilli (Coban et al. 2006).

Forty-nine *Mycobacterium tuberculosis* clinical isolates, including 28 MDR isolates and the reference strains H37Rv (susceptible to all drugs), ATCC 35822 (INH resistant) and ATCC 35820 (streptomycin resistant), were tested in this study. Antibacterial resistance patterns of the clinical isolates are summarised in Table I. Drug susceptibility testing of all isolates was determined by Bactec 460 TB, which is considered the benchmark automated system and is commonly used in diagnostic laboratories. INH and RIF powders were obtained from Sigma-Aldrich,

TABLE II
Comparison of blood agar results and BACTEC 460-TB system results

Drugs	Results on blood agar	Results of BACTEC 460 TB system		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Agreement (%)
		Resistant	Susceptible					
INH	Resistant	33	1	92.8	94.2	86.6	97	93.8
	Susceptible	2	13	-	-	-	-	-
RIF	Resistant	27	2	90.4	96.4	95	93.1	93.8
	Susceptible	1	19	-	-	-	-	-

INH: isoniazid; NPV: negative predictive value; PPV: positive predictive value; RIF: rifampicin.

7H11 agarda NRT

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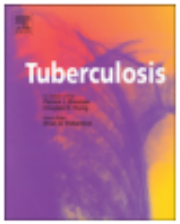

DIAGNOSTICS

Field evaluation of the direct detection of multidrug resistant *Mycobacterium tuberculosis* by nitrate reductase assay on 7H11 agar

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SUMMARY

In this study we evaluated the performance of colorimetric nitrate reductase assay (NRA) on Middlebrook 7H11 agar instead of Lowenstein–Jensen medium for detection of isoniazid (INH) and rifampin (RIF) resistance directly on 114 smear positive sputum specimens and compared the results with direct proportion method on LJ medium. The results of both methods were in 100% agreement for detection of RIF resistance while agreement for INH was 96.4%. The average turnaround time for NRA was 18.6 days and majority of the specimens gave positive results within 21 days. Thus direct NRA testing on smear positive sputum specimens by using 7H11 agar could be used as a fast, reliable and inexpensive method in resource starved settings.

7H11 agarda NRT

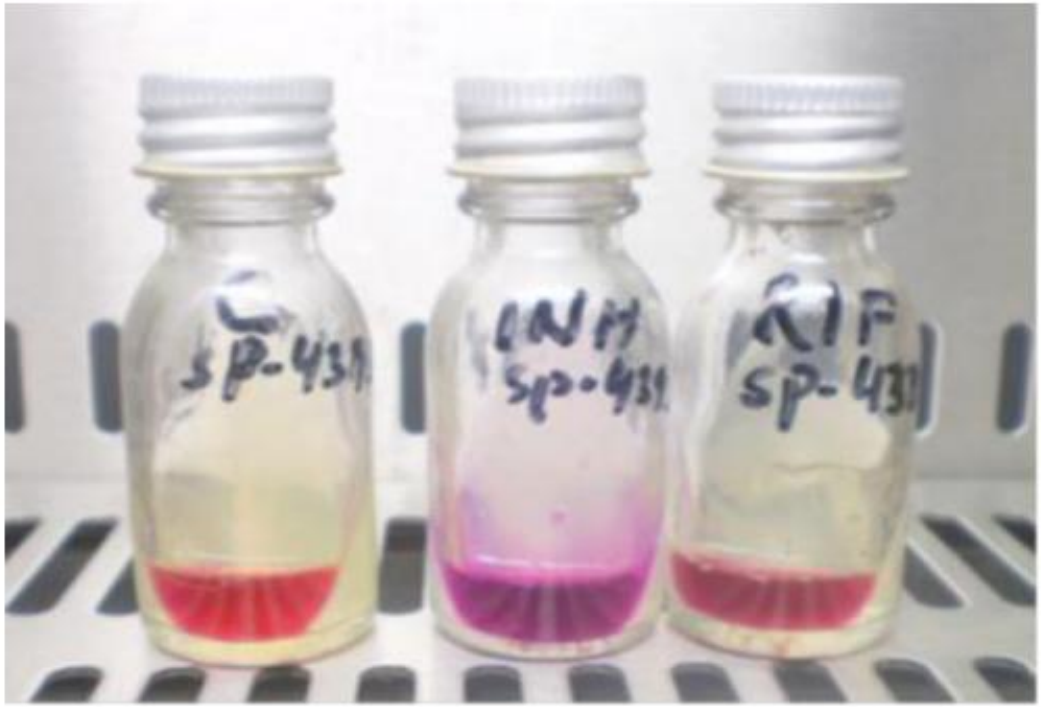

Figure 1. A *M. tuberculosis* strain showing resistance to both INH and RIF. Note that the color change in drug containing bottles is greater than that in control bottle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Comparison of results of direct LJ proportion method with results of sensitivity on 7H11 agar by NRA.

Drugs	LJ results	7H11 agar		%				
		R	S	Sensitivity	Specificity	PPV	NPV	Agreement
RIF	R	24	0	100	100	100	100	100
	S	0	88					
INH	R	28	4	100	95.2	88	100	96.4
	S	0	80					

R = resistant; S = susceptible; PPV = positive predictive value; NPV = negative predictive value.

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Prospective multicentre evaluation of the direct nitrate reductase assay for the rapid detection of extensively drug-resistant tuberculosis

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Objectives: To perform a multicentre study evaluating the performance of the direct nitrate reductase assay (NRA) for the detection of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis in sputum samples.

Methods: The study was conducted in six laboratories performing tuberculosis diagnosis that were located in six different countries. The NRA was performed directly on sputum samples in parallel with the reference method used at each site. Detection of resistance was performed for rifampicin, isoniazid, ofloxacin and kanamycin.

Results: Excellent agreement was obtained for all drugs tested at the majority of sites. The accuracy was 93.7%–100% for rifampicin, 88.2%–100% for isoniazid, 94.6%–100% for ofloxacin and 100% for kanamycin. The majority of NRA results were available at day 21 for sites 1, 2 and 5. Site 3 had a turnaround time of 13.9 days, at site 4 it was 18.4 days and at site 6 it was 16.2 days. The contamination rate ranged between 2.5% and 12%.

Conclusions: Rapid detection of drug resistance by the direct NRA on sputum smear-positive samples was accurate and easy to implement in clinical diagnostic laboratories, making it a good alternative for rapid screening for MDR and XDR tuberculosis.

Table 1. Susceptibility results for the direct NRA compared with the MGIT 960 system or LJ proportion method for each site

		MGIT 960 system or LJ proportion method							
		rifampicin		isoniazid		ofloxacin		kanamycin	
Site	Direct NRA	R	S	R	S	R	S	R	S
1	R	18	3	20	1	11	0	7	0
	S	0	31	0	31	0	41	0	45
2	R	12	0	15	0	1	0	1	0
	S	1	37	2	33	0	49	0	49
3	R	4	0	9	0	2	0	5	0
	S	0	53	0	48	0	55	0	52
4	R	0	0	6	0	2	1	0	0
	S	0	52	2	44	1	48	0	52
5	R	3	0	4	1	0	0	0	0
	S	1	12	1	11	0	17	0	17
6	R	2	0	2	0	0	2	0	0
	S	0	35	0	35	0	35	0	37

R, resistant; S, susceptible.

Table 2. Sensitivity, specificity, PPV, NPV and accuracy of the direct NRA for each drug tested at each study site

	Rifampicin (%)					Isoniazid (%)					Ofloxacin (%)					Kanamycin (%)				
Site	sens	spec	PPV	NPV	A	sens	spec	PPV	NPV	A	sens	spec	PPV	NPV	A	sens	spec	PPV	NPV	A
1	100	91.1	85.7	100	94.2	100	96.8	95.2	100	98.0	100	100	100	100	100	100	100	100	100	100
2	92.3	100	100	97.3	98.0	88.2	100	100	94.2	96.0	100	100	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4	NA	100	NA	NA	100	75	100	100	95.6	96.2	66.7	98	66.6	97.9	96.2	NA	100	NA	100	100
5	75	100	100	92.3	93.7	80	91.6	80	91.6	88.2	NA	100	NA	100	100	NA	100	NA	100	100
6	100	100	100	100	100	100	100	100	100	100	NA	94.6	NA	100	94.6	NA	100	NA	100	100

sens, sensitivity; spec, specificity; A, accuracy; NA, not applicable.

Journal of Clinical Microbiology

Nitrate Reductase Assay for Rapid Detection of Isoniazid, Rifampin, Ethambutol, and Streptomycin Resistance in Mycobacterium tuberculosis: a Systematic Review and Meta-Analysis

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Nitrate Reductase Assay for Rapid Detection of Isoniazid, Rifampin, Ethambutol, and Streptomycin Resistance in *Mycobacterium tuberculosis*: a Systematic Review and Meta-Analysis

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Colorimetric phenotypic tests recently gained interest because traditional primary drug susceptibility testing of *Mycobacterium tuberculosis* isolates takes a long time. We used meta-analysis techniques to review the reliability and accuracy of the nitrate reductase assay (NRA), which is one of the most popular colorimetric methods to detect resistance to first-line drugs. Medline, PubMed, ISI Web, Web of Science, and Google Scholar were used to search for studies enrolled in the meta-analysis. The analysis included 35 studies for isoniazid (INH), 38 for rifampin (RIF), and 22 for ethambutol (EMB) and streptomycin (STR). Summary receiver operating characteristic (SROC) curves were applied to summarize diagnostic accuracy. The meta-analyses were performed by the use of Meta-DiSc software (version 1.4) and were focused on sensitivity and specificity values for measurements of accuracy. The pooled sensitivities were 96% for INH, 97% for RIF, 90% for EMB, and 82% for STR. The pooled specificities for INH, RIF, EMB, and STR were 99%, 100%, 98%, and 96%, respectively. The times required to obtain results were between 5 and 28 days by the direct NRA and between 5 and 14 days by the indirect test. In conclusion, the present meta-analysis showed that the NRA is a reliable low-cost rapid colorimetric susceptibility test that can be used for the detection of multidrug-resistant (MDR) tuberculosis, including detection of EMB resistance. However, the test appears to have a relatively low sensitivity for STR and needs further improvement.

Table S1. Description of studies included in the meta-analysis of INH resistance detection.

References	Countries	Reference test	Samples	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Angeby et al.[1]	Sweden	Bactec 460 TB	56 isolates	0.97 (0.84-1.00)	0.96 (0.79-1.00)	7-14
Syre et al.[2]	Norway	Bactec 460 TB	74 isolates	1.00 (0.89-1.00)	0.95 (0.84-0.99)	5.4
Coban et al.[3]	Turkey	PM on LJ	80 isolates	1.00 (0.75-1.00)	1.00 (0.95-1.00)	7-14
Montoro et al.[4]	Cuba/Belgium	PM on LJ	100 isolates	0.96 (0.85-0.99)	1.00 (0.94-1.00)	7-14
Martin et al.[5] Site 1	Belgium	PM on LJ	30 isolates	1.00 (0.74-1.00)	1.00 (0.81-1.00)	7-14
Site 2	Cuba	PM on LJ	30 isolates	0.92 (0.62-1.00)	0.94 (0.73-1.00)	7-14
Site 3	Argentina	PM on LJ	30 isolates	1.00 (0.74-1.00)	0.94 (0.73-1.00)	7-14
Site 4	Chile	PM on LJ	30 isolates	0.92 (0.62-1.00)	0.94 (0.73-1.00)	7-14
Site 5	Peru	PM on LJ	30 isolates	1.00 (0.74-1.00)	1.00 (0.81-1.00)	7-14
Musa et al.*[6]	Argentina/Sweden	PM on LJ	130 sputum samples	0.93 (0.66-1.00)	1.00 (0.97-1.00)	10-18
Solis et al.*[7]	Peru/USA	PM on LJ	192 sputum samples	0.99 (0.95-1.00)	1.00 (0.95-1.00)	28
Lemus et al.[8]	Cuba/Belgium	PM on LJ	320 isolates	0.92 (0.82-0.97)	1.00 (0.99-1.00)	10-14
Affolabi et al.[9]	Benin/Belgium/France	PM on LJ	151 isolates	1.00 (0.85-1.00)	1.00 (0.97-1.00)	8-18
Affolabi et al.*[10]	Benin/Belgium/France	PM on LJ	144 sputum samples	1.00 (0.77-1.00)	0.99 (0.96-1.00)	5-18
Ani et al.[11]	Nigeria	PM on LJ	70 isolates	1.00 (0.59-1.00)	1.00 (0.94-1.00)	10-14
Visalakshi et al.*[12]	India	PM on LJ	108 sputum samples	0.95 (0.87-0.99)	1.00 (0.92-1.00)	10-21
Rosales et al.[13]	Honduras/Sweden	Bactec 460TB/PM on LJ	192 isolates	0.98 (0.91-1.00)	1.00 (0.97-1.00)	7-14
Bwanga et al.[14]	Uganda/Sweden	PM on LJ	31 isolates	1.00 (0.80-1.00)	1.00 (0.77-1.00)	7-10
Gupta and Anupurba*[15]	India	PM on LJ	45 sputum samples	1.00 (0.80-1.00)	0.93 (0.76-0.99)	21-28
Gupta et al.[16]	India	PM on LJ	286 isolates	0.97 (0.92-0.99)	1.00 (0.98-1.00)	7-14
Bwanga et al.*[17]	Uganda/Sweden	PM on LJ	210 sputum samples	0.93 (0.84-0.98)	0.96 (0.91-0.99)	10-21
Coban et al.[18]	Turkey	Bactec 460 TB	49 isolates	0.94 (0.81-0.99)	0.93 (0.66-1.00)	7-10
Dixit et al.[19]	India	PM on LJ	105 isolates	0.98 (0.90-1.00)	1.00 (0.93-1.00)	8-14
Lopez et al.[20]	Argentina	PM on LJ	302 isolates	0.85 (0.75-0.92)	1.00 (0.98-1.00)	7-14
Fonseca et al.[21]	Brasil	PM on LJ	106 isolates	0.91 (0.80-0.97)	0.98 (0.89-1.00)	8.8
Mansur et al.[22]	Brasil	PM on LJ	57 isolates	1.00 (0.74-1.00)	1.00 (0.92-1.00)	10
Ramos et al.[23]	Belgium	PM on LJ	147 isolates	0.99 (0.95-1.00)	0.95 (0.83-0.99)	10-14
Rosales et al.*[24]	Honduras/Sweden	PM on LJ	108 sputum samples	1.00 (0.69-1.00)	0.99 (0.94-1.00)	10-28
Gupta et al.*[25]	India	PM on LJ	100 sputum samples	0.98 (0.91-1.00)	0.98 (0.87-1.00)	7-14
Coban et al.[26]	Turkey	Bactec 460 TB/MGIT 960	73 isolates	1.00 (0.89-1.00)	0.98 (0.87-1.00)	7
Sethi et al.[27]	India	PM on LJ	100 isolates	0.97 (0.84-1.00)	1.00 (0.95-1.00)	7-14
Kumar et al.[28]	India/USA	PM on 7H10 agar	42 isolates	0.87 (0.60-0.98)	1.00 (0.87-1.00)	8
Mengatto et al.[29]	Argentina	PM on LJ	64 isolates	0.92 (0.75-0.99)	1.00 (0.91-1.00)	7-14
Shikama et al.[30]	Brasil/Belgium	PM on LJ/Bactec MGIT 960	120 isolates	0.98 (0.92-1.00)	0.98 (0.90-1.00)	7-14
Mendoza et al.[31]	Venezuella	PM on LJ	59 isolates	1.00 (0.03-1.00)	1.00 (0.92-1.00)	10-14

*Direct test, TTP: Time to positivity.

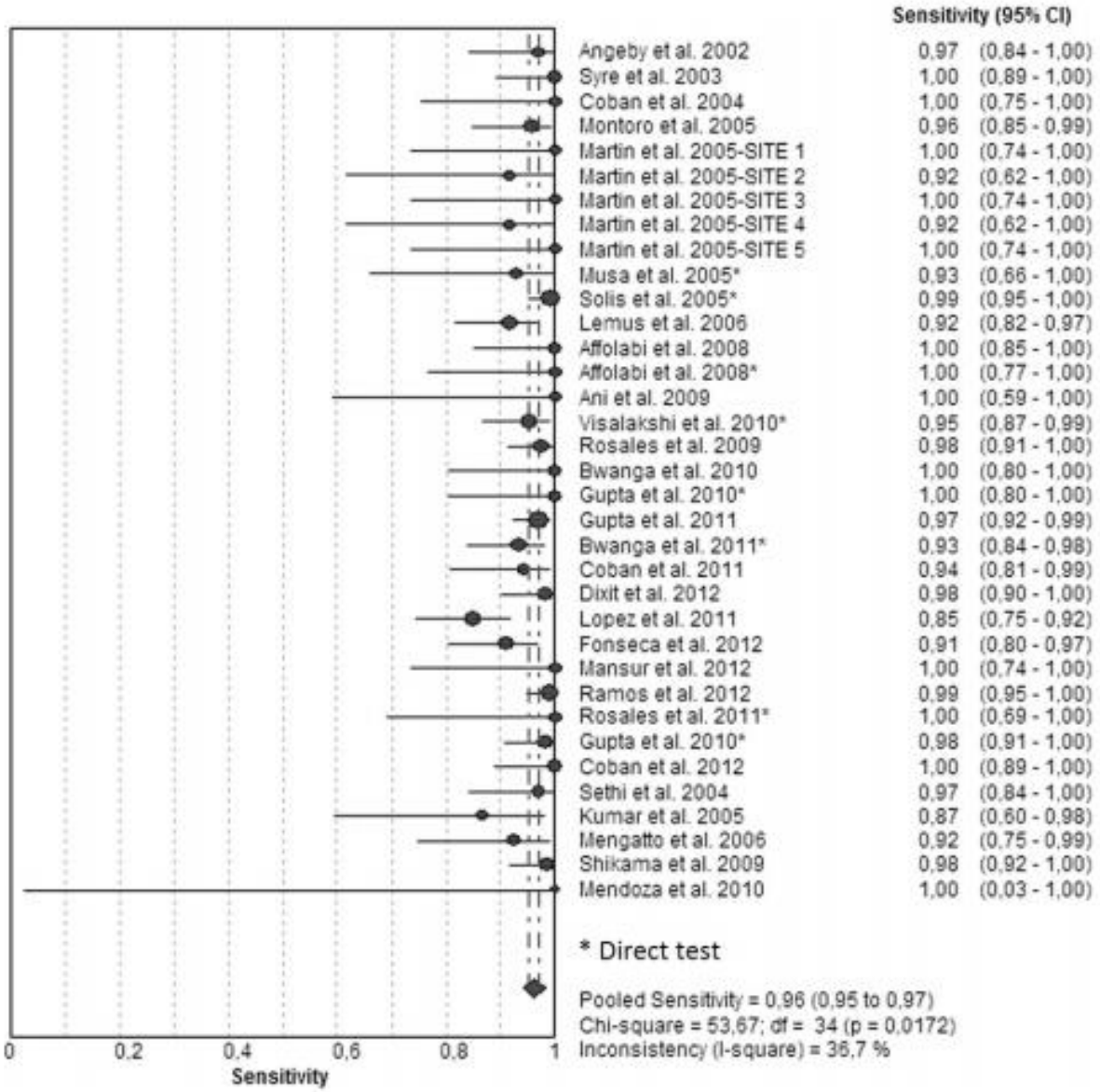

FIG 1 Forest plot of the sensitivities for INH assays. The point estimates of sensitivities from each study are shown as circles. Error bars indicate 95% confidence intervals.

Duyarlılık: %85-100

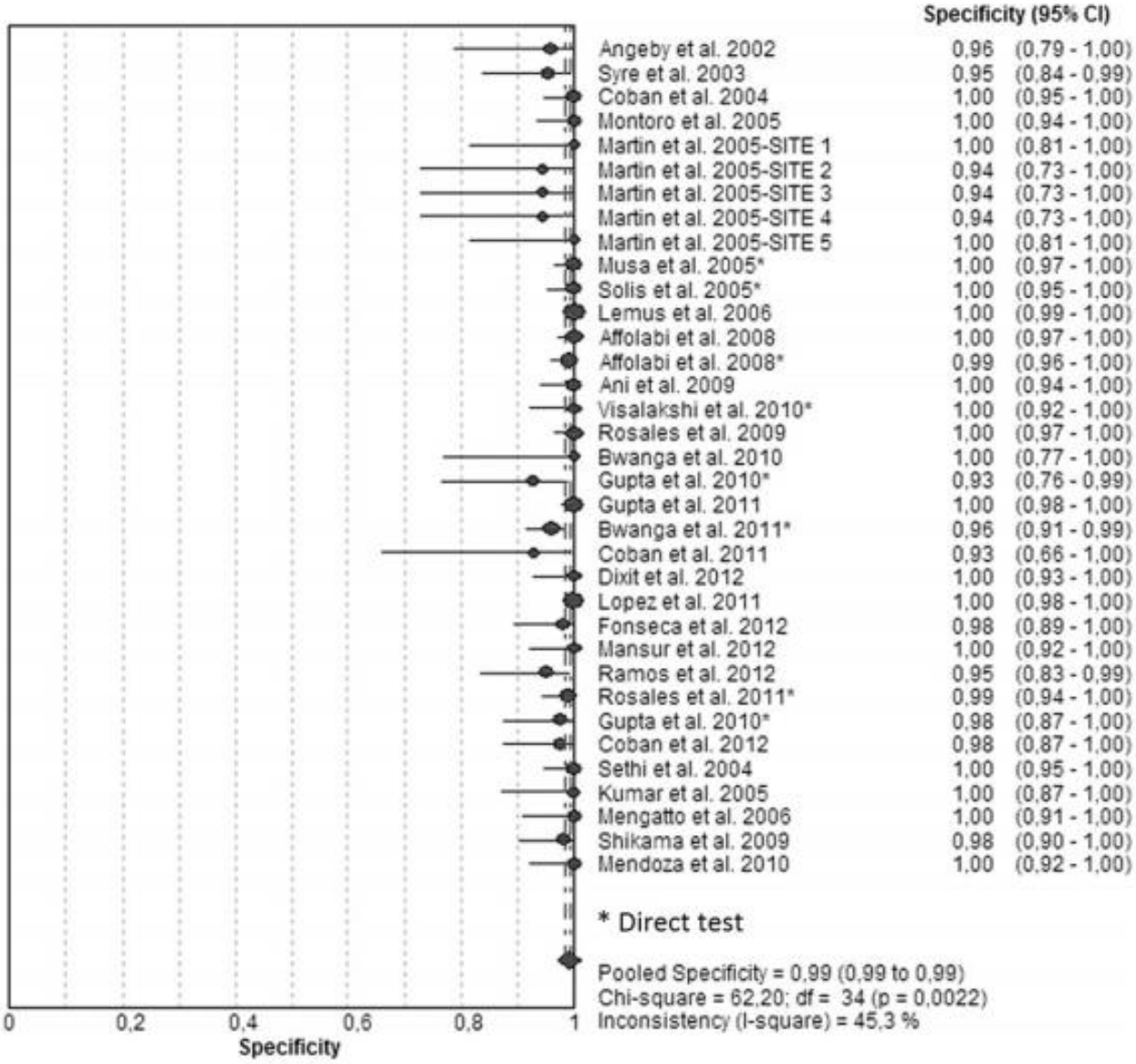

FIG 2 Forest plot of the specificities for INH assays. The point estimates of specificities from each study are shown as circles. Error bars indicate 95% confidence intervals.

Özgüllük: %96,4-100

Table S2. Description of studies included in the meta-analysis of RIF resistance detection.

References	Countries	Reference test	Samples	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Angeby et al.[1"]	Sweden	Bactec 460 TB	56 isolates	1.00 (0.89-1.00)	1.00 (0.87-1.00)	7-14
Syre et al.[2]	Norway	Bactec 460 TB	74 isolates	0.94 (0.71-1.00)	1.00 (0.94-1.00)	5.4
Coban et al.[3]	Turkey	PM on LJ	80 isolates	1.00 (0.63-1.00)	1.00 (0.95-1.00)	7-14
Montoro et al.[4]	Cuba/Belgium	PM on LJ	100 isolates	1.00 (0.91-1.00)	1.00 (0.94-1.00)	7-14
Martin et al.[5] Site 1	Belgium	PM on LJ	30 isolates	1.00 (0.78-1.00)	1.00 (0.78-1.00)	7-14
Site 2	Cuba	PM on LJ	30 isolates	1.00 (0.78-1.00)	1.00 (0.78-1.00)	7-14
Site 3	Argentina	PM on LJ	30 isolates	1.00 (0.78-1.00)	1.00 (0.78-1.00)	7-14
Site 4	Chile	PM on LJ	30 isolates	0.93 (0.68-1.00)	1.00 (0.78-1.00)	7-14
Site 5	Peru	PM on LJ	30 isolates	0.87 (0.60-0.98)	1.00 (0.78-1.00)	7-14
Musa et al.*[6]	Argentina/Sweden	PM on LJ	130 sputum samples	1.00 (0.72-1.00)	1.00 (0.97-1.00)	10-18
Solis et al.*[7]	Peru/USA	PM on LJ	192 sputum samples	0.94 (0.87-0.97)	1.00 (0.96-1.00)	28
Lemus et al.[8]	Cuba/Belgium	PM on LJ	320 isolates	0.94 (0.83-0.99)	1.00 (0.99-1.00)	10-14
Affolabi et al.*[32]	Benin/Belgium	PM on LJ	177 sputum samples	0.88 (0.47-1.00)	1.00 (0.98-1.00)	10-28
Affolabi et al.[9]	Benin/Belgium/France	PM on LJ	151 isolates	1.00 (0.79-1.00)	1.00 (0.97-1.00)	8-18
Affolabi et al.*[10]	Benin/Belgium/France	PM on LJ	144 sputum samples	1.00 (0.54-1.00)	0.99 (0.96-1.00)	5-18
Shikama et al.*[33]	Brasil/Belgium	PM on LJ	210 sputum samples	1.00 (0.54-1.00)	1.00 (0.98-1.00)	10-20
Ani et al.[11]	Nigeria	PM on LJ	70 isolates	0.90 (0.55-1.00)	0.97 (0.88-1.00)	10-14
Visalakshi et al.*[12]	India	PM on LJ	108 sputum samples	1.00 (0.92-1.00)	0.98 (0.91-1.00)	10-21
Rosales et al.[13]	Honduras/Sweden	Bactec 460TB/PM on LJ	192 isolates	0.95 (0.87-0.99)	1.00 (0.97-1.00)	7-14
Bwanga et al.[14]	Uganda/Sweden	PM on LJ	31 isolates	1.00 (0.77-1.00)	1.00 (0.80-1.00)	7-10
Gupta and Anupurba*[15]	India	PM on LJ	45 sputum samples	1.00 (0.83-1.00)	1.00 (0.86-1.00)	21-28
Gupta et al.[16]	India	PM on LJ	286 isolates	0.98 (0.94-1.00)	1.00 (0.98-1.00)	7-14
Bwanga et al.*[17]	Uganda/Sweden	PM on LJ	210 sputum samples	0.98 (0.87-1.00)	0.98 (0.94-0.99)	10-21
Coban et al.[18]	Turkey	Bactec 460 TB	49 isolates	0.96 (0.82-1.00)	0.90 (0.70-0.99)	7-10
Dixit et al.[19]	India	PM on LJ	105 isolates	1.00 (0.94-1.00)	1.00 (0.93-1.00)	8-14
Lopez et al.[20]	Argentina	PM on LJ	302 isolates	0.87 (0.76-0.94)	1.00 (0.98-1.00)	7-14
Fonseca et al.[21]	Brasil	PM on LJ	106 isolates	0.97 (0.87-1.00)	1.00 (0.95-1.00)	8.8
Mansur et al.[22]	Brasil	PM on LJ	57 isolates	1.00 (0.72-1.00)	1.00 (0.92-1.00)	10
Ramos et al.[23]	Belgium	PM on LJ	147 isolates	0.97 (0.92-0.99)	1.00 (0.92-1.00)	10-14
Rosales et al.*[24]	Honduras/Sweden	PM on LJ	108 sputum samples	0.80 (0.28-0.99)	1.00 (0.96-1.00)	10-28
Gupta et al.*[25]	India	PM on LJ	100 sputum samples	1.00 (0.94-1.00)	1.00 (0.92-1.00)	7-14
Coban et al.[26]	Turkey	Bactec 460 TB/MGIT 960	73 isolates	0.96 (0.78-1.00)	1.00 (0.93-1.00)	7
Sethi et al.[27]	India	PM on LJ	100 isolates	1.00 (0.90-1.00)	1.00 (0.94-1.00)	7-14
Kumar et al.[28]	India/USA	PM on 7H10 agar	42 isolates	1.00 (0.69-1.00)	1.00 (0.89-1.00)	8
Mengatto et al.[29]	Argentina	PM on LJ	64 isolates	1.00 (0.86-1.00)	1.00 (0.91-1.00)	7-14
Shikama et al.[30]	Brasil/Belgium	PM on LJ/Bactec MGIT 960	120 isolates	0.99 (0.93-1.00)	0.98 (0.88-1.00)	7-14
Mendoza et al.[31]	Venezuella	PM on LJ	59 isolates	1.00 (0.03-1.00)	1.00 (0.92-1.00)	10-14
Lemus et al.[34]	Cuba/Belgium	PM on LJ/Bactec 460 TB	20 isolates	1.00 (0.69-1.00)	1.00 (0.69-1.00)	7-14

*Direct test, TTP: Time to positivity.

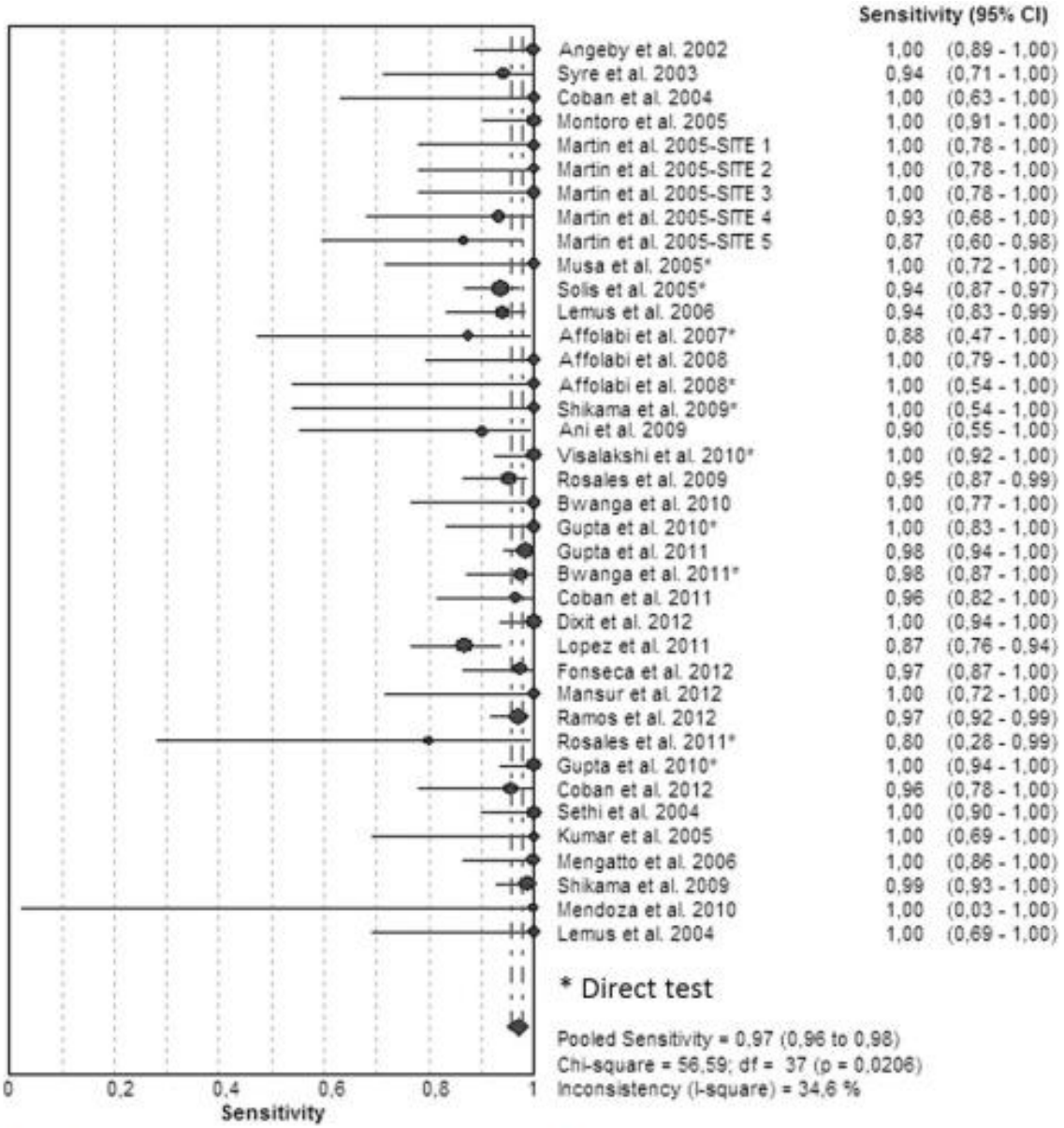

FIG 3 Forest plot of the sensitivities for RIF analyses. The point estimates of sensitivities from each study are shown as circles. Error bars indicate 95% confidence intervals.

Duyarlılık: %80-100

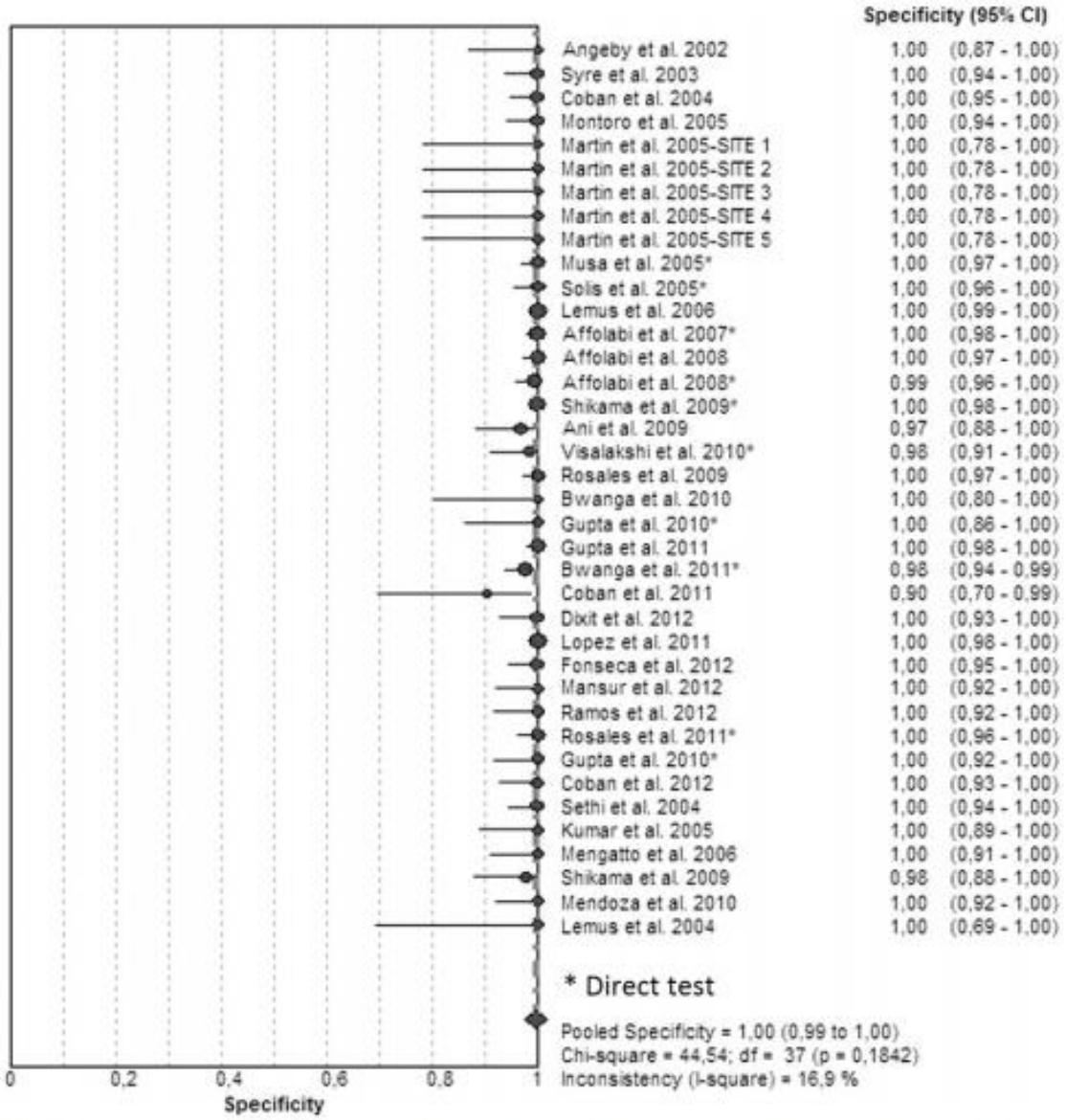

FIG 4 Forest plot of the specificities for RIF analyses. The point estimates of specificities from each study are shown as circles. Error bars indicate 95% confidence intervals.

Özgüllük: %96,4-100

Table S3. Description of studies included in the meta-analysis of EMB resistance detection.

References	Countries	Reference test	Samples	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Angeby et al.[1]	Sweden	Bactec 460 TB	56 isolates	0.75 (0.43-0.95)	0.98 (0.88-1.00)	7-14
Coban et al.[3]	Turkey	PM on LJ	80 isolates	1.00 (0.29-1.00)	0.92 (0.84-0.97)	7-14
Montoro et al.[4]	Cuba/Belgium	PM on LJ	100 isolates	1.00 (0.80-1.00)	0.99 (0.93-1.00)	7-14
Martin et al.[5] Site 1	Belgium	PM on LJ	30 isolates	1.00 (0.66-1.00)	0.95 (0.76-1.00)	7-14
Site 2	Cuba	PM on LJ	30 isolates	1.00 (0.66-1.00)	1.00 (0.84-1.00)	7-14
Site 3	Argentina	PM on LJ	30 isolates	0.89 (0.52-1.00)	1.00 (0.84-1.00)	7-14
Site 4	Chile	PM on LJ	30 isolates	1.00 (0.66-1.00)	1.00 (0.84-1.00)	7-14
Site 5	Peru	PM on LJ	30 isolates	0.89 (0.52-1.00)	1.00 (0.84-1.00)	7-14
Musa et al.*[6]	Argentina/Sweden	PM on LJ	130 sputum samples	0.56 (0.21-0.86)	0.99 (0.95-1.00)	10-18
Syre et al.[35]	Norway	Bactec 460TB	89 isolates	0.64 (0.43-0.82)	0.92 (0.83-0.97)	3-9
Lemus et al.[8]	Cuba/Belgium	PM on LJ	320 isolates	0.88 (0.69-0.97)	1.00 (0.99-1.00)	10-14
Ani et al.[11]	Nigeria	PM on LJ	70 isolates	0.75 (0.19-0.99)	1.00 (0.95-1.00)	10-14
Gupta and Anupurba*[15]	India	PM on LJ	45 sputum samples	1.00 (0.79-1.00)	1.00 (0.88-1.00)	21-28
Gupta et al.[16]	India	PM on LJ	286 isolates	0.94 (0.89-0.98)	0.99 (0.96-1.00)	7-14
Dixit et al.[19]	India	PM on LJ	105 isolates	0.98 (0.90-1.00)	0.98 (0.89-1.00)	8-14
Lopez et al.[20]	Argentina	PM on LJ	302 isolates	0.55 (0.23-0.83)	1.00 (0.98-1.00)	7-14
Fonseca et al.[21]	Brasil	PM on LJ	106 isolates	0.86 (0.64-0.97)	0.99 (0.94-1.00)	8.8
Mansur et al.[22]	Brasil	PM on LJ	57 isolates	1.00 (0.16-1.00)	0.98 (0.90-1.00)	10
Gupta et al.*[25]	India	PM on LJ	100 sputum samples	0.75 (0.60-0.86)	0.98 (0.89-1.00)	7-14
Sethi et al.[27]	India	PM on LJ	100 isolates	0.94 (0.70-1.00)	1.00 (0.96-1.00)	7-14
Kumar et al.[28]	India/USA	PM on 7H10 agar	42 isolates	1.00 (0.16-1.00)	1.00 (0.91-1.00)	8
Mengatto et al.[29]	Argentina	PM on LJ	64 isolates	0.75 (0.35-0.97)	0.98 (0.90-1.00)	7-14
Shikama et al.[30]	Brasil/Belgium	PM on LJ/Bactec MGIT 960	120 isolates	0.97 (0.92-0.99)	0.71 (0.44-0.90)	7-14

*Direct test, TTP: Time to positivity.

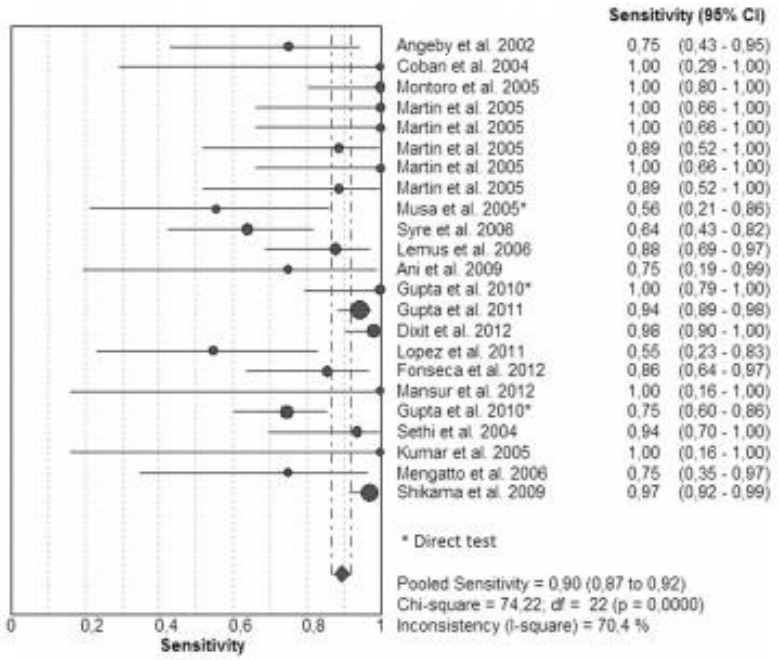

FIG 5 Forest plot of the sensitivities for EMB. The point estimates of sensitivities from each study are shown as circles. Error bars indicate 95% confidence intervals.

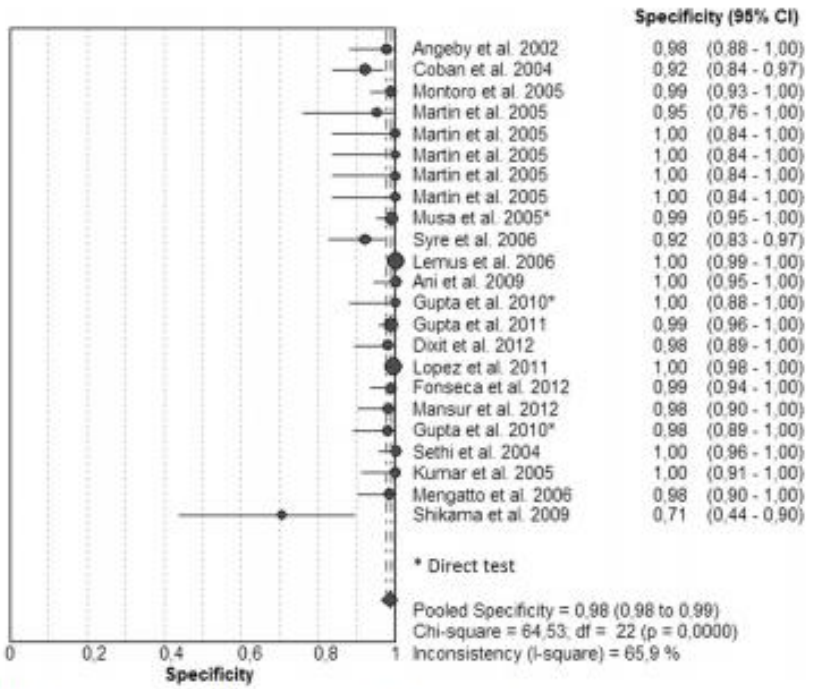

FIG 6 Forest plot of the specificities for EMB. The point estimates of specificities from each study are shown as circles. Error bars indicate 95% confidence intervals.

Duyarlılık: %55-100

Özgüllük: %75-100

Table S4. Description of studies included in the meta-analysis of STR resistance detection.

References	Countries	Reference test	Samples	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Angeby et al.[1]	Sweden	Bactec 460 TB	56 isolates	0.95 (0.77-1.00)	0.83 (0.66-0.93)	7-14
Coban et al.[3]	Turkey	PM on LJ	80 isolates	0.92 (0.64-1.00)	0.82 (0.71-0.90)	7-14
Montoro et al.[4]	Cuba/Belgium	PM on LJ	100 isolates	0.94 (0.83-0.99)	0.98 (0.90-1.00)	7-14
Martin et al.[5] Site 1	Belgium	PM on LJ	30 isolates	0.75 (0.43-0.95)	0.94 (0.73-1.00)	7-14
Site 2	Cuba	PM on LJ	30 isolates	0.12 (0.06-0.21)	1.00 (0.81-1.00)	7-14
Site 3	Argentina	PM on LJ	30 isolates	0.58 (0.28-0.85)	1.00 (0.81-1.00)	7-14
Site 4	Chile	PM on LJ	30 isolates	0.92 (0.62-1.00)	0.89 (0.65-0.99)	7-14
Site 5	Peru	PM on LJ	30 isolates	0.42 (0.15-0.72)	0.94 (0.73-1.00)	7-14
Musa et al.*[6]	Argentina/Sweden	PM on LJ	130 sputum samples	0.76 (0.53-0.92)	1.00 (0.96-1.00)	10-18
Syre et al.[35]	Norway	Bactec 460TB	89 isolates	0.73 (0.57-0.86)	1.00 (0.93-1.00)	3-9
Lemus et al.[8]	Cuba/Belgium	PM on LJ	320 isolates	0.97 (0.90-0.99)	0.99 (0.97-1.00)	10-14
Ani et al.[11]	Nigeria	PM on LJ	70 isolates	0.67 (0.35-0.90)	0.98 (0.91-1.00)	10-14
Gupta and Anupurba*[15]	India	PM on LJ	45 sputum samples	0.95 (0.75-1.00)	0.96 (0.80-1.00)	21-28
Gupta et al.[16]	India	PM on LJ	286 isolates	0.89 (0.82-0.93)	0.94 (0.88-0.97)	7-14
Dixit et al.[19]	India	PM on LJ	105 isolates	0.98 (0.90-1.00)	1.00 (0.93-1.00)	8-14
Lopez et al.[20]	Argentina	PM on LJ	302 isolates	0.59 (0.41-0.75)	0.99 (0.97-1.00)	7-14
Fonseca et al.[21]	Brasil	PM on LJ	106 isolates	0.94 (0.79-0.99)	0.89 (0.80-0.95)	8.8
Mansur et al.[22]	Brasil	PM on LJ	57 isolates	0.89 (0.52-1.00)	0.98 (0.89-1.00)	10
Gupta et al.*[25]	India	PM on LJ	100 sputum samples	0.96 (0.87-1.00)	0.84 (0.70-0.93)	7-14
Sethi et al.[27]	India	PM on LJ	100 isolates	1.00 (0.94-1.00)	1.00 (0.94-1.00)	7-14
Mengatto et al.[29]	Argentina	PM on LJ	64 isolates	0.84 (0.60-0.97)	1.00 (0.92-1.00)	7-14
Shikama et al.[30]	Brasil/Belgium	PM on LJ/Bactec MGIT 960	120 isolates	0.99 (0.93-1.00)	0.78 (0.63-0.89)	7-14
Mendoza et al.[31]	Venezuella	PM on LJ	59 isolates	0.33 (0.01-0.91)	1.00 (0.92-1.00)	7-14

*Direct test, TTP: Time to positivity.

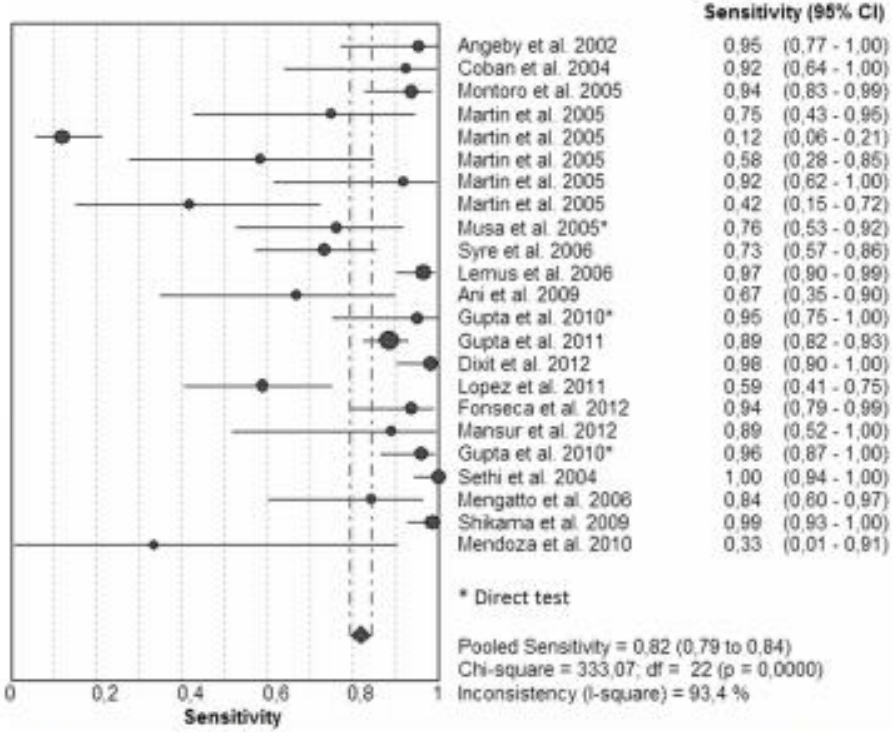

FIG 7 Forest plot of the sensitivities for STR. The point estimates of sensitivities from each study are shown as circles. Error bars indicate 95% confidence intervals.

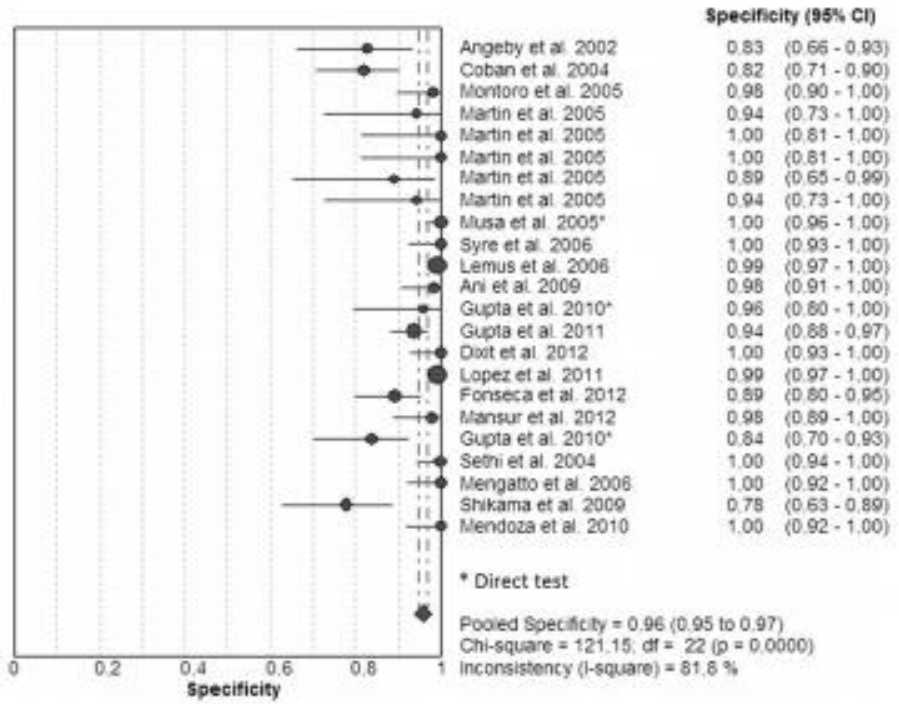

FIG 8 Forest plot of the specificities for STR. The point estimates of specificities from each study are shown as circles. Error bars indicate 95% confidence intervals.

Duyarlılık: %52,9-100

Özgüllük: %78-100

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doi: 10.5578/mb.10339

Çok İlaca Dirençli Tüberkülozun Hızlı Tespiti İçin Dolaylı Nitrat Redüktaz Testinin Çok Merkezli Değerlendirilmesi*

Multicenter Evaluation of the Indirect Nitrate Reductase Assay for the Rapid Detection of Multidrug-Resistant Tuberculosis

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Tablo I. Çalışmaya Katılan Dokuz Merkezde Test Edilen İzolatların Referans Yöntemlerle Direnç Durumları

Merkezler		INH-dirençli	RIF-dirençli	ÇİD	Duyarlı	Toplam suş sayısı
No	Kurum					
1	Ondokuz Mayıs Üniv. Tıp Fak. Tıbbi Mikrobiyoloji AD	12	0	30	51	93
2	İstanbul Üniv. İstanbul Tıp Fak. Tıbbi Mikrobiyoloji AD	-	-	9	10	19
3	Atatürk Göğüs Hastalıkları ve Göğüs Cerrahisi EAH Mikrobiyoloji Laboratuvarı	2	4	8	6	20
4	Dr. Suat Seren Göğüs Hastalıkları ve Göğüs Cerrahisi EAH Mikrobiyoloji Laboratuvarı	1	-	9	10	20
5	Gülhane Askeri Tıp Akademisi Tıbbi Mikrobiyoloji AD	-	-	5	15	20
6	Celal Bayar Üniv. Tıp Fak. Tıbbi Mikrobiyoloji AD	-	1	9	10	20
7	Dokuz Eylül Üniv. Tıp Fak. Tıbbi Mikrobiyoloji AD	-	2	8	10	20
8	Türkiye Halk Sağlığı Kurumu Tüberküloz Referans Laboratuvarı	1	2	11	3	17
9	Hacettepe Üniv. Tıp Fak. Tıbbi Mikrobiyoloji AD	-	-	4	4	8
Toplam		16	9	93	119	237

AD: Anabilim Dalı; EAH: Eğitim ve Araştırma Hastanesi; INH: İzoniazid; RIF: Rifampisin; ÇİD: Çok ilaca dirençli (hem RIF hem INH'ye dirençli).

Tablo II. Referans Yöntem ile NRT Sonuçlarının Karşılaştırılması

İlaçlar	NRT sonuçları	Referans yöntem		Duyarlılık (%)	Özgüllük (%)	PPD (%)	NPD (%)	Uyum (%)
		R	S					
INH	R	104	8	95.4	93.7	92.8	96	94.5
	S	5	120					
RIF	R	101	3	99	97.8	97.1	99.2	98.3
	S	1	132					

NRT: Nitrat redüktaz testi, INH: İzoniazid; RIF: Rifampisin; PPD: Pozitif prediktif değer; NPD: Negatif prediktif değer; R: Dirençli; S: Duyarlı.

KRİM

- **Kolorimetrik redoks indikatör metotları (KRİM)**
  - ***Alamar mavisi ile duyarlılık testi***
  - ***Rezasurin ile duyarlılık testi***
    - Mikrodilüsyon yöntemi ile
    - Tüpte kritik konsantrasyon kullanılarak
  - ***Tetrazolium tuzları ile duyarlılık testi***
    - MTT
    - XTT
  - ***Malaşit yeşili renksizleştirme testi (MYRT)***
  - ***Kristal viyole renksizleştirme testi (KVRT)***

Alamar mavisi kullanılarak duyarlılık testi

- Duyarlılık testi için kullanılan ilk redoks indikatörlerden biri
- Okside durumda mavi renkli
- Bakteriyel metabolizma sonucu redükte olunca pembe renge dönüyor
- Renk ayrımı çıplak gözle yapılabiliyor

- Çalışmalarda;
  - INH için;
    - Duyarlılık %94-100
    - Özgüllük %88-100
  - RIF için;
    - Duyarlılık %89-100
    - Özgüllük %97-100
  - EMB için;
    - Duyarlılık %95.8
    - Özgüllük %91.7
  - STR için;
    - Duyarlılık %91.2
    - Özgüllük %97.8

Rezasurin ile duyarlılık testi

- 2000'li yılların başında;
- Nükleik manyetik rezonans ve
- Kütle spektrometre analizleri ile
- Alamar mavisi ve rezasurinin aynı madde olduğu anlaşıldı

- Rezasurin;
  - Hücre canlılığı ve
  - Bakteriyel kontaminasyonu analiz etmek için kullanılmakta
  - Okside durumda floresans vermeyen mavi bir renk
  - Redükte olunca güçlü floresans veren kırmızı rezorufine döner
  - Renk dönüşümü çıplak gözle kolayca ayrılabilir
  - İndirekt duyarlılık testi olarak üremiş kültürlerde çalışılmaktadır

Rezasurin mikrodilüsyon yöntemi ile duyarlılık testleri

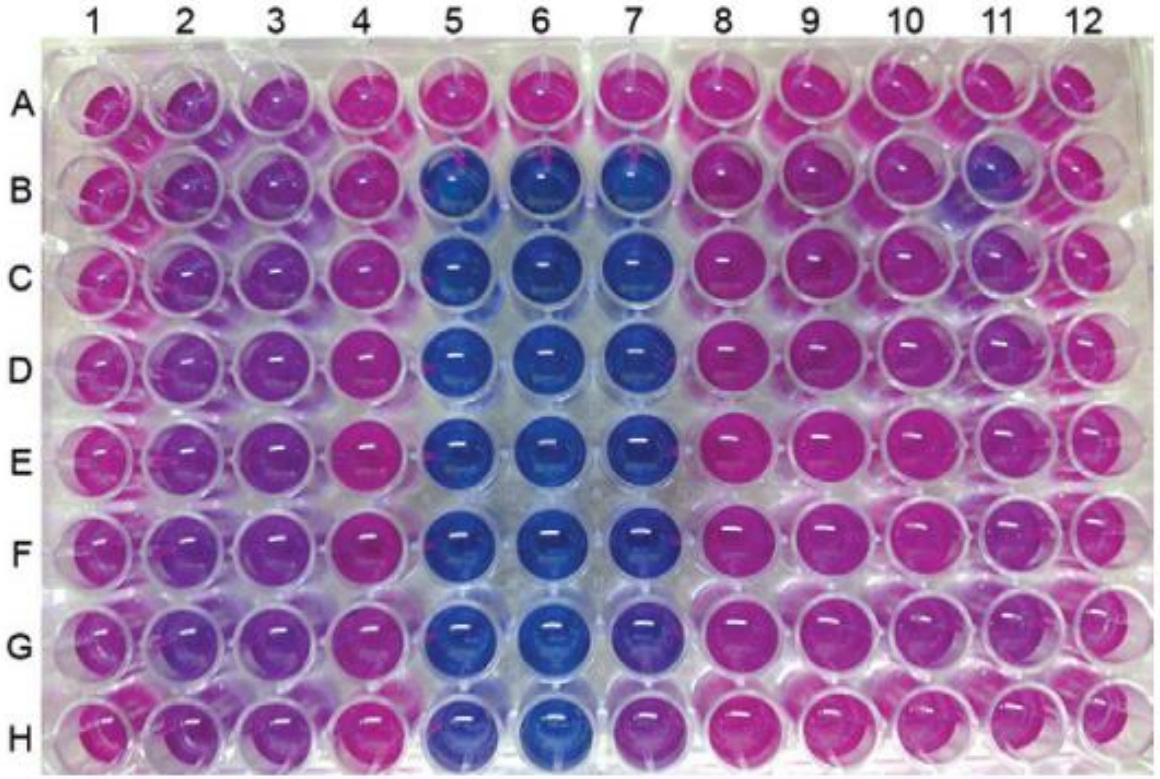
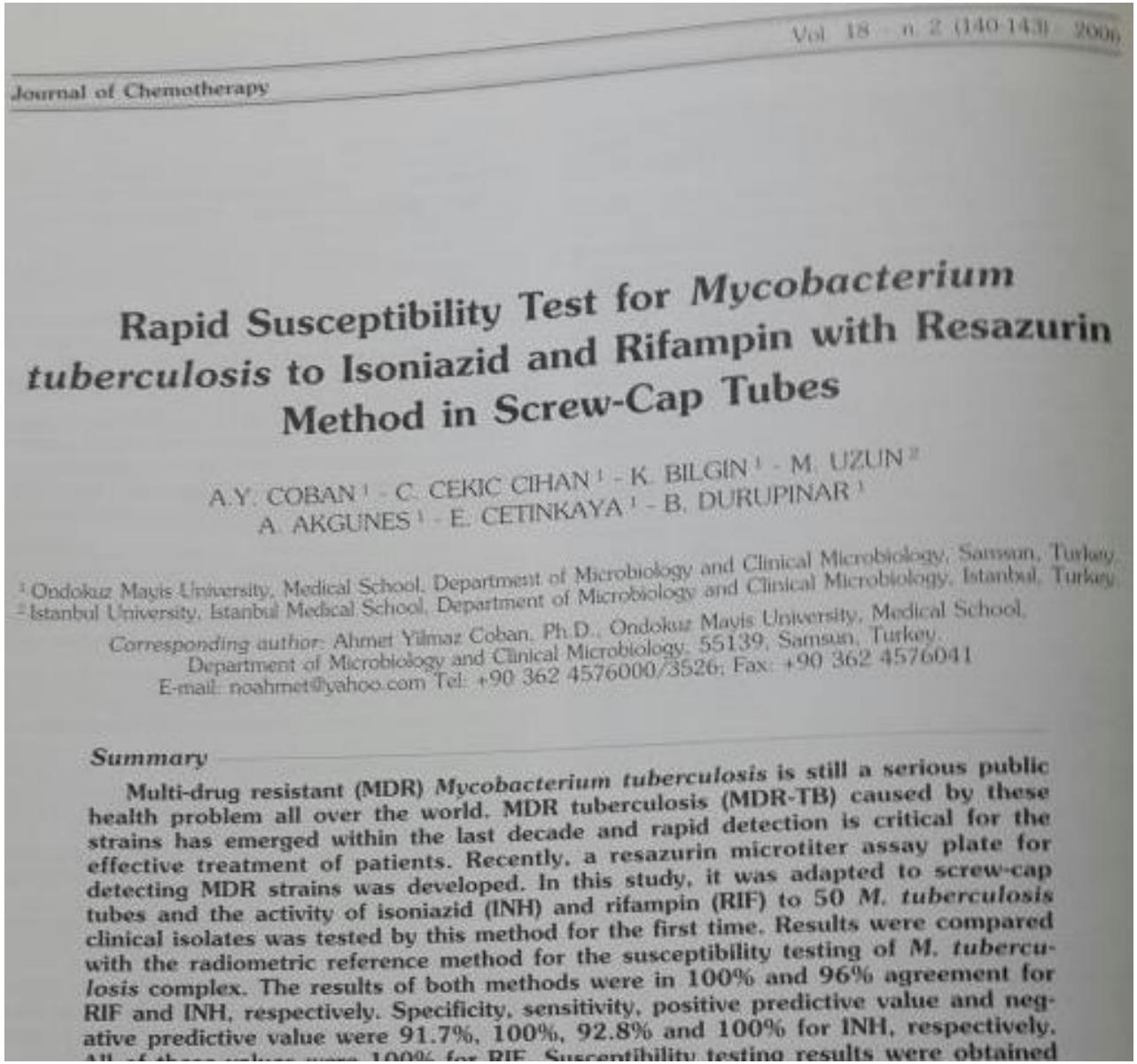


Fig. 1: assessment of rezasurin microtitre assay (REMA) results. A1-12: positive control wells; B1-12: 1.0 µg/mL for isoniazid (INH) and 2.0 µg/mL for rifampicin (RIF): C1-12: 0.5 µg/mL for INH and 1.0 µg/mL for RIF: D1-12: 0.25 µg/mL for INH and 0.5 µg/mL for RIF; E1-12: 0.125 µg/mL for INH and 0.25 µg/mL for RIF; F1-12: 0.06 µg/mL for INH and 0.125 µg/mL for RIF; G1-12: 0.03 µg/mL for INH and 0.06 µg/mL for RIF; H1-12: 0.01 µg/mL for INH and 0.03 µg/mL for RIF.

Tüpte kritik konsantrasyon kullanılarak



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Rapid Susceptibility Test for *Mycobacterium tuberculosis* to Isoniazid and Rifampin with Resazurin Method in Screw-Cap Tubes

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Summary

Multi-drug resistant (MDR) *Mycobacterium tuberculosis* is still a serious public health problem all over the world. MDR tuberculosis (MDR-TB) caused by these strains has emerged within the last decade and rapid detection is critical for the effective treatment of patients. Recently, a resazurin microtiter assay plate for detecting MDR strains was developed. In this study, it was adapted to screw-cap tubes and the activity of isoniazid (INH) and rifampin (RIF) to 50 *M. tuberculosis* clinical isolates was tested by this method for the first time. Results were compared with the radiometric reference method for the susceptibility testing of *M. tuberculosis* complex. The results of both methods were in 100% and 96% agreement for RIF and INH, respectively. Specificity, sensitivity, positive predictive value and negative predictive value were 91.7%, 100%, 92.8% and 100% for INH, respectively. All of these values were 100% for RIF. Susceptibility testing results were obtained

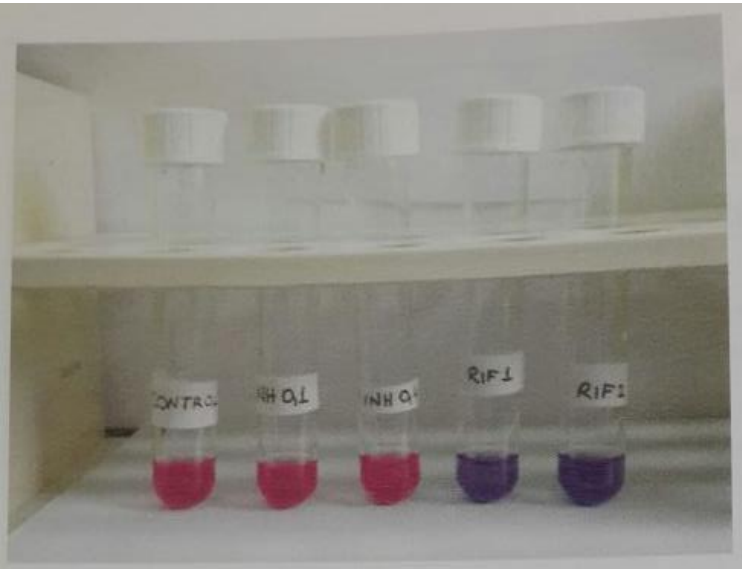

FIGURE 1C - A strain resistant to INH but susceptible to RIF.

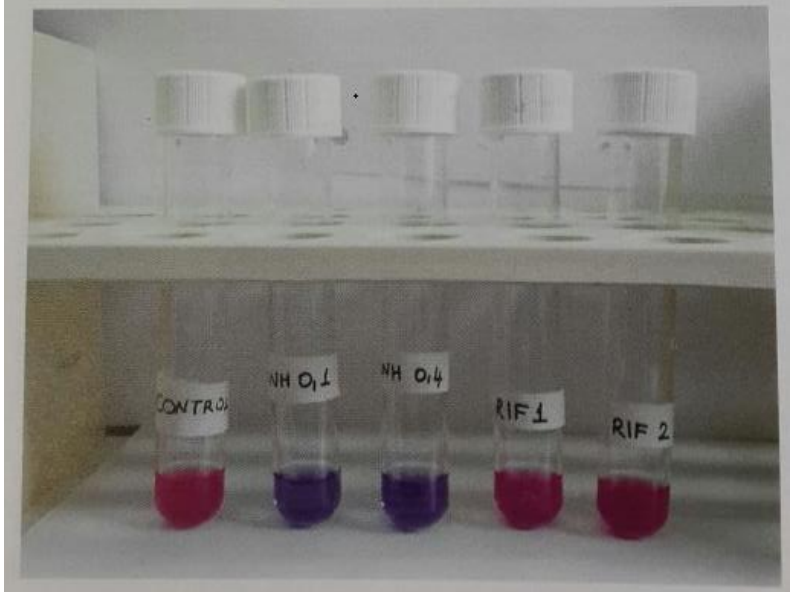

FIGURE 1D - A strain susceptible to INH but resistant to RIF.

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Review

Resazurin microtiter assay for isoniazid, rifampicin, ethambutol and streptomycin resistance detection in *Mycobacterium tuberculosis*: Updated meta-analysis

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ABSTRACT

Aims: The present meta-analysis aims to assess the evidence regarding the diagnostic accuracy and performance characteristics of the colorimetric redox indicator (CRI) assay with a special emphasis on the use of the resazurin microtiter assay (REMA) for determination of primary anti-tuberculosis drug resistance.

Subject and methods: By updating previous literature searches in Medline PubMed, ISI Web, Web of Science and Google academic databases of the REMA test for determination of primary anti-tuberculosis drug resistance, this meta-analysis includes 14 studies for isoniazid (INH); 15 studies for rifampicin (RIF); 6 studies for streptomycin (STR); and 5 studies for ethambutol (EMB). SROC curve analysis was performed for meta-analysis and diagnostic accuracy was summarized.

Results: Pooled sensitivity was 96% (94–98%) for INH, 97% (95–98%) for RIF, 92% (87–96%) for EMB and 92% (88–95%) for STR. Pooled specificity for INH, RIF, EMB and STR was 96% (95–98%), 99% (98–99%), 86% (81–89%) and 90% (87–93%), respectively. Susceptibility testing results had been obtained in 8–9 days.

Conclusion: In conclusion, REMA seems to be a reliable test for the determination of multidrug resistant (MDR) isolates in laboratories with limited resources. However, few studies for STR and EMB have been found, and cost-effectiveness studies need to be determined to recommend its widespread use.

Table 1 – Description of studies included in the meta-analysis of INH resistance detection.

References	Countries	Reference test	No. isolates	Sensitivity (95% CI)	Specificity (95% CI)	TTP (day)
Dixit et al. [12]	India	PM on LJ	105	0.93 (0.82–0.98)	0.98 (0.89–1.00)	8
Bwanga et al. [14]	Uganda/Sweden	PM on LJ	31	0.88 (0.64–0.99)	0.57 (0.29–0.82)	8
Montoro et al. [15]	Cuba/Belgium	PM on LJ	100	1.00 (0.92–1.00)	0.96 (0.87–1.00)	8
Palomino et al. [16]	Belgium/Bolivia/Peru	PM on LJ	80	1.00 (0.93–1.00)	0.96 (0.80–1.00)	8
Martin et al. [10]						
Site 1	Belgium	PM on LJ	30	1.00 (0.74–1.00)	1.00 (0.81–1.00)	8
Site 2	Argentina	PM on LJ	30	1.00 (0.74–1.00)	1.00 (0.81–1.00)	8
Site 3	Cuba	PM on LJ	30	1.00 (0.74–1.00)	0.94 (0.73–1.00)	8
Site 4	Brazil	PM on LJ	30	1.00 (0.74–1.00)	0.94 (0.73–1.00)	8
Site 5	Colombia	PM on LJ	30	1.00 (0.69–1.00)	1.00 (0.75–1.00)	8
Site 6	Chile	PM on LJ	30	0.92 (0.62–1.00)	1.00 (0.81–1.00)	8
Site 7	Nicaragua	PM on LJ	30	0.92 (0.62–1.00)	0.94 (0.73–1.00)	8
Rivoire et al. [17]	Madagascar/Belgium	PM on LJ	77	0.95 (0.83–0.99)	0.97 (0.86–1.00)	8
Affolabi et al. [18]	Benin/Belgium/France	PM on LJ	151	1.00 (0.85–1.00)	1.00 (0.97–1.00)	8
Rondon et al. [19]	Venezuela/USA	PM on 7H10 agar	155	0.79 (0.66–0.89)	0.96 (0.90–0.99)	8
Martin et al. [20]	Belgium/Argentina/ Colombia/Sweden	PM on LJ	149	0.99 (0.95–1.00)	1.00 (0.88–1.00)	8
Coban et al. [11]	Turkey	Bactec 460 TB	50	1.00 (0.87–1.00)	0.92 (0.73–0.99)	8–9
Iglesia et al. [21]	Argentina	PM on 7H11 agar	69	1.00 (0.85–1.00)	0.94 (0.82–0.99)	8
Coban et al. [22]	Turkey	Bactec 460 TB/MGIT 960	73	1.00 (0.89–1.00)	0.90 (0.77–0.97)	8
Nateche et al. [23]	Algeria/Belgium	PM on LJ	136	1.00 (0.80–1.00)	0.98 (0.94–1.00)	8
Banfi et al. [24]	Italy	PM on 7H11 agar	13	1.00 (0.48–1.00)	1.00 (0.63–1.00)	8

TTP: Time to positivity.

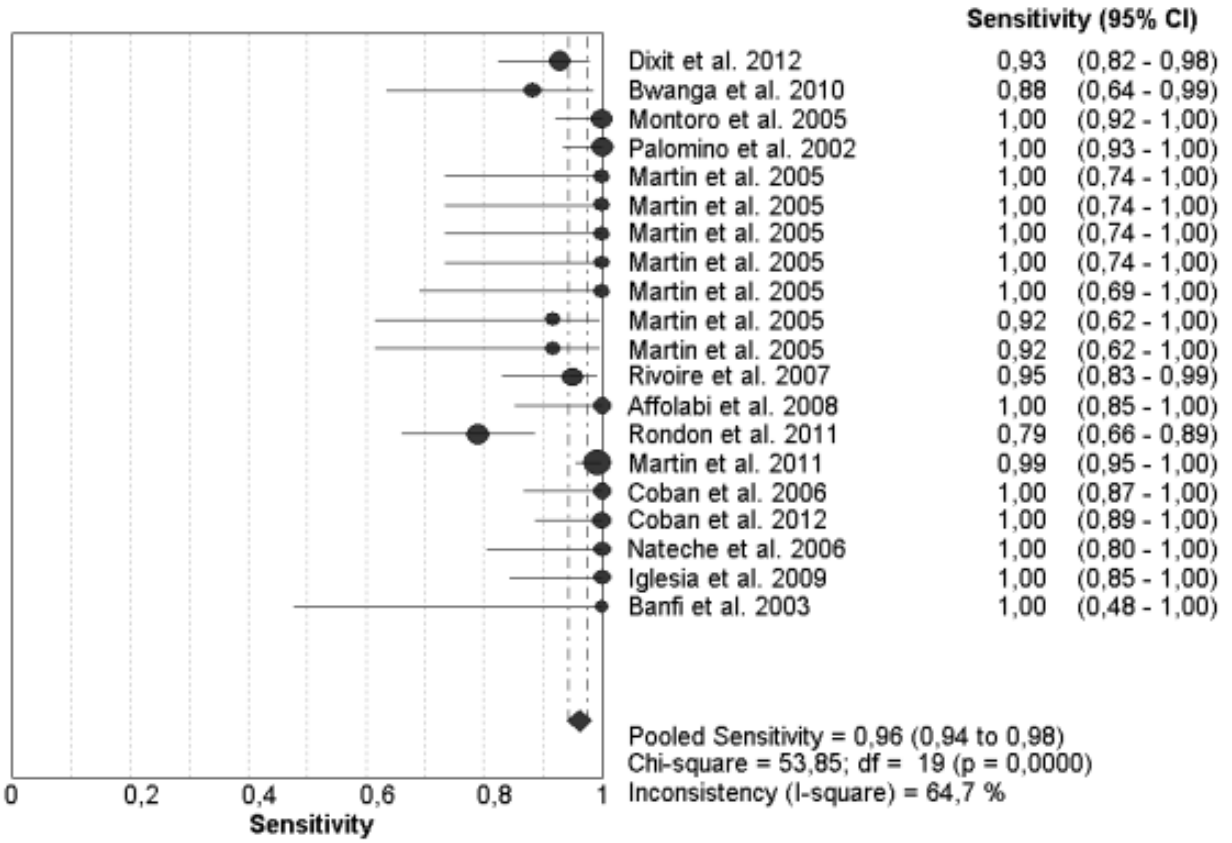
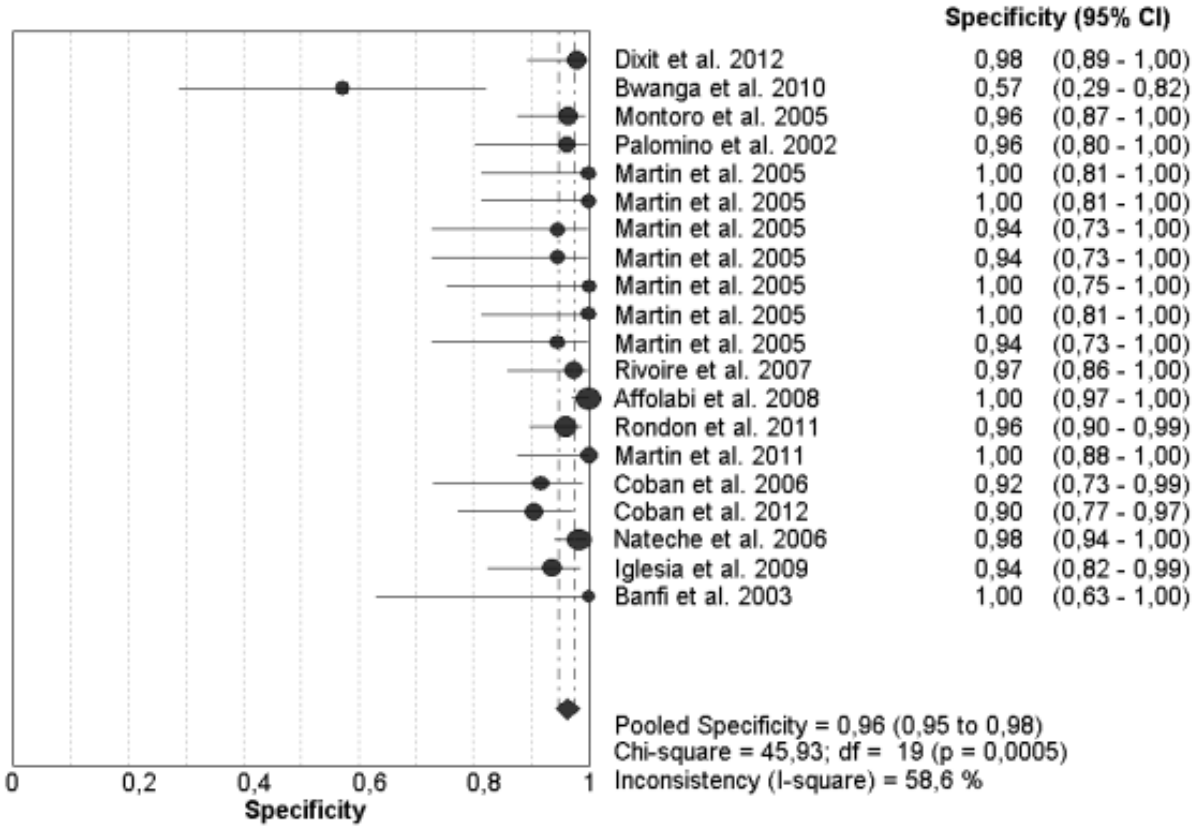


Figure 1. Forest plot of the sensitivity for INH assay. The point estimates of sensitivity from each study are shown as circles. Error bars 95% confidence intervals.

Duyarlılık: %88-100



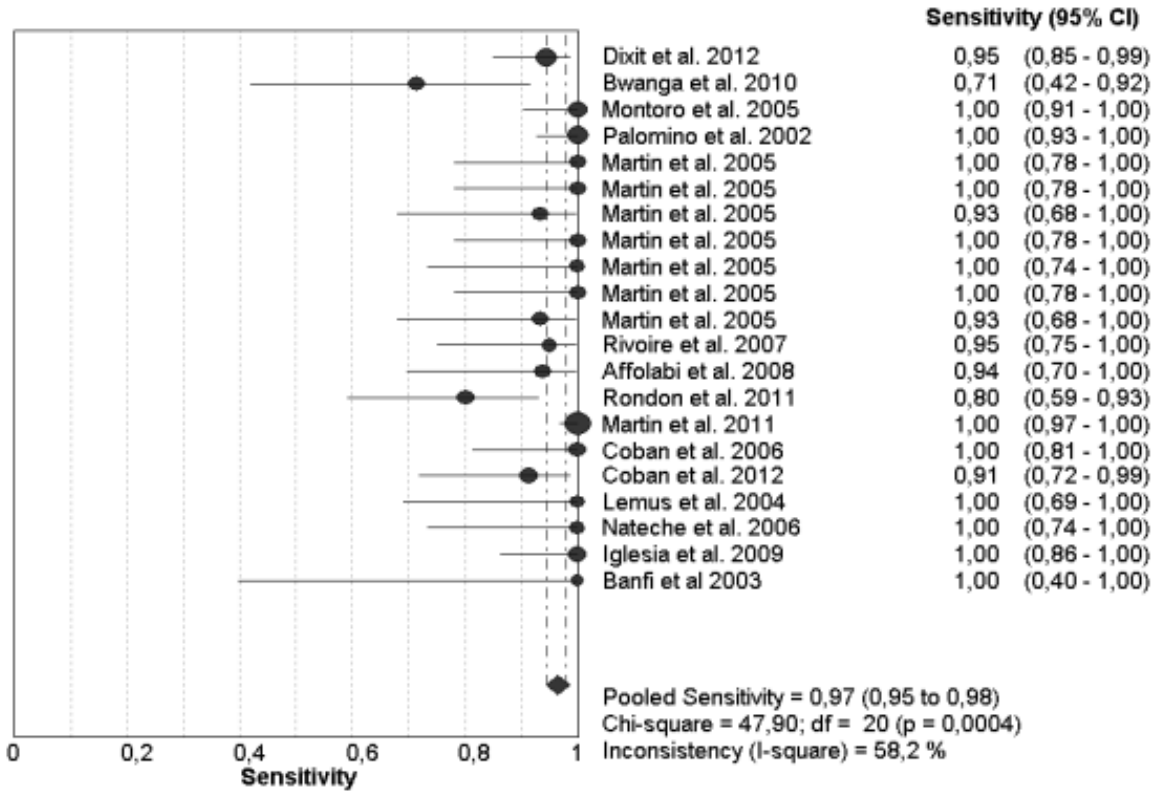
Özgüllük: %57-100

Figure 2. Forest plot of the specificity for INH assay. The point estimates of specificity from each study are shown as circles. Error bars 95% confidence intervals.

Table 2 – Description of studies included in the meta-analysis of RIF resistance detection.

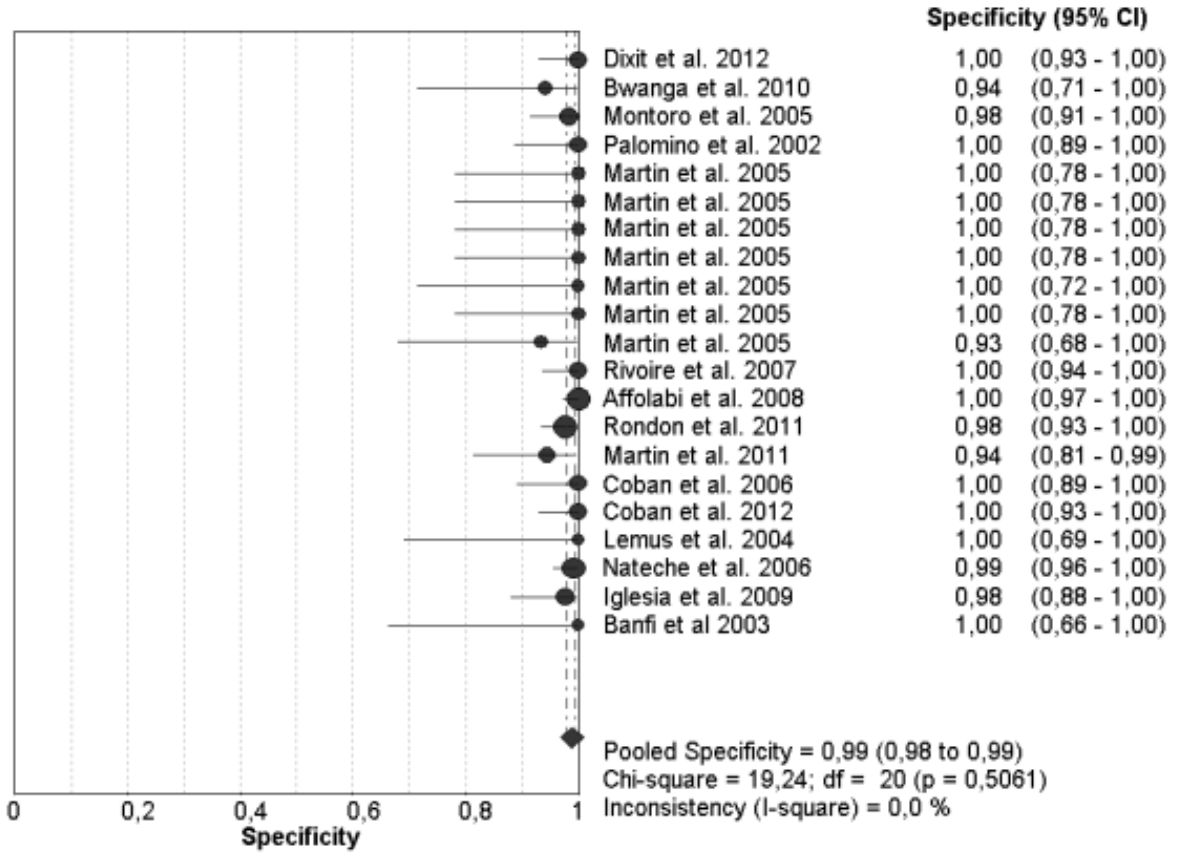
References	Countries	Reference test	No. isolates	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Dixit et al. [12]	India	PM on LJ	105	0.95 (0.85–0.99)	1.00 (0.93–1.00)	8
Bwanga et al. [14]	Uganda/Sweden	PM on LJ	31	0.71 (0.42–0.92)	0.94 (0.71–1.00)	8
Montoro et al. [15]	Cuba/Belgium	PM on LJ	100	1.00 (0.91–1.00)	0.98 (0.91–1.00)	8
Palomino et al. [16]	Belgium/Bolivia/Peru	PM on LJ	80	1.00 (0.93–1.00)	1.00 (0.89–1.00)	8
Martin et al. [10]						
Site 1	Belgium	PM on LJ	30	1.00 (0.78–1.00)	1.00 (0.78–1.00)	8
Site 2	Argentina	PM on LJ	30	1.00 (0.78–1.00)	1.00 (0.78–1.00)	8
Site 3	Cuba	PM on LJ	30	0.93 (0.68–1.00)	1.00 (0.78–1.00)	8
Site 4	Brazil	PM on LJ	30	1.00 (0.78–1.00)	1.00 (0.78–1.00)	8
Site 5	Colombia	PM on LJ	30	1.00 (0.74–1.00)	1.00 (0.72–1.00)	8
Site 6	Chile	PM on LJ	30	1.00 (0.78–1.00)	1.00 (0.78–1.00)	8
Site 7	Nicaragua	PM on LJ	30	0.93 (0.68–1.00)	0.93 (0.68–1.00)	8
Rivoire et al. [17]	Madagascar/Belgium	PM on LJ	77	0.95 (0.75–1.00)	1.00 (0.94–1.00)	8
Affolabi et al. [18]	Benin/Belgium/France	PM on LJ	151	0.94 (0.70–1.00)	1.00 (0.97–1.00)	8
Rondon et al. [19]	Venezuella/USA	PM on 7H10 agar	155	0.80 (0.59–0.93)	0.98 (0.93–1.00)	8
Martin et al. [20]	Belgium/Argentina/ Colombia/Sweden	PM on LJ	149	1.00 (0.97–1.00)	0.94 (0.81–0.99)	8
Coban et al. [11]	Turkey	Bactec 460 TB	50	1.00 (0.81–1.00)	1.00 (0.89–1.00)	8–9
Iglesia et al. [21]	Argentina	PM on 7H11 agar	69	1.00 (0.86–1.00)	0.98 (0.88–1.00)	8
Coban et al. [22]	Turkey	Bactec 460 TB/MGIT 960	73	0.91 (0.72–0.99)	1.00 (0.93–1.00)	8
Nateche et al. [23]	Algeria/Belgium	PM on LJ	136	1.00 (0.74–1.00)	0.99 (0.96–1.00)	8
Banfi et al. [24]	Italy	PM on 7H11 agar	13	1.00 (0,39–1.00)	1.00 (0.66–1.00)	9
Lemus et al. [25]	Cuba/Belgium	PM on LJ	20	1.00 (0.69–1.00)	1.00 (0.69–1.00)	8

TTP: Time to positivity.



Duyarlılık: %71-100

Figure 4. Forest plot of the sensitivity for RIF analysis. The point estimates of sensitivity from each study are shown as circles. Error bars 95% confidence intervals.



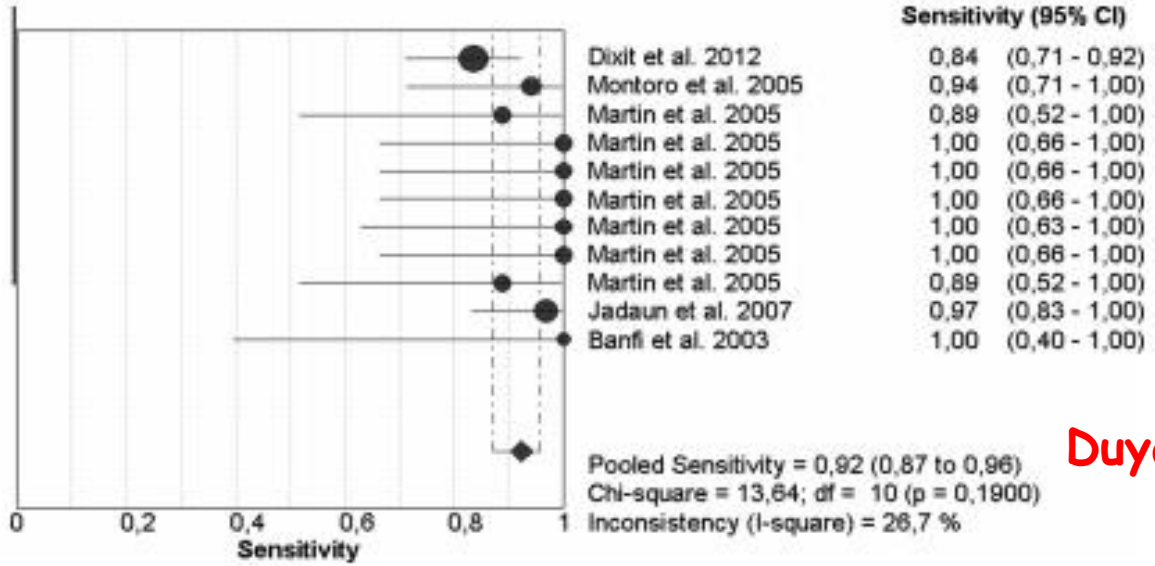
Özgüllük: %93-100

Figure 5. Forest plot of the specificity for RIF analysis. The point estimates of specificity from each study are shown as circles. Error bars 95% confidence intervals.

Table 3 – Description of studies included in the meta-analysis of EMB resistance detection.

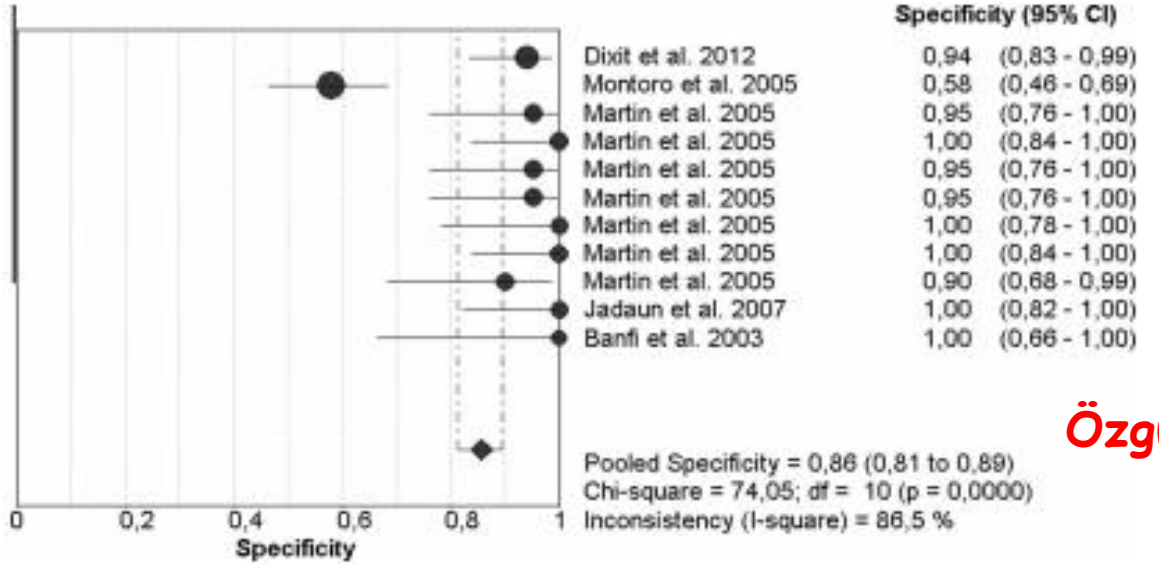
References	Countries	Reference test	No. isolates	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Dixit et al. [12]	India	PM on LJ	105	0.84 (0.71–0.92)	0.94 (0.83–0.99)	8
Montoro et al. [15]	Cuba/Belgium	PM on LJ	100	0.94 (0.71–1.00)	0.58 (0.46–0.69)	8
Martin et al. [10]						
Site 1	Belgium	PM on LJ	30	0.89 (0.52–1.00)	0.95 (0.76–1.00)	8
Site 2	Argentina	PM on LJ	30	1.00 (0.66–1.00)	1.00 (0.84–1.00)	8
Site 3	Cuba	PM on LJ	30	1.00 (0.66–1.00)	0.95 (0.76–1.00)	8
Site 4	Brazil	PM on LJ	30	1.00 (0.66–1.00)	0.95 (0.76–1.00)	8
Site 5	Colombia	PM on LJ	30	1.00 (0.63–1.00)	1.00 (0.78–1.00)	8
Site 6	Chile	PM on LJ	30	1.00 (0.66–1.00)	1.00 (0.84–1.00)	8
Site 7	Nicaragua	PM on LJ	30	0.89 (0.52–1.00)	0.90 (0.68–0.99)	8
Banfi et al. [24]	Italy	PM on 7H11 agar	13	1.00 (0.39–1.00)	1.00 (0.66–1.00)	9
Jadaun et al. [26]	India	PM on LJ	50	0.97 (0.83–1.00)	1.00 (0.82–1.00)	8–9

TTP: Time to positivity.



Duyarlılık: %75-100

Fig. 7 – Forest plot of the sensitivity for EMB. The point estimates of sensitivity from each study are shown as circles. Error bars 95% confidence intervals.



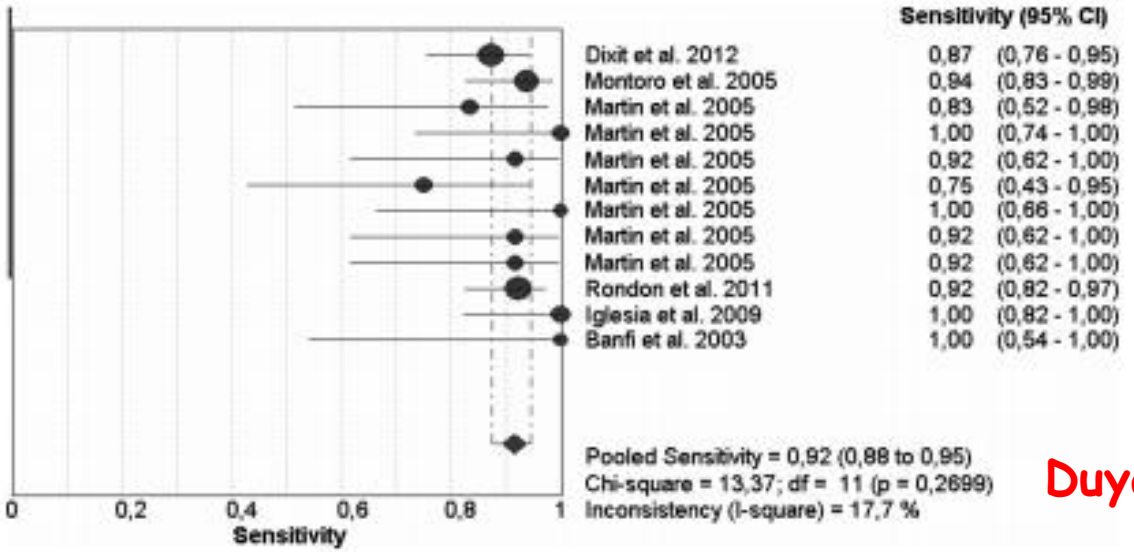
Özgüllük: %57.8-100

Fig. 8 – Forest plot of the specificity for EMB. The point estimates of specificity from each study are shown as circles. Error bars 95% confidence intervals.

Table 4 – Description of studies included in the meta-analysis of STR resistance detection.

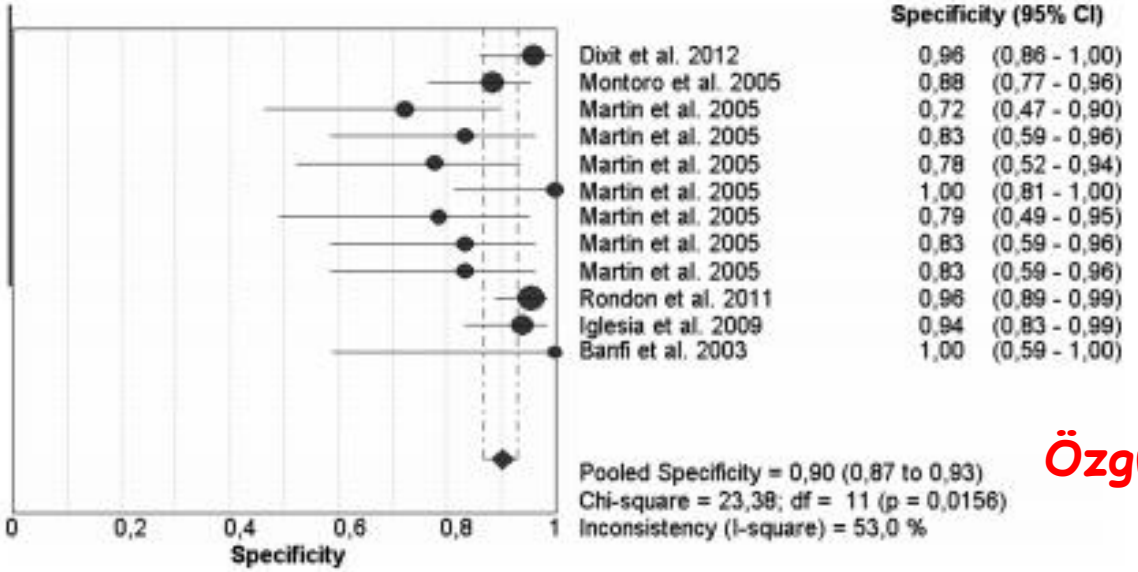
References	Countries	Reference test	No. isolates	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Dixit et al. [12]	India	PM on LJ	105	0.87 (0.76–0.95)	0.96 (0.86–1.00)	8
Montoro et al. [15]	Cuba/Belgium	PM on LJ	100	0.94 (0.83–0.99)	0.88 (0.77–0.96)	8
Martin et al. [10]						
Site 1	Belgium	PM on LJ	30	0.83 (0.52–0.98)	0.72 (0.47–0.90)	8
Site 2	Argentina	PM on LJ	30	1.00 (0.74–1.00)	0.83 (0.59–0.96)	8
Site 3	Cuba	PM on LJ	30	0.92 (0.62–1.00)	0.78 (0.52–0.94)	8
Site 4	Brazil	PM on LJ	30	0.75 (0.43–0.95)	1.00 (0.81–1.00)	8
Site 5	Colombia	PM on LJ	30	1.00 (0.66–1.00)	0.79 (0.49–0.95)	8
Site 6	Chile	PM on LJ	30	0.92 (0.62–1.00)	0.83 (0.59–0.96)	8
Site 7	Nicaragua	PM on LJ	30	0.92 (0.62–1.00)	0.83 (0.59–0.96)	8
Rondón et al. [19]	Venezuella/USA	PM on 7H10 agar	155	0.92 (0.82–0.97)	0.96 (0.89–0.99)	8
Iglesia et al. [21]	Argentina	PM on 7H11 agar	69	1.00 (0.82–1.00)	0.94 (0.83–0.99)	8
Banfi et al. [24]	Italy	PM on 7H11 agar	13	1.00 (0.54–1.00)	1.00 (0.59–1.00)	9

TTP: Time to positivity.



Duyarlılık: %75-100

Fig. 10 – Forest plot of the sensitivity for STR. The point estimates of sensitivity from each study are shown as circles. Error bars 95% confidence intervals.



Özgüllük: %72.2-100

Fig. 11 – Forest plot of the specificity for STR. The point estimates of specificity from each study are shown as circles. Error bars 95% confidence intervals.

- PZA;
  - Duyarlılık: %97-100
  - Özgüllük: %97.7-100
- İkinci kuşak antitüberküloz ilaçlar için de uygun olduğu raporlanmış

Tetrazolium tuzları kullanılarak duyarlılık testleri

- **MTT** (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium bromide);
  - Okside durumda sarı renkli
  - Metabolik aktif hücrelerin dehidrogenazı ile redüklenince,
    - Çözülmeyen mor MTT formazon kristelleri oluşuyor
    - Çözüldükten sonra 570nm'de ölçülebiliyor
      (Çözücü SDS ile dimetilformamid karışımı)
    - Çıplak gözle de sarı renkten mor renge dönüşüm gözlenerek te test sonuçlandırılabiliyor

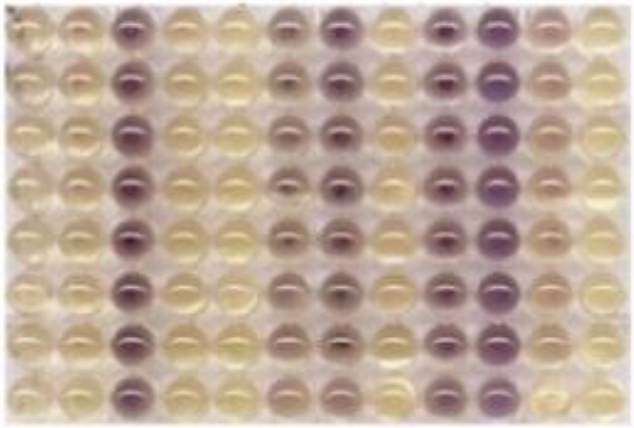
- 7 günlük inkübasyon sonrası
  - 10 µl MTT (5 g/L) solüsyonu eklenir
  - Bir gece inkübasyon
  - Mor formazon kristalleri var ise 50 µl SDS/DMF eklenir
  - 3 saat inkübasyon sarı renkten mor renge dönüşüm bakteriyel üremeyi gösterir

- Çalışmalarda;
  - INH için;
    - Duyarlılık %77-100
    - Özgüllük %86-100
  - RIF için;
    - Duyarlılık %71-100
    - Özgüllük %93.2-100
  - İndirekt duyarlılık test sonuçları 4-17 günde elde edilmiş
  - Direkt test 69 balgam örneğinde RIF için bir çalışmada uygulanmış
    - Sonuçlar 7-21 günde elde edilmiş
    - Uyum %100

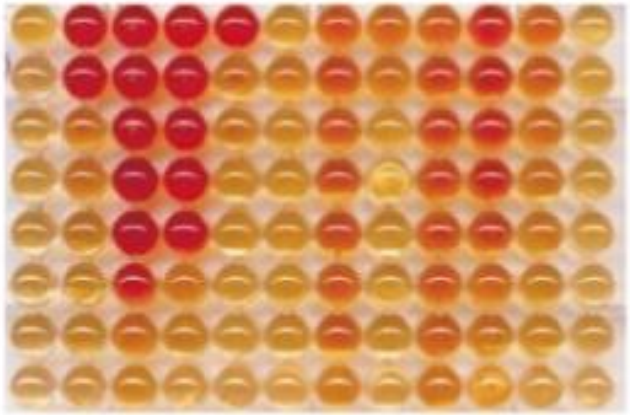
- PZA;
  - Duyarlılık: %97
  - Özgüllük: %99.6
- EMB;
  - Duyarlılık: %80-100
  - Özgüllük: %71.1-100
- STR;
  - Duyarlılık: %75-100
  - Özgüllük: %78.6-100
- İkinci kuşak ilaçlar;
  - Duyarlılık: %85.7-100
  - Özgüllük: %90-100

- **XTT** (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)car-bonyl]-2H-tetrazoliumhydroxide);
  - Redükte olunca suda çözünen formazon oluşuyor
  - Suda çözünmesi ilave işlem gerektirmeden değerlendirmeyi sağlıyor
  - Literatürde 3 çalışma mevcut
  - INH ve RIF için uyum %100
  - Sonuçlar;
    - RIF için 3-6 günde
    - INH için 8 günde elde edilmiş
  - MTT'ye üstünlüğü;
    - Oluşan formazon kristallerinin suda çözünür olması
    - İlave işlem gerektirmez

Example: MTT and XTT



MTT



XTT

Malaşit yeşili renksizleştirme testi (MYRT)

- Malaşit yeşili bir trifenilmetan boyasıdır
  - Memeli hücrelerine toksiktir
  - Antimikrobiyal etkisi vardır
  - Antifungal özelliği de vardır
  - Mikobakteriler dirençlidir

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Colorimetric Detection of Multidrug-Resistant or Extensively Drug-Resistant Tuberculosis by Use of Malachite Green Indicator Dye▽

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The malachite green microtube (MGMT) susceptibility assay was performed directly on sputum specimens ($n = 80$) and indirectly on *Mycobacterium tuberculosis* clinical isolates ($n = 60$). The technique is based on the malachite green dye, which changes color in response to *M. tuberculosis* growth. The MGMT assay is simple and rapid and does not require expensive instruments.

Recent advances in technology have introduced many rapid and reliable methods to differentiate between susceptible and resistant *Mycobacterium tuberculosis* strains (7, 10, 12, 16). However, due to their high cost and equipment requirement, these new methods are not feasible in the clinical laboratories of developing countries in the diagnosis of tuberculosis (TB). Instead, these countries use the proportional method, which is very time-consuming (2, 11). Consequently, physicians base their diagnosis of TB on microscopy results. Therefore, supplementary rapid and reliable methods are highly needed for clinical laboratories with limited resources. In 1995, Yajko et al. (15) used an oxidation-reduction indicator, Alamar blue, which changes color in response to the growing bacteria. In 2004, we demonstrated the viability of *M. tuberculosis* in sputum

accuracy and feasibility of the MGMT assay were compared to those of the standard LJ culture method for drug susceptibility testing.

Sputum specimens were digested and decontaminated by the Petroff method with a 2% final concentration of NaOH. Following digestion for 15 min, the samples were centrifuged at 3,000 × *g* for 30 min and decanted, leaving 1 to 2 ml of sediment (2, 11). The remaining sediments were reconstituted in 2 ml of sterile phosphate-buffered saline. Two hundred microliters of this suspension was inoculated onto each LJ culture slant, and 100 μl was inoculated into each microtube containing100 μl of $7H_9$GC broth with or without added drugs (4). All cultures were incubated at 37°C for up to 8 weeks. The

FIG. 1. In response to bacterial growth, the malachite green indicator changes in color from green to the total loss of color.

(4). All cultures were incubated at 37°C for up to 8 weeks. The drugs and their final concentrations in the malachite green microtubes were as follows: isonizid (INH), 0.2 μg/ml; streptomycin,10 μg/ml; ethambutol, 2 μg/ml; rifampin (RF), 40 μg/ml; caperomycin, 10 μg/ml; ciprofloxacin, 2 μg/ml; cycloserine, 30 μg/ml; ethionamide, 20 μg/ml; kanamycin, 20 μg/ml; and ofloxacin, 2 μg/ml (14). The drug concentrations were chosen according to the protocols of the World Health Organization (14) and Franzblau et al. (5). As the length of incubation needed for sufficient metabolic activity to occur can vary from strain to strain, three control microtubes were included in each test run. The samples were tested after 7, 14, and 21 days of incubation by adding 50 μl of a 0.02-μg/ml solution of malachite green (Merck, Germany) to the control tubes and documenting whether a color change occurred. If the green color disappeared (tube contents

TABLE 2. Comparison of malachite green microtube assay results with proportional method results (60 stock cultures)

Drug[a]	No. of strains with the following result[b]:				% Agreement in isolated strains	Sensitivity (%)[c]	Specificity (%)[d]
	Susceptible by both	Resistant by both	Resistant by MG, sensitive by LJ	Sensitive by MG, resistant by LJ			
SM	25	29	3	4	90	87	89
INH	30	30	0	0	100	100	100
RF	30	30	0	0	100	100	100
ETB	24	28	5	3	86	90	82
CIP	53	7	0	0	100	100	100
DC	55	3	1	1	96	75	98
OF	53	3	3		93	75	94
CAP	55	3	1	1	96.6	75	98
ETH	49	3	6	2	86	60	89
PAZ	55	3	1	1	96	75	98
KAN	54	4	1	1	96	80	98
AM	51	3	3	3	90	50	94

[a] Abbreviations: AM, ampicillin; CAP, caperomycin; CIP, ciprofloxacin; DC, doxycycline; ETB, ethambutol; ETH, erythromycin; INH, isonizid; KAN, kanamycin; OF, ofloxacin; PAZ, pyrazinamide; RF, rifampin; SM, streptomycin.

[b] MG, malachite green microtube susceptibility assay; LJ, Löwenstein-Jensen culture medium.

[c] The average sensitivity for all drugs studied was 80.5%.

[d] The average specificy for all drugs studied was 95%.

Rapid detection of multidrug-resistant *Mycobacterium tuberculosis* using the malachite green decolourisation assay

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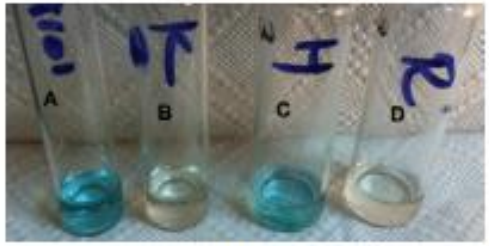
Early detection of drug resistance in Mycobacterium tuberculosis *isolates allows for earlier and more effective treatment of patients. The aim of this study was to investigate the performance of the malachite green decolourisation assay (MGDA) in detecting isoniazid (INH) and rifampicin (RIF) resistance in* M. tuberculosis *clinical isolates. Fifty* M. tuberculosis *isolates, including 19 multidrug-resistant, eight INH-resistant and 23 INH and RIF-susceptible samples, were tested. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and agreement of the assay for INH were 92.5%, 91.3%, 92.5%, 91.3% and 92%, respectively. Similarly, the sensitivity, specificity, PPV, NPV and agreement of the assay for RIF were 94.7%, 100%, 100%, 96.8% and 98%, respectively. There was a major discrepancy in the tests of two isolates, as they were sensitive to INH by the MGDA test, but resistant by the reference method. There was a minor discrepancy in the tests of two additional isolates, as they were sensitive to INH by the reference method, but resistant by the MGDA test. The drug susceptibility test results were obtained within eight-nine days. In conclusion, the MGDA test is a reliable and accurate method for the rapid detection of INH and RIF resistance compared with the reference method and the MGDA test additionally requires less time to obtain results.*

Key words: *Mycobacterium tuberculosis* - malachite green decolourisation assay - susceptibility testing - isoniazid - rifampicin



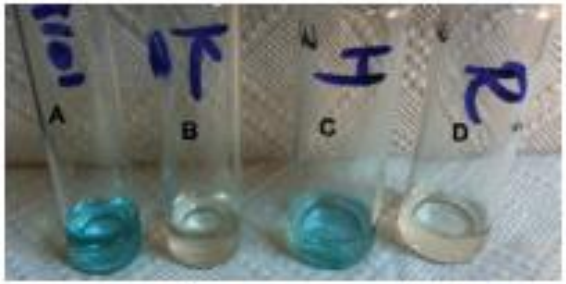
A: negative control (not contain drug and bacteria); B: positive growth control; C: isoniazid-susceptible (there is no growth, colour was not decolourised); D: rifampicin-resistant (decolourisation was observed due to bacterial growth).

Early detection of drug resistance in *Mycobacterium tuberculosis* isolates allows for appropriate and timely treatment (Martin et al. 2008). There are many well-defined phenotypic methods for testing the drug susceptibility of *M. tuberculosis*. The proportion method, performed on Löwenstein-Jensen and 7H10 or 7H11 agar media, is recommended as a reference method. However, this method requires at least three-six weeks to obtain results (Kent & Kubica 1985). There are also rapid automated systems for drug susceptibility testing. The ported that the colourimetric malachite green decolourisation assay (MGDA) could be used for the rapid detection of resistance.

The aim of this study was to investigate the performance of the MGDA test for the detection of isoniazid (INH) and rifampicin (RIF) resistance in *M. tuberculosis* clinical isolates.

MATERIALS AND METHODS

Bacterial isolates - Fifty *M. tuberculosis* isolates



A: negative control (not contain drug and bacteria); B: positive growth control; C: isoniazid-susceptible (there is no growth, colour was not decolourised); D: rifampicin-resistant (decolourisation was observed due to bacterial growth).

sequestration in the lipid fraction of the cells (Jones & Falkinham 2003).

Gelman et al. (2012) reported that MG interfered with the recovery of mycobacteria on solid culture media following exposure to certain antibiotics, including INH and ethionamide (ETM), that target cell wall biogenesis. This interference did not affect the test results for INH sensitivity because MG was used to determine the viability of bacteria in this test.

Farnia et al. (2008) reported that MGDA could be used for the rapid detection of drug susceptibilities of *M.*

TABLE

Comparing the results of malachite green decolourisation assay (MGDA) with those obtained with reference method

Drug	MGDA	Reference method (n)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Agreement (%)
		R	S					
INH	R	25	2	92.5	91.3	92.5	91.3	92
	S	2	21					
RIF	R	18	0	94.7	100	100	96.8	98
	S	1	31					

INH: isoniazid; NPV: negative predictive value; PPV: positive predictive value; R: resistant; RIF: rifampicin; S: susceptible.

Kristal viyole renksizleştirme testi

- Kristal viyole (Gentin viyole) bir trifenilmetan boyasıdır
  - Memeli hücrelerine toksiktir
  - Antimikrobiyal etkisi vadır
  - Antifungal özelliği vardır
  - Mikobakteriler dirençlidir
- Histolojik preparatlarda ve Gram boyamasında kullanılır
- Topikal antiseptiktir

A new rapid colourimetric method for testing *Mycobacterium tuberculosis* susceptibility to isoniazid and rifampicin: a crystal violet decolourisation assay

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The aim of this study was to investigate the performance of a new and accurate method for the detection of isoniazid (INH) and rifampicin (RIF) resistance among Mycobacterium tuberculosis *isolates using a crystal violet decolourisation assay (CVDA). Fifty-five* M. tuberculosis *isolates obtained from culture stocks stored at -80ºC were tested. After bacterial inoculation, the samples were incubated at 37ºC for seven days and 100 µL of CV (25 mg/L stock solution) was then added to the control and sample tubes. The tubes were incubated for an additional 24-48 h. CV (blue/purple) was decolourised in the presence of bacterial growth; thus, if CV lost its colour in a sample containing a drug, the tested isolate was reported as resistant. The sensitivity, specificity, positive predictive value, negative predictive value and agreement for INH were 92.5%, 96.4%, 96.1%, 93.1% and 94.5%, respectively, and 88.8%, 100%, 100%, 94.8% and 96.3%, respectively, for RIF. The results were obtained within eight-nine days. This study shows that CVDA is an effective method to detect* M. tuberculosis *resistance to INH and RIF in developing countries. This method is rapid, simple and inexpensive. Nonetheless, further studies are necessary before routine laboratory implementation.*

Key words: *Mycobacterium tuberculosis* - multidrug resistance - susceptibility testing - isoniazid - rifampicin - crystal violet decolourisation assay

Tuberculosis is a major public health problem, particularly in developing countries. *Mycobacterium tuberculosis* isolates that are resistant to at least rifampicin (RIF) and isoniazid (INH) are defined as multidrug resistant (MDR). As drug resistance is a serious problem for control programmes, there is a need for new, rapid and accurate drug susceptibility tests for the diagnosis of MDR tuberculosis isolates (Martin et al. 2007). Indeed, the early detection of drug resistance in *M. tuberculosis* iso-

non-radiometric method, but has a high cost, which is the main disadvantage (Martin et al. 2007).

Recently, inexpensive, rapid and reliable colourimetric methods have attracted increased interest; of these, the resazurin microplate method and the nitrate reductase assay are the most popular (Angeby et al. 2002, Palomino et al. 2002, Syre et al. 2003, Coban et al. 2004, Martin et al. 2005, Montoro et al. 2005, Bwanga et al. 2010, Dixit et al. 2012). Crystal violet (CV) is a triph-

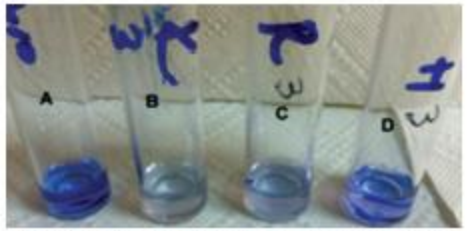

Fig. 1: blue/purple colour - there is no any bacterial growth. Decolourisation - there is bacterial growth. ATCC 35838 - rifampicin (RIF)-resistant standard strain. A: negative control tube without drug or bacteria; B: positive control tube with bacteria without drug; C: test tube with RIF (resistant to RIF); D: test tube with isoniazid (INH) (susceptible to INH).

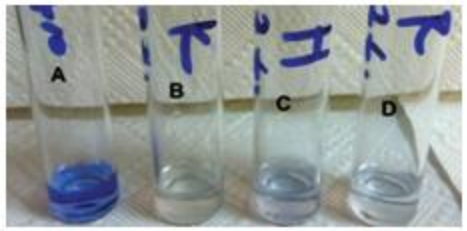

Fig. 2: blue/purple colour - there is no any bacterial growth. Decolourisation - there is bacterial growth. A clinical multidrug resistant isolate - resistant to isoniazid (INH) and rifampicin (RIF). A: negative control tube without drug or bacteria; B: positive control tube with bacteria without drug; C: test tube with INH (resistant to INH); D: test tube with RIF (resistant to RIF).

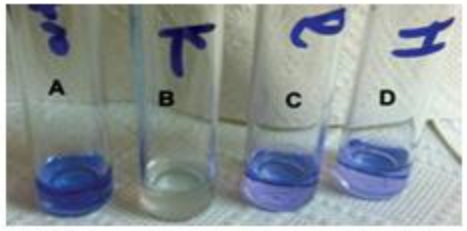

Fig. 3: blue/purple colour - there is no any bacterial growth. Decolourisation - there is bacterial growth. A clinical isolate - susceptible to to isoniazid (INH) and rifampicin (RIF). A: negative control tube without drug or bacteria; B: positive control tube with bacteria without drug; C: test tube with RIF (susceptible to RIF); D: test tube with INH (susceptible to INH).

TABLE

Comparing the results of crystal violet decolourisation assay (CVDA) with those obtained with reference method

Drug	CVDA	Reference method[a] 460TB/MGIT R	Reference method[a] 460TB/MGIT S	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Agreement (%)
INH	R	18/7	0/1	92.5	96.4	96.1	93.1	94.5
	S	0/2	27	-	-	-	-	-
RIF	R	16/0	0/0	88.8	100	100	94.8	96.3
	S	2/0	0/37	-	-	-	-	-

a: automated systems included BACTEC 460 TB and MGIT 960 were used as reference methods; INH: isoniazid; NPV: negative predictive value; PPV: positive predictive value; R: resistant; RIF: rifampicin; S: sensitive.

Evaluation of four colourimetric susceptibility tests for the rapid detection of multidrug-resistant *Mycobacterium tuberculosis* isolates

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[2]Istanbul University, Istanbul Medical School, Department of Medical Microbiology, Istanbul, Turkey

The purpose of this study is to evaluate four rapid colourimetric methods, including the resazurin microtitre assay (REMA), malachite green decolourisation assay (MGDA), microplate nitrate reductase assay (MNRA) and crystal violet decolourisation assay (CVDA), for the rapid detection of multidrug-resistant (MDR) tuberculosis. Fifty Mycobacterium tuberculosis *isolates were used in this study. Eighteen isolates were MDR, two isolates were only resistant to isoniazid (INH) and the remaining isolates were susceptible to both INH and rifampicin (RIF). INH and RIF were tested in 0.25 µg/mL and 0.5 µg/mL, respectively. The agar proportion method was used as a reference method. MNRA and REMA were performed with some modifications. MGDA and CVDA were performed as defined in the literature. The agreements of the MNRA for INH and RIF were 96% and 94%, respectively, while the agreement of the other assays for INH and RIF were 98%. In this study, while the specificities of the REMA, MGDA and CVDA were 100%, the specificity of the MNRA was lower than the others (93.3% for INH and 90.9% for RIF). In addition, while the sensitivity of the MNRA was 100%, the sensitivities of the others were lower than that of the MNRA (from 94.1-95%). The results were reported on the seventh-10th day of the incubation. All methods are reliable, easy to perform, inexpensive and easy to evaluate and do not require special equipment.*

Key words: resazurin microtitre assay - malachite green decolourisation assay - microplate nitrate reductase assay - crystal violet decolourisation assay - multidrug-resistant tuberculosis

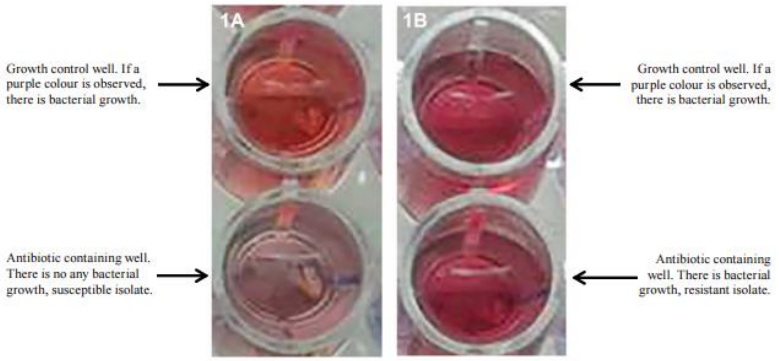

Fig. 1: microtitre nitrate reductase assay.

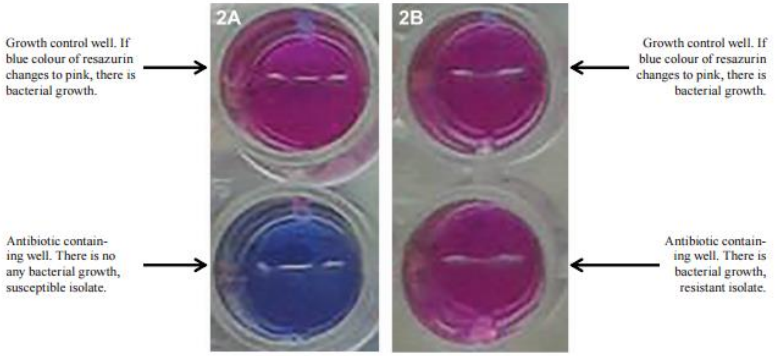

Fig. 2: resazurin microtitre assay.

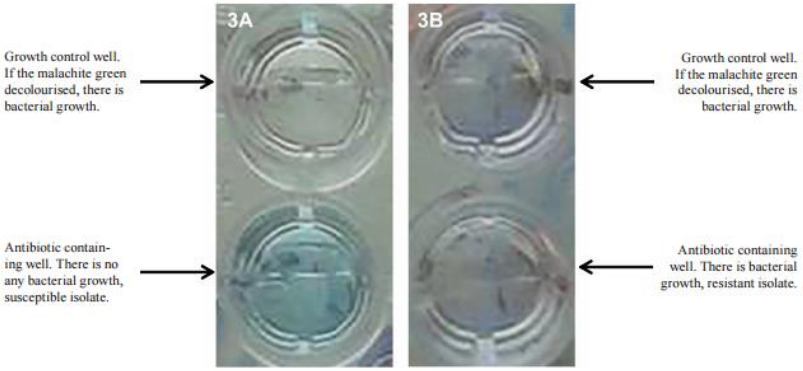

Fig. 3: malachite green decolourisation assay.

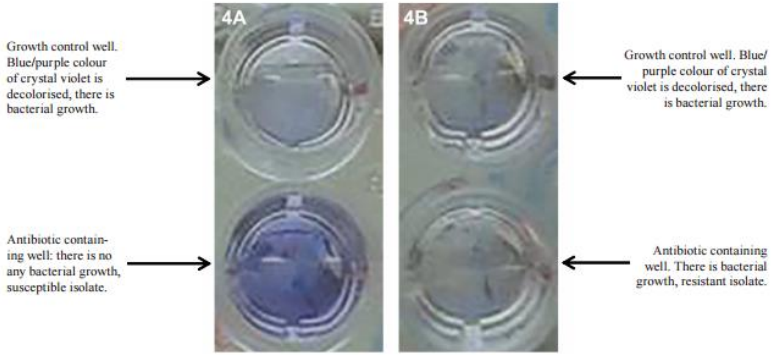

Fig. 4: crystal violet decolourisation assay.

TABLE

Comparing the results of colourimetric methods with those obtained with reference method

Drug (%)		Reference method[a] R	S	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)	Agreement (%)
INH	R	20	2	93.3	100	90.9	100	96
96	S	0	28					
RIF	R	17	3	90.9	100	85	100	94
94	S	0	30					
	REMA							
INH	R	19	0	100	95	100	96.7	98
98	S	1	30					
RIF	R	16	0	100	94.1	100	97	98
98	S	1	33					
	MGDA							
INH	R	19	0	100	95	100	96.7	98
98	S	1	30					
RIF	R	16	0	100	94.1	100	97	98
98	S	1	33					
	CVDA							
INH	R	19	0	100	95	100	96.7	98
98	S	1	30					
RIF	R	16	0	100	94.1	100	97	98
98	S	1	33					

a: agar proportion method on 7H11 agar; CVDA: crystal violet decolourisation assay; INH: isoniazid; MGDA: malachite green decolourisation assay; MNRA: microtitre nitrate reductase assay; NPV: negative predictive value; PPV: positive predictive value; R: resistant; REMA: resazurin microtitre assay; RIF: rifampicin; S: susceptible.

Evaluation of crystal violet decolorization assay for minimal inhibitory concentration detection of primary antituberculosis drugs against *Mycobacterium tuberculosis* isolates*

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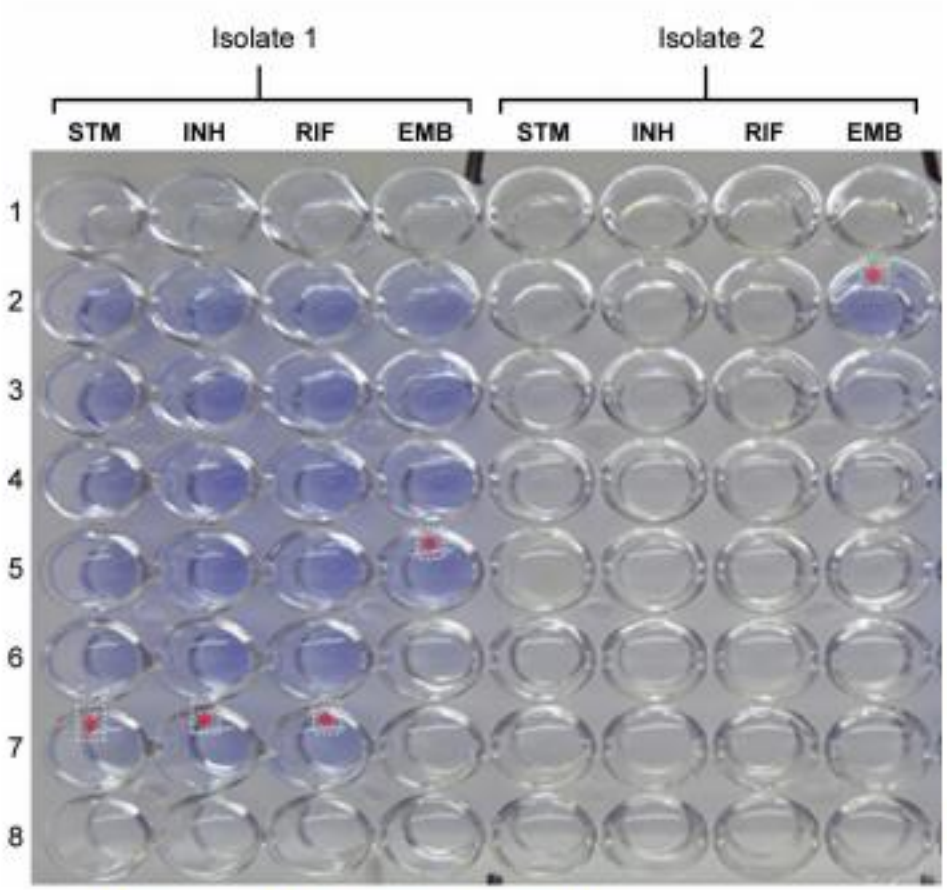
In this study we evaluated the crystal violet decolorization assay (CVDA) for detection of minimum inhibitory concentration (MIC) of antituberculosis drugs. 53 isolates were tested in this study and 13 of them were multidrug resistant (MDR) isolates. The antibiotics concentrations were 2-0.06 mg/L for isoniazid (INH) and rifampicin (RIF) and were 16-0.25 mg/L for streptomycin (STM) and ethambutol (EMB). Crystal violet (CV-25 mg/L) was added into the microwells on the seventh day of incubation and incubation was continued until decolorization. Decolorization of CV was the predictor of bacterial growth. Overall agreements for four drugs were detected as 98.1%, and the average time was detected as 9.5 ± 0.89 day after inoculation. One isolate for INH and two isolates for STM were determined resistant in the reference method, but susceptible by the CVDA. One isolate was susceptible to EMB by the reference method, but resistant by the CVDA. All results were concordant for RIF. This study shows that CVDA is a rapid, reliable and suitable for determination of MIC values of Mycobacterium tuberculosis. *And it can be used easily especially in countries with limited-sources.*

Key words: *Mycobacterium tuberculosis* - antituberculosis drugs - susceptibility testing - multi drug resistance - crystal violet decolorization assay



Evaluation of minimal inhibitory concentration plate.

streptomycin (STM) 1, isoniazid (INH) 1, rifampicin (RIF) 1, ethambutol (EMB) 1: Growth control (without antibiotic)

STM 2, EMB 2: 16 mg/L	**INH 2, RIF 2:** 2 mg/L
STM 3, EMB 3: 8 mg/L	**INH 3, RIF 3:** 1 mg/L
STM 4, EMB 4: 4 mg/L	**INH 4, RIF 4:** 0.5 mg/L
STM 5, EMB 5: 2 mg/L	**INH 5, RIF 5:** 0.25 mg/L
STM 6, EMB 6: 1 mg/L	**INH 6, RIF 6:** 0.125 mg/L
STM 7, EMB 7: 0.5 mg/L	**INH 7, RIF 7:** 0.06 mg/L
STM 8, EMB 8: 0.25 mg/L	**INH 8, RIF 8:** 0.03 mg/L

*: Minimal inhibitory concentration (MIC) values

MIC values for isolate 1; STM: 0.5 mg/L, INH: 0.06 mg/L, RIF: 0.06 mg/L, EMB: 2 mg/L (susceptible to all tested antibiotics)

MIC values for isolate 2; STM: > 16 mg/L, INH: > 2 mg/L, RIF: > 2 mg/L, EMB: 16 mg/L (resistant to all tested antibiotics-multidrug resistant isolate)

• If there is a bacterial growth, blue/purple color of crystal violet was decolorized. MIC was defined as the last well with blue/purple colour. The bacteria was considered to be resistant, if MIC value was > 0.125, >0.5, > 2 and > 4 mg/L for INH, RIF, STM and EMB, respectively.

TABLE IV

Comparison of the result of crystal violet decolorization assay (CVDA) with those obtained with reference method

Drugs	CVDA	Reference method*		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Agreement (%)
		R	S					
INH	R	26	0	96.3	100	100	96.3	98.1
	S	1	26					
RIF	R	14	0	100	100	100	100	100
	S	0	39					
STM	R	21	0	91.3	100	100	93.7	96.2
	S	2	30					
EMB	R	10	1	100	97.6	90.9	100	98.1
	S	0	42					

*Reference method: Bactec MGIT 960; EMB: ethambutol; INH: isoniazid; NPV: negative predictive value; PPV: positive predictive value; R: resistant; RIF: rifampicin; S: susceptible; STM: streptomycin.

Multicenter evaluation of crystal violet decolorization assay (CVDA) for rapid detection of isoniazid and rifampicin resistance in *Mycobacterium tuberculosis* *

Short title: CVDA for rapid detection of MDR-TB

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Table 2. Comparison of the CVDA and the reference method results.

Drugs		CVRT	Reference method		Sensitivity	Specificity	PPV	NPV	Agreement
			R	S	(%)	(%)	(%)	(%)	(%)
	INH	R	42	1	97.7	95.2	93.3	98.3	96.2
Phase I		S	3	60					
	RIF	R	41	0	100	96.9	95.3	100	98.1
		S	2	63					
	INH	R	67	2	97.1	96.5	95.7	97.7	96.8
Phase II		S	3	84					
	RIF	R	60	2	96.8	100	100	97.9	98.7
		S	0	94					

INH: isoniazid; RIF: rifampicin; PPV: positive predictive value; NPV: negative predictive value

XXXVII. Türk Mikrobiyoloji Kongresi

Mycobacterium tuberculosis'de etambutol ve streptomisin direncinin hızlı tespiti için yeni bir yöntem; KVRT

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Çalışmada etambutol (EMB) ve streptomisin (SM) direncinin hızlı tespiti için yeni geliştirilmiş olan kristal viyole renksizleştirme testi (KVRT)'nin değerlendirilmesi amaçlandı.

Bu amaçla, 9 farklı merkezden gönderilen 140 adet izolat test edildi. Her bir izolat ETM için 5 mg/L ve SM için 2 mg/L kritik konsantrasyonlarında test edildi.

Çalışma sonucunda, EMB için özgüllük %98.2, duyarlılık %77.7, PPD %91.3 ve NPD %94.8 ve uyum %94.2 olarak saptandı. SM için özgüllük %94.8, duyarlılık %81.3, PPD %87.5 ve NPD %92 ve uyum %90.7 olarak tespit edildi. Çalışmada sonuçlar 11.33±2.68 günde (8-21 gün) elde edildi.

Sonuç olarak KVRT, EMB ve SM direncinin hızlı tespiti için kullanılabilecek kolorimetrik bir yöntem olarak görülmekle birlikte daha fazla izolat ile çok merkezli çalışmalara gerek vardır.

Keywords : M. tuberculosis, etambutol, streptomisin, KVRT

KVRT		Referans Yöntem (MGIT 960)						
		Dirençli	Duyarlı	Duyarlılık(%)	Özgüllük(%)	PPV(%)	NPV(%)	Uyum(%)
STR	Dirençli	35	5	81,3	94,8	87,5	92	90,7
	Duyarlı	8	92					
EMB	Dirençli	21	2	77,7	98,2	91,3	94,8	94,2
	Duyarlı	6	111					

MİDT/MODS

- ARB pozitif klinik örneklerden ya da
- Üremiş kültürden çalışılabilir
- Mikrokolonilerin mikroskobik olarak incelenmesi temeline dayanır
- İlaçsız besiyerinde üreme, pozitif kültürü gösterir
- Aynı anda ilaçlı besiyerinde de üreme varsa direnci gösterir

- 7H9 besiyerinde mikroplaklarda kord formasyonunun inverted mikroskop ile belirli aralıklarla gözlenmesiyle uygulanır

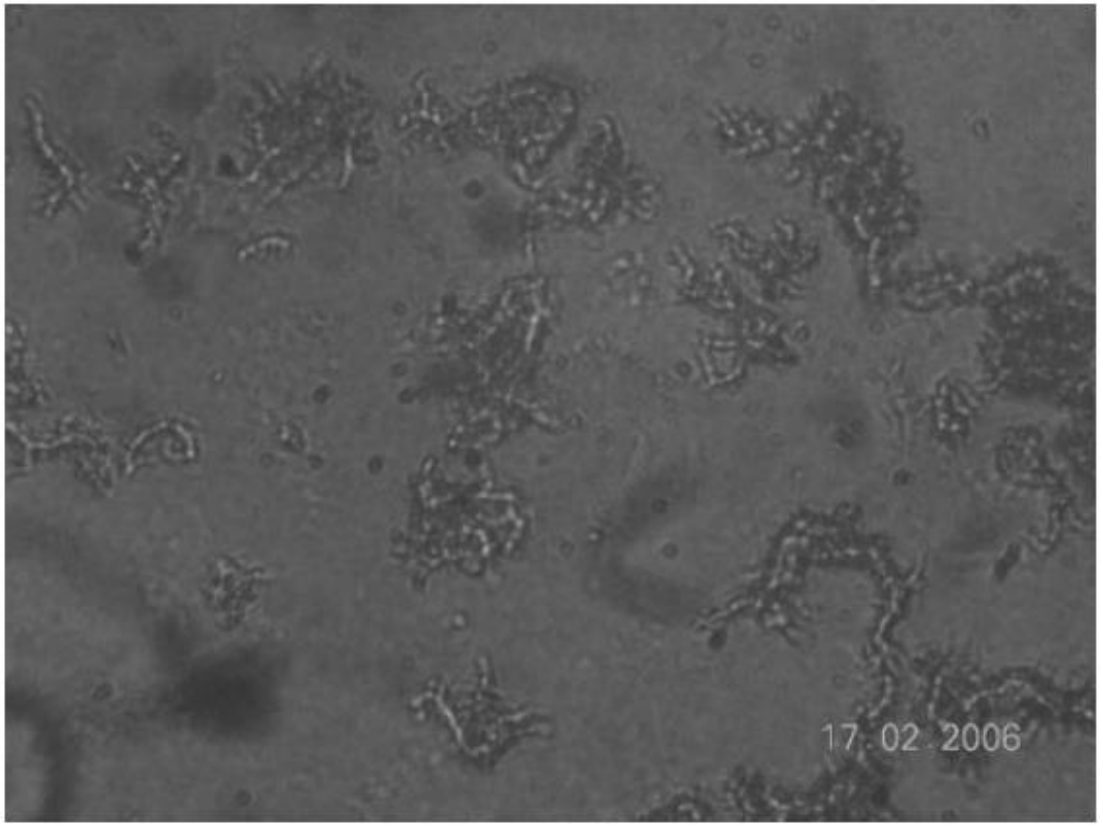

FIG. 1. Characteristic serpentine structure of young *M. tuberculosis* colonies grown in Middlebrook 7H9 broth for MODS, as seen under an inverted-light microscope (original magnification, ×20).

- Çalışmalarda;
  - INH için;
    - Duyarlılık %72.6-100
    - Özgüllük %78.4-100
  - RIF için;
    - Duyarlılık %72.7-100
    - Özgüllük %75-100
  - EMB için;
    - Duyarlılık %73.7-100
    - Özgüllük %88.2-91.5
  - STR için;
    - Duyarlılık %87.5-96
    - Özgüllük %96.4-97.6
  - PZA için;
    - Duyarlılık %95.5-100
    - Özgüllük %93.3-100

- İkinci kuşak ilaçlar;
  - Duyarlılık: %91.5-96.2
  - Özgüllük: %98.7-100
- Sonuçlar;
  - İndirekt test ile 5-18 gün
  - Direkt test ile 5-38 günde elde ediliyor

İTAT/TLA

- Genel olarak kültürde tüberküloz basillerinin erken tanısı için kullanılır
- Kültürler konvansiyonel mikroskopta incelenir
- Duyarlılık testinin uygulandığı çalışma sınırlıdır
- Bir indirekt duyarlılık testinde;
  - RIF için;
    - Duyarlılık ve özgüllük %100
- Üç tane direkt duyarlılık testinde;
  - Hem INH hem de RIF için;
    - Duyarlılık ve özgüllük %100
- Sonuçlar 8-14 günde elde edilmiştir

- Bir çalışmada;
  - INH;
    - Duyarlılık: %98
    - Özgüllük: %100
  - RIF;
    - Duyarlılık: %98
    - Özgüllük: %88
  - CIP;
    - Duyarlılık: %98
    - Özgüllük: %91

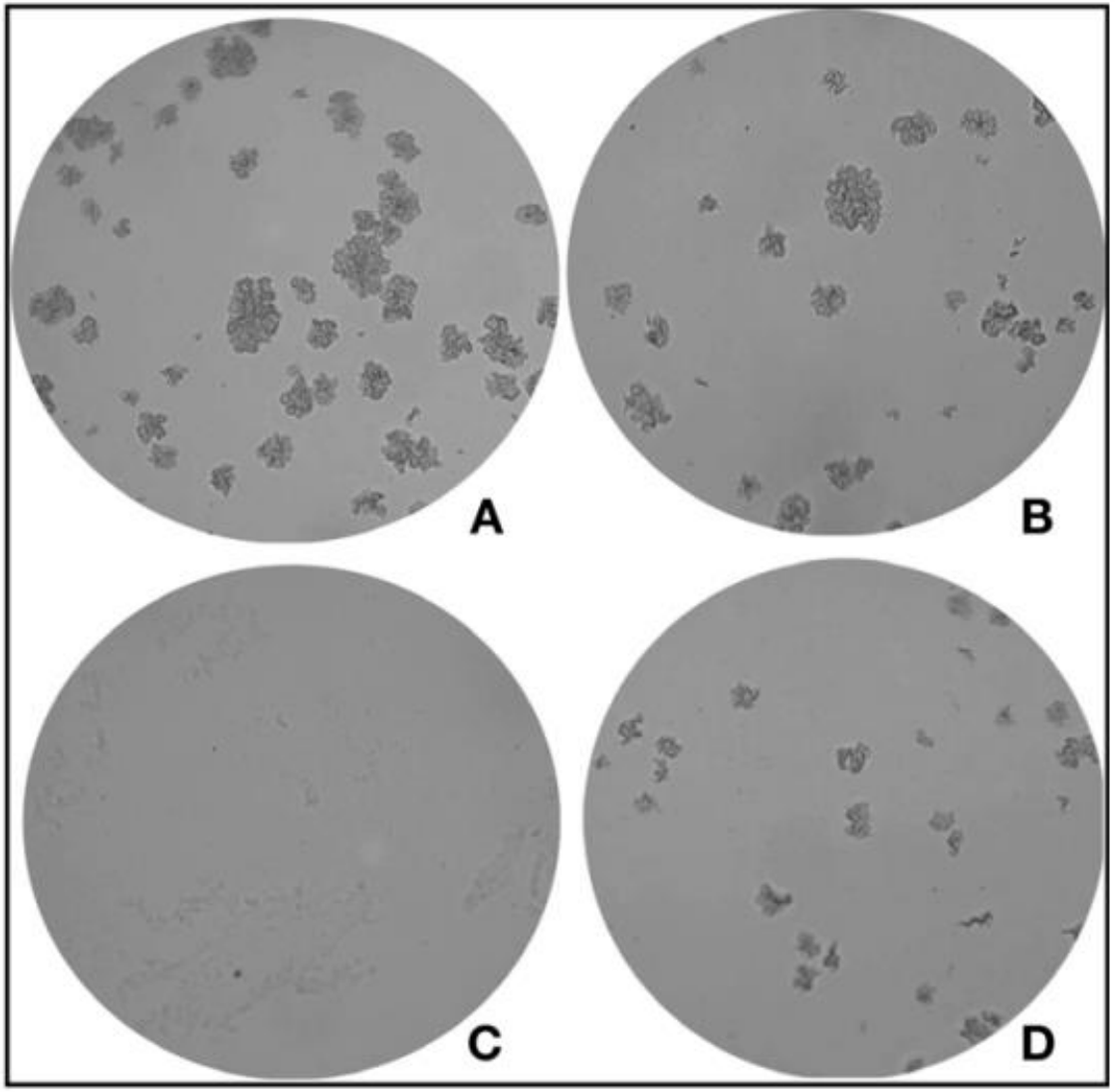

Figure Thin layer agar quadrant plate: **A**) positive growth control; **B**) rifampicin-resistant; **C**) ofloxacin-susceptible; **D**) kanamycin-resistant.

Broth mikrodilüsyon testi

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Susceptibility Testing of Slowly Growing Mycobacteria by a Microdilution MIC Method with 7H9 Broth

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Based on previous success with rapidly growing mycobacteria, a microdilution MIC system was devised for slowly growing mycobacterial species using 7H9 broth. Test drugs included isoniazid, rifampin, ethambutol, streptomycin, clofazamine, and sulfamethoxazole. Sixty isolates of four mycobacterial species, including *Mycobacterium tuberculosis*, from patients who had never received drug therapy were evaluated in the system, as well as 25 drug-resistant isolates and 11 control strains. MICs were read when good macroscopic control growth was evident, a period which varied with each species. Most species exhibited a narrow range of MICs with easily discernible growth endpoints. The aminoglycosides, ethambutol, clofazamine, and sulfamethoxazole were the only drugs with activity against all species at clinically achievable levels in serum. Correlation between susceptibilities by the proportion method in agar with single drug concentrations and the broth method were excellent for *M. tuberculosis*, *M. kansasii*, and *M. marinum* for isoniazid, rifampin, and ethambutol. Isolates of the *M. avium* complex were much more susceptible in broth than in agar for rifampin, ethambutol, and streptomycin. Given the successful transition of most microbiology laboratories to MIC plates for other bacterial species, this method would allow for testing of multiple drugs at multiple concentrations and has good potential for evaluation of drug combinations and drug-resistant isolates.

Current mycobacterial susceptibility testing in the United States uses the proportion method, whereby the number of

system are that many mycobacteria, including *M. tuberculosis*, grow more rapidly in broth, and the system allows for

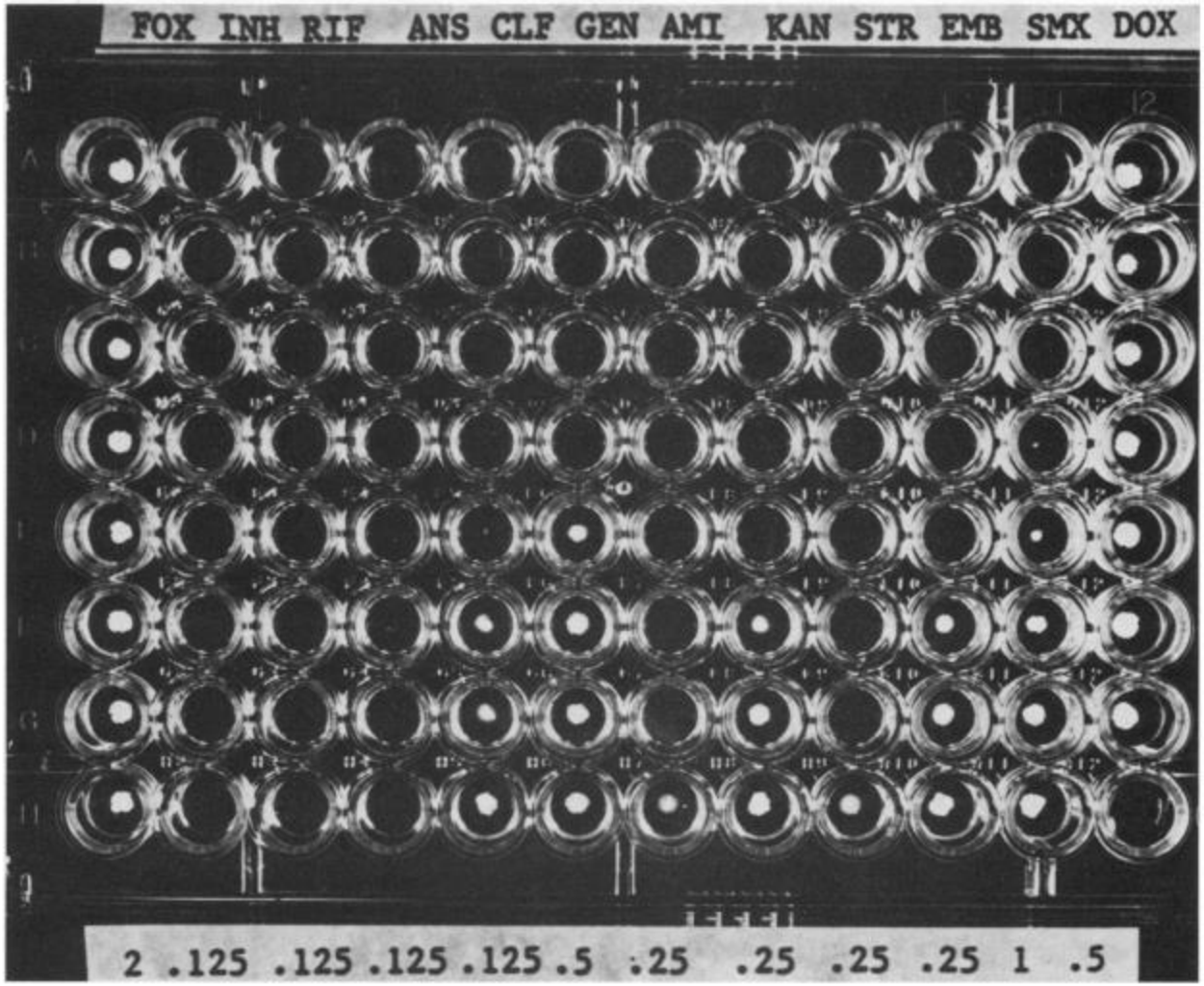

FIG. 1. Microdilution MIC plate for a susceptible strain of *M. tuberculosis*. The wells contained twofold dilutions (rows A to H) of antimicrobial agents, with the lowest drug concentration (row H) listed in micrograms per milliliter. The well in the upper left corner is the positive control, whereas the well in the lower right corner is the broth control. Note the sharp endpoints for all drugs except SMX. FOX = Cefoxitin; ANS, ansamycin; CLF, clofazamine; DOX, doxycycline.

TABLE 4. Comparison of susceptible and resistant strains tested in broth and in agar by the standard proportion method

Organism (no. of strains)	Drug (concn in agar [μg/ml])[a]	No. of strains classified as[b]:				% Agreement
		A	B	C	D	
M. tuberculosis (21)	INH (1)	10	11	0	0	100
	RIF (1)	13	8	0	0	100
	EMB (5)	17	4	0	0	100
	STR (2)	17	4	0	0	100
M. kansasii (27)	INH (1)	1	20	5	1	78
	RIF (1)	16	10	1	0	96
	EMB (5)	20	6	0	1	96
	STR (2)	9	2	16	0	59
M. marinum (17)	INH (1)	0	17	0	0	100
	RIF (1)	17	0	0	0	100
	EMB (5)	17	0	0	0	100
	STR (2)	6	1	9	1	41
M. avium complex (20)	INH (1)	0	20	0	0	100
	RIF (1)	0	5	15	0	30
	EMB (5)	5	3	12	0	40
	STR (2)	0	13	7	0	65

[a] Comparative concentrations in broth were the same as in agar except for EMB, for which 8 μg/ml was used as the resistance breakpoint.
[b] A, Susceptible by both methods; B, resistant by both methods; C, susceptible in broth but resistant in agar; D, resistant in broth but susceptible in agar.

Drug Susceptibility Testing of *Mycobacterium tuberculosis* by the Broth Microdilution Method with 7H9 Broth

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In this study, we have evaluated the broth microdilution method (BMM) for susceptibility testing of Mycobacterium tuberculosis. *A total of 43 clinical isolates of* M. tuberculosis *and H37Rv as a control strain were studied. All isolates were tested by the proportion method and the BMM for isoniazid (INH), rifampicin (RIF), streptomycin (STR), and ethambutol (ETM). The proportion method was carried out according to the National Committee for Clinical Laboratory Standards (NCCLS) on Löwenstein-Jensen (LJ) medium. The BMM was carried out using 7H9 broth with 96 well-plates. All strains were tested at 3.2-0.05 µg/ml, 16-0.25 µg/ml, 32-0.5 µg/ml, and 32-0.5 µg/ml concentrations for INH, RIF, STR, and ETM, respectively. When the BMM was compared with the proportion method, sensitivity was 100, 100, 96.9, and 90.2%, while specificity was 100, 85.7, 90.9, and 100% for INH, RIF, STR, and ETM, respectively. The plates were examined 7, 10, 14, and 21 days after incubation. The majority of the result were obtained at 14th days after incubation, while the proportion method result were ended in 21-28 days. According to our results, it may be suggested that the BMM is suitable for early determining of multidrug-resistance-*M. tuberculosis *strains in developed or developing countries.*

Key words: *Mycobacterium tuberculosis* - broth microdilution method - susceptibility testing

Tuberculosis is an important public health problem in developed and especially in developing countries. The incidence of multidrug-resistant (MDR) *Mycobacterium tuberculosis* has increased in recent years. Mycobacterial cultures and susceptibility testing must be rapidly concluded for effective treatment and control of the disease (Zapata et al. 1999).

The two methods most commonly used for susceptibility testing of *M. tuberculosis* include the proportion method performed on Löwenstein-Jensen medium (LJ) and Middlebrook 7H10-11 agar, and the Bactec TB 460 system (Becton Dickinson Microbiology System, Cockeysville, (Yamane et al. 1998, 1999, Leite et al. 2000). In addition, it has been disclosed that the broth microdilution method is very close to the Center for Disease Control and Prevention (CDC), Atlanta, US (1994) guidelines, except for isolation and identification of *M. tuberculosis* (Yamane et al. 1999).

Our aim was to compare the proportion method with LJ medium and the broth microdilution method (BMM) for susceptibility testing of 43 *M. tuberculosis* clinical isolates.

MATERIALS AND METHODS

TABLE

The results of susceptibility testing by the broth microdilution method (BMM) and the proportion method

Drug	Results of proportion method	The BMM results: Nr of resistant strains	Nr of susceptible strains	% sensitivity	% specificity	% positive predictive value	% negative predictive value
INH	Resistant	9	0	100	100	100	100
	Susceptible	0	34				
RIF	Resistant	6	1	100	85.7	97.3	100
	Susceptible	0	36				
STR	Resistant	10	1	96.9	90.9	96.9	90.9
	Susceptible	1	31				
EMB	Resistant	2	0	90.2	100	100	33.3
	Susceptible	4	37				

INH: isoniazid; RIF: rifampicin; STR: streptomycin; ETM: ethambutol

- INH;
  - Duyarlılık: %100
  - Özgüllük: %91-100
- RIF;
  - Duyarlılık: %100
  - Özgüllük: %85.7-100
- EMB;
  - Duyarlılık: %90.2-100
  - Özgüllük: %96-100
- STR;
  - Duyarlılık: %96.9-100
  - Özgüllük: %90.9-98
- PZA;
  - Duyarlılık: %100
  - Özgüllük: %95-100

E test

- Antibiyotik emdirilmiş stripler kullanılır
- McFarland 3 bulanıklığında inokülüm kullanılır
- Sonuçlar 5-10 gün arasında elde edilir
- İndirekt test olarak uygulanır
- Bir çalışmada RIF direncinin hızlı tespiti için direkt test uygulanmış
  - Uyum %100
  - Sonuçlar 14-37 günde (ortalama 20 gün) elde edilmiş

E test

- Çalışmalarda;
  - INH;
    - Duyarlılık: %55.6-94.6
    - Özgüllük: %94.8-98.6
  - RIF;
    - Duyarlılık: %62.5-100
    - Özgüllük: %97.3-10
  - EMB;
    - Duyarlılık: %50-85.7
    - Özgüllük: %97.7-98.8
  - STR;
    - Duyarlılık: %5.9-85.7
    - Özgüllük: %85.1-100

- 7H10 ve 11 agarda uygulanır
- Kanlı ve çukulatamsı agarda da test edilmiştir

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Comparative Study for Determination of *Mycobacterium tuberculosis* Susceptibility to First- and Second-Line Antituberculosis Drugs by the Etest Using 7H11, Blood, and Chocolate Agar▽

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We investigated the performance of blood and chocolate agar as alternatives to Middlebrook 7H11 agar for testing the susceptibility of *Mycobacterium tuberculosis* to first-and second-line drugs by the Etest method. A total of 39 strains of *M. tuberculosis* including 22 multidrug-resistant *M. tuberculosis* strains and 17 susceptible strains were tested. In conclusion, our results showed that chocolate agar gave insufficient growth, needing up to 21 days of incubation, while results on blood agar were comparable to those on Middlebrook 7H11 agar and can be further explored as an alternative for Etest-based susceptibility testing of *M. tuberculosis*.

Early clinical diagnosis of tuberculosis, identification of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains, and appropriate treatment are the most effective strategies to control the spread of MDR tuberculosis (18). Therefore, rapid and efficient methods are needed to accurately diagnose and control this disease efficiently. The CLSI reference method for susceptibility testing of *M. tuberculosis* is the agar proportion method (22) performed with Middlebrook 7H10 to -11 agar. On the other hand, there are semiautomated and automated systems (BACTEC 460 TB and BACTEC MGIT [mycobacte-

In this study, the performance of blood and chocolate agar was compared to that of Middlebrook 7H11 agar with the aim to evaluate them as possible alternatives for susceptibility testing of *M. tuberculosis* clinical isolates against IZ, RI, SM, EB, ofloxacin (OF), ciprofloxacin (CI), levofloxacin (LE), linezolid (LZ), and ethionamide (ET) by Etest.

A total of 39 strains of *M. tuberculosis* and H37Rv (control strain susceptible to all antituberculous drugs) were tested. While 22 MDR *M. tuberculosis* strains were isolated from spu-

TABLE 1. Comparison of categorical results between Etest on Middlebrook 7H11 and BACTEC 460 TB

Radiometric proportion method result[a]	No. with result by Etest on 7H11 agar[b]		% Agreement
	Resistant	Susceptible	
SM			
Resistant	8	1	94.9
Susceptible	1	29	
IZ			
Resistant	24	1	97.4
Susceptible	0	14	
RI			
Resistant	22	0	100
Susceptible	0	17	
EB			
Resistant	10	3	89.7
Susceptible	1	25	

[a] Day of detection, day 8.
[b] Day of detection, day 10.

TABLE 2. Comparison of categorical results for Etest on blood and chocolate agar to results with BACTEC 460 TB and Middlebrook 7H11 agar

Comparison	% Agreement				Day of detection
	SM	IZ	RI	EB	
BACTEC 460 TB vs[a]:					
Etest					
Blood agar	94.9	97.4	100	82.1	10
Chocolate agar	94.9	97.4	97.4	89.7	21
7H11 agar vs[b]:					
Etest					
Blood agar	94.9	100	100	92.3	10
Chocolate agar	94.9	100	97.4	89.7	21

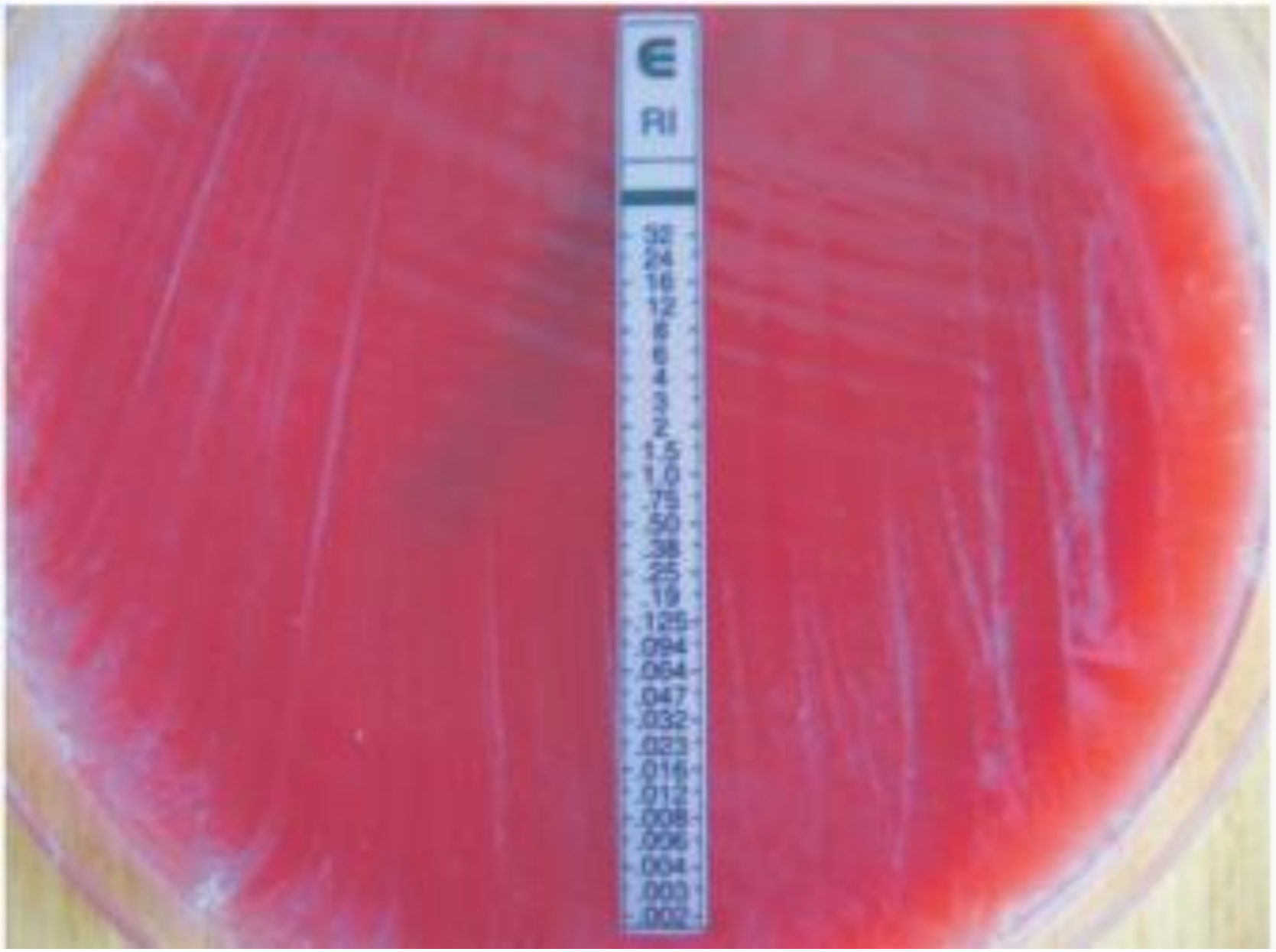
[a] Day of detection, day 8.
[b] Day of detection, day 10.

TABLE 3. Comparison of categorical results of Etest on blood and chocolate agar versus results with Middlebrook 7H11 agar for second-line drugs

Etest parameter	% Agreement compared with 7H11 agar[a]					Day of detection
	ET	OF	LE	CI	LZ	
Blood agar	93.3	90	100	96.6	100	10
Chocolate agar	56.6	86.6	100	96.6	100	21

[a] Day of detection, day 10.


E
RI


E
RI
32
24
16
12
8
6
4
3
2
1.5
1.0
.75
.50
.38
.25
.19
.125
.094
.064
.047
.032
.023
.016
.012
.008
.006
.004
.003
.002

Kanlı agarda proporsiyon yöntemi ile duyarlılık testi

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2005, p. 1930–1931 Vol. 43, No. 4
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Susceptibilities of *Mycobacterium tuberculosis* to Isoniazid and Rifampin on Blood Agar

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In this study, blood agar was used instead of 7H10 agar for the susceptibility testing of 34 clinical isolates of *Mycobacterium tuberculosis* to isoniazid (INH) and rifampin (RIF) in accordance with the NCCLS. The BACTEC 460 TB system (Becton Dickinson, Sparks, Md.) was used as a "gold standard." Results for both media were in agreement for RIF and INH at 100 and 94.1%, respectively. For INH, the specificity, sensitivity, positive predictive value, and negative predictive value were found to be 71.4, 100, 93.1, and 100%, respectively, while these values were 100% for RIF. In addition, the results of the susceptibility test performed with blood agar were obtained on day 14 of incubation. In conclusion, results were obtained much earlier with blood agar (2 weeks) than with 7H10 agar (3 weeks), and the results of this study suggest that blood agar may be used as an alternative medium for the susceptibility testing of *M. tuberculosis* to INH and RIF.

The increasing incidence of multidrug-resistant tuberculosis (MDR-TB) produces serious problems in developed and especially in developing countries. Detecting tuberculosis and identifying MDR *Mycobacterium tuberculosis* strains by conventional methods is difficult because of the low growth rate of the causative agent. Therefore, rapid and efficient methods are needed for the control of this disease (3–6, 13).

Several manufacturers have directed considerable effort toward the development of rapid and efficient systems for the growth, detection, and susceptibility testing of mycobacteria.

isoniazid (INH) and rifampin (RIF) by using the proportion method.

Bacterial isolates. Thirty-four clinical isolates of *M. tuberculosis* were examined in this study, and H37Rv and H37Ra were also included as control strains. Drug susceptibility patterns of all isolates were previously detected by the BACTEC 460 TB system, and a standard protocol in accordance with the manufacturer's instructions was followed. Seven isolates were susceptible to INH and RIF. Eight were resistant only to INH, while the rest (19 strains) were resistant to both drugs.

TABLE 1. Comparison of radiometric proportion method results and blood agar results

Drug[a]	Result on blood agar	Results of proportion method					
		No. of samples that were:		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
		Resistant	Susceptible				
INH	Resistant	27	2	100	71.4	93.1	100
	Susceptible	0	5				
RIF	Resistant	19	0	100	100	100	100
	Susceptible	0	15				

[a] INH at 0.2 μg/ml; RIF at 1 μg/ml.

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Blood agar for susceptibility testing of *Mycobacterium tuberculosis* against first-line drugs

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SUMMARY

OBJECTIVE: To evaluate the performance of blood agar for the susceptibility testing of 50 *Mycobacterium tuberculosis* clinical isolates against isoniazid (INH), rifampicin (RMP), streptomycin (SM) and ethambutol (EMB).

DESIGN: The activity of the drugs was determined by the proportion method on blood agar instead of Middlebrook 7H10 agar according to Clinical Laboratory Standard Institute recommendations. The final concentrations of INH, RMP, SM and EMB were 0.2 μg/ml, 1 μg/ml, 2 μg/ml and 5 μg/ml, respectively.

RESULTS: The results were compared with the radiometric proportion method as the reference, and the agreements were determined as 100% for INH and RMP, 92% for SM and 96% for EMB. The specificity, sensitivity, positive predictive value and negative predictive value were 90.4% and 97.5%, 100% and 90%, 66.6% and 90% and 100% and 97.5% for SM and EMB, respectively, while these values were 100% for INH and RMP. The results of susceptibility testing were obtained on the 14th day of incubation.

CONCLUSION: According to this preliminary study, our results suggest that blood agar can be used as an alternative medium for the susceptibility testing of *M. tuberculosis* strains against INH, RMP, SM and EMB in resource-limited countries. However, further studies are needed before implementating the method in diagnostic laboratories.

KEY WORDS: *Mycobacterium tuberculosis*; proportion method; blood agar

THE SPREAD of multidrug-resistant tuberculosis (MDR-TB, defined as resistance to at least isoniazid

intensive, expensive, generate radioactive waste and are not always available, many investigators have

Journal of Chemotherapy Vol. 20 - n. 3 (307) - 2008

BRIEF COMMUNICATION

Evaluation of Blood Agar for Susceptibility Testing of *Mycobacterium tuberculosis* against First-Line Antituberculous Drugs: Results from Two Centers

A.Y. COBAN [1] - A. MARTIN [2] - M. UZUN [3]
K. BILGIN [3] - J.C. PALOMINO [2]
B. DURUPINAR [1]

Tuberculosis remains one of the major unresolved global health problems and the major causes of death from a single infectious agent worldwide [1,2]. In addi-

Institute of Tropical Medicine, Antwerp. The other 100 strains (50 strains from Ondokuz Mayis University Medical School, Department of Microbiology and Clinical Microbiology and 50 strains from Istanbul University Istanbul Medical School, Department of Microbiology and Clinical Microbiology) were tested in Ondokuz Mayis University Medical School, Department of Microbiology and Clinical Microbiology. *M. tuberculosis* H37Rv (ATCC 27294) sensitive to first-line drugs, strains resistant to INH (ATCC 35822) and STR (ATCC 35820) were used as control strains. All strains were sub-cultured on Löwenstein-Jensen medium. Resistance profiles of *M. tuberculosis* strains used in this study are summarized in *Table 1*.

TABLE 1 - *Resistance profiles of M. tuberculosis isolate that used in this study.*

	Isolates (n) (Belgium) By the proportion method	Isolates (n) (Turkey) By the BACTEC 460 TB

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Testing Susceptibility of Multidrug-Resistant *Mycobacterium tuberculosis* to Second-Line Drugs by Use of Blood Agar▽

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In this study, the susceptibilities of 35 multidrug-resistant (MDR) *Mycobacterium tuberculosis* clinical isolates to second-line drugs, including kanamycin (KM), rifabutin (RBU), ofloxacin (OFX), *p*-aminosalicylic acid (PAS), capreomycin (CAP), clofazimine (CFM), and ethionamide (ETH), were investigated on blood agar according to CLSI recommendations. Compared with the results of the Bactec 460 TB system, agreement was 100, 100, 97, 100, 100, 100, and 86% for KM, RBU, OFX, PAS, CAP, CFM, and ETH, respectively. Compared with the results of the proportion method, agreement was 100, 100, 97, 100, 97, 100, and 77% for KM, RBU, OFX, PAS, CAP, CFM, and ETH, respectively.

Multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are major public health problems, especially in developing countries (13, 18, 21). Rapid susceptibility testing is critical for early diagnosis of MDR- and XDR-TB and the initiation of effective regimens (14). The agar proportion method performed on Middlebrook 7H10 and 7H11 agars is the reference method for susceptibility of *Mycobacterium tuberculosis* according to CLSI recommendations (16). The Bactec 460 TB system (Becton Dickinson Diagnostic Systems, Sparks, MD), Bactec MGIT 960 (Becton Dickinson Diagnostic Systems, Sparks, MD), and Versa TREK system (formerly known as ESP II; Trek Diagnostic Systems, West Lake, OH) are cleared for used by the U.S. FDA for testing *M. tuberculosis* susceptibility to first-line drugs (16). It has recently been demonstrated that blood agar can be used for routine culture and testing of *M. tuberculosis* susceptibility to first-line drugs (1–4, 6, 7, 15).

In this study, the susceptibilities of 35 MDR *M. tuberculosis*

lutions except CFM, which was stored in the dark at room temperature, were stored at −70°C in small aliquots.

The agar proportion method using Middlebrook 7H10 and blood agar was performed with concentrations of 10, 5, 5, 1, 2, 1, and 2 μg/ml for CAP, ETH, KM, CFM, OFX, RBU, and PAS, respectively (9, 10, 12, 16, 17).

Middlebrook 7H12 broth (Bactec 12B; Becton Dickinson Microbiology Systems) was used for radiometric testing (5, 11, 16, 19, 20). Second-line drugs (KM, RBU, PAS, CAP, CFM, OFX, and ETH) were tested by using single critical concentrations. Concentrations of second-line drugs tested in the Bactec 460 TB system were 1.25, 1.25, 5, 0.5, 2, 0.5, and 4 μg/ml for CAP, ETH, KM, CFM, OFX, RBU, and PAS, respectively (9, 10, 12, 16, 17).

The inoculum was prepared from freshly grown colonies on Löwenstein-Jensen medium. The supernatant of each isolate was adjusted to a 1 McFarland standard. The agar proportion method was performed on both Middlebrook 7H10 agar and

TABLE 1. Comparison of blood agar results and Bactec 460 TB system results[a]

Drug	Result on blood agar	Bactec 460 TB system			% specificity	PPV (%)	NPV (%)	% agreement
		No. of resistant isolates	No. of susceptible isolates	% sensitivity				
KM	Resistant	0	0	100		100		100
	Susceptible	0	35					
RBU	Resistant	27	0	100	100	100	100	100
	Susceptible	0	8					
OFX	Resistant	1	1	97	100	100	50	97
	Susceptible	0	33					
PAS	Resistant	3	0	100	100	100	100	100
	Susceptible	0	32					
CAP	Resistant	2	0	100	100	100	100	100
	Susceptible	0	33					
CFM	Resistant	0	0	100		100		100
	Susceptible	0	35					
ETH	Resistant	17	5	72	100	100	77	86
	Susceptible	0	13					

[a] KM, kanamycin; RBU, rifabutin; OFX, ofloxacin; PAS, *p*-aminosalicylic acid; CAP, capreomycin; CFM, clofazimine; ETM, ethionamide; PPV, positive predictive value; NPV, negative predictive value.

TABLE 2. Comparison of blood agar results and Middlebrook 7H10 agar results[a]

Drug	Result on blood agar	7H10 agar			% specificity	PPV (%)	NPV (%)	% agreement
		No. of resistant isolates	No. of susceptible isolates	% sensitivity				
KM	Resistant	0	0	100		100		100
	Susceptible	0	35					
RBU	Resistant	27	0	100	100	100	100	100
	Susceptible	0	8					
OFX	Resistant	1	1	97	100	100	50	97
	Susceptible	0	33					
PAS	Resistant	3	0	100	100	100	100	100
	Susceptible	0	32					
CAP	Resistant	1	1	97	100	100	50	97
	Susceptible	0	33					
CFM	Resistant	0	0	100		100		100
	Susceptible	0	35					
ETH	Resistant	14	8	61.9	100	100	63.6	77
	Susceptible	0	13					

[a] KM, kanamycin; RBU, rifabutin; OFX, ofloxacin; PAS, *p*-aminosalicylic acid; CAP, capreomycin; CFM, clofazimine; ETM, ethionamide; PPV, positive predictive value; NPV, negative predictive value.

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Rapid Direct Testing of Susceptibility of *Mycobacterium tuberculosis* to Isoniazid and Rifampin on Nutrient and Blood Agar in Resource-Starved Settings

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In this study, we evaluated the performance of blood agar (by macroscopic growth) and nutrient agar (by a microcolony detection method) for drug susceptibility testing of *Mycobacterium tuberculosis* against rifampin (RIF) and isoniazid (INH), using 67 smear-positive sputum specimens. The direct proportion method on Lowenstein-Jensen (LJ) medium was used as the "gold standard." Compared with LJ medium, results for both media were in 100% agreement for RIF, while for INH the agreement levels for blood agar and nutrient agar were 98% and 95%, respectively. Within 2 weeks, 100% of specimens yielded results on blood agar, while 96.8% of specimens yielded results on nutrient agar. Our study showed that blood agar and nutrient agar can be used as alternative media for direct susceptibility testing of RIF and INH, especially in resource-poor settings.

Despite recent advancements in the field of mycobacteriology, multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) still remain public health nuisances (9, 19). Conventional culture methods are slow, requiring about 6 to 8 weeks for diagnosis and subsequent drug susceptibility testing (DST). In the last decade, the Bactec MGIT 960 automated system was introduced for early diagnosis and DST of *Mycobacterium tuberculosis* from various clinical specimens. However, this system is costly, its consumables are not easily available, and it requires technical expertise. Parallel to the progress in automated systems, much work has also been published on the development of rapid but economical methods which are equally

Remaining sediment was diluted in 3 ml of sterile distilled water and used subsequently for inoculation.

Preparation of nutrient agar and blood agar with drugs. Autoclaved nutrient agar (Oxoid, United Kingdom) was divided into two equal parts, and 5% sheep blood was added to one part. Both were further subdivided into four equal parts; *para*-nitrobenzoic acid (PNB) (500 μg/ml), INH (0.2 μg/ml), and RIF (1.0 μg/ml) were added to the first three parts, while the fourth part was used without antibiotic (control). About 5 ml of respective medium was poured into the quadrants of sterile labeled petri dishes.

Inoculation of sputum specimens on media. In order to ensure adequate but not excessive growth, concentrated sputum specimens were diluted as follows: 10^{-1} for 1 to 9 AFB/10 fields (2+), 10^{-2} for 1 to 9

TABLE 1 Comparison of results of LJ proportion method with results of sensitivity on blood agar[a]

Drug	LJ result	No. of samples with blood agar result		% Sensitivity	% Specificity	PPV (%)	NPV (%)	Agreement (%)
		R	S					
RIF	R	11	0	100	100	100	100	100
	S	0	52					
INH	R	14	1	100	98	94	100	98
	S	0	48					

[a] R, resistant; S, susceptible; PPV, positive predictive value; NPV, negative predictive value.

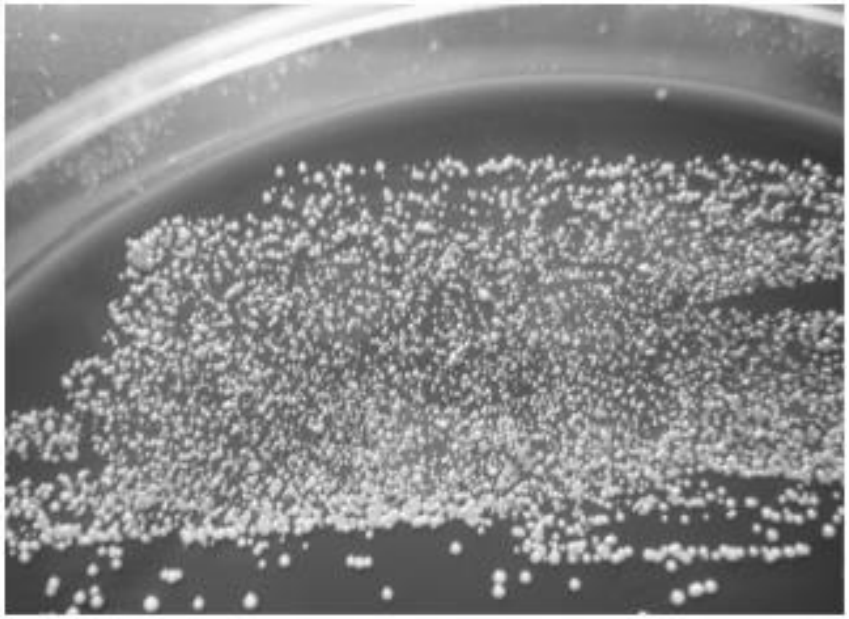

FIG 2 Colonies of *M. tuberculosis* on blood agar plate after 14 days of incu bation.

TABLE 2 Comparison of results of LJ proportion method with results of sensitivity on nutrient agar (by microcolony detection method)[a]

Drug	LJ result	No. of samples with nutrient agar result		% Sensitivity	% Specificity	PPV (%)	NPV (%)	Agreement (%)
		R	S					
RIF	R	11	0	100	100	100	100	100
	S	0	52					
INH	R	12	3	100	95	80	100	95
	S	0	48					

[a] R, resistant; S, susceptible; PPV, positive predictive value; NPV, negative predictive value.

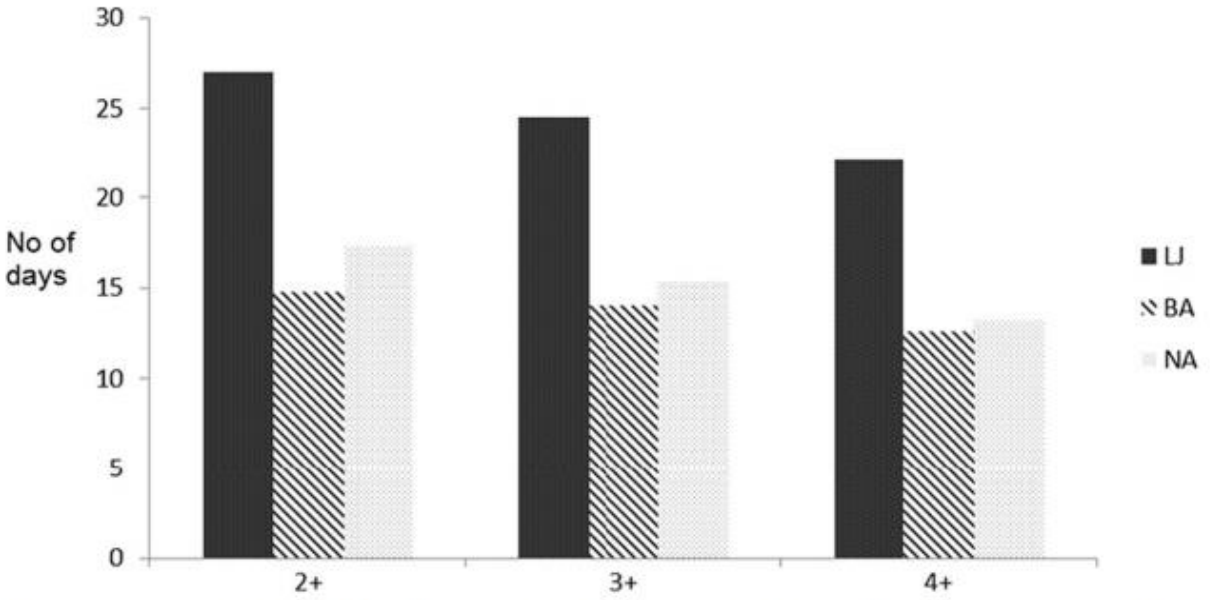

FIG 1 Average time (in days) for detection of growth by three culture methods, grouped by bacillary smear load. BA, blood agar; NA, nutrient agar.

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PLOS ONE

Blood Agar Validation for Susceptibility Testing of Isoniazid, Rifampicin, Ethambutol, and Streptomycin to *Mycobacterium tuberculosis* Isolates

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Abstract

In recent studies, it was shown that blood agar can be used at least as effectively as Löwenstein-Jensen medium for growing *Mycobacterium tuberculosis*. It was also shown that susceptibility testing can be performed on blood agar. Additional validation of blood agar was performed on regional *M. tuberculosis* isolates from Turkey to determine critical concentrations of isoniazid (INH), rifampicin (RIF), ethambutol (ETM), and streptomycin (STR). In the current study, 40 *M. tuberculosis* clinical isolates were tested. H37Rv, which is susceptible to all antituberculosis agents, ATCC 35822 (INH-resistant), ATCC 35838 (RIF-resistant), ATCC 35837 (ETM-resistant), and ATCC 35820 (STR-resistant) quality control strains were used as control strains. Proportion method on 7H11 agar was considered as gold standard in the study. MIC values of the control strains and clinical isolates were detected on blood and 7H11 agar. Categorical agreements were 100% for each antibiotic, and essential agreements were 100%, 97.5%, 82.5%, and 95% for INH, RIF, ETM, and STR, respectively. According to the data, 0.2 µg/mL for INH, 1 µg/mL for RIF, 4 µg/mL for ETM, and 2 µg/mL for STR were appropriate breakpoint values for susceptibility testing on blood agar. Blood agar may be recommended for use in both developed and developing countries for the susceptibility testing of *M. tuberculosis* isolates to primary antituberculosis drugs.

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Competing Interests: The author has declared that no competing interest exist.

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Table 1. MIC values of standard strains on both media in the preliminary study.

Strains	INH (μg/ml)		RIF (μg/ml)		ETM (μg/ml)		STR (μg/ml)	
	BA	7H11A	BA	7H11A	BA	7H11A	BA	7H11A
H37Rv	0.125	0.125	1	1	4	4	2	1
ATCC 35822	>32	>32	1	1	4	4	2	1
ATCC 35838	0.125	0.125	>32	>32	4	4	2	1
ATCC 35820	0.125	0.125	1	1	4	4	>32	>32
ATCC 35837	0.06	0.06	0.5	0.25	16	16	1	1

INH: isoniazid; RIF: rifampicin; ETM: ethambutol; STR: streptomycin; BA: Blood agar; 7H11A: 7H11 agar; S: susceptible; R: resistant.

Table 2. Comparison of the MIC values of each antibiotics on blood agar with 7H11 agar.

Drugs	0 dilution (n)	±1 dilution (n)	±2 dilution (n)	±3 dilution (n)	EA (%)	CA (%)
INH	22	18	–	–	100	100
RIF	31	8	1	–	97.5	100
ETM	18	15	5	2	82.5	100
STR	22	16	2	–	95	100

EA: essential agreement, CA: Categorical agreement; n: number of isolates.

agreements were 100%, 97.5%, 82.5%, and 95% for INH, RIF, ETM, and STR, respectively. According to the data, 0.2 μg/mL for INH, 1 μg/mL for RIF, 4 μg/mL for ETM, and 2 μg/mL for STR were appropriate breakpoint values for susceptibility testing on blood agar. Blood agar may be recommended for use in both developed and developing countries for the susceptibility testing of *M. tuberculosis* isolates to primary antituberculosis drugs.

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Evaluation of Agar-Based Medium with Sheep Sera for Testing of Drug Susceptibility of *Mycobacterium tuberculosis* to Isoniazid, Rifampin, Ethambutol, and Streptomycin

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The performance of sheep sera instead of sheep blood in agar-based media was investigated for susceptibility testing of *Mycobacterium tuberculosis* against primary drugs. The levels of agreement between agar-based medium supplemented with sheep sera and the proportion method on Middlebrook 7H11 agar as the reference method for determining susceptibility to isoniazid (INH), rifampin (RIF), ethambutol (EMB), and streptomycin (STR) were 98.4, 98.4, 95.3, and 100%, respectively.

Tuberculosis is a major public health problem, particularly in developing countries. Multidrug-resistant tuberculosis (MDR-TB) is one of the factors that reduce the efficacy of tuberculosis control programs (1). Early identification of drug-resistant *Mycobacterium tuberculosis* is the first and most crucial step in the fight against tuberculosis. The existence of an available and cheap method for susceptibility testing may help to prevent and reduce dissemination of MDR tuberculosis and may increase as the efficacy of tuberculosis control programs as well (2).

The agar proportion method performed with Middlebrook 7H10-11 agar is used as a reference method for susceptibility testing of *Mycobacterium tuberculosis*. The results are calculated by comparing the colony number in a drug-containing tube to the colony number in a drug-free tube. If this ratio is >1%, the bacteria are considered resistant to the drug (3). It was reported that min-dextrose-catalase [OADC]) was added at 10%. Each antibiotic was added from stock solutions. The final concentrations of INH, RIF, EMB, and STR were 0.2 μg/ml, 1 μg/ml, 7.5 μg/ml, and 2 μg/ml, respectively (3). Once the drug was added, 1 ml of medium with drug was dispensed into 24-well plates (TPP; Tissue Culture Testplate, Switzerland) and this was repeated for each drug. Growth control medium without drug was also prepared in 24-well plates for each bacterium. All plates were stored at +4°C until use.

Blood agar-based medium (bioMérieux, Marcy l'Etoile, France) was prepared according to the manufacturer's instructions; after it was sterilized, sheep sera (Pendik Veteriner Kontrol Enstitusu; Pendik, Istanbul, Turkey) were added to the medium at 5%. Each antibiotic was added from stock solutions. The final concentrations of INH, RIF, EMB, and STR were 0.2 μg/ml, 1

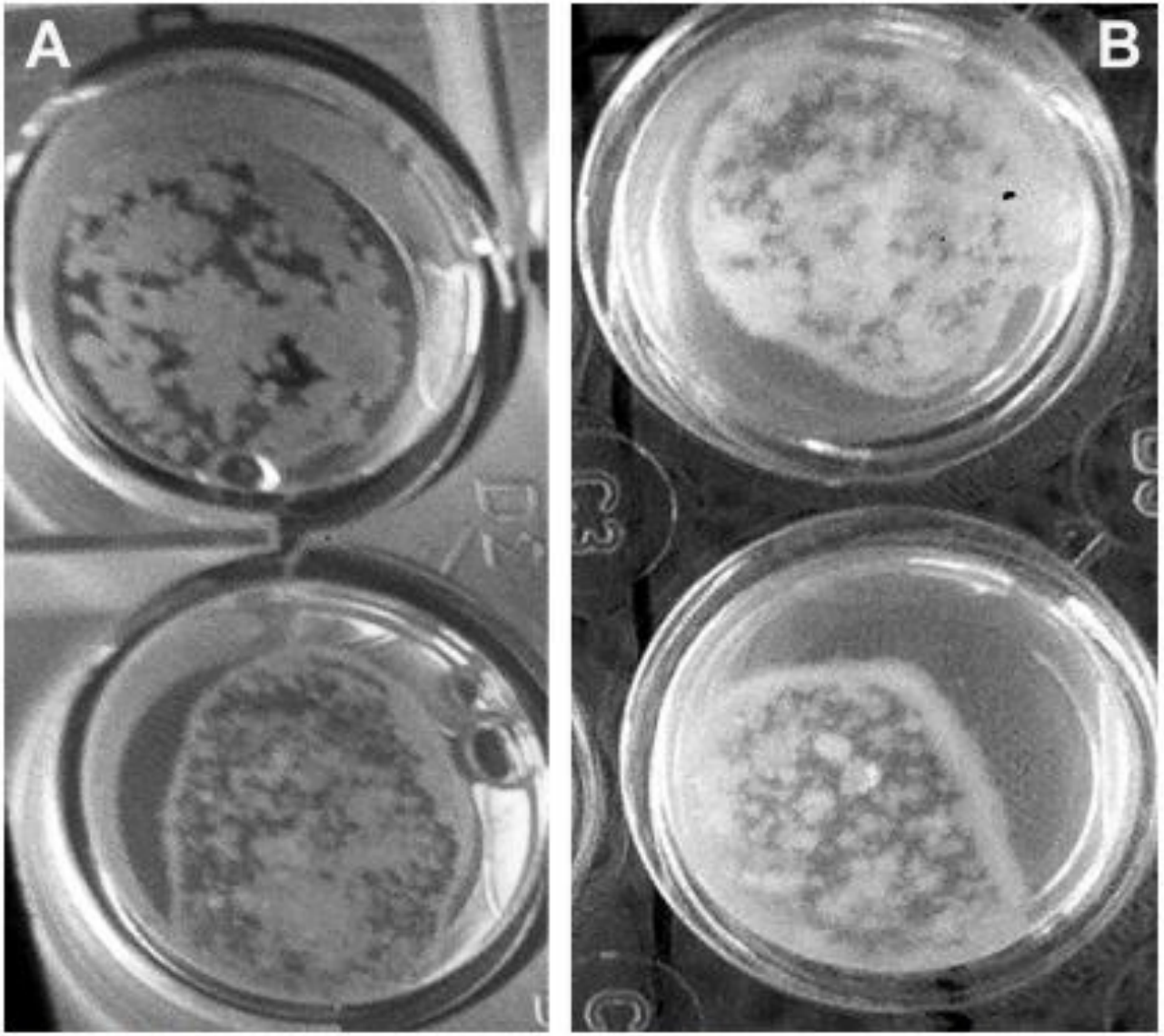

FIG 1 Bacterial growth on blood agar base medium with sheep sera (A) and on 7H11 agar (B).

TABLE 1 Results determined using blood agar-based media with sheep sera compared with results by the reference method, agar proportion on 7H11 agar[a]

		Reference method (agar proportion on 7H11 agar)						
Drug	BBMSS	S	R	Sensitivity (%)	Specificity (%)	Major discrepancy (%)	Very major discrepancy (%)	Agreement (%)
INH	S	40	1	100	95.8	0	2.5	98.4
	R	0	23					
RIF	S	44	1	100	95	0	2.2	98.4
	R	0	19					
EMB	S	55	3	100	66.7	0	5.4	95.3
	R	0	6					
STR	S	51	0	100	100	0	0	100
	R	0	13					

[a] BBMSS, blood agar-based medium with sheep sera; INH, isoniazid; RIF, rifampin; EMB, ethambutol; STR, streptomycin; S, susceptible; R, resistant.

Çalışma	Duyarlılık(%)				Özgüllük(%)				Uyum(%)				Yöntem	Referans Yöntem
	STR	INH	RIF	EMB	STR	INH	RIF	EMB	STR	INH	RIF	EMB		
Lopez-Roa ve ark	-	-	-	85,7	-	-	-	73,7	-	-	-	76,9	MGIT 960	Proportion
Said ve ark	95	-	-	92	37	-	-	37	61	-	-	44	MGIT 960	Proportion
Zhao ve ark	93,3	96,9	98,4	94,6	96,9	98,2	98,4	95,5	95,6	97,6	98	95,1	MGIT 960	Proportion

World Health Organization

Noncommercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis

Executive summary

Commercial liquid culture systems and molecular line-probe assays have been endorsed by the World Health Organization (WHO) as gold standards for rapid detection of multidrug-resistant (MDR) tuberculosis (TB); however, because of technical complexity, cost and the requirement for sophisticated laboratory infrastructure, use of these techniques has been limited in many resource-constrained settings. Several noncommercial culture and drug-susceptibility testing (DST) methods have been developed specifically for settings with limited access to sophisticated laboratory infrastructure and technical expertise. Several rapid, inexpensive methods have shown initial promise. The most advanced are microscopic observation of drug susceptibility (MODS), colorimetric redox indicator (CRI) methods, thin-layer agar methods, the nitrate reductase assay (NRA) and mycobacteriophage-based assays.

- *MODS*: a microcolony direct method in liquid culture, based on inoculation of specimens into drug-free and drug-containing media, followed by microscopic examination of early growth;
- *thin-layer agar*: a microcolony direct method on solid culture, based on inoculation of specimens into drug-free and drug-containing media, followed by microscopic examination of early growth;
- *CRI methods*: indirect methods based on the reduction of a coloured indicator added to liquid culture medium on a microtitre plate after exposure of *Mycobacterium tuberculosis* strains to anti-TB drugs in vitro;
- *NRA*: a direct or indirect method based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a colour reaction; and
- *phage-based assays*: assays in which bacteriophages are used to infect and detect the presence of viable *M. tuberculosis* in clinical specimens and culture isolates.

References

1. http://www.gradeworkinggroup.org
2. http://www.who.int/tb/laboratory/policy_statements/en/index.html
3. http://www.who.int/tb/advisory_bodies/stag/en/index.html
4. http://www.tbevidence.org/rescentre/sop.htm

Sonuç

- Literatür incelendiğinde;
  - İlaç direncinin hızlı tespiti için;
    - Kolay uygulanabilen
    - Ucuz
    - Hızlı
    - Güvenilir

      Fenotipik/kolorimetrik yöntemler bulunmaktadır
  - Klinik mikobakteriyoloji laboratuvarları bu yöntemlerden kendileri için uygun olanı seçip kullanabilir
  - Ayrıca;
    - Bu yöntemleri içeren çok merkezli validasyon çalışmaları ile en uygun yöntem belirlenip adres gösterilebilir.