



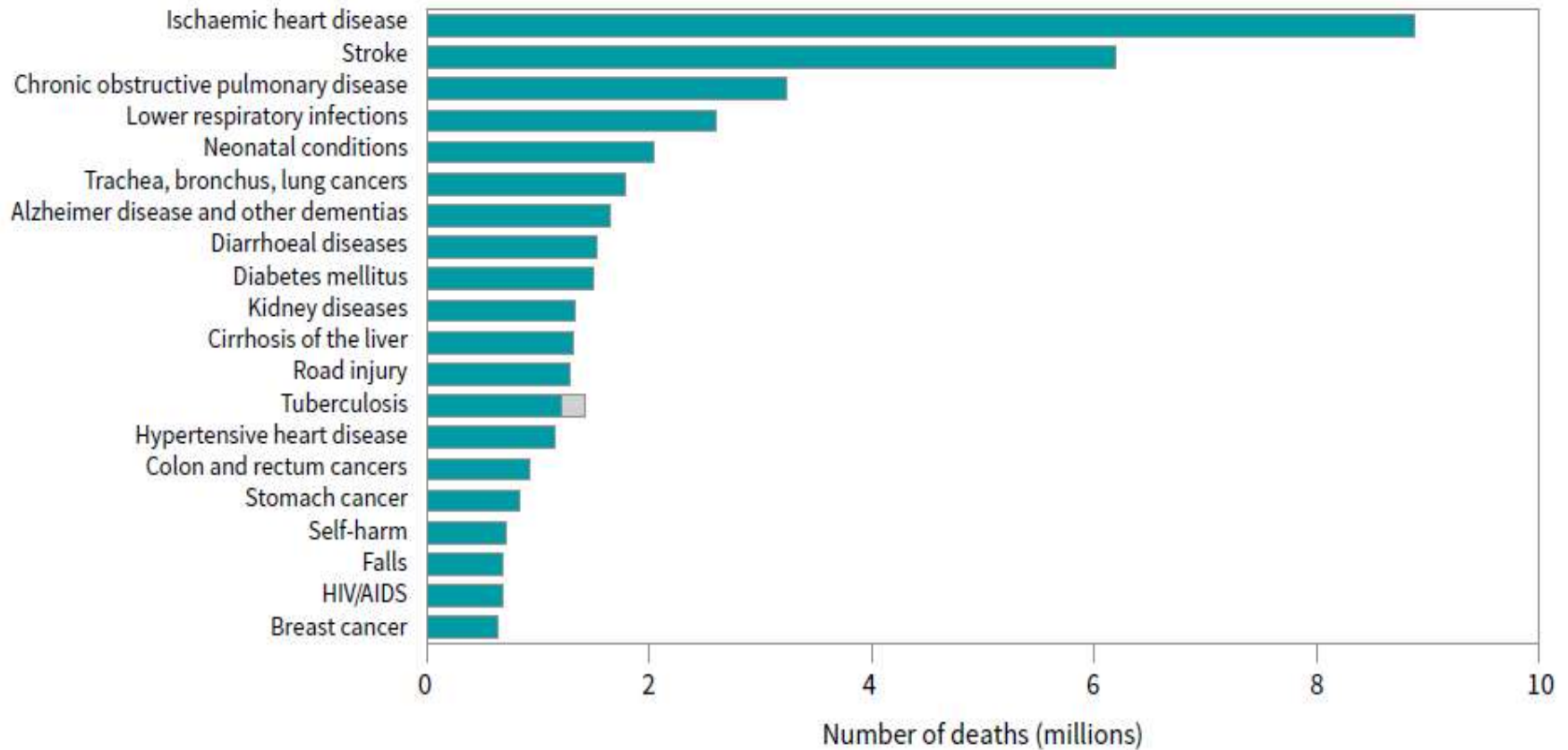
TÜBERKÜLOZ TANISINDA YENİLİKLER

YUSUF ZİYA DEMİROĞLU
BAŞKENT ÜNİVERSİTESİ TIP FAKÜLTESİ
ENFEKSİYON HASTALIKLARI VE KLİNİK
MİKROBİYOLOJİ

TB-Epidemiyoloji

Top causes of death worldwide in 2019^{a,b}

Deaths from TB among HIV-positive people are shown in grey.



Global tuberculosis report 2022. Geneva: WHO

TB-Epidemiyoloji

Yeni tanı alan TB

- 2019; 7.1 milyon kişi
- 2020; 5.8 milyon
- 2021; 6.4 milyon

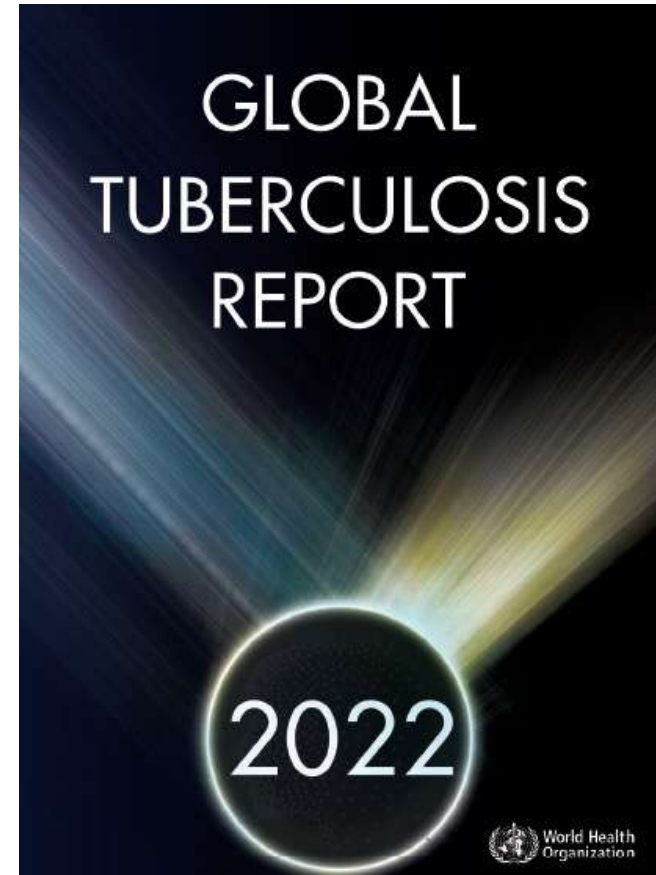
WHO tahminleri

- 2020;10.1 milyon kişi
- 2021;10.6 milyon kişi

2020'den 2021'e insidans %3,6 ↑

Son 2 dekat olan yıllık %2 azalma ters döndü.

2015-2021 net düşüş %10

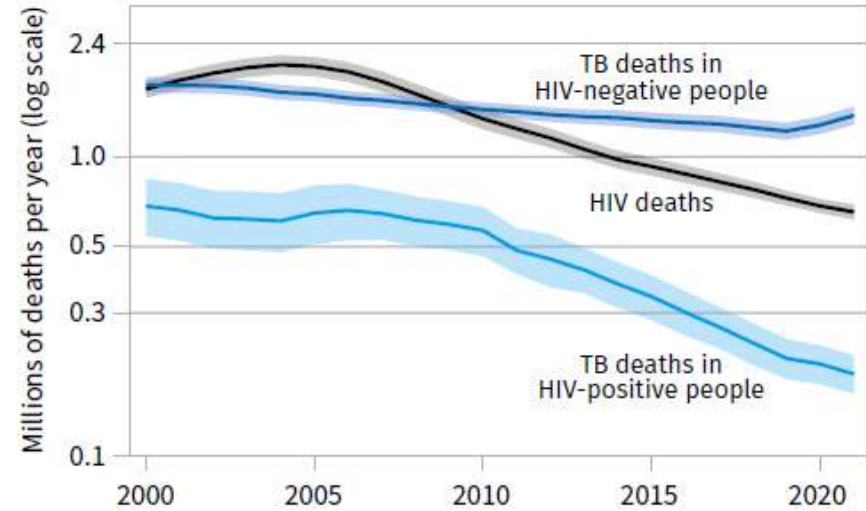


TB-Epidemiyoloji

Global trend in case notifications of people newly diagnosed with TB, 2015–2021



Global trends in the estimated number of deaths caused by TB and HIV, 2000–2021^{a,b}
Shaded areas represent 95% uncertainty intervals.



COVID-19'un olumsuz etkisi

- Tanı konulamayan/ bildirilmeyen olgular ölümlerin artması

Tuberculosis in Türkiye



POPULATION 2021
85 million

See also the detailed TB profile

End TB Strategy milestones for 2020



TB DEATHS 2021 VS 2015
87% increase
(Target is 35% reduction by 2020)



TB INCIDENCE 2021 VS 2015
3.8% reduction
(Target is 20% reduction by 2020)

TB deaths (including in people living with HIV)



Tuberculosis profile: Türkiye

Population 2021: 85 million

Estimates of TB burden*, 2021

| | Number | (Rate per 100 000 population) |
|---------------------------|------------------------|-------------------------------|
| Total TB incidence | 15 000 (12 000-18 000) | 18 (15-21) |
| HIV-positive TB incidence | 180 (130-230) | 0.21 (0.16-0.27) |
| MDR/RR-TB incidence** | 420 (330-510) | 0.5 (0.39-0.61) |
| HIV-negative TB mortality | 970 (870-1 100) | 1.1 (1-1.3) |
| HIV-positive TB mortality | 83 (74-92) | 0.1 (0.09-0.11) |

Estimated proportion of TB cases with MDR/RR-TB*, 2021

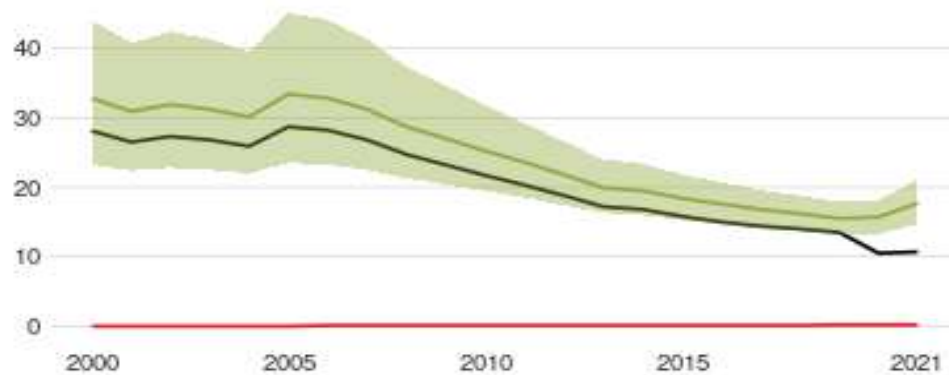
| | |
|--------------------------|----------------|
| New cases | 2.4% (2.1-2.7) |
| Previously treated cases | 8% (6.6-9.9) |

Universal health coverage and social protection*

| | |
|--|-------------|
| TB treatment coverage (notified/estimated incidence), 2021 | 60% (51-73) |
| TB patients facing catastrophic total costs | |
| TB case fatality ratio (estimated mortality/estimated incidence), 2021 | 7% (6-9) |

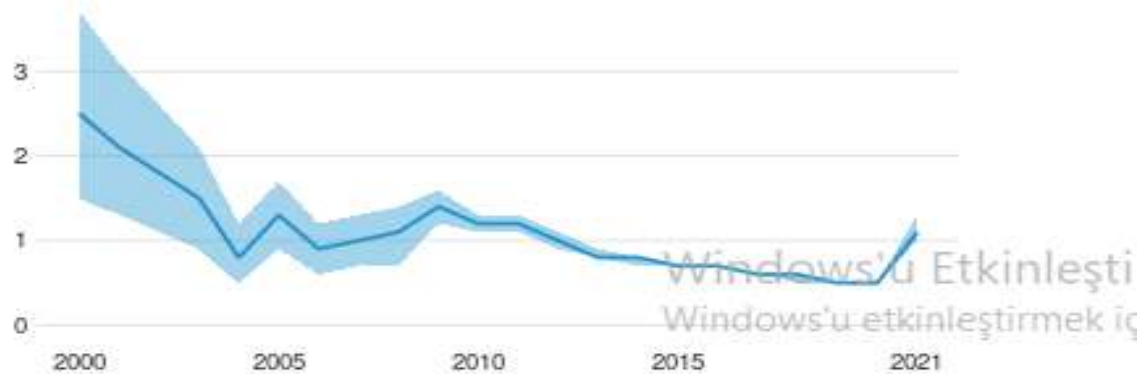
Incidence, New and relapse TB cases notified, HIV-positive TB incidence

(Rate per 100 000 population per year)



HIV-negative TB mortality

(Rate per 100 000 population per year)



WHO ne hedefliyor?

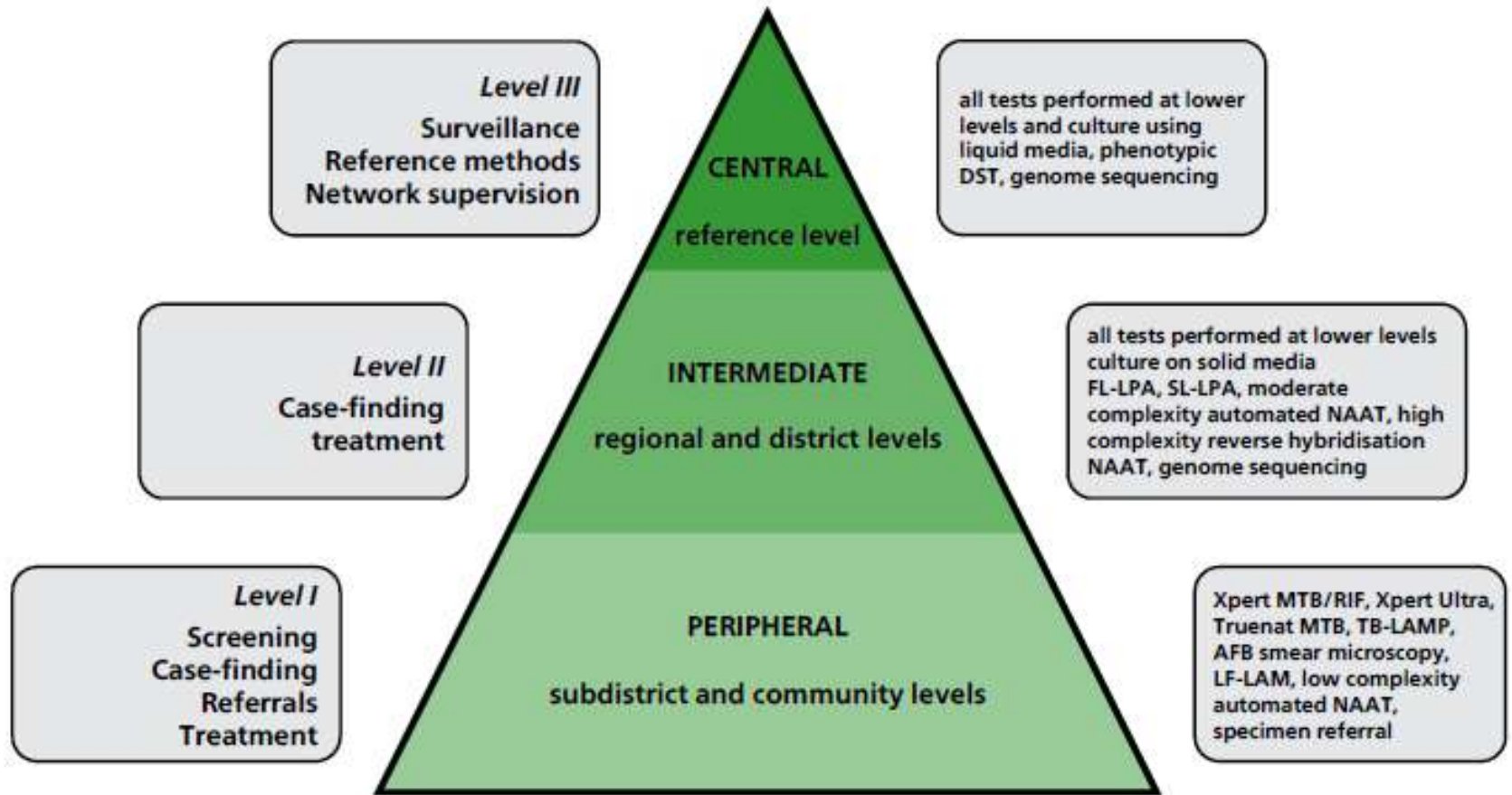
End-TB; 2015-2030

- Erken ve hızlı tanı
- Bakteriyolojik konfirme edilen olgulara en azından RIF için direnç testi, RIF direnci varsa FQ ve 2. seçenek enjektabl ilaçlara direnç testi
- Bu amaçla bazı moleküler testleri tavsiye ediyor.
- (molecular WHO-recommended rapid diagnostic tests (mWRDs))

WHO operational handbook on tuberculosis.

Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update

Fig. 3.1. Organization of a TB diagnostic network



AFB: acid-fast bacilli; DST: drug susceptibility testing; FL: first-line; LAMP: loop-mediated isothermal amplification; LF-LAM: lateral flow lipoarabinomannan assay; LPA: line-probe assay; NAAT: nucleic acid amplification test; SL: second-line; TB: tuberculosis.

WHO operational handbook on tuberculosis.

Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update

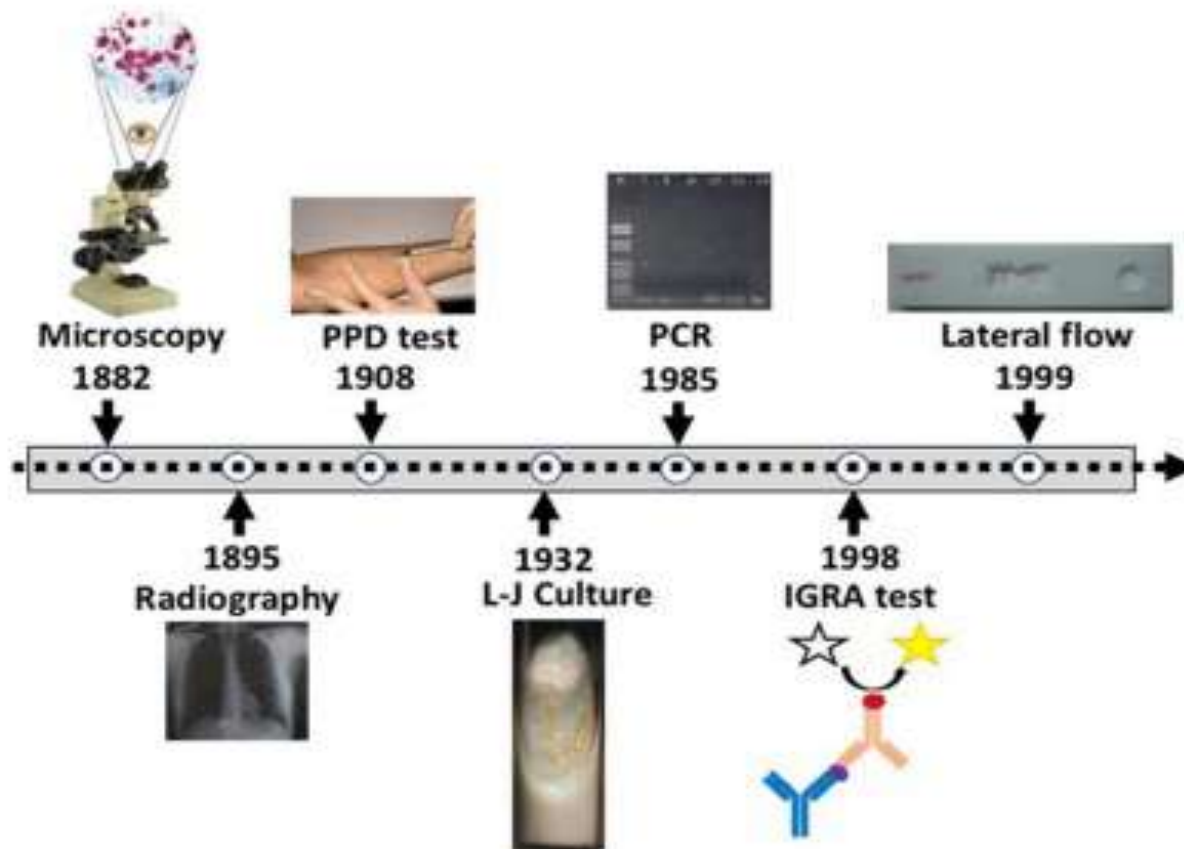


Fig. 1 Milestone on the evolution of tuberculosis diagnostic assays. The dotted line indicates the time line but not in scale. *IGRA* interferon-gamma release assay, *L-J* Lowenstein–Jensen, *PCR* polymerase chain reaction, *PPD* purified protein derivative. (Color figure online)

Etkenin gösterilmesi

I-TB basilinin mikroskopik olarak araştırılması

a-Işık mikroskop

- Ziehl-Neelsen boyama
- Kinyoun

b-Floresan mikroskop

- Auramin O
 - Auramin-Rodamin
- } Floresan boyalar

c-LED (Light emitting diode) mikroskop

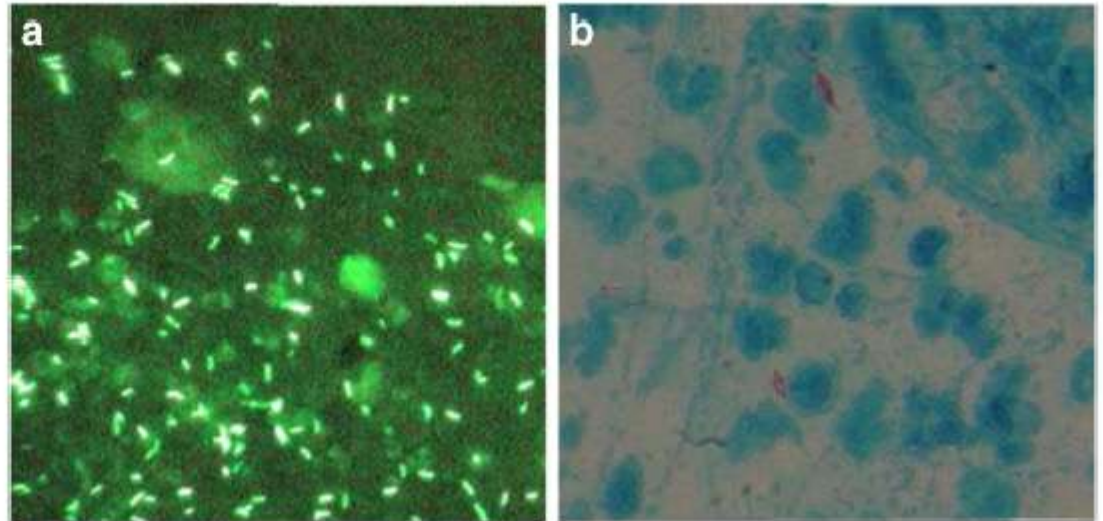
- Floresan boyama ile

Etkenin gösterilmesi

c-LED mikroskopi

- 2009'dan beri WHO önerisi
- Duyarlılık (%84), Özgüllük (%98) (Kültüre göre)
- Duyarlılık EZN'den %6 daha fazla, özgüllük aynı
- Duyarlılık konvansiyonel FM'den %5 fazla, %1 daha özgül
- Florasan mikroskoptan daha ucuz
- Karanlık oda ve UV gerektirmiyor
- Taşınabilir batarya ile daha az güçle çalışıyor
- Daha hızlı inceleme

Fig. 1 (A) Photomicrograph of auramine-o stained sputum smear showing TB bacilli (AFB +++) by light emitting diode (LED) fluorescence microscopy ($\times 200$); (B) photomicrograph of Ziehl-Neelsen stain sputum smear showing TB bacilli (AFB +++) ($\times 1000$) [8]



Mikroskopi aşamasında yenilikler

Biyogüvenlik ve duyarlılık arttıran yöntemler

- 1-TB*Detec*
- 2-SafeTain

Etkenin gösterilmesi

I-TB basilinin mikroskopik olarak araştırılması

RESEARCH ARTICLE

Development and evaluation of novel bio-safe filter paper-based kits for sputum microscopy and transport to directly detect *Mycobacterium tuberculosis* and associated drug resistance

Divya Anthwal^{1e}, Surabhi Lavania^{2e}, Rakesh Kumar Gupta^{1a}, Ajoy Verma³, Vithal Prasad Myneedu³, Prem Prakash Sharma³, Hitesh Verma⁴, Vivekshel Malhotra⁴, Ashawant Gupta⁵, Nalini Kant Gupta⁵, Rohit Sarin³, Sagarika Halder^{1a}, Jaya Sivaswami Tyagi^{1,2}

TB Detec

(A)



(B)



(C)



Fig 1. Developed kits: (A) 'TB detect' kit; (B) 'TB concentration & transport' kit; and (C) 'TB DNA extraction' kit.

TBDetec

(A) 'TB Detect' kit



Mix Dissolving
Solution + sputum
Incubate for 30 min



Filter through
BioFM-Filter



Staining &
Disinfection



Observe under
LED-microscope

- * Balgamın likefaksiyonunu sağlayan, bakterilerin yoğunlaşmasını sağlayan bir membran kullanılan filtre sistemi
 - * Dışardan vakum yada basınca ihtiyaç duymadan yerçekiminin etkisi ve ped bazlı kapiller hareket ile eriyen balgamı filtreden geçirirken bakterileri filtre üzerinde tutar.
- TBDetec kit siyah non-floresan membran ve siyah non floresans plastik cihaz içerir.
- * Çözücü solüsyon; Guanidinyum hidroklorür, Tris tamponu, Triton X-100 ve DTT'ye benzeyen işleve sahip indirgeyici ajan



Original article

Evaluation of 'TBDetect' sputum microscopy kit for improved detection of *Mycobacterium tuberculosis*: a multi-centric validation study

Divya Anthwal^{1,2}, Rakesh Kumar Gupta^{1,2}, Narayan Sivaramakrishnan Gomathi³, Srikanth Prasad Tripathy³, Dasarathi Das⁴, Sanghamitra Pati⁴, Nikita Panwalkar⁵, Prabha Desikan⁵, Kiran Bala⁶, Urvashi B. Singh⁶, Manpreet Bhalla⁷, Ritu Singhal⁷, Ajoy Kumar Verma⁷, Khalid Umar Khayyam⁷, Vithal Prasad Myneedu⁷, Rohit Sarin⁷, Sandeep Sharma⁸, Avi Kumar Bansal⁸, Umesh D. Gupta⁸, Sripad A. Patil⁸, Abhinav Goyal⁹, Ashawant Gupta⁹, Manjula Singh¹⁰, Nalini Kant Gupta⁹, Sagarika Halder^{1,2,*,*}, Jaya Sivaswami Tyagi^{1,11,*}

- Çift kör, Hindistanda 6 farklı LAB (5'i ulusal referans LAB)
- 2086 Balgam örneği

| | Pozitif | |
|--------------|---------|-----|
| TBDetect | 417 | %20 |
| LED-FM | 337 | %16 |
| Zieh-Neelsen | 333 | %16 |

Table 2

Sensitivity and specificity of TBDetect kit, light-emitting diode fluorescence microscopy (LED-FM) and Ziehl Neelsen (ZN) smear microscopy. vs Mycobacterium Growth Indicator Tube culture

| Site | TBDetect | | LED-FM | | ZN | |
|---|----------------|---------------|---------------|---------------|----------------|----------------|
| Total no. of samples with culture result/total no. of samples included in the study | Sn % (95% CI) | Sp % (95% CI) | Sn % (95% CI) | Sp % (95% CI) | Sn % (95% CI) | Sp % (95% CI) |
| Total (<i>n</i> = 1949/2086) [#] | 55.0* (50, 60) | 88 (86, 90) | 52.0 (47, 57) | 91.8 (90, 93) | 50.9* (46, 56) | 92 (90, 93) |
| NIRT, Chennai (<i>n</i> = 311/358) | 65.7 (48, 81) | 93 (90, 96) | 62.9 (45, 79) | 96.7 (94, 99) | 60 (42, 76) | 98.2 (96, 99) |
| RMRC, Bhubaneswar (<i>n</i> = 316/353) | 74.2 (62, 85) | 96.4 (93, 98) | 72.6 (60, 83) | 96.4 (93, 98) | 72.6 (60, 83) | 98 (96, 99) |
| BMHRC, Bhopal (<i>n</i> = 308/341) | 37.8 (27, 49) | 96 (93, 98) | 37.8 (27, 49) | 96 (93, 98) | 35.4 (25, 47) | 97 (94, 99) |
| AIIMS, New Delhi (<i>n</i> = 345/345) | 42.8 (1, 82) | 72.1 (67, 77) | 42.8 (1, 82) | 73.4 (68, 78) | 57.1 (18, 90) | 70.4 (65, 75) |
| NITRD, New Delhi (<i>n</i> = 344/348) | 48.2 (37, 59) | 87.7 (83, 92) | 47 (36, 58) | 98 (96, 99) | 44.6 (34, 56) | 99.2 (97, 100) |
| NJILOMD, Agra (<i>n</i> = 325/341) | 60 (50, 70) | 88.5 (84, 92) | 52 (42, 62) | 97 (94, 99) | 52 (42, 62) | 97 (94, 99) |

AIIMS, All India Institute of Medical Sciences; BMHRC, Bhopal Memorial Hospital Research Centre; CI, confidence interval (rounded off to nearest whole number).

NIRT, National Institute for Research in Tuberculosis; NITRD, National Institute of Tuberculosis and Respiratory Diseases; NJILOMD, National JALMA Institute of Leprosy and other Mycobacterial Diseases; RMRC, Regional Medical Research Centre. Sn, sensitivity; Sp, specificity.

[#] For some samples, cultures were contaminated or results were unavailable.

* The increment in sensitivity of TBDetect vs ZN smear was significant ($p < 0.05$); but not between TBDetect vs LED-FM ($p 0.14$).

| | Yöntem | Duyarlılık | Özgüllük |
|-------------------|--------------------|-------------------|-----------------|
| Balgam mikroskopi | Ziehl-Neelsen | 32-94 | 50-99 |
| LED-FM | Auramine | 52-97 | 94-100 |
| TBDetec kit | BioFM-Filtre bazlı | 55 | 88 |

Etkenin gösterilmesi

I-TB basilinin mikroskopik olarak araştırılması

SafeTAIN TB boyama seti

- Hem EZN ve hem Kinyoun boyamada kullanılan karbolfuksin çözeltisindeki fenolün laboratuvardaki toksik etkilerini azaltmak için
- Karbolfuksin boyasının mikolik asitlerin arasına girmesini kolaylaştıran alkol, deterjan ve surfaktanların uygun karışımı ile oluşan boya
- Kinyoun boyası ile benzer sonuçlar

Etkenin gösterilmesi

II-Kültür yöntemleri

Katı besiyerleri: Yumurta temelli ve agar temelli

| Besiyeri | Bileşenleri | İnhibitör Ajanlar |
|-------------------|---|---|
| Löwenstein-Jensen | Tam yumurta, tuzlar, gliserol, patates unu | 0,025gr/100ml Malaşit yeşili |
| Petragnani | Tam yumurta, yumurta sarısı, tam süt, patates, patates unu, | 0,052gr/ 100ml gliserol Malaşit yeşili |
| ATS Besiyeri | Koagüle taze yumurta sarısı, gliserol, patates unu | Malaşit yeşili, 0,02 g /100 ml |
| Middlebrook 7H10 | Bazı tuzlar, vitaminler, kofaktörler, oleik asit, albümin, katalaz, gliserol, dextroz | Malaşit yeşili, 0.0025g/ 100ml |

Etkenin gösterilmesi

II-Kültür yöntemleri

Katı besiyerleri: Yumurta temelli ve agar temelli

| Besiyeri | Bileşenleri | İnhibitör Ajanlar |
|------------------|---|--|
| Middlebrook 7H11 | Bazı tuzlar, Vit.ler, kofaktörler, oleik asit, albümin, katalaz, gliserol, dextroz, %0,1 kazein hidrolizat Malaşit yeşili | Malaşit yeşili, 0.0025g/100ml |
| Modifiye L-J | Tam yumurta, tuzlar, gliserol, patates unu, 5mg/100ml RNA | 0,025gr/100ml Malaşit yeşili, 50U/ml penisilin, 35mg/ml nalidiksik asit |
| Middlebrook 7H10 | Bazı tuzlar, vitaminler, kofaktörler, oleik asit, albümin, katalaz, gliserol, glikoz | 0,0025gr/100ml Malaşit yeşili, 360µg/ml sikloheksimid, 2 µg/ml linkomisin, 20µg/ml nalidiksik asit |

Etkenin gösterilmesi

II-Kültür yöntemleri

Katı besiyerleri: Yumurta temelli ve agar temelli

| Besiyeri | Bileşenleri | İnhibitör Ajanlar |
|---|--|--|
| Selektif 7H11, (Mitchison's besiyeri) | Tuzlar, vitaminler, kofaktörler oleik asit, albümin, katalaz, glikoz, kazein hidrolizat gliserol, | 50µg/ml karbenisilin 10µg/ml amfoterisin B 200U/ml polimiksin B, 20µg/ml trimethoprim laktat |

Etkenin gösterilmesi

II-Kültür yöntemleri

Sıvı besiyerleri, yarı otomatik, tam otomatik

1-MGIT (Becton Dickinson, Biosciences)

Modifiye Middlebrook 7H9 sıvı by.

2-MB Redox (Heipha Diagnostica Biotest)

3-BACTEC 460TB (Becton Dickinson, Biosciences)

4- MGIT 960 (Becton Dickinson, Biosciences)

5- BACTEC 9000 MB (Becton Dickinson, Biosciences)

6-ESP II Kültür Sistemi (Trek Diagnostics)

7-MB/BacT Sistemi (Organon Teknika, İrlanda)

8-TK Medium / Mycolor TK

Etkenin gösterilmesi

III-Klinik Örneklerden Nükleik Asit Amplifikasyon Testi (NAAT) ile MTB tespiti

- Kültür ve mikroskopi negatif örneklerde
- TB basilinin atipik mikobakterilerden ayrımında
- İlaç direncinin genetik olarak saptanmasında

Etkenin gösterilmesi

III-Klinik Örneklerden Nükleik Asit Amplifikasyon Testi (NAAT) ile MTB tespiti

- 1- Xpert MTB/RIF Assay** (Cepheid, Sunnyvale, USA)
- 2- Xpert MTB/RIF Ultra Assay** (Cepheid, Sunnyvale, USA)
- 3-Truenat MTB, MTB plus and MTB-RIF Dx Assay** (Molbio Diagnostics, Goa, India)
- 4-TB-LAMP Assay** (Eiken Chemical, Tokyo, Japan)
(Loop-mediated isothermal amplification)
- 5-Line Probe Assay (LPA)**

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Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update

1-Xpert MTB/RIF Assay

- Kartuş tabanlı, real time PCR
- MTB varlığını ve RIF direncini (rpo B) 2 saatten az sürede

WHO önerisi

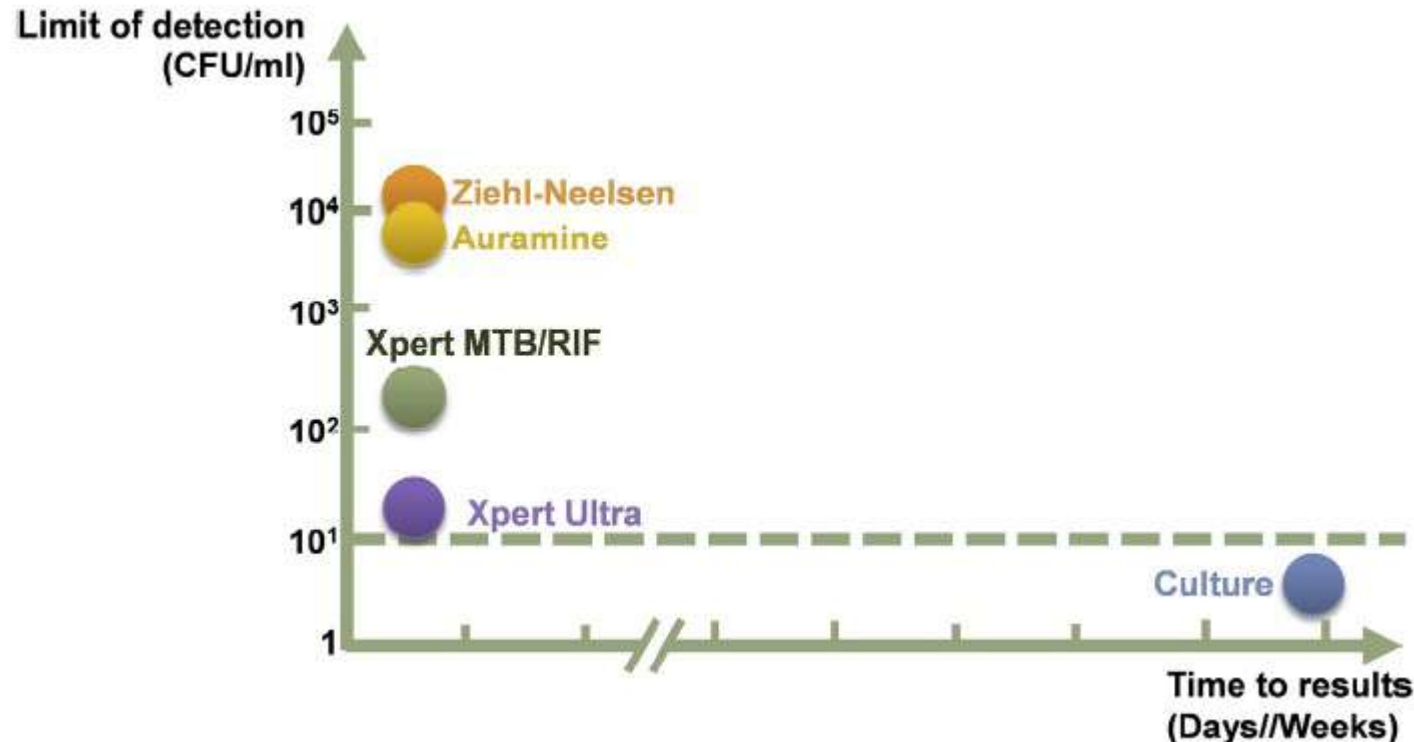
- Balgam, gastrik aspirat, nazofarengeal aspirat veya dışkı, BOS, lenf nodu biyopsisi, plevral effüzyon, peritoneal sıvı, perikardial sıvı, eklem sıvısı, idrar
- Erişkin ve çocuklarda kültür ve fenotipik duyarlılık testi yerine
- HIV (+) dissemine TB'da kan testi



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2-Xpert MTB/RIF Ultra Assay

- 2 kopyalı amplifikasyon hedefi (IS6110 ve IS1810)
- rpoB geni
- Daha hassas (16 cfu/ml iken Xpert TB 131 cfu/ml)



[Diagnostic Test Accuracy Review]

Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults

David J Horne^{1a}, Mikashmi Kohli^{2b}, Jerry S Zifodya³, Ian Schiller⁴, Nandini Dendukuri⁴, Deanna Tollefson⁵, Samuel G Schumacher⁶, Eleanor A Ochodo⁷, Madhukar Pai², Karen R Steingart⁸

- 86 çalışma (42,091 hasta, 45'i TB insidansı yüksek, 50'si HIV/TB insidansı yüksek)

Xpert MTB/RIF

- Duyarlılık %85; Özgüllük % 98 (CrI %95)
- Yalancı Pozitif %17, Yalancı Negatif %2

Xpert MTB/RIF Ultra

- Duyarlılık %88; Özgüllük % 96 (CrI %95)

[Diagnostic Test Accuracy Review]

Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults

David J Horne^{1a}, Mikashmi Kohli^{2b}, Jerry S Zifodya³, Ian Schiller⁴, Nandini Dendukuri⁴, Deanna Tollefson⁵, Samuel G Schumacher⁶, Eleanor A Ochodo⁷, Madhukar Pai², Karen R Steingart⁸

| Xpert MTB/RIF | Duyarlılık |
|---------------|------------|
| ARB (+) | %98 |
| ARB (-) | %67 |
| HIV (-) | %88 |
| HIV (+) | %81 |

| Xpert MTB/RIF Direnç tespiti | Duyarlılık |
|---------------------------------|------------|
| Duyarlılık | % 96 |
| Özgüllük | % 98 |
| Yalancı Pozitif | % 16 |
| Yalancı Negatif | % 0.4 |

Xpert Ultra RIF direnci tespitinde; Duyarlılık % 95
Özgüllük % 98

[Diagnostic Test Accuracy Review]

Xpert MTB/RIF Ultra and Xpert MTB/RIF assays for extrapulmonary tuberculosis and rifampicin resistance in adults

Mikashmi Kohli¹, Ian Schiller², Nandini Dendukuri², Mandy Yao², Keertan Dheda^{3,4}, Claudia M Denkinger^{5,6}, Samuel G Schumacher⁵, Karen R Steingart⁷

¹Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada. ²Centre for Outcomes Research, McGill University Health Centre - Research Institute, Montreal, Canada. ³Centre for Lung Infection and Immunity Unit, Department of Medicine and UCT Lung Institute, University of Cape Town, Cape Town, South Africa. ⁴Faculty of Infectious and Tropical Diseases, Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, UK. ⁵FIND, Geneva, Switzerland. ⁶Division of Tropical Medicine, Centre for Infectious Diseases, University Hospital Heidelberg, Heidelberg, Germany. ⁷Honorary Research Fellow, Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK

- 69 Ç; 67 Xpert MTB/RIF, 11 Xpert Ultra, 9'u her ikisi
- Çin, Hindistan, Güney Afrika ve Uganda

BOS alıřmaları

Xpert Ultra ; 6 alıřma

- Duyarlılık % 89.4 , zgllk % 91,2
- Yalancı Pozitif %47
- Yalancı Negatif %1

Xpert MTB/RIF ; 30 alıřma

- Duyarlılık %71.1, zgllk 96,9
 - Yalancı Pozitif % 28
 - Yalancı Negatif %3
-
- Referans yntem: sıvı yada katı kltr

Plevral sıvı alıřmaları

Xpert Ultra ; 4 alıřma

- Duyarlılık % 75 , zgllk %87
- Yalancı Pozitif %61
- Yalancı Negatif %3

Xpert MTB/RIF ; 25 alıřma

- Duyarlılık %49.5, zgllk % 98.9
- Yalancı Pozitif % 17
- Yalancı Negatif % 5
- Referans yntem: Sıvı yada katı kltr

Lenf nodu Aspiratı alıřmaları

Xpert Ultra ; 1 alıřma (Kltre gre)

- Duyarlılık % 70 (70 test (+) tm TB)
- zgllk % 100 (30 TB dahil 930 negatif)
- Yalancı Pozitif % 0
- Yalancı Negatif % 3

Xpert MTB/RIF ; 4 alıřma

- Duyarlılık % 81.6, zgllk % 96.4
 - Yalancı pozitif % 31
 - Yalancı Negatif %2
-
- Referans test: Komposit referans standart

Rifampisin Direnci alıřmaları

Xpert Ultra ; 4 alıřma

- Duyarlılık % 100, Özgüllük % 100

Xpert MTB/RIF ; 19 alıřma

- Duyarlılık % 97 (105 pozitif, 8'inde R yok)
- Özgüllük % 99 (895 test negatif, 3'ünde R var)
- Referans yöntem; Sıvı yada katı kültür bazlı duyarlılık testleri yada Line Probe Assay

3-Truenat MTB, MTB Plus ve MTB RIF Dx Assay

- Truenat MTB ve MTB plus chip bazlı, real-time PCR, balgamdan direkt olarak 1 saatten kısa sürede tespit
- Pille çalışabilir
- Minimum eğitim ve teknik gerektirir



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3-Truenat MTB, MTB Plus ve MTB RIF Dx Assay

WHO önerisi

- AC TB düşündürülen semptomları olan erişkin ve çocuklarda mikroskopi yada kültür yerine Truenat MTB veya MTB Plus tercih edilebilir.
- Truenat MTB veya MTB Plus pozitif çıkması durumunda RIF direncini tespit için kültür veya fenotipik duyarlılık testleri yerine MTB RIF Dx kullanılabilir.

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Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update

Truenat MTB, MTB Plus ve MTB RIF Dx Assay

- Çok merkezli, prospektif
- 19 merkez, 7 referans LAB (Peru, Hindistan, Etopya, Papua Yeni Gine)
- AC TB tanısında kültür, fenotipik duyarlılık testleri, Xpert MTB/RIF veya Ultra ile karşılaştırılma
- 1807 TB bulguları olan hasta, %24 kültür pozitif, %15 RIF dirençli

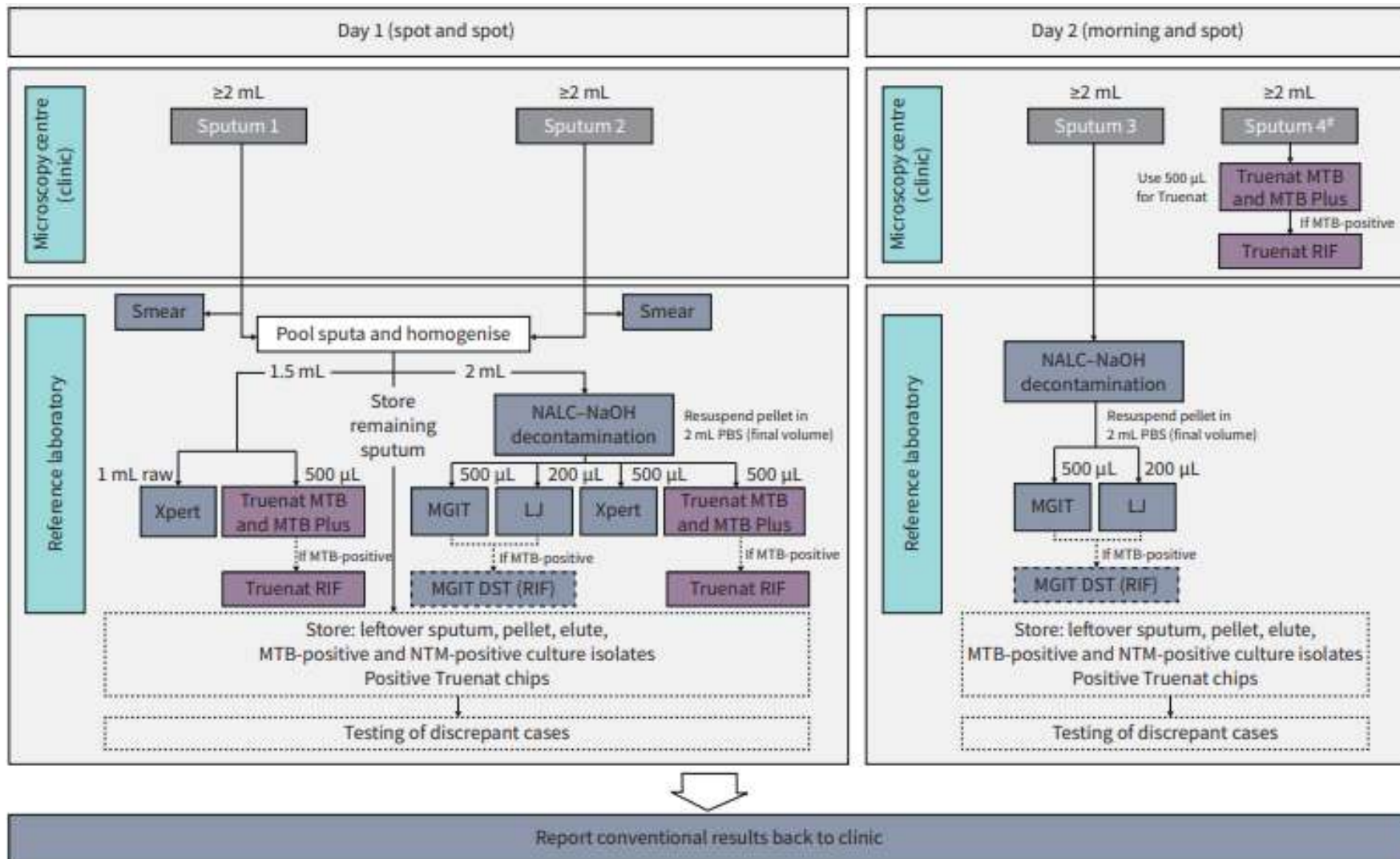
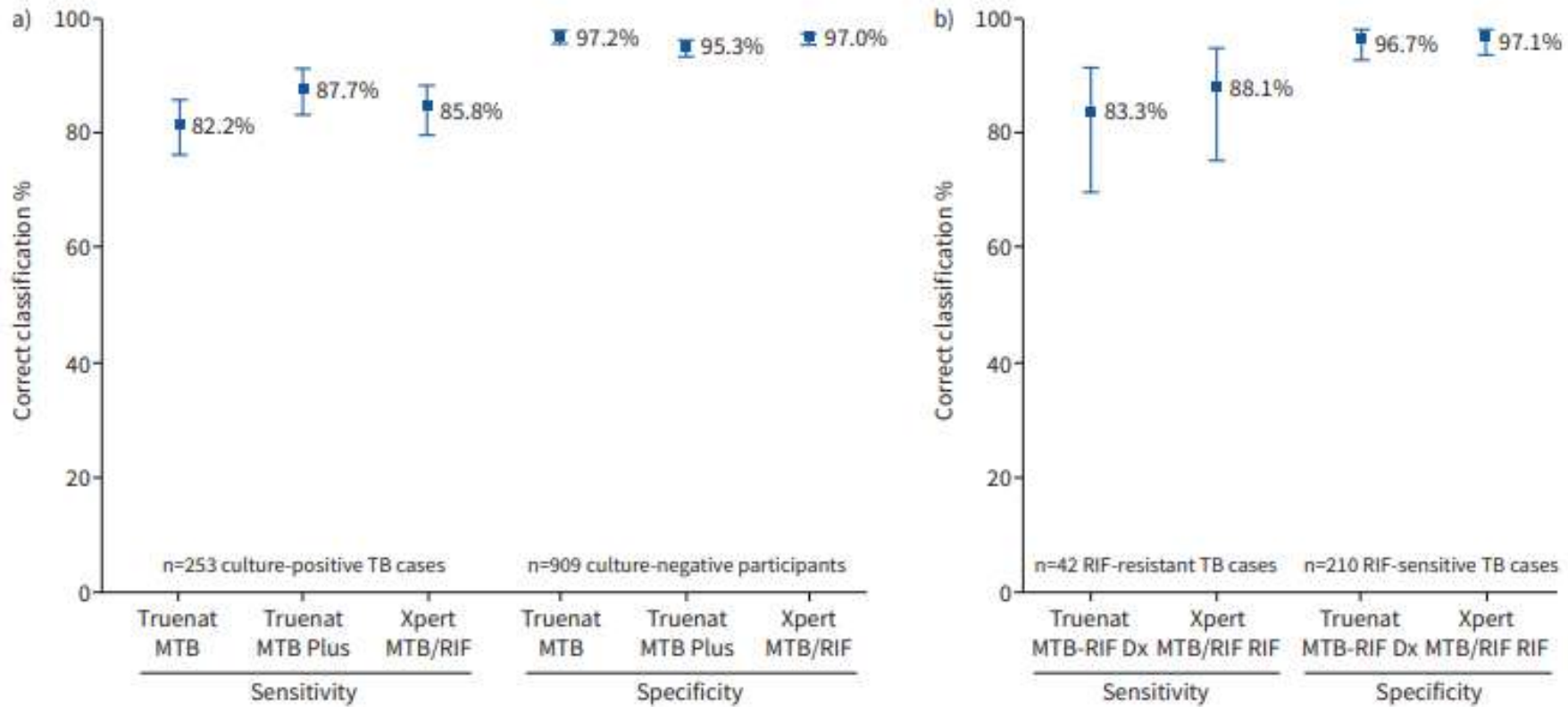


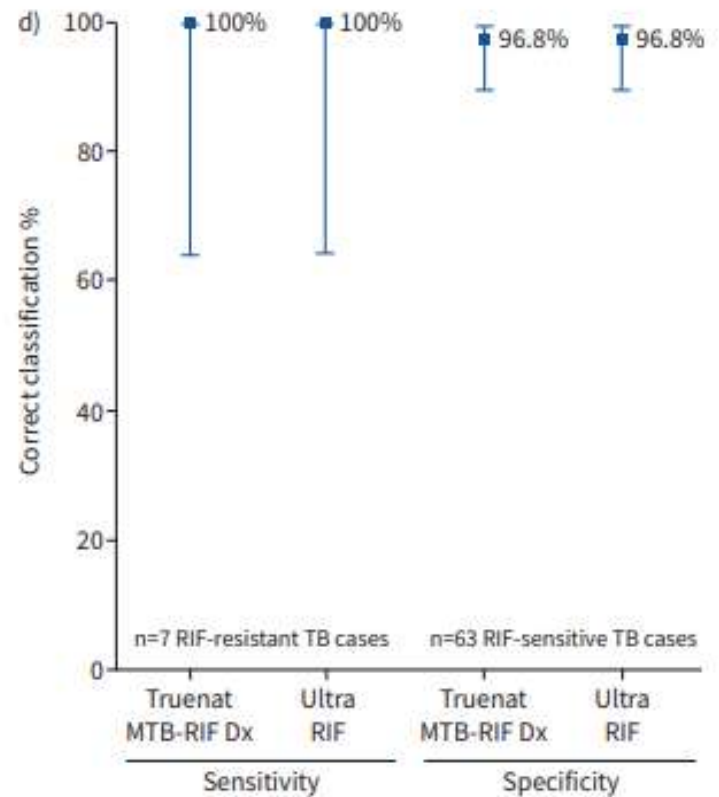
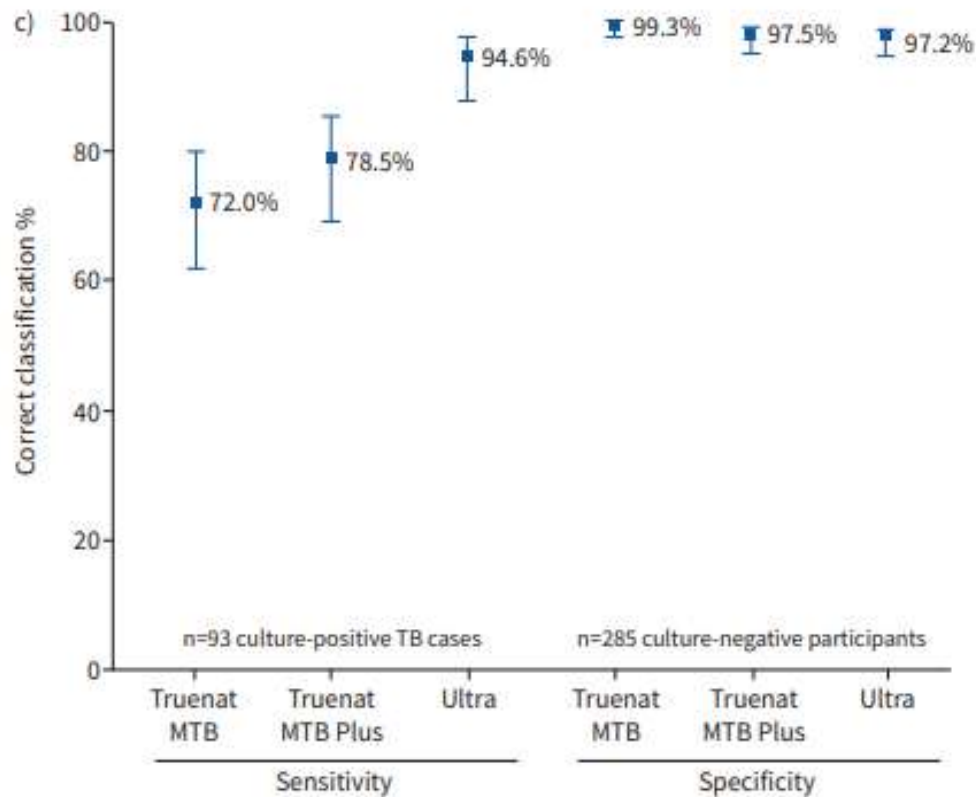
TABLE 2 Performance of Truenat assays for tuberculosis and for rifampicin resistance detection at the primary healthcare centre (microscopy centre) and the reference laboratory

| | N | True positive | False positive | False negative | True negative | Sensitivity % (95% CI) | Sensitivity % smear-positive (95% CI) | Sensitivity % smear-negative (95% CI) | Specificity % (95% CI) |
|------------------------------------|------|---------------|----------------|----------------|---------------|------------------------|---------------------------------------|---------------------------------------|------------------------|
| Microscopy centre sputum | | | | | | | | | |
| Truenat MTB | 1356 | 192 | 25 | 71 | 1068 | 73.0 (67.3–78.0) | 91.0 (85.8–94.4) (n=177) | 36.0 (26.7–46.6) (n=86) | 97.7 (96.7–98.5) |
| Truenat MTB Plus | 1356 | 210 | 40 | 53 | 1053 | 79.8 (74.6–84.2) | 96.0 (92.1–98.1) (n=177) | 46.5 (36.4–57.0) (n=86) | 96.3 (95.1–97.3) |
| Truenat MTB-RIF Dx | 190 | 16 | 9 | 3 | 162 | 84.2 (62.4–94.5) | 87.5 (64.0–96.5) (n=16) | 66.7 (20.8–93.8) (n=3) | 94.7 (90.3–97.2) |
| Reference laboratory sputum | | | | | | | | | |
| Truenat MTB | 1541 | 275 | 27 | 71 | 1168 | 79.5 (74.9–83.4) | 95.8 (92.4–97.7) (n=236) | 44.5 (35.6–53.9) (n=110) | 97.7 (96.7–98.4) |
| Truenat MTB Plus | 1541 | 295 | 51 | 51 | 1144 | 85.3 (81.1–88.6) | 98.3 (95.7–99.3) (n=236) | 57.3 (47.9–66.1) (n=110) | 95.7 (94.4–96.7) |
| Truenat MTB-RIF Dx | 332 | 44 | 9 | 8 | 271 | 84.6 (72.5–92.0) | 86.7 (73.8–93.7) (n=45) | 71.4 (35.9–91.8) (n=7) | 96.8 (94.0–98.3) |

Analysis of Truenat performance is shown on specimens tested at the microscopy centre and at the reference laboratory separately, with valid results available for both the Truenat MTB assay and the Truenat MTB Plus assay; denominators differ as two sites (PD Hinduja Hospital and Papua New Guinea) only had reference laboratory facilities available. Comparative performance of each assay performed on samples processed in the microscopy centre or the reference laboratory is shown in supplementary table S5.



Nicholson AP. *Eur Respir J.* 2021 Nov 4;58(5):2100526.



Nicholson AP. *Eur Respir J.* 2021 Nov 4;58(5):2100526.

4-TB-LAMP

(Loop-Mediated isothermal Amplifikasyon)

- Hızlı, kolay, ucuz, yüksek oranda spesifik
- 6 farklı hedef bölgeye özgü en az 4 primer kullanılır
- Duyarlılık %76-80 ve %97-98
- Tek adımlı sarmal yer değiştirme reaksiyonu
- 65 °C sabit sıcaklıkta 15-60 dk. amplifikasyon
- Çoğalma floresans veya gözle tüplerde bulanıklık olması ile tespit edilir.
- Kaynakları sınırlı, endemik bölgelerde önerilir.
- Ekstrapulmoner TB'da daha duyarlı.

4-TB-LAMP

Performance of Loop-Mediated Isothermal Amplification Assay for Diagnosis of Extrapulmonary Tuberculosis and Antituberculosis Treatment Initiation

Priyanka Singh, Swapna Kanade, Gita Nataraj

Department of Microbiology, Seth G. S. Medical College and King Edward Memorial Hospital, Mumbai, Maharashtra, India

- 100 hasta, 52 E, 48 K
- 31-45 yaş
- 38 örnekte herhangi bir yöntem ile MTB
- LAMP (kültüre göre)
- Duyarlılık % 85.7
- Özgüllük % 88.89

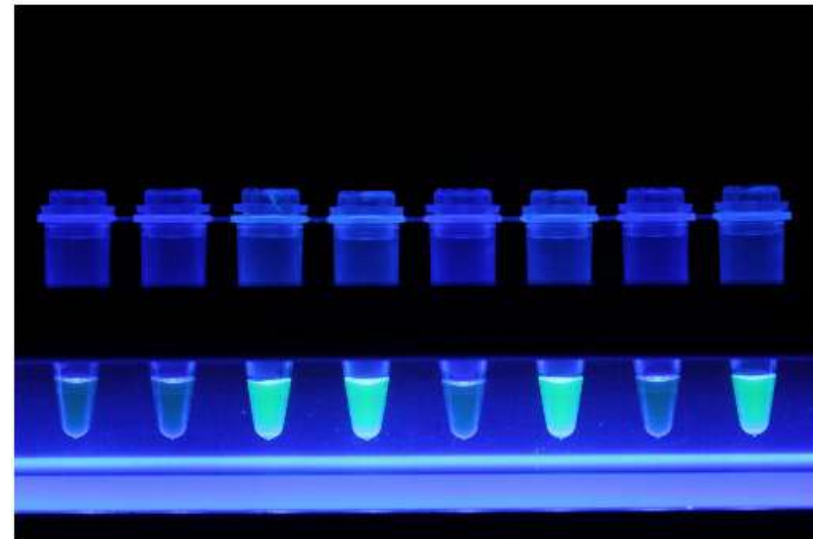


Table 2: Mycobacterium tuberculosis detection in different specimens (n=100)

| Specimen type | Total tested | MTB detected by any test, n (%) | MTB detected | | | |
|-------------------|--------------|---------------------------------|--------------|-------------|-------------------|------------|
| | | | Microscopy | Xpert assay | Culture (LJ/MGIT) | LAMP assay |
| Pleural fluid | 35 | 12 (34.28) | 1 | 7 | 8 | 9 |
| Ascitic fluid | 21 | 4 (19.04) | 0 | 1 | 1 | 3 |
| Pericardial fluid | 4 | 0 (0) | 0 | 0 | 0 | 0 |
| CSF | 5 | 1 (20) | 1 | 1 | 1 | 1 |
| Pus | 19 | 15 (78.94) | 2 | 11 | 12 | 15 |
| Tissue/FNAC | 16 | 6 (37.5) | 1 | 5 | 6 | 4 |
| Total | 100 | 38 | 5 | 25 | 28 | 32 |

MTB: Mycobacterium tuberculosis, MGIT: Mycobacteria Growth Indicator Tube, LJ: Lowenstein Jenson, LAMP: Loop-mediated isothermal amplification, CSF: Cerebrospinal fluid, FNAC: Fine-needle aspiration cytology

Table 3: Comparison of Nu-loop-mediated isothermal amplification™ tuberculosis and culture results (n=100)

| Nu-LAMP™ TB | Culture positive | Culture negative | Total |
|-------------|------------------|------------------|-------|
| Positive | 24 | 8 | 32 |
| Negative | 4 | 64 | 68 |
| Total | 28 | 72 | 100 |

LAMP: Loop-mediated isothermal amplification, TB: Tuberculosis

Table 4: Comparison of Nu-loop-mediated isothermal amplification™ tuberculosis and Xpert assay results (n=100)

| Nu-LAMP™ TB | Xpert positive | Xpert negative | Total |
|-------------|----------------|----------------|-------|
| Positive | 22 | 10 | 32 |
| Negative | 3 | 65 | 68 |
| Total | 25 | 75 | 100 |

LAMP: Loop-mediated isothermal amplification, TB: Tuberculosis

Table 5: Comparison of Nu-loop-mediated isothermal amplification™ tuberculosis with composite reference standard (n=100)

| Nu-LAMP™ TB | CRS positive | CRS negative | Total |
|-------------|--------------|--------------|-------|
| Positive | 24 | 8 | 32 |
| Negative | 6 | 62 | 68 |
| Total | 30 | 70 | 100 |

LAMP: Loop-mediated isothermal amplification, TB: Tuberculosis, CRS: Composite reference standard

Xpert'e göre
Duyarlılık % 88

Özgüllük %86.67

Referans CRS
Duyarlılık %80

Özgüllük %88.6

Table 7: Comparison of antituberculosis treatment with specimen type

| Specimen type | Total number | Tested positive by any test | On ATT | Not on ATT |
|----------------------|---------------------|------------------------------------|---------------|-------------------|
| Pleural fluid | 35 | 12 | 19 | 16 |
| Ascitic fluid | 21 | 4 | 10 | 11 |
| Pus | 19 | 15 | 12 | 7 |
| Tissue/FNAC/biopsy | 16 | 6 | 10 | 6 |
| CSF | 5 | 1 | 3 | 2 |
| Pericardial fluid | 4 | 0 | 2 | 2 |
| Total | 100 | 38 | 56 | 44 |

CSF: Cerebrospinal fluid, FNAC: Fine-needle aspiration cytology, ATT: Anti-TB treatment.

5-Line Probe Assay (LPA)

- TB varlığını ve ilaç direncine yol açan mutasyonları PCR ve takiben DNA hibridizasyon (oligonükleotid prop)
- TB varlığında renkli bant oluşur
- 2008 yılından beri WHO onaylı,
- Genotype MTBDR *plus* version 2; RIF (*rpoB* geni) ve INH direnci (*inhA* ve *katG*)
- Genotype MTBDRs version 2; kinolon direnci (*gyrA* ve *gyrB*) ve Amikasin direnci (*rrs* ve *eis* promoter)

5-Line Probe Assay (LPA)

Sistemik meta-analizlerde

- ARB pozitif PTB 'da

TB tespit; duyarlılık %94,

RIF direncini doğru belirleme;

- Duyarlılık %96.7 (n:21225), Özgüllük %98.8

INH direnci

- Duyarlılık:%90.2(n:20954),Özgüllük:99.2

İdrar LF-LAM

(Lateral flow Urine lipoarabinomannan)

- LAM hücre duvarının ana bileşeni, bakteri kitlesinin %15'i
- Özellikle HIV-ko-enfekte çocuk hastalarda
- WHO HIV ko-enfekte hastalarda öneriyor
- Duyarlılık düşük olmasına rağmen hızlı olması (15 dk) ,yatak başı yapılabilmesi



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WHO operational handbook on tuberculosis.

Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update

WHO LF-LAM önerileri

Yatan HIV pozitif erişkin, adölesan ve çocuk hastanın TB tanısı için;

- TB semptomları olan (pulmoner ve extrapulmoner)
- İlerlemiş HIV hastalığı olanlarda
- Ağır hasta olanlarda yada TB ile uyumlu semptomlar olmasa bile CD4 <200 hücre/mm³ olanlarda

Ayaktan HIV pozitif erişkin, adölesan ve çocuk hastada aktif TB tanısı için

- TB semptomları olan (pulmoner ve extrapulmoner)
- İlerlemiş HIV hastalığı olanlarda
- Ağır hasta olanlarda yada TB ile uyumlu semptomlar olmasa bile CD4 <100 hücre/mm³ olanlarda

WHO operational handbook on tuberculosis.

Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update

WHO LF-LAM önerileri

Ayaktan HIV pozitif erişkin, adelösan ve çocuk hastada aktif TB tanısı için

- TB semptomları açısından değerlendirilmemiş
- CD4 sayısı bilinmeyen veya CD4 sayısı $> 100 \text{ mm}^3$

NOT

WHO balgam verebilen TB semptomları olan tüm hastalarda en az bir balgam örneğinin alınarak moleküler test yapılmasını öneriyor.

Diğer testlerle birlikte karara ek test olarak öneriyor

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FujiLAM TB

- LAM antikorlarına gümüş partiküller bağlanmış.
- 2 kat daha duyarlı, 30 kat daha düşük konsantrasyondaki LAM'ı tespit ediyor.
- Ocak 2019-Haziran 2019; Zambia, prospektif
- 151 hasta, 89 E, Yaş ort: 37 (28-43Y), 68 (%46) HIV
- 32 öyküde TB,
- %23 (34) kültür (+) PTB

FujiLAMTB

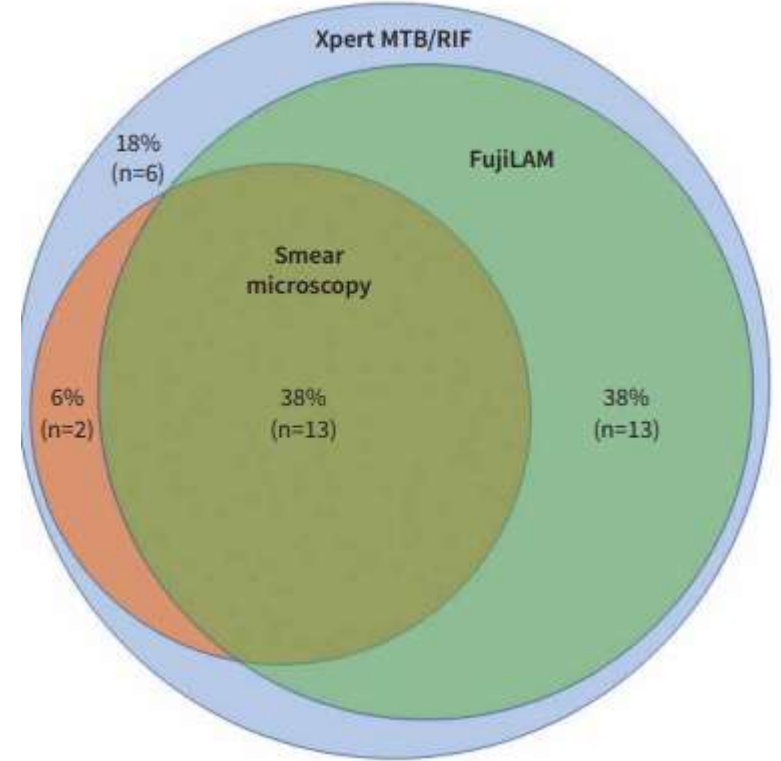
| | Duyarlılık | Özgüllük |
|---------|------------|----------|
| Toplam | %77 | %92 |
| HIV(+) | %75 | %89 |
| HIV(-) | %75 | %95 |
| ARB (+) | %87 | |
| ARB (-) | %68 | %92 |

PPD

Toplam; %74
HIV(+); %60
HIV(-); %83

NPD

Toplam; %93
HIV (-); % 92
HIV(+); % 94



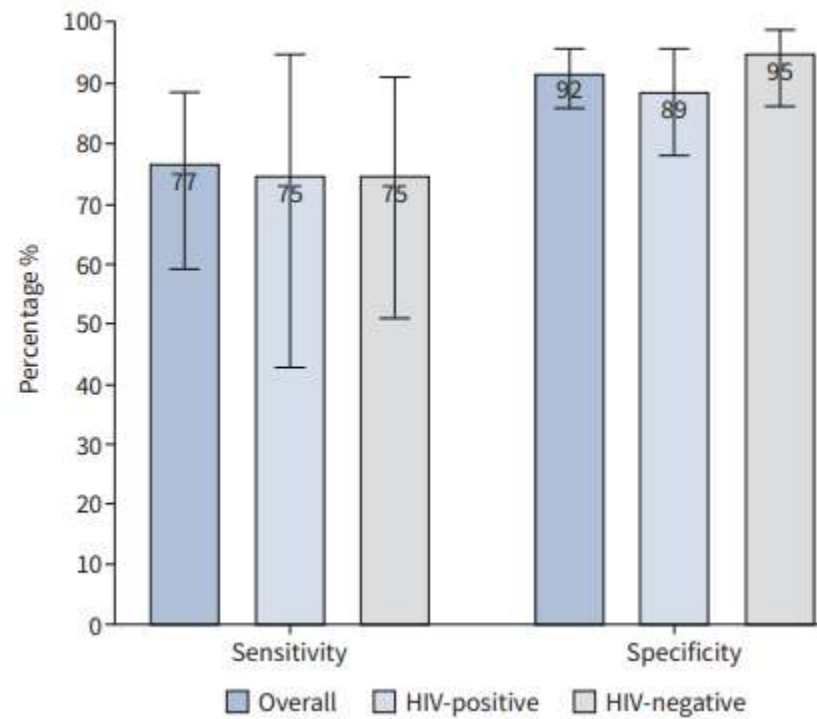


Table 1 Commonly used tests and WHO-endorsed methods including commercially available assay for the screening and diagnosis of active TB including drug resistance

| Test or platform | Test/assay principle | Use | Pooled sensitivity (%) | Pooled specificity (%) | TAT | Target setting | WHO-endorsed year | Comments |
|--|--|---|--|---|------------|--------------------------------|-------------------------------------|--|
| Chest X-ray ^a | Imaging | Active TB screening | 87 | 89 | Same day | Secondary and tertiary centers | – | In use since long back |
| Sputum smear microscopy | AFB staining and light microscopy | Active TB diagnosis | 32–94 | 50–99 | Same day | Peripheral and reference labs | – | In use since long back |
| LED Fluorescence smear microscopy | Auramine staining and fluorescence microscopy | Active TB diagnosis | 52–97 | 94–100 | Same day | Peripheral and reference labs | 2011 | |
| Liquid culture with DST | Liquid culture media | Active TB diagnosis and drug resistance | 89 (smear +ve) 73 (smear –ve) | >99 | 10–21 days | Reference lab | 2007 | |
| Tuberculin skin test (TST) | Immune response against TB in body | TB screening | 87–98 | 74–96 | 2–3 days | Secondary and tertiary centers | WHO made –ve recommendation in 2011 | False +ve in BCG vaccinated and NTM infected |
| Interferon gamma release assay (IGRA) | Immune reactivity against MTB Ag in blood | TB screening | QFT (75–84) | QFT (75–91%) | 2–3 days | Secondary and tertiary centers | – | As an alternative to TST |
| LAM lateral flow | Antigen detection | Active TB diagnosis in HIV +ve | 44 (all) 54 (HIV +ve) | 92 (all) 90 (in HIV +ve) | Same day | Peripheral lab | 2015 | |
| Xpert MTB/RIF | NAAT (qPCR) | Active TB diagnosis and rifampicin resistance | 98 (smear and culture +ve) 67 (smear –ve but culture +ve) 95 (rifampicin resistance) | 99 (smear and culture –ve) 98 (rifampicin resistance) | Same day | District or sub-district lab | 2010 | |
| First-line LiPA (Hain ^b GenoType MTBDR plus, NIPRO ^c NTM + MDR-TB detection kit) | NAA and hybridization (<i>rpoB</i> , <i>KatG</i> and <i>hbaA</i>) | Active TB diagnosis and drug resistance (rifampicin and isoniazid) | 98.1 (rifampicin resistance) 84 (Isoniazid resistance) | 98.7 (rifampicin resistance) >99 (isoniazid resistance) | 1–2 days | Reference laboratory | 2008 and 2016 | |
| Second-line LiPA (Hain ^b GenoType MTBDRsl) | NAA and hybridization (V1: <i>gyrA</i> , <i>rrs</i> , <i>embB</i>) (V2: <i>gyrA</i> , <i>gyrB</i> , <i>rrs</i> , <i>eis</i>) | Drug resistance (fluoroquinolones, aminoglycosides/ cyclic peptides and ethambutol) | 86 (fluoroquinolone resistance) 87 (other second-line drugs) | 98 (fluoroquinolone resistance) 99 (other second-line drugs) | 1–2 days | Reference laboratory | 2016 | |
| LAMP ^d | NAAT (LAMP-PCR) | Active TB diagnosis | 76–80 | 97–98 | Same day | Peripheral laboratory | 2016 | |

AFB acid fast bacilli, Ag antigen, BCG Bacille Calmette-Guerin, DST drug susceptibility testing, IGRA Interferon gamma release assay, LAM lipoarabinomannan, LAMP loop-mediated isothermal amplification, LED light-emitting diode, LiPA line probe assay, MTB *Mycobacterium tuberculosis*, NAAT nucleic acid amplification test, NTM nontuberculous mycobacteria, QFT QuantIFERON-TB Gold, qPCR quantitative PCR, TAT turnaround time, TB tuberculosis, TST tuberculin skin test; ^aRadiography is the screening test only; ^bHain lifescience, Germany; ^cNIPRO cooperation, Japan; ^dEiken Chemical, Japan; Pooled sensitivity and specificity were compared with reference to culture and culture-based phenotypic DST. Tuberculin Skin Test (TST) and Quantiferon-TB gold (QFT) are primarily used for TB screening only not for the active TB diagnosis [82] (Sources: Pai M et al., Tuberculosis. Nat Rev Dis Primers 2016; 2:16,076; and WHO Policy Statements (https://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/))

Mtb 85 Ag kompleksi

- 30-32 kD'luk 3 protein (Ag85A, Ag85B, Ag85C)
- Enzimatik mikoliltransferaz aktivitesi ile mikolik asitlerin hücre duvarı arabinogalaktan ile birleşmesini sağlayarak kord faktörü biyogenezinde rol oynar.
- Kan ve idrarda Ag85 kompleksinin belirlenmesi bu proteine olası bir prediktif biyobelirteç özelliği sağlamakla beraber farklı çalışmalarda oldukça değişken bir performans sağlamaktadır.

Mtb 85 Ag kompleksi tespitinde kullanılan yöntemler

1- ELİSA

2- İmmunoblott teknikler

3- Waveguide-based biyosensörler

4- Liquid kromatografik tandem spektroskopi

ORIGINAL ARTICLE

Diagnosis of osteoarticular tuberculosis by immuno-PCR assay based on mycobacterial antigen 85 complex detection

A. Khan¹ , R. Singh², S. Sharma³, V. Singh⁴, A. Sheoran⁵, A. Soni^{1,6}, V. Dhull⁷, P.S. Gill³, A. Yadav³, D. Chaudhary⁸, M.C. Gupta⁹ and P.K. Mehta¹ 

- 11 doku biyopsi, 30 sinoviyal sıvı, 18 abse
- Mart 2019-Mart 2020
- **8 konfime OATB** (ARB (+), LJ (+), GeneXpert (+), histopat.)
- **51 şüpheli OATB** (ppd, klinik, radyoloji, sitolojik bulgular, ATT yanıt)
- 33 non-TB kontrol
- Indirect immuno-PCR (I-PCR), RT-PCR ve ELISA ile Ag85

Table 1 Sensitivity and specificity at 95% CI by ELISA and I-PCR based on Ag85 detection in OATB patients

| Categories of OATB samples | ELISA | | | I-PCR | | |
|---------------------------------------|-------|-------------------|----------------|-------|-------------------|------------------|
| | (+) | % Sensitivity | % Specificity | (+) | % Sensitivity | % Specificity |
| Confirmed (<i>n</i> = 8) | 6 | 75 (34.9–96.8) | | 7 | 87.5 (47.3–99.6) | |
| Clinically suspected (<i>n</i> = 51) | 25 | 49 (34.7–63.4)* | | 36 | 70.5 (56.1–82.5)* | |
| Total (<i>n</i> = 59) | 31 | 52.5 (39.1–65.7)† | | 43 | 72.8 (59.7–83.6)† | |
| Non-TB controls (<i>n</i> = 33) | 3 | | 90.9 (75.6–98) | 2 | | 93.9 (79.7–99.2) |

Significant differences ($P < 0.05$ – 0.01) were observed between the sensitivities of *clinically suspected and †total OATB cases with ELISA as compared to I-PCR using exact symmetry test, taking CRS as the reference standard.

n, number of sample; OATB, osteoarticular tuberculosis; (+), positive cases.

Table 2 Sensitivity and specificity at 95% CI by GeneXpert, RT-I-PCR, I-PCR and ELISA based on Ag85 detection in OATB patients

| Categories of OATB samples | GeneXpert | | | RT-I-PCR | | | I-PCR | | | ELISA | | |
|---------------------------------------|-----------|-------------------|----------------|----------|-------------------|------------------|-------|-------------------|------------------|-------|-------------------|------------------|
| | (+) | % Sensitivity | % Specificity | (+) | % Sensitivity | % Specificity | (+) | % Sensitivity | % Specificity | (+) | % Sensitivity | % Specificity |
| Confirmed (<i>n</i> = 7) | 5 | 71.4 (29.4–96.3) | | 6 | 85.7 (42.1–99.6) | | 6 | 85.7 (42.1–99.6) | | 5 | 71.4 (29.4–96.3) | |
| Clinically suspected (<i>n</i> = 23) | 9 | 39.1 (19.7–61.4)* | | 17 | 73.9 (51.5–89.7)* | | 16 | 69.5 (47–86.7)* | | 10 | 43.4 (23.1–65.5)* | |
| Total (<i>n</i> = 30) | 14 | 46.6 (28.3–65.6)† | | 23 | 76.6 (57.7–90)† | | 22 | 73.3 (54.1–87.7)† | | 15 | 50 (31.3–68.7)† | |
| Non-TB controls (<i>n</i> = 23) | 0 | | 100 (58.1–100) | 2 | | 91.3 (72.9–98.9) | 2 | | 91.3 (72.9–98.9) | 2 | | 91.3 (72.9–98.9) |

Significant differences ($P < 0.05$ – 0.01) were observed between the sensitivities of *clinically suspected and †total OATB cases with GeneXpert vs RT-I-PCR, ELISA vs RT-I-PCR, GeneXpert vs I-PCR and ELISA vs I-PCR using exact symmetry test, taking CRS as the reference standard.

n, number of sample; OATB, osteoarticular tuberculosis; (+), positive cases.

Rapid electrochemical detection of *Mycobacterium tuberculosis* in sputum by measuring Ag85 activity with disposable carbon sensors

Elodie Barbier^a, Théo Fouchet^{a,b,c,d}, Alain Hartmann^a, Emmanuelle Cambau^{b,c},
Faiza Mougari^{b,c}, Clément Dubois^d, Maurice Lubetzki^d, Murielle Rochelet^{a,*}

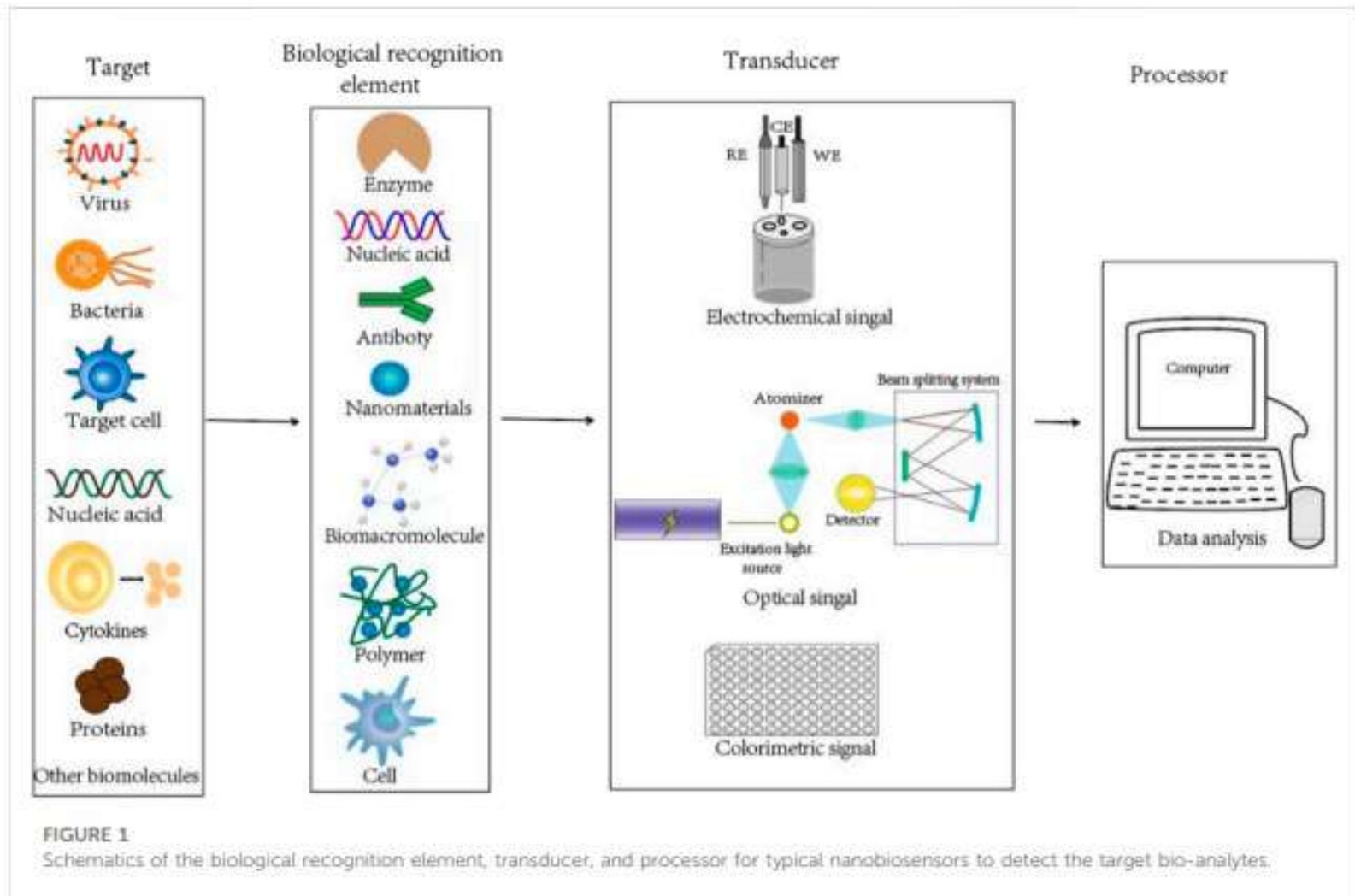
- 36 dondurulmuş ve dekontamine balgam (18 pozitif)
- Elektroaktif özellikleri olan 2 substrat P-aminofenil-6-O-oktamil 3-D glukopiranzidaz (p-APOG) ve p-aminofenil 6-3-D-glukopiranzidaz (p-APG)e
- Cut-off ; 1200 nA
- Duyarlılık %78, özgüllük % 89 (kültür ve mikroskopiye göre)
- Ucuz, hızlı ve taşınabilir

Biyosensörler

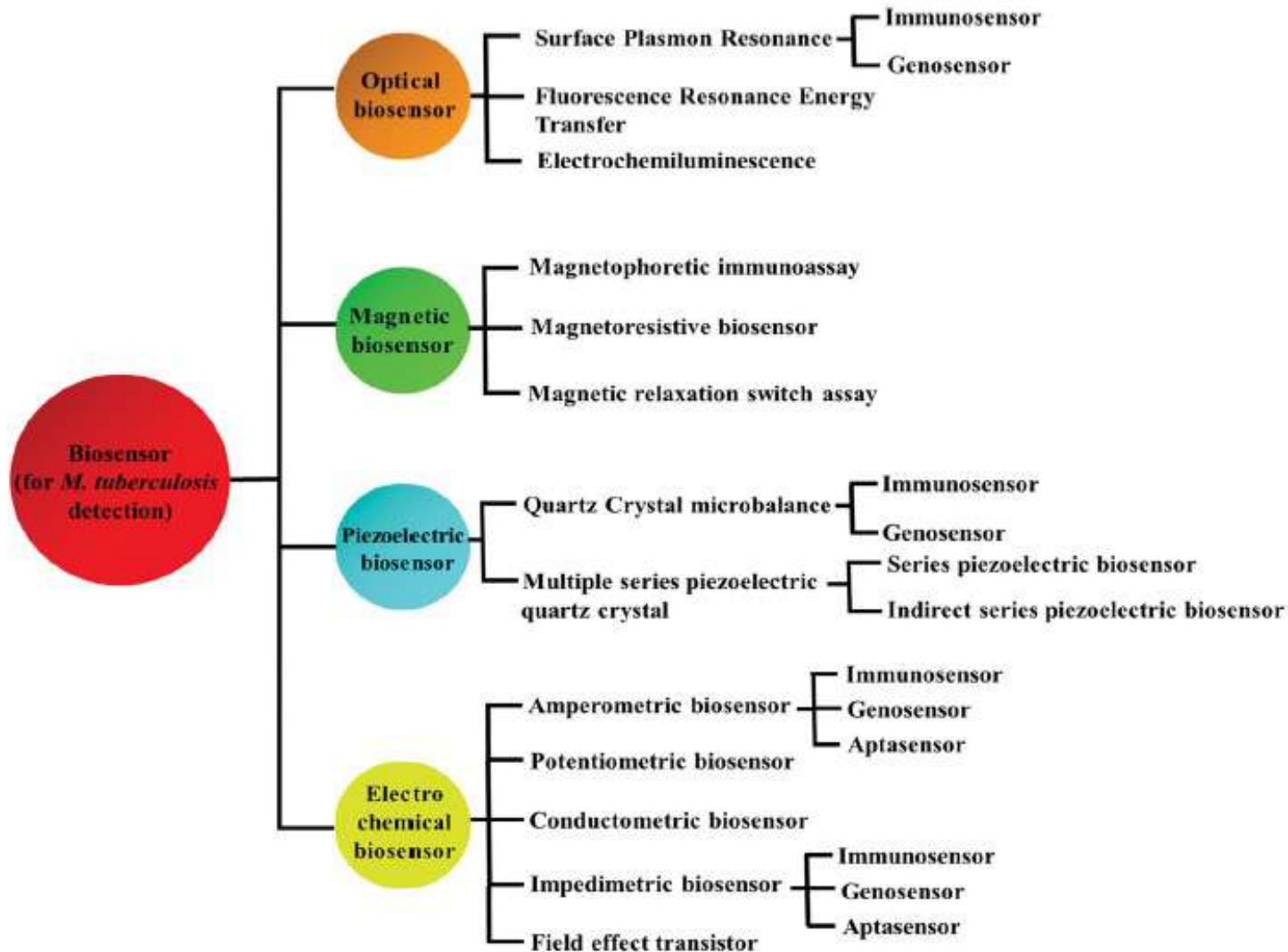
International Union Of Pure And Applied Chemistry
(Uluslararası Temel ve Uygulamalı Kimya Birliği)

Kimyasal bir bileşiğe verilen biyolojik yanıtı, optik, termal yada elektriksel sinyallere dönüştüren cihazlar

Biyosensörler-Nanobiyosensörler



TB tanısında biyosensörler



| Biosensing technique | Sample type | Biomarker | Response duration | Linear range | Detection limit | Ref. |
|---|-----------------|------------------|-------------------|--|---------------------------|---------------------------|
| <u>SPR-based optical biosensor</u> | | | | | | |
| PNA-based | Culture | Mtb DNA | 10 min | 5–50 ng ml ⁻¹ | 10 ng ml ⁻¹ | (Prabhakar et al. 2008) |
| DNA-based | Culture | Mtb DNA | 20 min | 0.05–2 μM | 0.05 μM | (Duman and Piskin 2010) |
| Immuno | Culture | CFP-10 | 200 sec | 100 ng ml ⁻¹ | 100 ng ml ⁻¹ | (Hong et al. 2011) |
| DNA-based | Culture | Mtb DNA | – | μg ml ⁻¹ | 115 ng ml ⁻¹ | (Hsu et al. 2013) |
| Immuno | Culture, Sputum | Ag85B | 15 min | – | 10 ng ml ⁻¹ | (Trzaskowski et al. 2018) |
| <u>FRET-based optical biosensor</u> | | | | | | |
| Immuno | Sputum | ESAT-6 | 4 min | 10 fg–200 fg | 10 fg | (Shojaei et al. 2014) |
| DNA-based | Culture | IS6110 sequence | 15 min | 10 ² –10 ⁶ copies/μl | 10 ² copies/μl | (Hwang et al. 2014) |
| <u>Electrochemiluminescence-based optical biosensor</u> | | | | | | |
| DNA-based | Culture | <i>rpoB</i> gene | 60 min | – | 1 pM | (Sweeney et al. 2004) |
| DNA-based | Culture | IS6110 sequence | 60 min | 10 fM–1 pM | 6.7 fM | (Jiang et al. 2011) |
| DNA-based | Serum | IS6110 sequence | 5 min | 1 fM–1 pM | 0.33 fM | (Yu et al. 2012) |
| DNA-based | Culture | <i>rpoB</i> gene | 30 min | 0.1–100 nM | 0.04 nM | (Li et al. 2014) |

Magnetic biosensor

| | | | | | | |
|-----------|---------|--------------------|--------|--|---|--------------------------|
| DNA-based | Sputum | Mtb DNA | - | 10^4 -109 CFU ml ⁻¹ | 10^4 CFU ml ⁻¹ | (Pang et al. 2008) |
| Immuno | Culture | Ag85B | 60 min | 28-491 CFU μ l ⁻¹ | 8 CFU μ l ⁻¹ | (Liang et al. 2012) |
| DNA-based | Sputum | <i>fadE15</i> gene | 2.5 h | 0-107 CFU ml ⁻¹ | 10^3 CFU ml ⁻¹ | (Liong et al. 2013) |
| Immuno | Culture | CFP-10 | 2 h | 1 μ g ml ⁻¹ -1 pg ml ⁻¹ | 0.051 pg ml ⁻¹ | (Zou et al. 2015) |
| Immuno | Sputum | CFP-10 | 2 h | 2.3 pM-0.23 μ M | 0.32 pM | (Kim et al. 2017) |
| Immuno | Culture | Mtb cell | 30 min | - | 10.79 and 13.13 cells ml ⁻¹ | (Barroso et al. 2018) |

Piezoelectric biosensor

| | | | | | | |
|-----------|----------------|----------------------------------|---------|---|--|-----------------------------|
| Immuno | Sputum, Saliva | Mtb cell | 20 min | 10^5 -108 cells ml ⁻¹ | 10^5 cells ml ⁻¹ | (He and Zhang 2002) |
| MSPQC | Culture | NH ₃ +CO ₂ | 192 h | 10^2 -107 CFU ml ⁻¹ | 10 CFU ml ⁻¹ | (Ren et al. 2008) |
| DNA-based | Culture | IS6110 sequence | 20 min | 0-1.5 μ M | 0.25 μ M | (Kaewphinit et al. 2010) |
| PA- MSPQC | Culture | NH ₃ +CO ₂ | 30 h | 10 -104 CFU ml ⁻¹ | 100 CFU ml ⁻¹ | (Mi et al. 2012) |
| Immuno | Culture | Mtb cell α -LAM | 20 min | 8.7×10^4 - 8.7×10^7 cells ml ⁻¹ | 8.7×10^6 cells ml ⁻¹ 8.7×10^5 cells ml ⁻¹ | (Hiatt and Cliffel 2012) |
| ISP | Culture | Mtb cell | 111.7 h | 10^3 -108 CFU ml ⁻¹ | 10 -100 CFU ml ⁻¹ | (Ren et al. 2013) |
| MSPQC | Sputum | CFP10-ESAT6 | 96.3 h | 10^3 -108 CFU ml ⁻¹ | 10^3 CFU ml ⁻¹ | (He et al. 2016) |
| MSPQC | Sputum | 16S rDNA sequence | 3 h | 10^2 -108 CFU ml ⁻¹ | 20 CFU ml ⁻¹ | (Zhang et al. 2019) |

Amperometric-based electrochemical biosensor

| | | | | | | |
|---------------------|------------------------------|-----------------------------|-----------|--------------------------------------|--------------------------|-------------------------------------|
| Immuno (CV and SWV) | Culture | Ag231 | 90 min | 0.005-0.1 $\mu\text{g ml}^{-1}$ | 1 ng ml^{-1} | (Díaz-González et al. 2005) |
| Immuno (DPV) | Culture | LAM | 30 min | 0.0156-1 $\mu\text{g ml}^{-1}$ | 5.3 ng ml^{-1} | (Wang et al. 2012 Oct 1) |
| Immuno (SWASV) | Serum | IFN- γ | 60 min | 1-500 pg ml^{-1} | 0.34 pg ml^{-1} | (Huang et al. 2015) |
| Immuno | Sputum | Mtb cell | 60 min | 10^2 - 10^5 CFU ml^{-1} | 100 CFU ml^{-1} | (Hiraiwa et al. 2015) |
| Immuno (SWV) | Serum, Sputum | ESAT-6 | 30 min | 10 - 10^3 ng ml^{-1} | 7.1 ng ml^{-1} | (Diouani et al. 2017) |
| Immuno (CV) | Culture | MPT-64 | 100 min | 0.3-50 ng ml^{-1} | 0.43 ng ml^{-1} | (Chutichetpong et al. 2018) |
| Immuno (DPV) | Urine | CFP-10 | 2 h | 0.005-500 ng ml^{-1} | 0.33 ng ml^{-1} | (Tufa et al. 2018) |
| Immuno (DPV) | Sputum | CFP-10 | 60 min | 20-100 ng ml^{-1} | 15 ng ml^{-1} | (Mohd Azmi et al. 2018) |
| Immuno (DPV) | Culture | CFP10-ESAT6 | 2 h | 40-100 ng ml^{-1} | 0.15 ng ml^{-1} | (Mohd Bakhori et al. 2019) |
| PNA-based (SWV) | Serum | Mtb 16S-23S spacer sequence | 60 min | – | 2.5 pg ml^{-1} | (Prabhakar et al. 2008) |
| PNA-based (SWV) | Culture | Mtb DNA | 90 sec | 0.1–50 fM | 0.1 fM | (Prabhakar et al. 2010) |
| DNA-based (DPV) | Culture | Mtb IS6110 sequence | 2 h | 0.01–10 ng ml^{-1} | 0.01 ng ml^{-1} | (Torres-Chavolla and Alocilja 2011) |
| DNA-based | Sputum | Mtb DNA | 10 min | 1.25–50 ng ml^{-1} | 1.25 ng ml^{-1} | (Thirupathiraja et al. 2011) |
| DNA-based (SWV) | Culture | Mtb DNA | – | 30 pM–100 nM | 300 pM | (Zhang et al. 2012) |
| DNA-based (SWV) | Culture | Mtb DNA | 2 h | 10 fM–1 nM | 8.7 fM | (Zhang et al. 2015) |
| DNA-based | Sputum, Pleural fluid, Urine | Mtb IS6110 sequence | 90 min | 0.5–100 aM | 0.5 aM | (Barreda-García et al. 2015) |
| DNA-based (DPV) | Sputum | Mtb IS6110 sequence | 4 h | 1 fM–1 nM | – | (Liu et al. 2014) |
| DNA-based (SWV) | Culture | Mtb DNA | 40 min | 0.5 fM–500 pM | 0.2 fM | (Yan et al. 2015) |
| DNA-based (CV) | Culture | <i>rpoB</i> gene | – | 0.1 fM–1 pM | 70 fM | (Zribi et al. 2016) |
| DNA-based (SWV) | Culture | Mtb DNA | 60-90 min | 1.5–12.5 nM | 1.3 nM | (Yesil et al. 2016) |
| PNA-based (DPV) | Sputum | Mtb DNA | 50 min | 10 pM–100 nM | 0.89 pM | (Zaid et al. 2017) |
| DNA-based (DPV) | Sputum | Mtb IS6110 sequence | – | 0.1 pM–10 nM | 50 fM | (Chen et al. 2017) |

| | | Target sequence | | | | |
|---|---------|------------------|---------------|--|-------------------------------|---------------------------------|
| DNA-based (DPV) | Sputum | Mtb DNA | 60 min | 1 fM–10 nM | 0.33 fM | (Chen et al. 2018) |
| DNA-based (SWV) | Culture | <i>rpoB</i> gene | 30 min | 1 aM–100 fM | 0.36 aM | (Khoder and Korri-Youssef 2020) |
| DNA aptamer (SWV) | Serum | IFN- γ | 15 min | 0.06–10 nM | 0.06 nM | (Liu et al. 2010) |
| DNA aptamer (DPV) | Serum | IFN- γ | 60 min | 0.1–0.7 pM | 0.065 pM | (Yan et al. 2013) |
| DNA aptamer (DPV) | Culture | IFN- γ | 4 h | 0.01–50 ng ml ⁻¹ | 0.003 ng ml ⁻¹ | (Liu et al. 2015) |
| DNA aptamer (DPV) | Serum | MPT64 | 30 min 2 h | 0.02–1000 pg ml ⁻¹ | 20 fg ml ⁻¹ | (Bai et al. 2017) |
| DNA aptamer (DPV) | Serum | MPT64 | 15 min | 10 ⁷ –10 ³ fg ml ⁻¹ | 0.5 fg ml ⁻¹ | (Thakur et al. 2017) |
| DNA aptamer (DPV) | Serum | IFN- γ | 50 min | 10–1500 pg ml ⁻¹ | 3 pg ml ⁻¹ | (Abnous et al. 2017) |
| DNA aptamer (CV) | Serum | ESAT6 | 60 min | 100 fg ml ⁻¹ –200 ng ml ⁻¹ | 33 fg ml ⁻¹ | (Li et al. 2018) |
| DNA aptamer (DPV) | Serum | MPT64 | 3 h | 0.02–1000 pg ml ⁻¹ | 10 fg ml ⁻¹ | (Li et al. 2018) |
| DNA aptamer (DPV) | Serum | MPT64 | 4 h | 5 fg ml ⁻¹ –1 ng ml ⁻¹ | 0.34 fg ml ⁻¹ | (Gou et al. 2018) |
| Field-effect-based electrochemical biosensors | | | | | | |
| DNA aptamer | Culture | IFN- γ | – | – | 83 pM | (Farid et al. 2015) |
| Immuno | Culture | Ag85B | 60 min | 0.12–1 μ g ml ⁻¹ | 0.12 μ g ml ⁻¹ | (Saengdee et al. 2016) |
| DNA-based | Sputum | Mtb DNA | 40 min | – | 0.3 nM | (Bronder et al. 2018 May 17) |

Impedimetric-based electrochemical biosensor

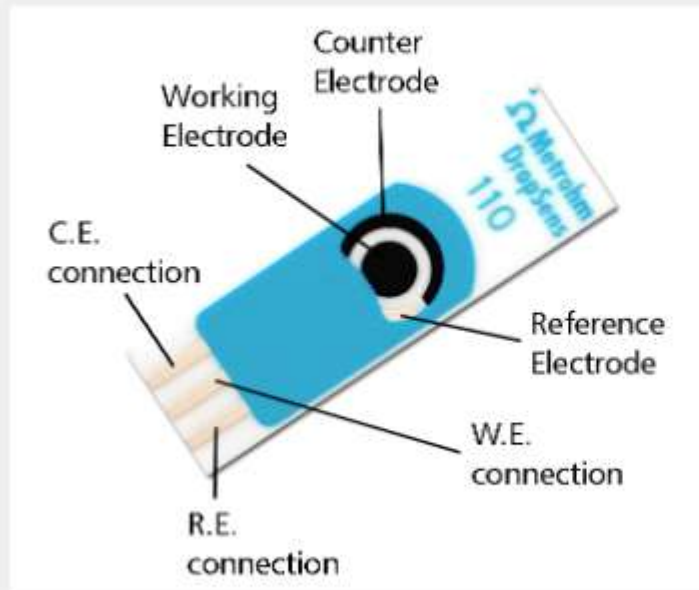
| | | | | | | |
|-------------|---------------|------------------------------|--------|--------------------------------|----------------------------|--------------------------|
| Immuno | Culture | IFN- γ | - | 0-12 pg ml ⁻¹ | 0.02 fg ml ⁻¹ | (Dijksma et al. 2001) |
| Immuno | Culture | IFN- γ | 80 min | 0.0001-0.1 ng ml ⁻¹ | 0.1 pg ml ⁻¹ | (Yang et al. 2015) |
| Immuno | Culture | 16kDa HSP | 30 min | 100fM-1 nM | 100 fM | (Gopinath et al. 2016) |
| Immuno | Culture | ESAT6 | 1 h | - | - | (Sepulveda et al. 2017) |
| Immuno | Culture | IFN- γ | - | 0.0001-0.1 ng ml ⁻¹ | 0.12 pg ml ⁻¹ | (Wang et al. 2017) |
| Immuno | Culture | PPD | 2 min | - | 10ng ml ⁻¹ | (Cui et al. 2013) |
| DNA-based | Culture | Mtb 16S rRNA spacer sequence | 20 min | 0.01 mM-0.01 nM | 0.01 nM | (Das et al. 2011) |
| DNA-based | Culture | Mtb DNA | - | 6-40 ng μ l ⁻¹ | 6 ng μ l ⁻¹ | (Costa et al. 2014) |
| DNA-based | Culture | Mtb IS6110 sequence | 1 min | 10 fM-0.1 μ M | 10fM | (Perumal et al. 2018) |
| DNA aptamer | Culture | IFN- γ | 35 min | 22.22 pM-0.11 nM | 11.56 pM | (Ding et al. 2017) |
| DNA aptamer | Serum, Sputum | MPT64 | 15 min | 0.1 fM - 5nM | 4.1 fM | (Sypabekova et al. 2019) |

Elektrokimyasal immunosensörler

- Karbon pasta elektrot (CPE; Carbon paste electrodes-CPE)
- Camsi karbon elektrot (GCE; glassy carbon electrodes)
- Altın elektrot (gold electrodes)
- Screen-printed carbon electrodes (SPCE)

Screen-printed electrodes

SCREEN-PRINTED ELECTRODES (SPEs)



Screen-printed electrodes based on carbon, gold, platinum, silver or carbon nanotubes inks. Innovative strips manufactured for electrochemical analysis in environmental, clinical or agri-food areas.

Low cost, disposable devices specially designed to work with microvolumes of sample. Ideal for quality control or research purposes and also for teaching electrochemistry.

Strip's general dimensions: 3.4 x 1.0 x 0.05 cm. Reference electrode and electric contacts made of silver (unless otherwise stated).

Personalized SPEs can be manufactured following customer's specifications on materials and patterns.



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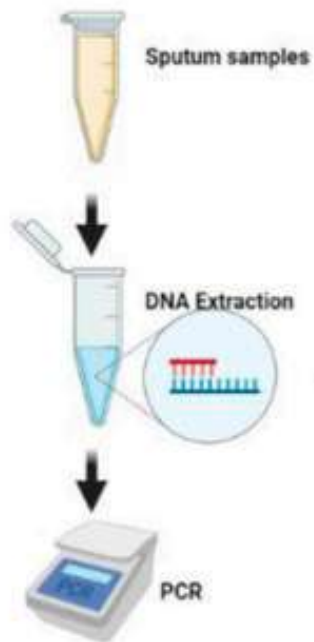
Tuberculosis detection from raw sputum samples using Au-electroplated screen-printed electrodes as E-DNA sensor

M. N. Sharif^{1,2†}, S. Taufiq^{1,2}, M. Sohail³ and S. R. Abbas^{1,2*†}

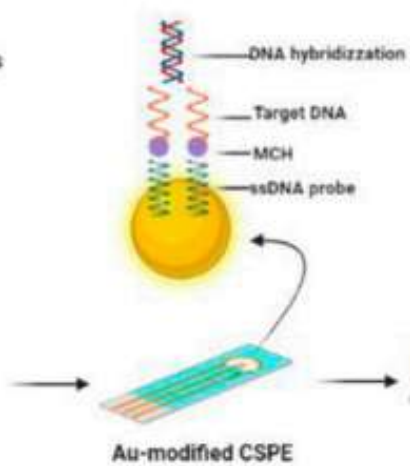
¹Biosensors and Therapeutics Lab, School of Interdisciplinary Engineering and Sciences (SINES), Islamabad, Pakistan, ²Department of Industrial Biotechnology, Atta Ur Rahman School of Applied

- Ultrasensitif, elektrokimyasal DNA biyosensörü
- Screen printed elektrotlar nanopartikül olarak altın
- Elektron mikroskopi/Enerji Dispersif x-ray analiz (SEM/EDX)
- Siklik voltametri ve Değişken atımlı voltametri

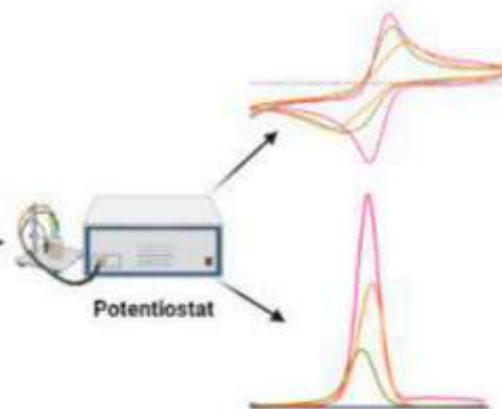
Sample preparation



Electrochemical sensor



Read Out



GRAPHICAL ABSTRACT

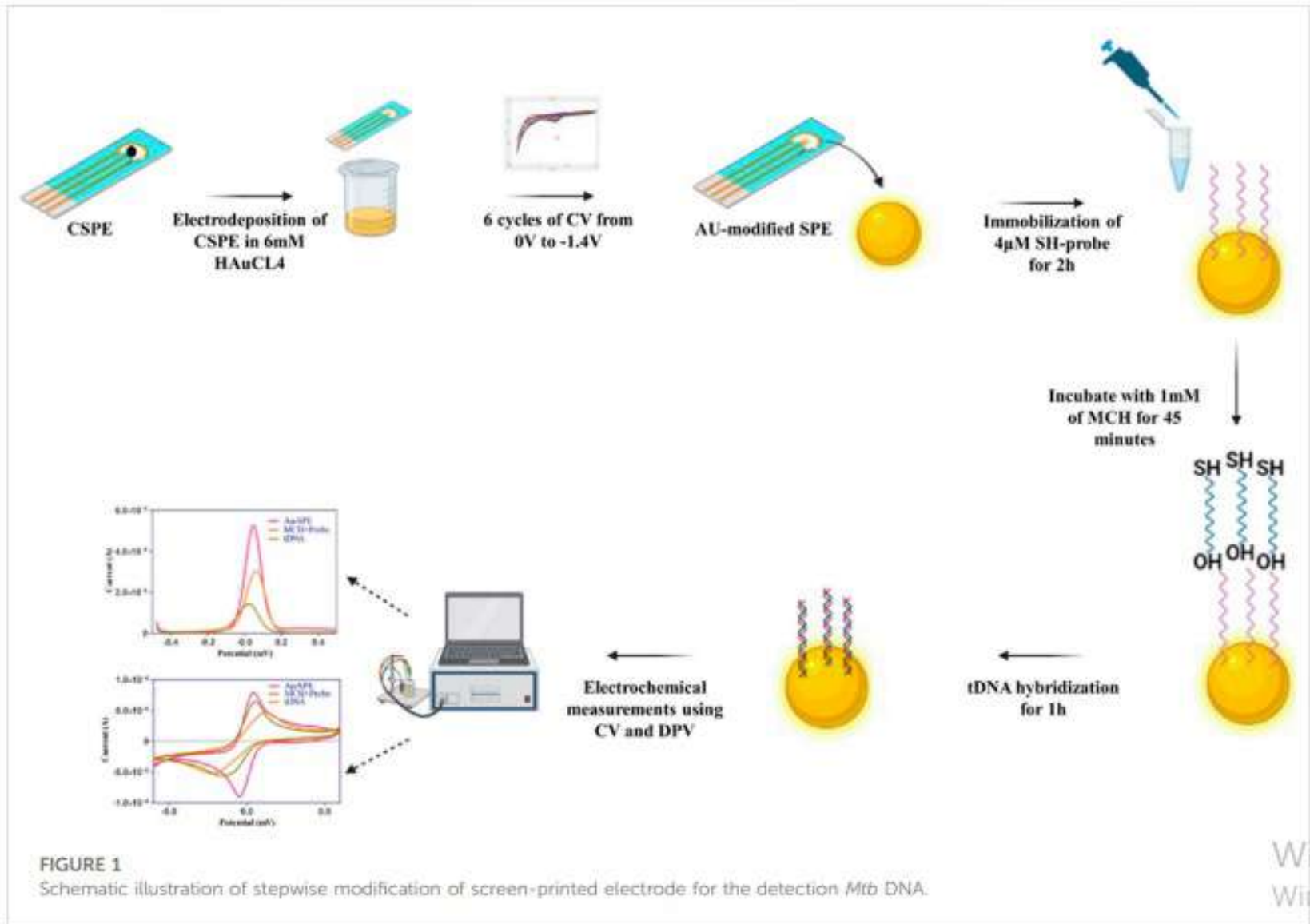


FIGURE 1
Schematic illustration of stepwise modification of screen-printed electrode for the detection *Mtb* DNA.

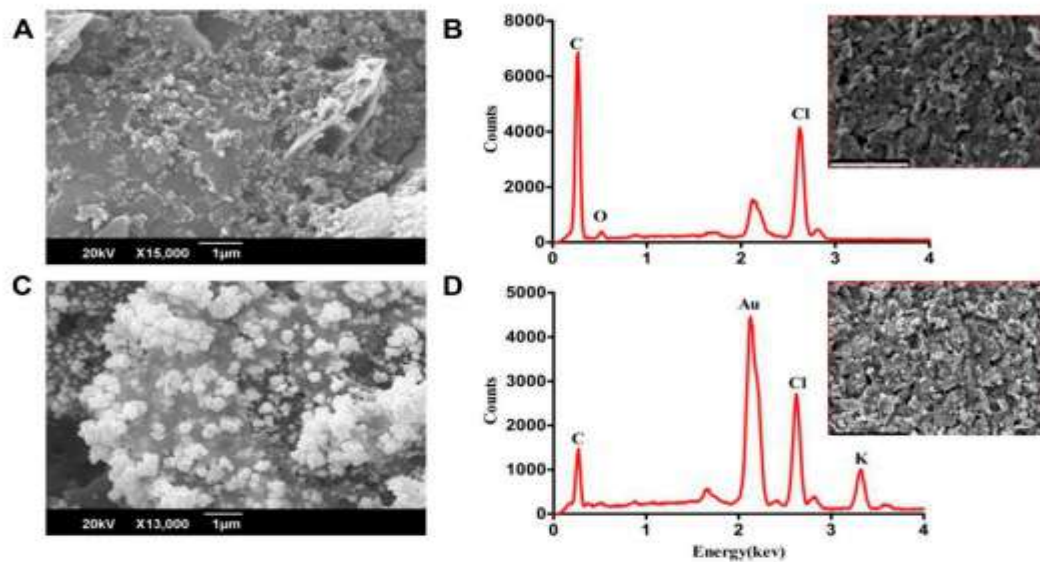


FIGURE 2
SEM and EDX analysis of carbon SPE (A,B) and electrodeposited gold SPE (C,D).

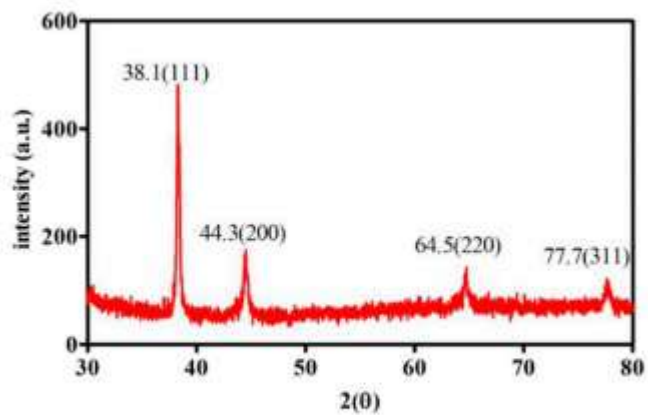
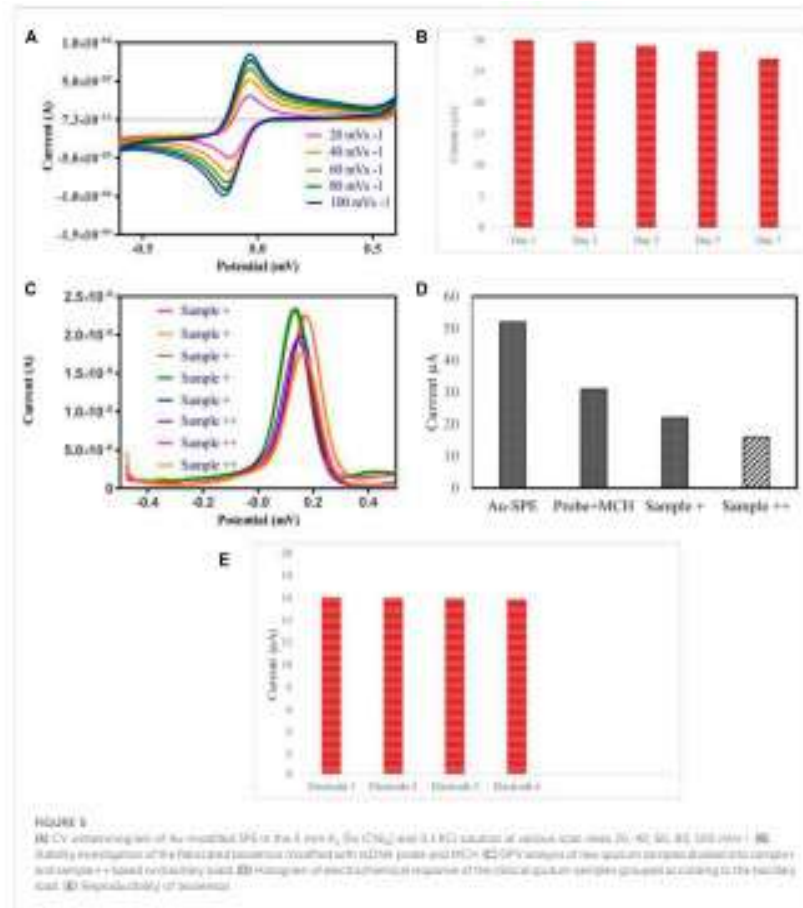
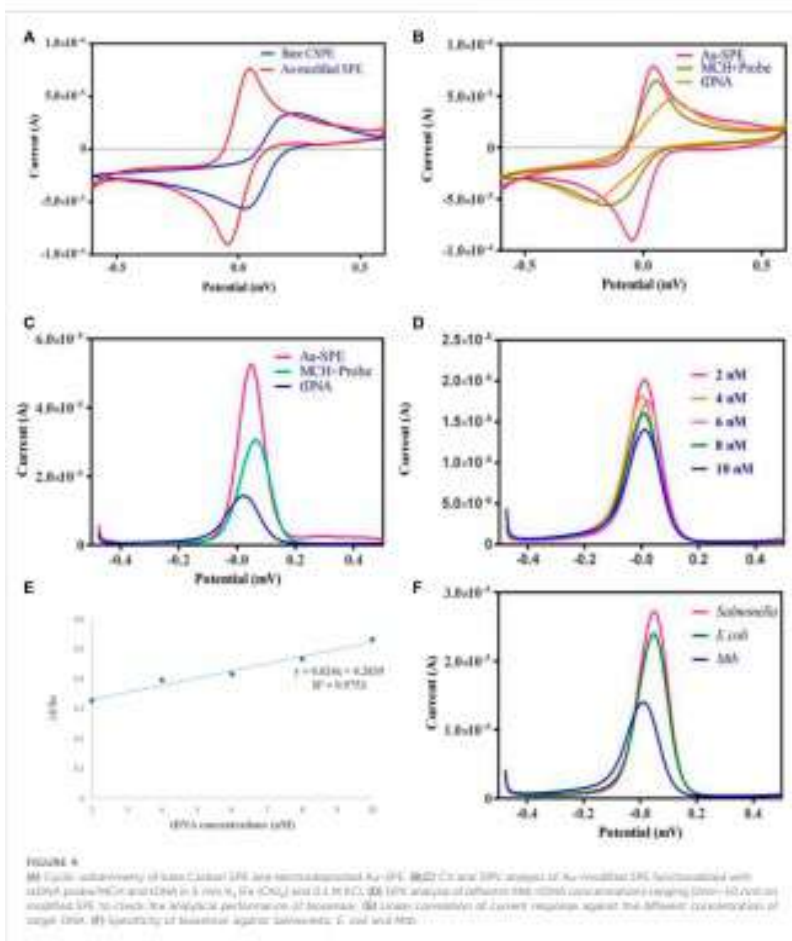


FIGURE 3
XRD analysis of Au-modified SPE.

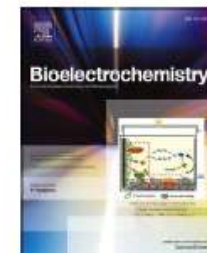




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Towards portable rapid TB biosensor: Detecting *Mycobacterium tuberculosis* in raw sputum samples using functionalized screen printed electrodes

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İşlenmemiş balgamdan IS6110'u marker olarak kullanan DNA hibridizasyona dayalı, etiketsiz, ticari olarak sağlanabilen screen Printed electrode (DropSens-Zensors)

- Elektron mikroskopi
- X-ray spektroskopisi
- Siklik voltametri ve Diferansiyel Pulse Voltametri

Sonuç

- Biyosensörler konusunda hız kazanmış olmakla birlikte henüz WHO tanı klavuzlarında onaylanmış biyosensör yok
- İdrarda LAM tespiti ve Mtb 85 Ag ile ilgili çalışmalarda duyarlılık konusundaki olumlu veriler artarsa WHO klavuzlarında öneri genişleyebilir.





**BAŞIMIZ
SAĞ OLSUN**