



Diagnosis and misdiagnosis of tuberculosis: an overview of new tools and emerging technologies

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TROPICAL AND
PARASITIC DISEASES
A REVIEW OF CLINICAL AND LABORATORY MEDICINE

A tale of two worlds

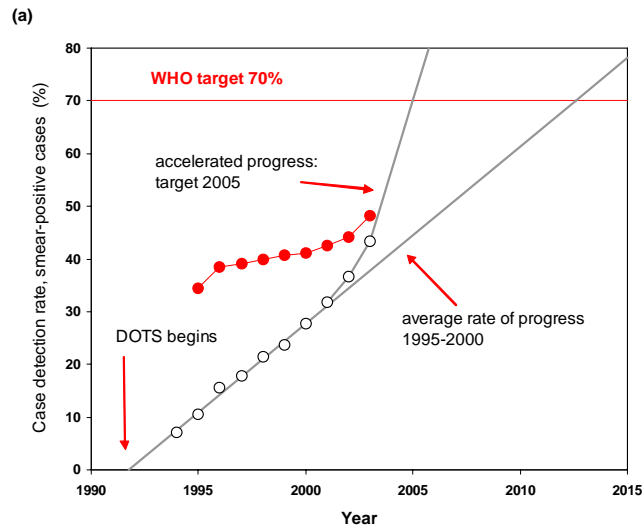


- There are two worlds when it comes to the diagnosis of TB
- One world has only smear microscopy at its disposal (and perhaps radiology)
- The other world has all modern techniques available including culture, NAAT, molecular diagnostics, and sophisticated radiological techniques such as CT and PET scanning.
- The ability to diagnose or misdiagnose TB will vary across these two worlds.

Davies PDO & Pai M (2008)



Diagnosis remains a major concern for global TB control



Source: WHO



Conventional TB diagnostics: badly in need of upgrade



- Latent TB (LTBI)
 - Tuberculin skin test [1890]
- Active TB
 - Sputum microscopy [1882]
 - Mycobacterial culture [1882]
 - Chest X-rays [1896]



TB diagnostics
1882

Stalled TB technology exemplifies a system-wide neglect of diagnostics for diseases of poverty



TB diagnostics
2008



Resurgence of interest in new tools



Stop TB Partnership

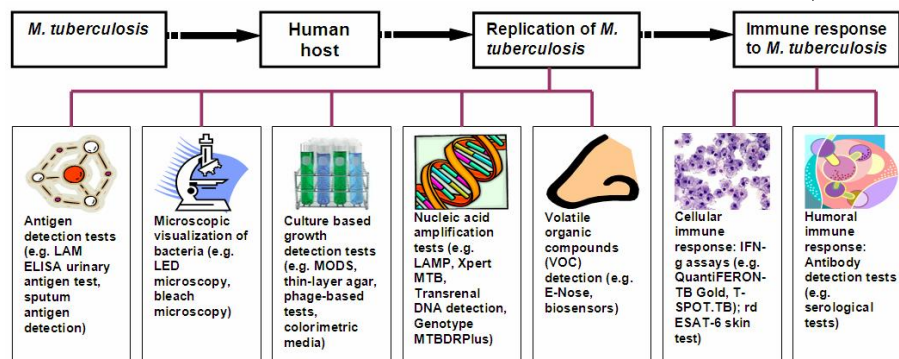


Latent TB



The end of tuberculin skin testing?

New diagnostics pipeline



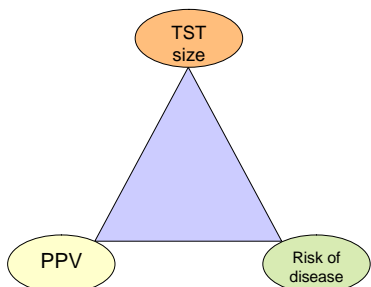
Tuberculin skin test (TST)

- TST
 - Measures cell-mediated immune response (CMI)
 - Uses PPD: a crude antigenic mixture
- Limitations of TST:
 - fairly high proportion of false positives and false negatives
 - technical problems in administration and interpretation
 - difficulty in separating true infection from the effects of BCG and non-tuberculous mycobacteria (NTM)
 - repeated TST boosts the immune response
 - requires a 3-dimensional interpretation



Thinking in three dimensions: a web-based algorithm to aid the interpretation of tuberculin skin test results

D. Menzies,* G. Gardiner,** M. Farhat,** C. Greenaway,** M. Pai**
*Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute, McGill University, Montreal, †Massachusetts General Hospital, Harvard University, Boston, Massachusetts, USA; ‡Division of Infectious Disease Microbiology, Sir Mortimer B Davis Jewish General Hospital, McGill University, Montreal, †Department of Epidemiology and Biostatistics, McGill University, Montreal, Canada



What do we know about T-cell based IGRA performance in general?

Annals of Internal Medicine

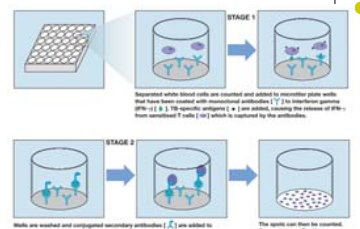
Meta-analysis: New Tests for the Diagnosis of Latent Tuberculosis Infection: Areas of Uncertainty and Recommendations for Research

Background: Until recently, the tuberculin skin test was the only test for detecting latent tuberculosis (LTB) infection. But 2 new tests, interferon-gamma release assays (IGRAs), emerged and emerged. IGRAs are more sensitive than the tuberculin skin test and are not affected by BCG vaccination. However, IGRAs are more expensive and require laboratory support. Interferon-gamma release assays (IGRAs) are more sensitive than the tuberculin skin test and are not affected by BCG vaccination. However, IGRAs are more expensive and require laboratory support. Interferon-gamma release assays (IGRAs) are more sensitive than the tuberculin skin test and are not affected by BCG vaccination. However, IGRAs are more expensive and require laboratory support.

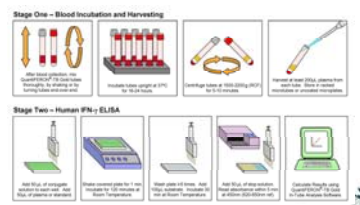


Menzies et al. *Ann Intern Med* 2007

Interferon-gamma release assays (IGRA)



T-SPOT.TB® [Oxford Immunotec, UK]



QuantiFERON-TB Gold® [Cellestis Ltd, Australia]

What do we know about TST and IGRA performance?

- IGRAs and TST cannot distinguish between LTBI and active TB
- TST is fairly sensitive in immunocompetent, but suboptimal in immunocompromised
- TST specificity is high in BCG non-vaccinated; but low and variable in BCG vaccinated
 - BCG after infancy and booster vaccinations lower specificity
- IGRAs have very high specificity (and definitely higher than TST)
 - IGRAs are not affected by BCG vaccination
- QFT-G is as sensitive as TST
- T-SPOT.TB is more sensitive than QFT-G and TST
- In immunocompromised:
 - T-SPOT.TB appears more sensitive than TST
 - QFT-G is more likely to produce indeterminate results, especially in low CD4+ counts
- IGRAs correlate well with TB exposure in low incidence settings
- Discordance between TST and IGRA is common
- Insufficient data on predictive value

Active TB



Optimization of microscopy



Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review

Karen R Steingart, Megan Henry, Vivienne Ng, Philip C Hopewell, Andrew Ramsay, Jane Cunningham, Richard Urbanczik, Mark Perkins, Mohamed Abdel Aziz, Madhukar Pai

Lancet Infect Dis 2006

Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review

Karen R Steingart, Vivienne Ng, Megan Henry, Philip C Hopewell, Andrew Ramsay, Jane Cunningham, Richard Urbanczik, Mark D Perkins, Mohamed Abdel Aziz, Madhukar Pai

Lancet Infect Dis 2006

Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review

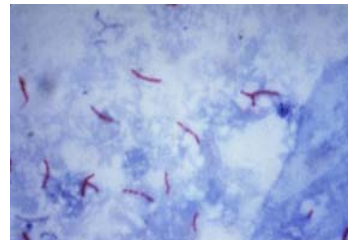
S. R. Mase,*† A. Ramsay,‡ V. Ng,§ M. Henry,¶ P. C. Hopewell,** J. Cunningham,† R. Urbanczik,* M. D. Perkins,** M. A. Aziz,†† M. Pai††

IJTL D 2007



Sputum smear microscopy

- Relies on microscopy
 - High specificity
 - Poor sensitivity
 - Smear-negative TB: a major problem in HIV-infected
 - Less helpful in extrapulmonary TB



New policy developments



Updated WHO policies



Mrs Eman Lottfi preparing DST (Egypt)

1. Revised definition of a new sputum smear positive pulmonary TB case
2. Reduction of the number of specimen for diagnosis of TB from three to two
3. Using liquid culture

[More information](#)

New WHO Policy on Microscopy and Culture



Request for Applications for Diagnostic Trial Sites

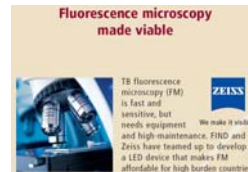
Improving the diagnosis of tuberculosis through optimization of sputum smear microscopy



LED based microscopy - 'Primostar iLED'



Courtesy: FIND, Geneva



Liquid Culture

- Liquid culture systems reduce delays in obtaining results to days rather than weeks
 - For DST, delay may be as little as 10 days vs. 28-42 days with solid media
- Liquid systems are more sensitive - may increase the case yield by ~10% over solid media
- Liquid systems are, however, more prone to contamination by other micro-organisms.
 - In experienced laboratories, ~5-10% of specimens cannot yield results because of contamination
 - Procedures to prevent cross-contamination should be strictly followed, especially in high-incidence countries

<http://www.who.int/tb/dots/laboratory/policy/en/index3.html>



Culture

- High sensitivity
- But tedious, time-consuming and expensive
- Liquid cultures have emerged as the standard
 - Push towards cultures was partly driven by the HIV epidemic



New policy developments



FIND-BD MGIT Demonstration Projects

Updated WHO policies

1. Revised definition of a new sputum smear positive pulmonary TB case
2. Reduction of the number of specimen for diagnosis of TB from three to two
3. Using liquid culture

Mrs Eman Lotfi preparing DST (Egypt)

[More information](#)



Use of Liquid TB Culture and Drug Susceptibility Testing (DST) in Low and Medium Income Settings

Summary report of the Expert Group Meeting on the use of liquid culture media, Geneva, 26 March 2007



Nucleic acid amplification tests (NAAT)

- NAATs have high specificity and PPV
- Sensitivity is lower and highly variable across studies
 - Sensitivity lower in extra-pulmonary and smear-neg pulmonary TB – thus, reducing applicability in HIV+
 - Negative test does not rule out TB
- Expensive; limited applicability in developing countries with high HIV prevalence

Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis

Madhukar Pai, Laura L Flores, Nitika Pai, Alan Hubbard, Lee W Riley, and John M Colford Jr

Research article

Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis
Madhukar Pai¹, Laura L Flores², Alan Hubbard³, Lee W Riley² and John M Colford Jr⁴

Open Access

Nucleic acid amplification tests for the diagnosis of tuberculous lymphadenitis: a systematic review

P. Daley,* S. Thomas,* M. Pai[†]

* Christian Medical College, Vellore, India; [†] McGill University, Montreal, Quebec, Canada

Research article

In-house nucleic acid amplification tests for the detection of *Mycobacterium tuberculosis* in sputum specimens: meta-analysis and meta-regression
Laura L Flores^{1,2,3}, Madhukar Pai^{1,3}, John M Colford Jr¹ and Lee W Riley⁴

Open Access

Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis

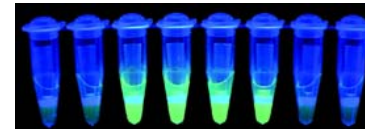
S Greco, E Girardi, A Navarra, C Salfini

Commercial Nucleic-Acid Amplification Tests for Diagnosis of Pulmonary Tuberculosis in Respiratory Specimens: Meta-Analysis and Meta-Regression

Daphne I Ling¹, Laura L Flores², Lee W Riley^{3*}, Madhukar Pai^{4*}
¹Division of Epidemiology, School of Public Health, University of California, Berkeley, California, United States of America; ²Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, San Francisco, California, United States of America; ³Division of Infectious Diseases, School of Public Health, University of California, Berkeley, California, United States of America; ⁴Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Quebec, Canada



LAMP (loop mediated isothermal amplification)



Rapid (1 hour), isothermal, high throughput, clinical samples

Feasible in high burden settings (hospitals)

Sens in culture+ = 97%

Spec in culture- = 99%

JOURNAL OF CLINICAL MICROBIOLOGY, June 2007, p. 1936-1940
0095-1137/07/060193-05 doi:10.1128/JCM.02352-06
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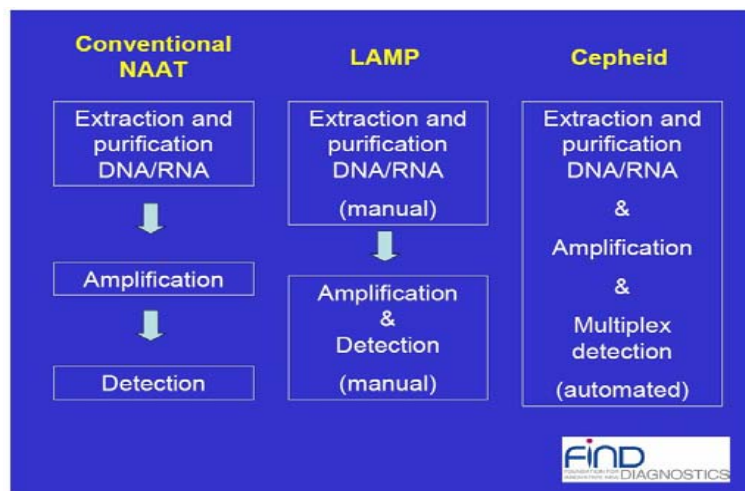
Vol. 45, No. 6

Operational Feasibility of Using Loop-Mediated Isothermal Amplification for Diagnosis of Pulmonary Tuberculosis in Microscopy Centers of Developing Countries¹

Catharina C. Boehme,^{1*} Pamela Nabeta,² German Henostroza,² Rubhana Raqib,³ Zeaur Rahim,³ Martina Gerhardt,⁴ Erica Sanga,⁵ Michael Hoelscher,² Tsugunori Notomi,⁶ Tetsu Hase,⁷ and Mark D. Perkins⁸

Courtesy: FIND, Geneva

Newer NAATs



Courtesy: FIND, Geneva

Serological tests for TB

- Attractive, especially if made into point of care (POC)
- Have been around for a long time
- Existing serological tests have failed

OPEN ACCESS Freely available online

PLOS MEDICINE

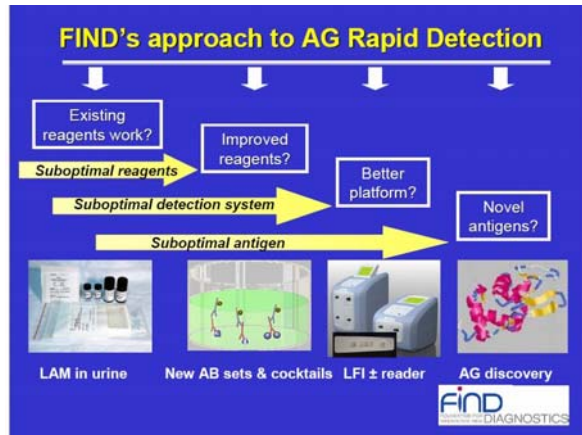
Commercial Serological Antibody Detection Tests for the Diagnosis of Pulmonary Tuberculosis: A Systematic Review

Karen R. Steingart^{1,2}, Megan Henry³, Suman Laal^{1,3,4}, Philip C. Hopewell^{1,2}, Andrew Ramsay⁵, Dick Menzies^{6,7}, Jane Cunningham⁸, Karin Welding⁹, Madhukar Pai^{1,10*}

A systematic review of commercial serological antibody detection tests for the diagnosis of extrapulmonary tuberculosis

Karen R Steingart, Megan Henry, Suman Laal, Philip C Hopewell, Andrew Ramsay, Dick Menzies, Jane Cunningham, Karin Welding, Madhukar Pai

Thorax 2007;62:911-918. doi: 10.1136/thx.2006.075754



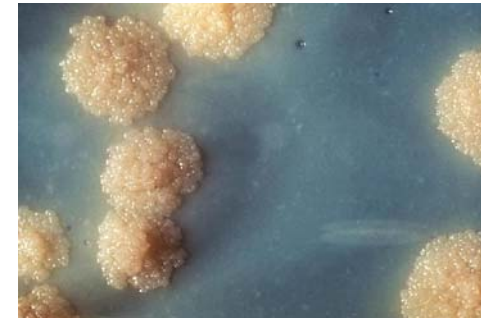
PrimaTB STAT-PAK Assay, a Novel, Rapid Lateral-Flow Test for Tuberculosis in Nonhuman Primates^{1,2}

Konstantin P. Lyashchenko,^{1*} Rema Greenwald,¹ Javan Esfandiari,¹ David Greenwald,¹ Carol A. Nacy,² Susan Gibson,² Peter J. Didier,^{2*} Marc Washington,² Peter Szczerba,² Sherri Motzel,¹ Larry Handt,² John M. Pollock,² James McNair,² Peter Anderson,² Jan A. M. Langermans,^{3,4} Frank Verreck,² Sean Ervin,⁵ Frank Ervin,⁶ and Candace McCombs²



Courtesy: FIND, Geneva

Drug susceptibility testing



Antigen detection tests

Urinary LAM antigen test

- Detects LAM antigen by ELISA in the urine
- Optimal specimen, rapid (2.5 hrs)
- Sensitivity: 80% vs 60% for smear in PTB (71% HIV+)
- N= 119 culture + HIV-TB (sensitivity- 81%)
Boehme C, *Trans Roy Soc Trop Med*, 2005
- N= 108 culture +, sensitivity 73%
Specificity 94% (69% HIV+)
McArthur C, *ASM*, 2007 (abstract)

Strip test under development



Diagnosis of drug resistance

- Relies on sputum culture:
 - solid media in most developing countries
 - Slow
 - Not widely available
 - Liquid media (BACTEC, MGIT):
 - rapid, but
 - expensive and beyond the reach of resource limited countries
- New tools for drug resistance:
 - Rapid cultures
 - Line probe assays
 - Phage-based assays
 - MODS



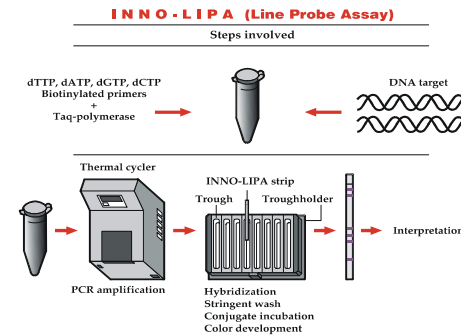
Rapid culture systems

- Liquid media (MGIT):
 - rapid and accurate, but
 - expensive and beyond the reach of resource limited countries
 - may be useful in high risk groups (HIV)
 - New WHO policy supports widespread use
- Colorimetric culture systems (TK Medium)
 - limited evidence, but may be more suitable for high burden countries



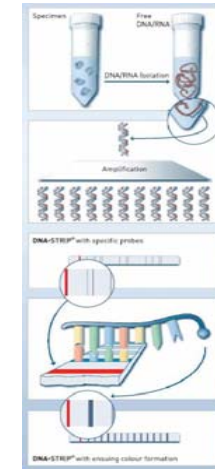
Line probe assays

Inno-LiPA assay



Courtesy: Innogenetics, Belgium

GenoType® MTBDR assay



[Hain LifeScience, Germany]



Microscopic observation drug susceptibility assay (MODS)

- Mycobacteria grow more rapidly in liquid media and detectable earlier-inverted microscope
- Sensitivity better than LJ slopes (98 vs 84%); 7 days for culture and DST, cheap, simple, accurate

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Microscopic-Observation Drug-Susceptibility Assay for the Diagnosis of TB

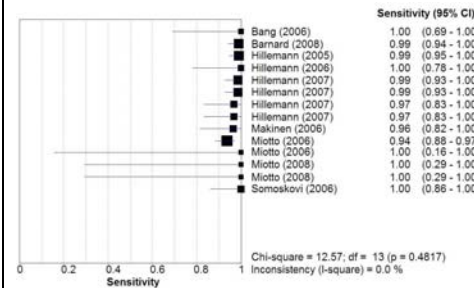
David A.J. Moore, M.D., Carlton A.W. Evans, M.D., Ph.D., Robert H. Gilman, M.D., Luz Caviedes, B.Sc., Jorge Coronel, B.Sc., Aldo Vivar, M.D., Eduardo Sanchez, M.D., Yvette Pinedo, M.D., Juan Carlos Saravia, M.D., Cayo Salazar, M.D., Richard Oberhelman, M.D., Maria-Graciela Hollm-Delgado, M.Sc., Doris LaChira, M.D., A. Rodenick Escombe, M.D., Ph.D., and Jon S. Friedland, M.D., Ph.D.



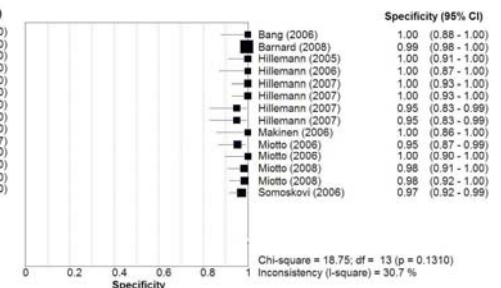
Courtesy: David Moore & Luz Caviedes



Meta-analysis of GenoType MTBDR studies



98% Sensitivity



99% Specificity



FASTPlaque-Response™

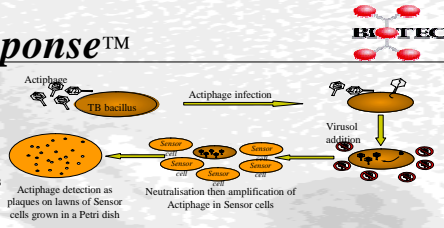


Introduction

- Bacteriophage-based test for the rapid detection of rifampicin resistance in smear-positive sputum specimens containing MTB.
- Results read by eye within 2 days.

Principle of assay

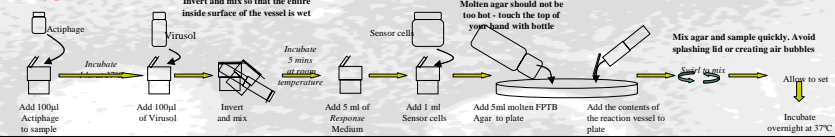
- Portions of decontaminated sputum incubated with and without rifampicin.
- Bacteriophage (Actiphage) infect viable TB bacilli in sputum.
- A potent virucide (Virusol) destroys all bacteriophage that have not infected TB bacilli.
- Bacteriophage replicating in TB bacilli survive and are detected as plaques (zones of clearing) after plating with non-pathogenic mycobacterial Sensor cells.
- Presence of viable TB bacilli following rifampicin treatment signifies



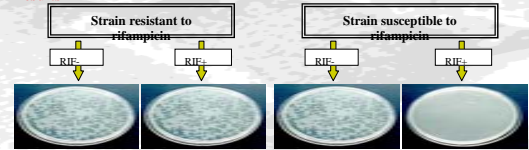
Specimen processing



Assay procedure



Results



Interpretation of results
 Positive control: 20 plaques or more
 Negative control: 10 plaques or less
 RIF- plate must have 100 plaques or more to interpret results
 RIF+ <50 plaques RIFAMPICIN SUSCEPTIBLE
 RIF+ ≥50 plaques or more RIFAMPICIN RESISTANT

Biotech Laboratories Ltd., 32 Anson Road, Martlesham Heath, Ipswich, IP5 3RG, UK.
 Tel: +44 1473 612158 Fax: +44 1473 611476 Email: FPTB@biotec.com Website: www.biotec.com

Rev 1, 2005.12.02

In conclusion, much progress has been made in improving TB diagnosis, but...



Journal of Infection (2005) 51, 175-187



www.elsevierhealth.com/journals/jinf



Bacteriophage-based assays for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a meta-analysis

Madhukar Pai^{a,b,*}, Shriprakash Kalantri^{a,c}, Lisa Pascopella^d, Lee W. Riley^a, Arthur L. Reingold^a

- When performed on culture isolates, phage assays have relatively high accuracy
 - 11 of 19 (58%) studies included in the review reported sensitivity and specificity estimates in ≥95%
 - Specificity estimates were slightly lower and more variable than sensitivity; 5 of 19 (26%) studies reported specificity <90%
- Only two studies performed phage assays directly on sputum specimens, with inconsistent results
 - Current evidence, therefore, is mostly restricted to the use of phage assays for the detection of rifampicin resistance in culture isolates
- When applied to isolates, these assays have relatively high sensitivity and specificity
- When applied directly to clinical specimens, phage assays seem to have lower sensitivity, presumably because of the lower bacillary load



will new tools reach those who really need it?



Until they do, the ability to diagnose or misdiagnose TB will vary across the two worlds

