

# BRN SEMINARS

Scientific workshops to foster collaborative research

## Recerca en diagnòstic ràpid de la tuberculosi

RECERCA EN TB

18-01-2018

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# Conflicto de intereses

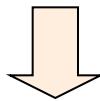
No tengo afiliación, relación contractual ni intereses económicos con ninguna organización relacionada con el tema tratado ni los productos citados en esta sesión

# Control de la TB

**RETRASO DIAGNÓSTICO (*Enfermedad* y *TB-R*)**  
gran obstáculo para el control (OMS)



**Diagnóstico RÁPIDO de TB activa y TB-R**



Intervención epidemiológica (aislamiento y ECC) y  
terapéutica precoz

# Diagnóstico Microbiológico – TB activa

## ***Microscopía*** (*ácido-alcohol resistencia*)

- Luz visible (Tinción de Ziehl-Neelsen)
- Luz ultravioleta (Auramina)

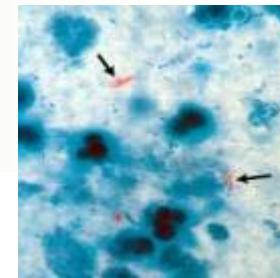
## ***Cultivo***

- Sólido y líquido

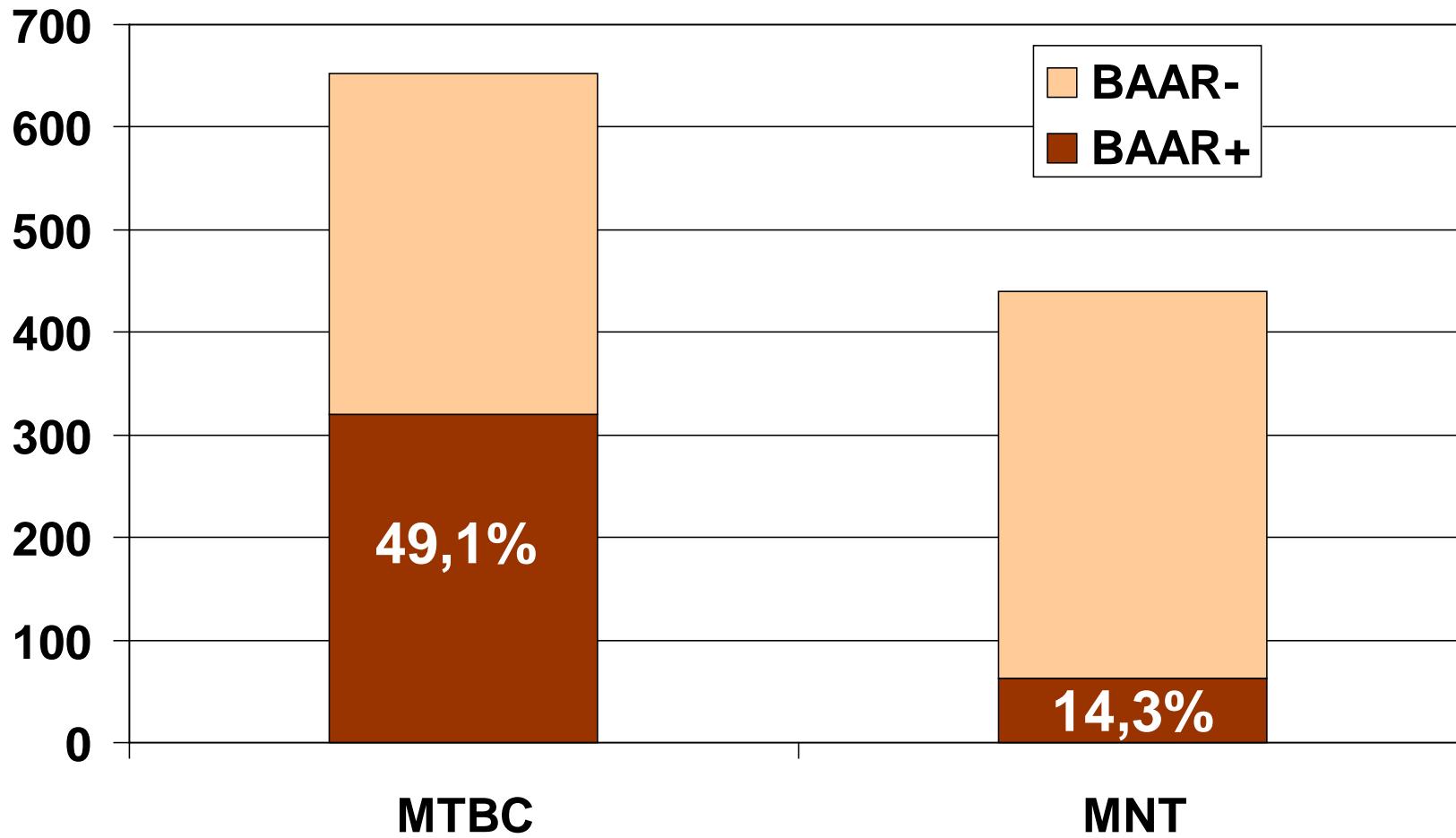
## ***Identificación*** (*fenotípica y/o molecular*)

- Indirecta (cultivo puro)
- **Directa (Muestra)**

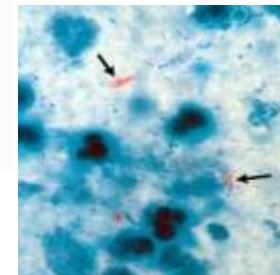
# Diagnóstico baciloscópico



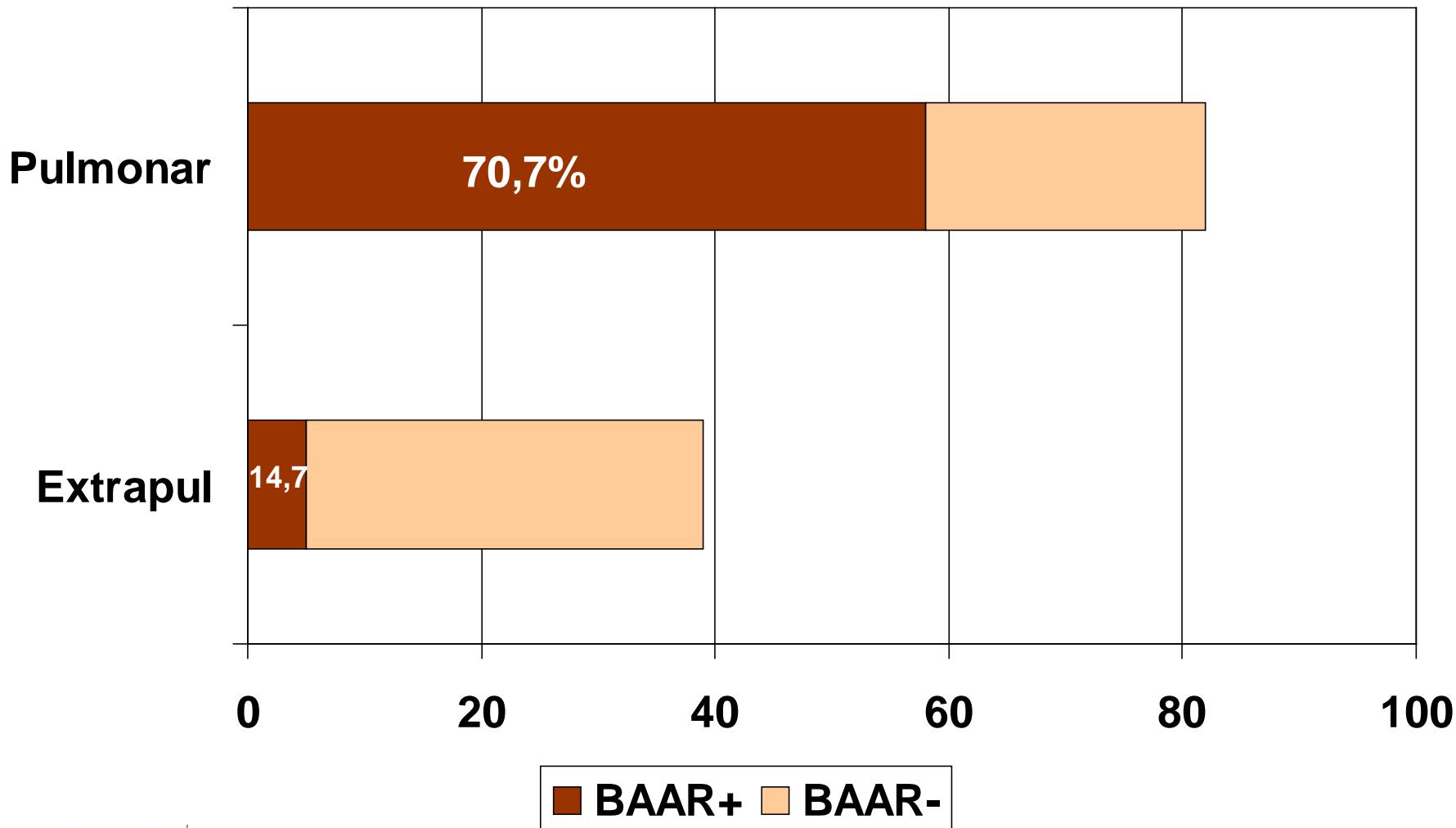
Pacientes HUB (2011-15)- Cultivos micobacterianos (n=1.092)



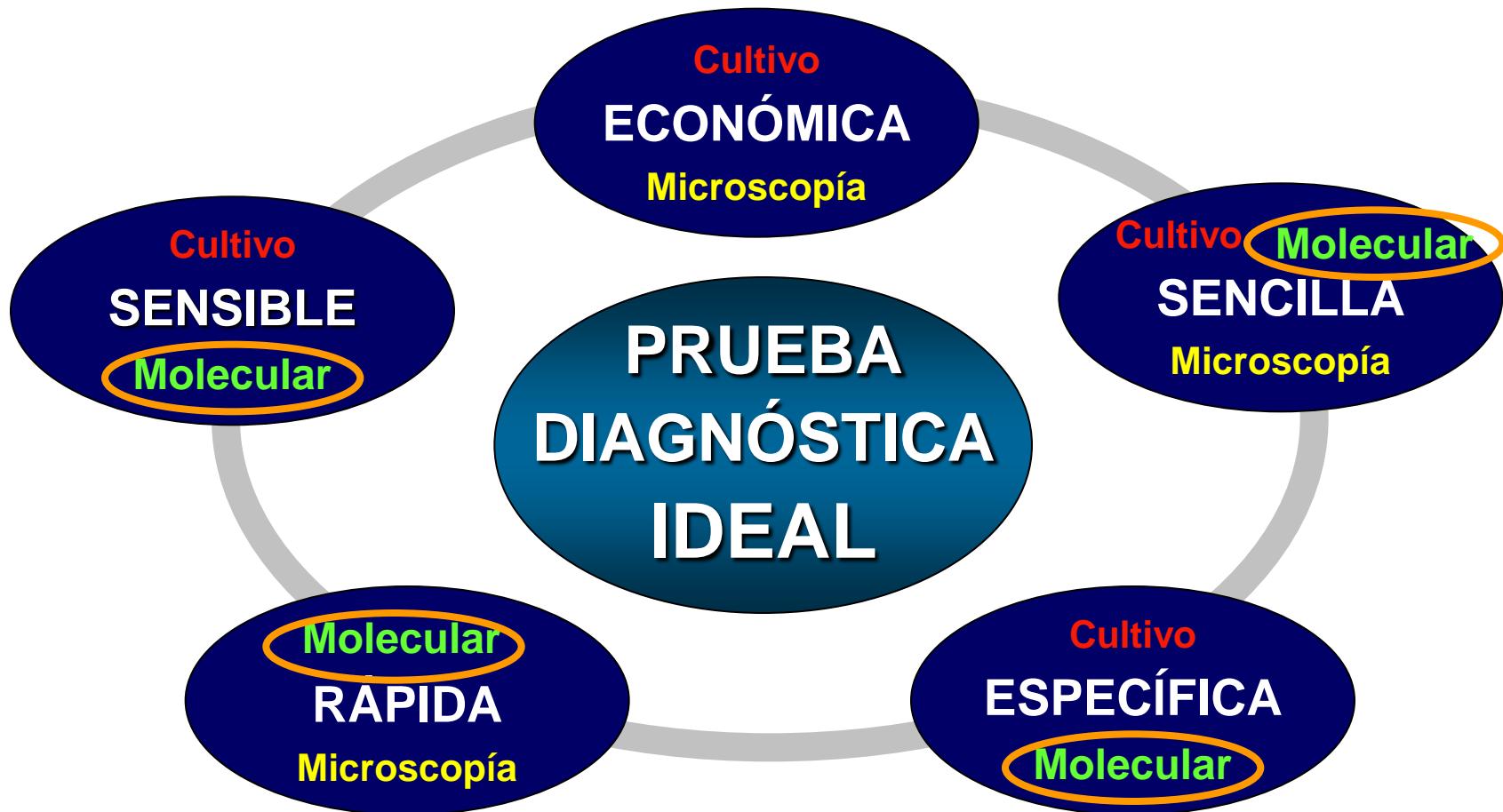
# Diagnóstico baciloscópico



Pacientes HUB (2013-14)- Tuberculosis (n=121)

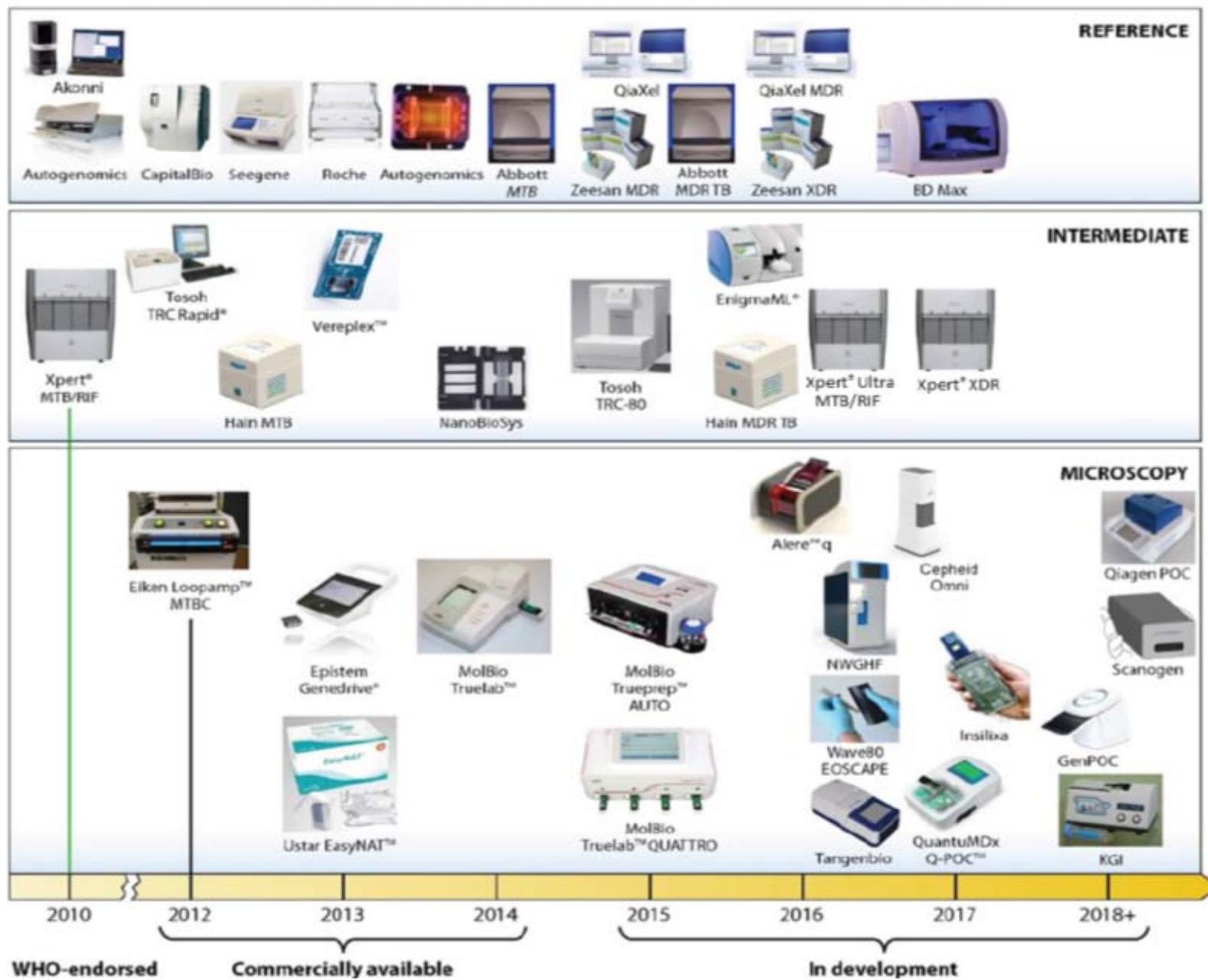


# Dilema en el Diagnóstico Microbiológico



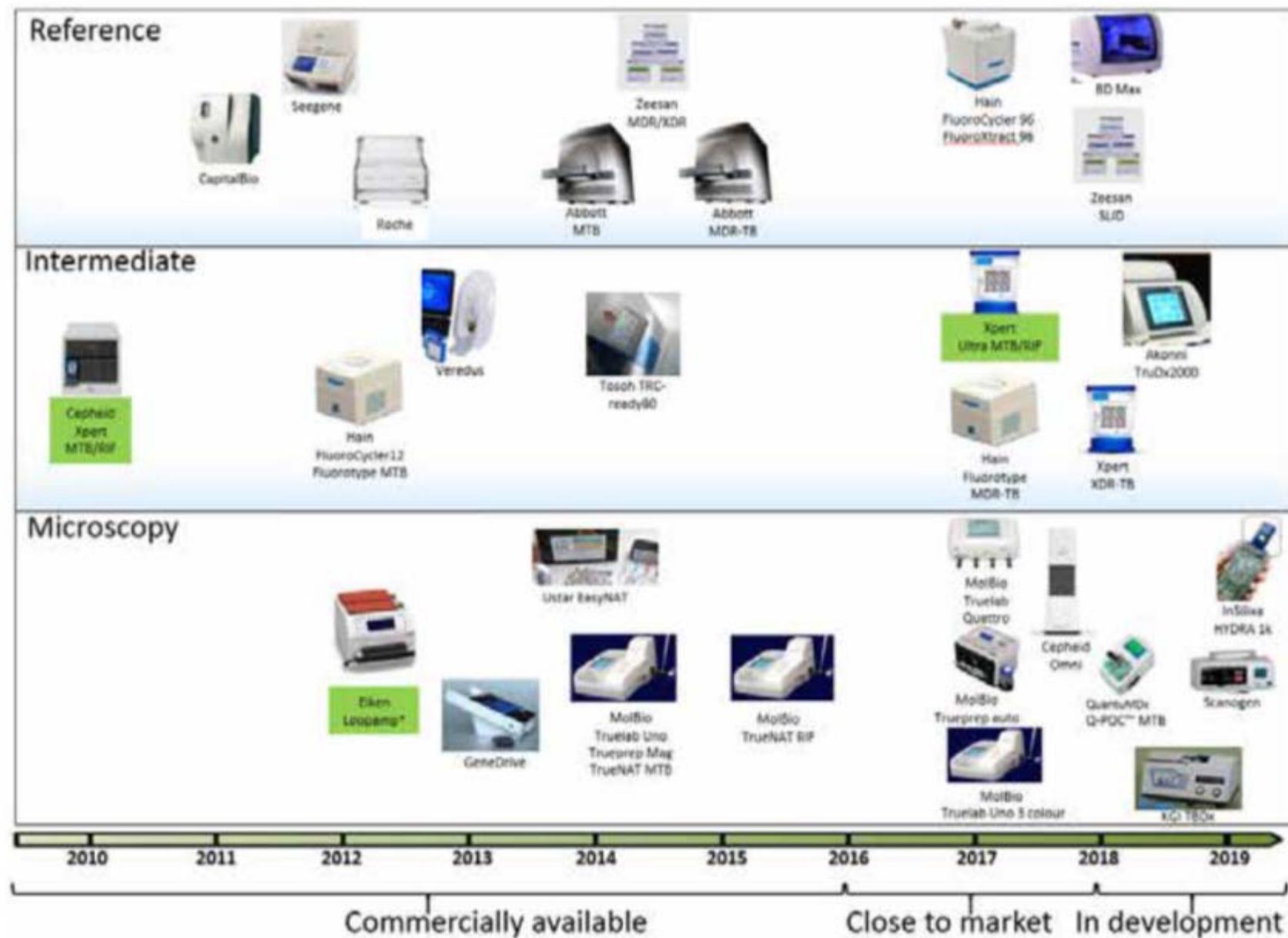
# UNITAID 2015 Tuberculosis Diagnostics Technology and Market Landscape

**Figure 13. Current and emerging automated, semi-modular or non-integrated TB NAATs; their intended laboratory location and release date (actual or anticipated)**



# UNITAID 2017 Tuberculosis Diagnostics Technology and Market Landscape

**Figure 7.** Current and emerging automated, semi-modular or non-integrated TB NAATs; their intended laboratory location and release date (actual or anticipated)



**Table 1.** Summary of NAATs relating their role in TB diagnosis in terms of intended location of use, throughput and other key factors

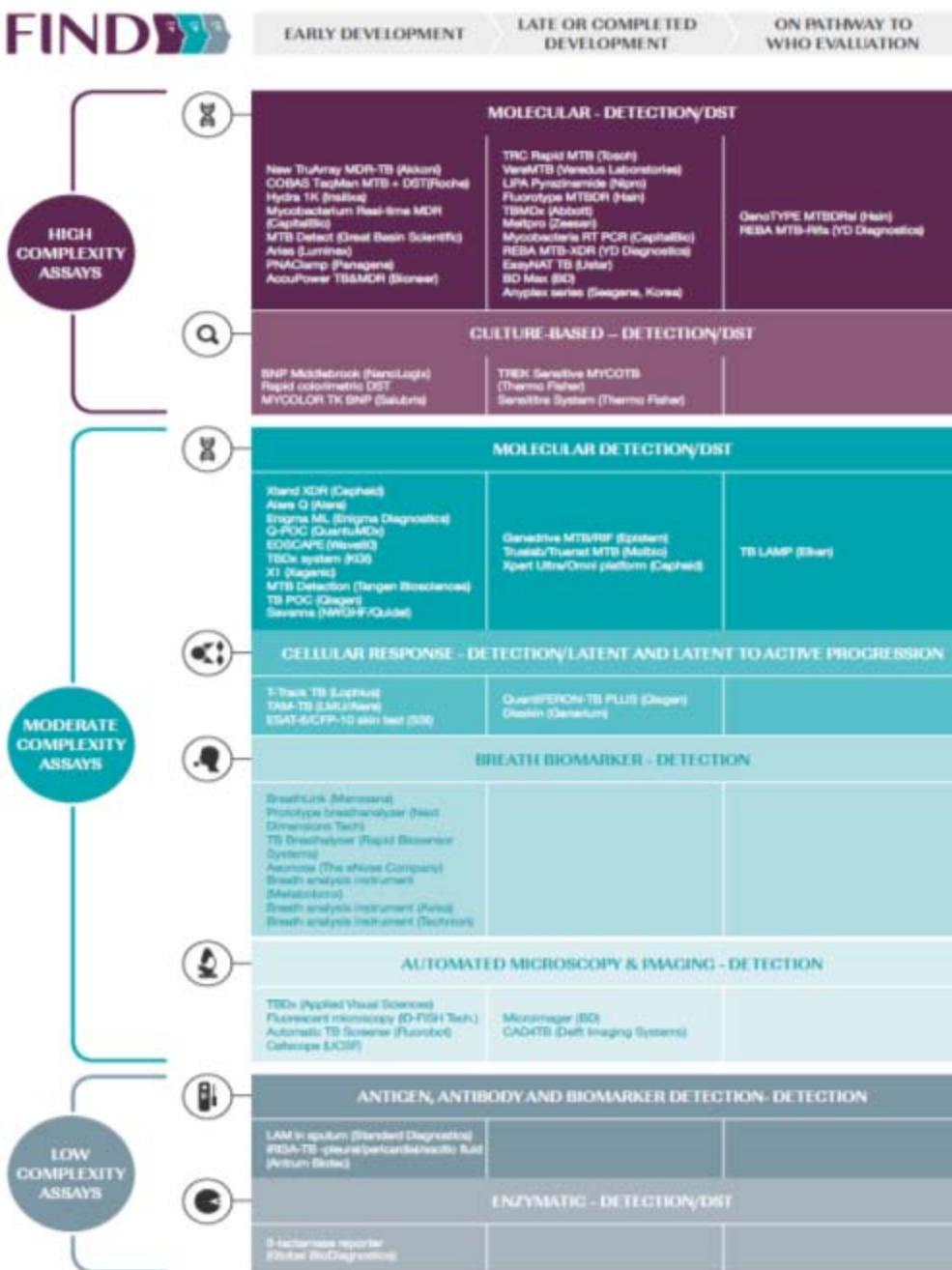
Test	Level	Throughput	Function	Complexity	Cost	Cost/test
<b>POC assays</b>	3, 2, 1	Moderate/low	TBDx, DST <sup>a</sup>	Low	Moderate/low	Low
<b>Modular NAATs</b>	3, 2, 1 <sup>b</sup>	Moderate	TBDx, DST	High	High	Moderate
<b>Microarrays</b>	3, 2	Moderate	MTB/NTM <sup>c</sup> Dx, DST <sup>a</sup>	High	High	High/moderate
<b>LPAs</b>	3, 2	Moderate	MTB/NTM <sup>c</sup> Dx, DST <sup>a</sup>	Medium	Medium	Medium
<b>Automated batched PCR</b>	3	High/moderate	MTB/NTM <sup>c</sup> Dx, DST <sup>a</sup>	High	High	Low
<b>Open PCR platforms</b>	3	Moderate	MTB Dx, DST <sup>a</sup>	High	High	Low
<b>NGS</b>	3	High	MTB/NTM <sup>c</sup> Dx, DST, mol epi	High	High	Moderate

<sup>a</sup> DST is genotyping of drug resistance that may be multiplexed with TB diagnosis or applied as a reflexive test after MTB infection is confirmed. Level 1 microscopy centre or community care centre, Level 2 hospital or regional clinical facility, Level 3 reference or tertiary hospital.<sup>14</sup>

<sup>b</sup> Testing at this level is performed in few LMICs.

<sup>c</sup> Some tests are available to rule in other common types of NTM.

**Figure 1.** Current FIND TB diagnostics pipeline listing the development phases and types of technologies in development or evaluation



4. CDC. Interim guidelines for the evaluation of infants born to mothers infected with West Nile virus during pregnancy. MMWR 2004; 53:154-7.
5. Pinon JM, Dumon H, Chemla C, et al. Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M, and A antibodies. J Clin Microbiol 2001;39:2267-71.
6. Jamieson DJ, Theiler RN, Rasmussen SA. Emerging infections and pregnancy. Emerg Infect Dis 2006;12:1638-43.
7. Cono J, Cragan JD, Jamieson DJ, Rasmussen SA. Prophylaxis and treatment of pregnant women for emerging infections and bioterrorism emergencies. Emerg Infect Dis 2006;12:1631-7.
8. Tsai TF. Congenital arboviral infections: something new, something old. Pediatrics 2006;117:936-9.
9. Orosio JE, Scheppe RJ, Yull TM. Effects of La Crosse infection on pregnant domestic rabbits and Mongolian gerbils. Am J Trop Med Hyg 1996;55:384-90.
10. Edwards JE, Karabatsos N, Collison EW, de la Concha Bermejillo A. Ovine fetal malformations induced by in utero inoculation with Main Drain, San Angelo, and LaCrosse viruses. Am J Trop Med Hyg 1997;56:171-6.

## Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis

Guidelines for the use of nucleic acid amplification (NAA) tests for the diagnosis of tuberculosis (TB) were published in 1996 (1) and updated in 2000 (2). Since then, NAA testing has become a routine procedure in many settings because NAA tests can reliably detect *Mycobacterium tuberculosis* bacteria in specimens 1 or more weeks earlier than culture (3). Earlier laboratory confirmation of TB can lead to earlier treatment initiation, improved patient outcomes, increased opportunities to interrupt transmission, and more effective public health interventions (4,5). Because of the increasing use of NAA tests and the potential impact on patient care and public health, in June 2008, CDC and the Association of Public Health Laboratories (APHL) convened a panel of clinicians, laboratorians, and TB control officials to assess existing guidelines (1,2) and make recommendations for using NAA tests for laboratory confirmation of TB. On the basis of the panel's report and consultations with the Advisory Council for the Elimination of TB (ACET),\* CDC recommends that NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities, such as contact

\*Additional information regarding ACET is available at <http://www.cdc.gov/mmwr/facmact.htm>.

investigations. These guidelines update the previously published guidelines (1,2).

### Background

Conventional tests for laboratory confirmation of TB include acid-fast bacilli (AFB) smear microscopy, which can produce results in 24 hours, and culture, which requires 2–6 weeks to produce results (5,6). Although rapid and inexpensive, AFB smear microscopy is limited by its poor sensitivity (45%–80% with culture-confirmed pulmonary TB cases) and its poor positive predictive value (50%–80%) for TB in settings in which nontuberculous mycobacteria are commonly isolated (3,6,7).

NAA tests can provide results within 24–48 hours. The Amplified *Mycobacterium tuberculosis* Direct Test (MTD, Gen-Probe, San Diego, California) was approved by the Food and Drug Administration (FDA) in 1995 for use with AFB smear-positive respiratory specimens, and in a supplement application, an enhanced MTD test was approved in 1999 for use with AFB smear-negative respiratory specimens from patients suspected to have TB. In addition, the Amplicor *Mycobacterium tuberculosis* Test (Amplicor, Roche Diagnostics, Basel, Switzerland) was approved by FDA in 1996 for use with AFB smear-positive respiratory specimens from patients suspected to have TB. NAA tests for TB that have not been FDA-approved also have been used clinically (e.g., NAA tests based on analyte specific reagents, often called "home-brew" or "in-house" tests) (8,9).

Compared with AFB smear microscopy, the added value of NAA testing lies in its 1) greater positive predictive value (>95%) with AFB smear-positive specimens in settings in which nontuberculous mycobacteria are common and 2) ability to confirm rapidly the presence of *M. tuberculosis* in 50%–80% of AFB smear-negative, culture-positive specimens (3,7–9). Compared with culture, NAA tests can detect the presence of *M. tuberculosis* bacteria in a specimen weeks earlier than culture for 80%–90% of patients suspected to have pulmonary TB whose TB is ultimately confirmed by culture (3,8,9). These advantages can impact patient care and TB control efforts, such as by avoiding unnecessary contact investigations or respiratory isolation for patients whose AFB smear-positive specimens do not contain *M. tuberculosis*.

Despite being commercially available for more than a decade (1), NAA tests for TB have not been widely used in the United States largely because of 1) an uncertainty as to whether NAA test results influence case-management decisions or TB control activities; 2) a lack of information on the overall cost-effectiveness of NAA testing for TB; and 3) a lack of demand from clinicians and public health authorities. However, recent

## Availability of an Assay for Detecting *Mycobacterium tuberculosis*, Including Rifampin-Resistant Strains, and Considerations for Its Use — United States, 2013

In August 2013, the Food and Drug Administration (FDA) permitted marketing of the Xpert MTB/RIF assay (Cepheid, Sunnyvale, California) to detect DNA of the *Mycobacterium tuberculosis* complex (MTBC) and genetic mutations associated with resistance to rifampin (RMP) in unprocessed sputum and concentrated sputum sediments (1). Along with clinical, radiographic, and other laboratory findings, results of the assay aid in the diagnosis of pulmonary tuberculosis (TB). The assay is a nucleic acid amplification-based (NAA)\* test using a disposable cartridge in conjunction with the GeneXpert Instrument System. Sensitivity and specificity of the Xpert MTB/RIF assay for detection of MTBC appear to be comparable with other FDA-approved NAA assays for this use, although direct comparison studies have not been performed. Sensitivity of detection of RMP resistance was 95% and specificity 99% in a multicenter study using archived and prospective specimens from subjects aged ≥18 years suspected of having TB who had 0–3 days of antituberculous treatment (1). CDC continues to recommend following published U.S. guidelines for TB diagnosis and infection control practice, including the use and interpretation of NAA test results (2–4). Providers and laboratories need to ensure that specimens are available for other recommended mycobacteriological testing. The Xpert MTB/RIF assay aids in the prompt diagnosis of TB and RMP-resistant disease. RMP resistance most often coexists with isoniazid (INH) resistance; TB that is resistant to both drugs is multidrug-resistant (MDR)<sup>†</sup> TB. Because the prevalence of RMP resistance is low in the United States (about 1.8% of TB cases) (5), a positive result indicating a mutation in the *rmpA2* gene of MTBC should be confirmed by rapid DNA sequencing.

\*"NAA tests" are only one type of "direct" molecular diagnostic devices that are applied to a clinical specimen (e.g., sputum) with or without processing. NAA testing to diagnose MTBC in patients should not be confused with testing using NAA assays applied to isolates for the rapid species identification of MTBC.  
†MDR TB is defined as TB caused by MTBC resistant to at least INH and RMP. Extensively drug-resistant TB (XDR TB) is a type of MDR TB that is additionally resistant to any fluoroquinolone and to at least one of three injectable second-line drug (i.e., amikacin, kanamycin, or capreomycin).

for prompt reassessment of the treatment regimen and followed by growth-based drug susceptibility testing (DST) (1,6,7). CDC offers these services free of charge.<sup>§</sup>

The World Health Organization has published guidance on use of the Xpert MTB/RIF assay aimed primarily at settings where the prevalence of TB and drug-resistant disease is much higher than in the United States (8).

### Detection of MTBC

In 2008, the Association of Public Health Laboratories and CDC convened a panel<sup>¶</sup> that recommended NAA testing as standard practice in the United States to aid in the initial diagnosis of patients with suspected TB. On the basis of the panel report (7) and consultation with the Advisory Council for the Elimination of TB, CDC published revised NAA guidelines, including a detailed testing and interpretation algorithm for initial diagnosis (4). Recent studies further support NAA test use in the United States to avoid delays in diagnosis and treatment, especially for patients with suspected TB and sputum smears negative for acid-fast bacilli on microscopy. Because of rapid results, NAA testing can help avoid unnecessary respiratory isolation, treatment, and contact investigation of patients without TB (9)\* and can contribute to system cost savings in patients with HIV infection, homelessness, or substance abuse, compared with smear microscopy alone (9).

CDC recommends that NAA testing be performed on at least one (preferably the first) respiratory specimen from each patient suspected of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB

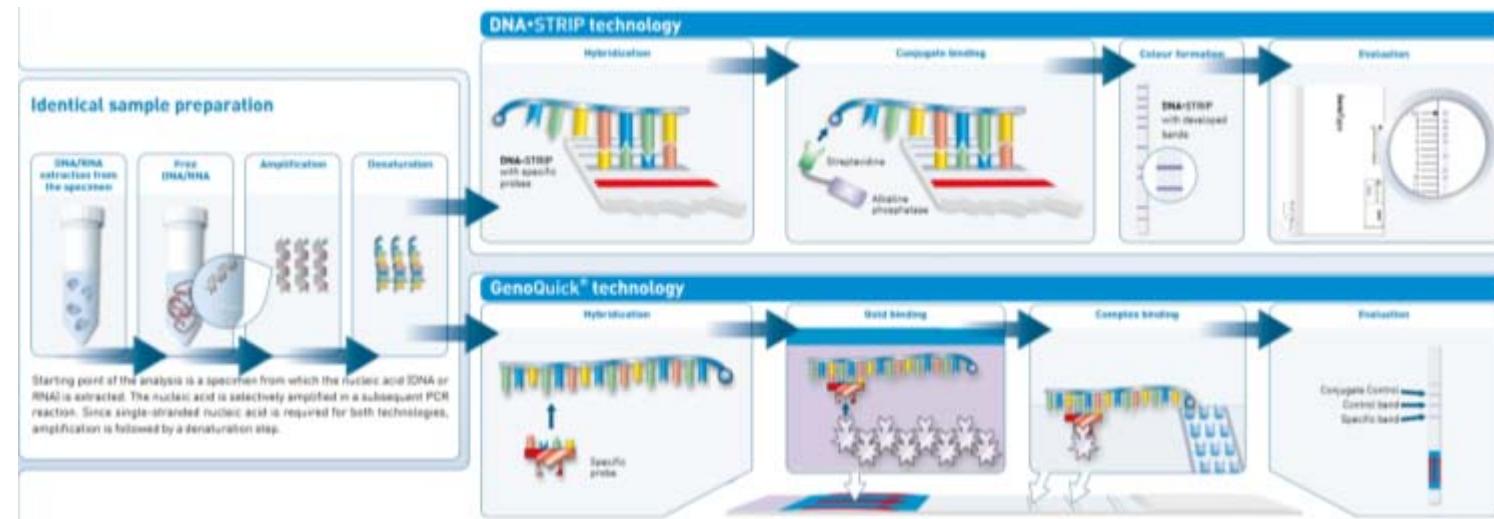
<sup>¶</sup>Information available at <http://www.cdc.gov/tb/topic/laboratory>.  
<sup>§</sup>In response to a recommendation by the Advisory Council for the Elimination of Tuberculosis.

<sup>¶</sup>Davis JL, Ho C, Catamanchi A, et al. The clinical and public health impact of automated nucleic acid testing for TB: evaluation in San Francisco. Abstract. American Thoracic Society International Conference: Denver, Colorado; 2011. Available at [http://www.atsjournals.org/doi/pdf/10.1164/atsci-conference.2011.113.1\\_meetingabstract.A5314](http://www.atsjournals.org/doi/pdf/10.1164/atsci-conference.2011.113.1_meetingabstract.A5314).

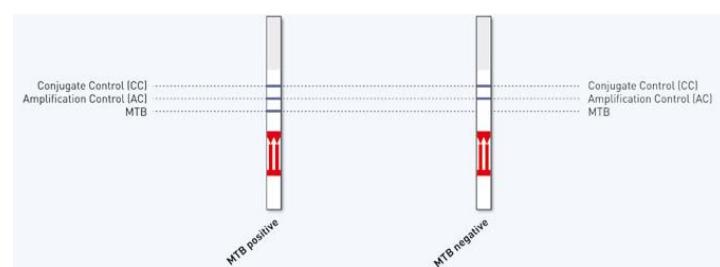


# Diagnóstico rápido – AAN / Hibridación reversa (directas de muestra)

## GenoQuick®-technology GenoQuick MTB



- **MTBC**
- **PCR + Rápida Hibridación (manual)**
- **Bastante Rápida y Sencilla**
- **Económica**
- **Reciente**



# Diagnóstico rápido TB – Tecnología GenoQuick



June 2012 Volume 50 Number 6

Journal of Clinical Microbiology p. 2089–2091

## Direct Detection of *Mycobacterium tuberculosis* Complex in Clinical Samples by a Molecular Method Based on GenoQuick Technology

Raquel Moure,<sup>a</sup> Miriam Torres,<sup>a</sup> Rogelio Martín,<sup>a,b</sup> and Fernando Alcaide<sup>a,b</sup>

Department of Microbiology, IDIBELL-Bellvitge Hospital, Feixa Llarga s/n, Hospitalet de Llobregat, Barcelona, Spain,<sup>a</sup> and Department of Pathology and Experimental Therapeutics, University of Barcelona, Feixa Llarga s/n, Hospitalet de Llobregat, Barcelona, Spain<sup>b</sup>

Several molecular systems for direct detection of *Mycobacterium tuberculosis* complex (MTBC) have recently been developed. The GenoQuick MTB assay (GQ-MTB) used in this study detected 82 of the 96 (85.4%) samples with MTBC, including 50 of 64 (78.1%) samples with negative acid-fast bacillus smears. Fifteen samples containing nontuberculous mycobacteria were also studied: 13 were GQ-MTB negative, one was positive, and one was indeterminate. GQ-MTB showed good effectiveness for the direct detection of MTBC from clinical samples.

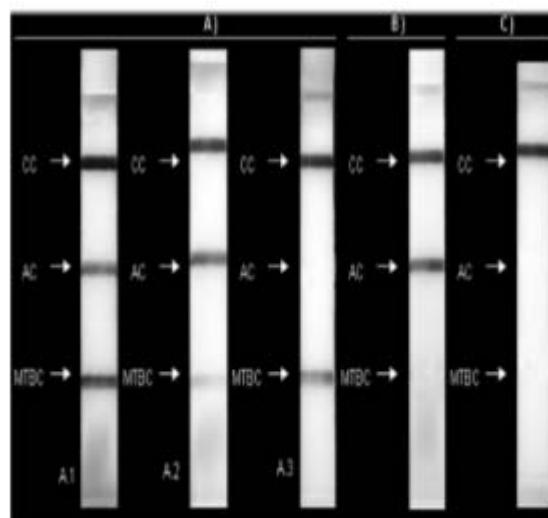
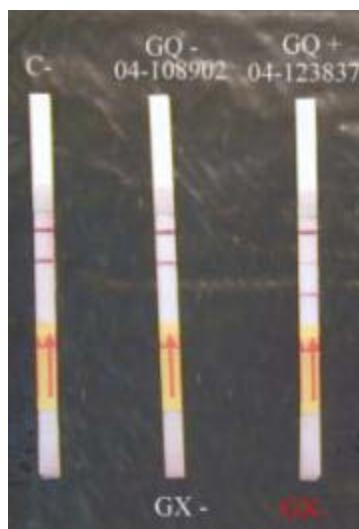


FIG 1 Images of the results obtained with the GQ-MTB technique. MTBC, *Mycobacterium tuberculosis* complex; AC, amplification control; CC, conjugate control. (A) Positive result. Three different possibilities were obtained: all bands were well marked (A.1), the MTBC band was present though faded (A.2), and the amplification control band was absent but the MTBC band was present (A.3). (B) Negative result (MTBC band completely missing). (C) Invalid result (both amplification control and MTBC bands were missing).

Sample type	No. of samples							
	MTBC culture positive		NTM <sup>b</sup> culture positive					
	GQ-MTB positive	GQ-MTB negative	GQ-MTB positive	GQ-MTB negative	S-P	S-N		
Sputum	25	46	0	11	1	0	8	2
Bronchial aspirate	3	2	0	1	0	0	0	0
Gastric aspirate	3	1	0	1	0	0	0	0
Urine	1	1	0	1	0	0	0	1
Other nonrespiratory <sup>c</sup>	0	0	0	0	0	0	2	0
Total	32	50	0	14	1	0	10	3

<sup>a</sup> Abbreviations: MTBC, *Mycobacterium tuberculosis* complex; NTM, nontuberculous mycobacteria; GQ-MTB, GenoQuick MTB; S-P, smear-positive specimens; S-N, smear-negative specimens.

<sup>b</sup> One smear-positive urine sample with a positive culture of *M. gordonae* showed an invalid GenoQuick MTB result, not included for calculation of specificity.

<sup>c</sup> One skin abscess and one colon biopsy.

# Diagnóstico Rápido TB

## PCR a tiempo Real (detección directa)- Xpert



N Engl J Med 2010.  
Rapid Molecular Detection of Tuberculosis  
and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D.,  
Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S.,  
Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D.,  
David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D.,  
David Allard, M.D., and Mark D. Perkins, M.D.

- Xpert MTB/RIF is an automated, cartridge-based nucleic amplification assay for the simultaneous detection of TB and rifampicin resistance directly from sputum in under two hours.
- The technology is based on the GeneXpert platform and was developed as a partnership between the **Foundation for Innovative New Diagnostics** (FIND), **Cepheid** Inc. and the **University of Medicine and Dentistry of New Jersey**, with support from the **US National Institutes of Health**.
- **WHO** recommended use of the technology in **December 2010** and is monitoring the global roll-out of the technology to promote coordination.

World Health Organization

Rapid Implementation  
of the Xpert MTB/RIF diagnostic test

Technical and operational 'How-to'  
Practical considerations



March 2011

# PCR a Tiempo Real - Xpert MTB/RIF



## Reactivos



### Cartucho Xpert MTB/RIF

- Reactivos extracción/PCR
- Sondas RT

### Líquido inactivante

## Software



### Módulos

### Lector código barras

### Monitor

# PCR a Tiempo Real - Xpert MTB/RIF



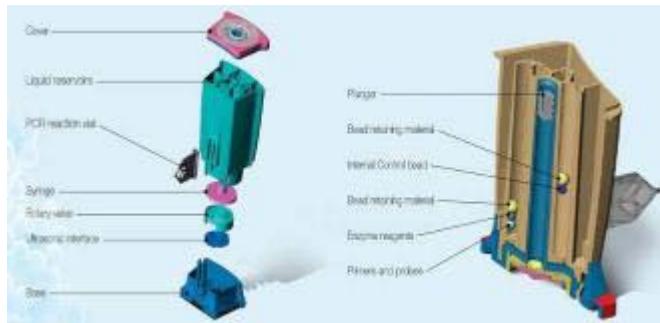
1) **inactivación** de la muestra (15 min)



2) **Dispensación** de la muestra en el cartucho



3) **Carga del cartucho** en el modulo



- 1) **Extracción DNA**
- 2) **AAN (Heminested PCR)**
- 3) **Hibridación** del amplificado con 5 sondas complementarias de la región core del *rpoB*

≈ total **2 h**  
(2-3 min  
“manipulación  
técnica”)

**Detección de MTBC y mutaciones en *rpoB* (MDR)**

# PCR a Tiempo Real - Xpert MTB/RIF



[Diagnostic Test Accuracy Review]

## Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults

Karen R Steingart<sup>1</sup>, Hojoon Sohn<sup>2</sup>, Ian Schiller<sup>3</sup>, Lorie A Kloda<sup>4</sup>, Catharina C Boehme<sup>5</sup>, Madhukar Pai<sup>2</sup>, Nandini Dendukuri<sup>2</sup>

<sup>1</sup>Department of Health Services, University of Washington, School of Public Health, Seattle, Washington, USA. <sup>2</sup>Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada. <sup>3</sup>Department of Clinical Epidemiology, McGill University Health Centre, Montreal, Canada. <sup>4</sup>Library, McGill University, Montreal, Canada. <sup>5</sup>Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland

Contact address: Karen R Steingart, Department of Health Services, University of Washington, School of Public Health, Seattle, Washington, 98195-7230, USA. [karenst@uw.edu](mailto:karenst@uw.edu).

**Editorial group:** Cochrane Infectious Diseases Group.

**Publication status and date:** Edited (no change to conclusions), published in Issue 2, 2013.

**Review content assessed as up-to-date:** 7 September 2012.

**Citation:** Steingart KR, Sohn H, Schiller I, Kloda LA, Boehme CC, Pai M, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database of Systematic Reviews* 2013, Issue 1. Art. No.: CD009593. DOI: 10.1002/14651858.CD009593.pub2.

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### Findings

Eighteen studies including 7816 participants (median number in the studies 117, Interquartile range (IQR) 93, 214), evaluated Xpert for TB detection. The prevalence of TB in the 18 studies ranged from 18.3% ([Lawn 2011](#)) to 100% ([Friedrich 2011](#)), median 37.4% (IQR 29.4, 59.8). Of the total 18 studies, 11 studies including 2340 participants provided data for 2 x 2 tables for rifampicin resistance detection. Of the remaining studies, four studies reported that all specimens were found to be drug sensitive (no rifampicin resistant specimens) ([Ciftci 2011](#); [Hanif 2011](#); [Marlowe 2011](#); [Rachow 2011](#)); two studies provided data jointly for pulmonary and extrapulmonary specimens ([Miller 2011](#); [Moure 2011](#)); and one study did not report information on rifampicin resistance ([Helb 2010](#)). The prevalence of rifampicin resistance in the 11 studies ranged from 0.8% ([Teo 2011](#)) to 29.4% ([Scott 2011](#)), median 7.3% (IQR 3.0, 19.6). All studies used a cross-sectional study design relevant to determining the diagnostic accuracy of Xpert. The majority of studies used expectorated (coughed-up) sputum not induced sputum.

# PCR a Tiempo Real - Xpert MTB/RIF

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2011, p. 1137–1139  
0095-1137/11/\$12.00 doi:10.1128/JCM.01831-10  
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Vol. 49, No. 3

## Rapid Detection of *Mycobacterium tuberculosis* Complex and Rifampin Resistance in Smear-Negative Clinical Samples by Use of an Integrated Real-Time PCR Method<sup>▽</sup>

Raquel Moure,<sup>1</sup> Laura Muñoz,<sup>2</sup> Miriam Torres,<sup>1</sup> Miguel Santin,<sup>2,4</sup>  
Rogelio Martín,<sup>1,3</sup> and Fernando Alcaide<sup>1,3\*</sup>

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Received 9 September 2010/Returned for modification 18 October 2010/Accepted 20 December 2010

Sixty-four of 85 (75.3%) smear-negative respiratory ( $n = 78$ ) and nonrespiratory ( $n = 7$ ) samples with positive cultures of *Mycobacterium tuberculosis* complex (MTC) were detected by the GeneXpert system using the Xpert MTB/RIF assay (GX). In addition, GX found *rpoB* mutations in all six of the rifampin-resistant strains detected. The test was negative in 20 culture-negative and 20 nontuberculous culture-positive samples (100% specificity). GX offers high potential for the diagnosis of tuberculosis due to its capacity for direct detection of MTC, its rapidity, and its simplicity.

TABLE 2. GX results for the detection of *M. tuberculosis* complex in 125 smear-negative clinical samples

GX result <sup>a</sup>	No. of MTC culture-positive specimens		No. of MTC culture-negative specimens		Total	Predictive value <sup>d</sup> (%)	
	Respiratory	Nonrespiratory	Positive for NTM <sup>b</sup> , respiratory	Negative for mycobacteria <sup>c</sup>			
				Respiratory	Nonrespiratory		
Positive	61	3	0	0	0	64	100
Negative	17	4	20	9	10	60	65

<sup>a</sup> The GX sensitivity was 75.3% (95% confidence interval [95% CI], 64.5 to 83.7%), and the specificity was 100% (95% CI, 88.8 to 100%).

<sup>b</sup> NTM, nontuberculous mycobacteria.

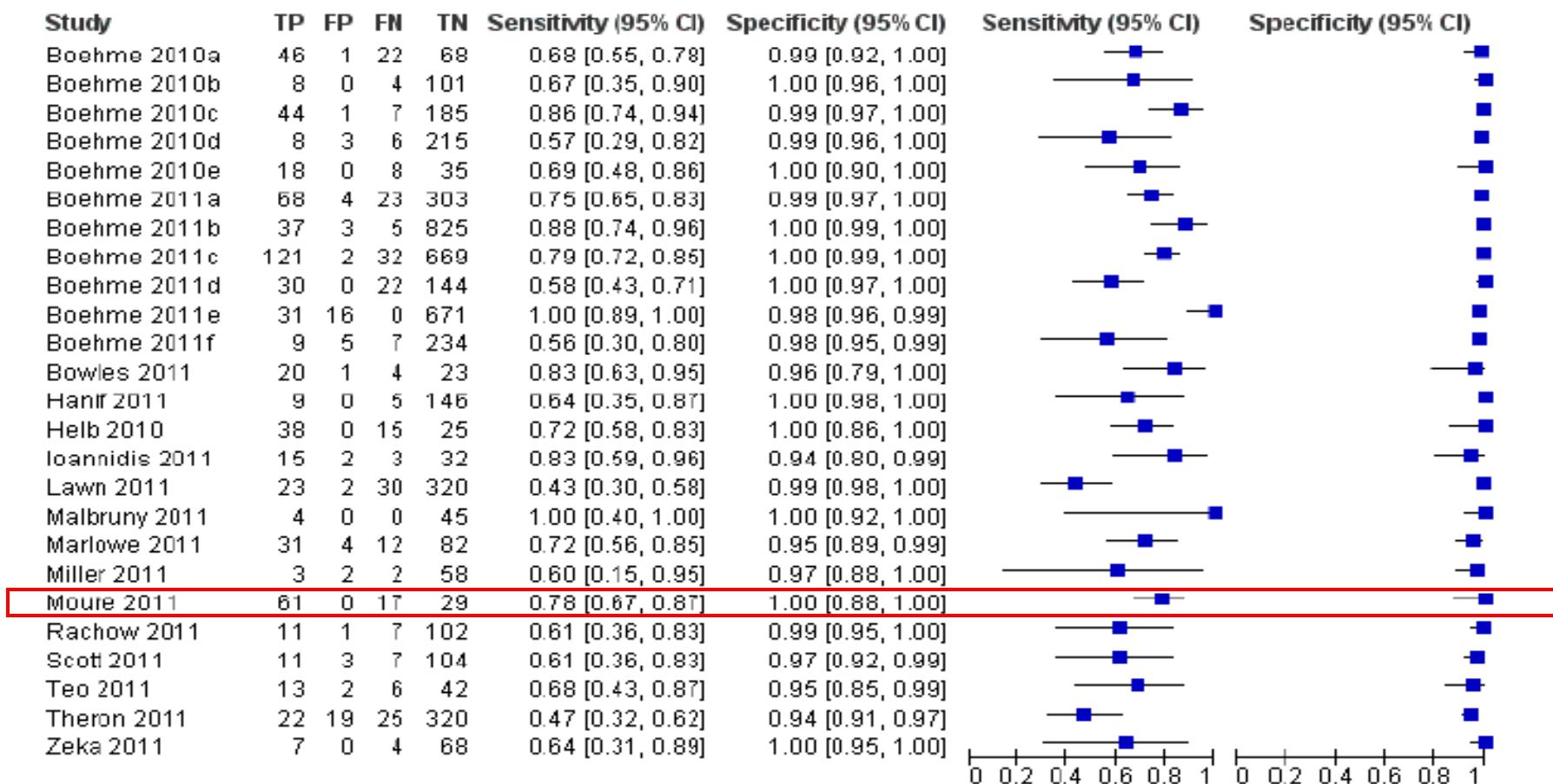
<sup>c</sup> One respiratory culture-negative sample had an invalid result using GX.

<sup>d</sup> The positive predictive value is given for the positive GX results, and the negative predictive value is given for the negative GX results.

# PCR a Tiempo Real - Xpert MTB/RIF



**Figure 7. Forest plots of Xpert for TB detection, Xpert used as an add-on test following a negative smear microscopy result. TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative. Between brackets the 95% CI of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).**



# PCR a Tiempo Real - Xpert MTB/RIF

Diagnostic Microbiology and Infectious Disease 75 (2013) 325–326

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## GeneXpert® for smear-negative pulmonary tuberculosis: does it play a role in low-burden countries?

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### ABSTRACT

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We performed a retrospective analysis of costs and time to treatment (TT) of 150 culture-confirmed TB cases: 100 sputum smear (SS) (+) and 50 SS(−). This group underwent GeneXpert® (GX) assay. Expenditures and TT of SS(−)/GX(+) cases were inferred from the SS(+) group. GX detected 68% of SS(−) cases.  
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**Table 2**

Cost per patient (€) according to AFB smear status.

	AFB negative, n = 48 (%) <sup>a</sup>	AFB positive, n = 100 (%)
All patients		
Mean (SD)	3260.52 (3406.4)	2097.0 (3224.78)
Median (IQR)	2426.58 (231.49–5269.65)	254.18 (218.61–3297.82)
Patients requiring hospitalization		
Mean (SD)	5788.72 (2698.23)	4989.51 (3603.31)
Median (IQR)	5090.92 (4542.52–7481.04)	3935.96 (1965.81–7404.91)
Patients not requiring hospitalization		
Mean (SD)	272.65 (191.39)	247.7 (142.62)
Median (IQR)	218.61 (193.96–281.38)	218.61 (171.47–244.89)

<sup>a</sup> Calculated from 48 patients (2 patients from the study population were excluded due to unexpectedly long hospitalizations).

**Table 1**  
Characteristics of the study population.

	AFB negative, n = 50 (%)	AFB positive, n = 100 (%)	P value
Male sex	35 (70)	75 (75)	0.51
Age (years), mean (SD)	49.3 (20.2)	41.3 (16.4)	0.02
Birth in a high-incidence TB country	12 (24)	31 (31)	0.24
Immunosuppressive condition <sup>a</sup>	14 (28)	21 (21)	0.22
Symptoms suggestive of TB <sup>b</sup>	25 (50)	82 (82)	<0.001
Radiographic features <sup>c</sup>			
Normal Rx	6 (12)	1 (1)	0.001
Parenchymal infiltrates	15 (30)	24 (24)	
Cavitation	20 (40)	67 (67)	
Other	9 (18)	5 (5)	
Patients requiring hospitalization	28 (56)	39 (39)	0.06
Days of hospitalization			
Mean (SD)	13.5 (23.3)	6.2 (10.9)	0.02
Median (IQR)	7.5 (0–16)	0 (0–9.3)	
Reasons for admission			
Diagnostic work-out	20 (71)	9 (23)	<0.001
Worsened clinical condition	1 (4)	12 (31)	0.005
Hemoptysis/respiratory failure	5 (18)	10 (26)	
Others	2 (7)	8 (20)	
Number of invasive tests required for diagnosis, per patient			
Bronchoscopy			
None	36 (72)	91 (91)	0.002
≥1	14 (28)	9 (9)	
Chest CT			
None	32 (64)	88 (88)	<0.001
≥1	18 (36)	12 (12)	
CT-guided puncture of the lung			
None	45 (90)	98 (98)	0.04
≥1	5 (10)	2 (2)	
Days to initiation of treatment			
Mean (SD)	17.3 (19.8)	3.9 (6.1)	<0.001
Median (IQR)	8.5 (2.25–25.3)	2 (1–4)	
Days to initiation of contact tracing			
Mean (SD)	24.1 (24.2)	7.9 (11.5)	<0.001
Median (IQR)	22 (2.2–32)	4 (1–11)	

AFB = Acid-fast bacilli; IQR = interquartile range; CT = computed tomography.

<sup>a</sup> Including HIV, drugs, cancer and impaired renal function (patients could present more than 1 condition).

<sup>b</sup> Included fever, cough, and weight loss for at least 2 weeks.

<sup>c</sup> Patients could present more than 1 condition.

# PCR a Tiempo Real - Xpert MTB/RIF

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## Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis

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**ABSTRACT:** Extrapulmonary tuberculosis (EPTB) accounts for more than 20% of tuberculosis (TB) cases. Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA, USA) is a fully automated amplification system, for which excellent results in the diagnosis of pulmonary TB in highly endemic countries have been recently reported. We aimed to assess the performance of the Xpert system in diagnosing EPTB in a low incidence setting.

We investigated with Xpert a large number of consecutive extrapulmonary clinical specimens (1,476, corresponding to 1,068 patients) including both paediatric (494) and adult samples.

We found, in comparison with a reference standard consisting of combination of culture and clinical diagnosis of TB, an overall sensitivity and specificity of 81.3% and 99.8% for Xpert, while the sensitivity of microscopy was 48%. For biopsies, urines, pus and cerebrospinal fluids the sensitivity exceeded 85%, while it was slightly under 80% for gastric aspirates. It was, in contrast, lower than 50% for cavitary fluids. High sensitivity and specificity (86.9% and 99.7%, respectively) were also obtained for paediatric specimens.

Although the role of culture remains central in the microbiological diagnosis of EPTB, the sensitivity of Xpert in rapidly diagnosing the disease makes it a much better choice compared to smear microscopy. The ability to rule out the disease still remains suboptimal.

### AFFILIATIONS

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Federica Piana\*\*#, Claudio Scarparo††, Luana Coltell‡, Giulia Lombardi§  
and Daniela M. Cirillo\*

**TABLE 1** Comparison of Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA, USA) results with culture and clinical data

Sample type	Xpert-positive and MTC-positive culture	Xpert-negative and MTC-positive culture	Xpert-positive, MTC-negative culture and TB diagnosis	Xpert-negative NTM-positive culture	Xpert-positive, MTC-negative culture and non-TB diagnosis	Xpert-negative, MTC-negative culture	Xpert-Indeterminate	Total (% proportion)
Biopsy specimen*	71	11	12	19	0	255	4	368 (25.0)
Pleural fluid	5	10	3	0	0	312	4	330 (24.4)
Gastric aspirate	45	13	3	14	0	149	0	224 (15.2)
Pus	40	7	8	22	0	118	4	195 (13.2)
CSF	11	2	1	0	1	118	5	133 (9.0)
Urines	11	2	3	6	1	107	0	130 (8.8)
Cavitory fluid†	5	5	0	0	0	84	0	94 (6.4)
<b>Total</b>	<b>188</b>	<b>50</b>	<b>30</b>	<b>61</b>	<b>2</b>	<b>1143</b>	<b>17</b>	<b>1474 (100)</b>

Data are presented as n, unless otherwise stated. Two samples that were Xpert-positive and *Mycobacterium tuberculosis* complex (MTC) culture-negative, from patients lacking a confident diagnosis, were not included in the table. TB: tuberculosis; NTM: nontuberculous mycobacteria; CSF: cerebrospinal fluid. \*: including 119 fine-needle-aspirates; †: including 60 peritoneal, 19 synovial and 15 pericardial fluid samples.

# PCR a Tiempo Real - Xpert MTB/RIF

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## Xpert MTB/RIF Assay for Diagnosis of Pleural Tuberculosis<sup>▽</sup>

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Received 12 August 2011/Returned for modification 9 September 2011/Accepted 3 October 2011

We prospectively investigated the diagnostic utility of the Xpert MTB/RIF (*Mycobacterium tuberculosis*/rifampin [RIF] resistance) assay in 20 cases with confirmed tuberculous pleural effusion. The sensitivity and specificity of the Xpert assay in pleural fluid were 25% and 100%, respectively. All cases positive by the Xpert assay were also positive by pleural fluid culture.

TABLE 1. Diagnostic accuracy of assays used on samples from 20 TB and 5 non-TB patients

Method	No. of samples that were:			Sensitivity (%)	Specificity (%)
	Positive	Negative	Not done		
Pleural fluid ADA	22	3	0	100	60.0
Pleural biopsy specimen	18	5	2	94.7	100
histology/ZN					
microscopy/liquid					
culture					
Sputum smear microscopy	6	7	12	54.5	100
Pleural fluid liquid culture	9	16	0	45.0	100
Pleural fluid Xpert assay	5	20	0	25.0	100

# PCR a Tiempo Real - Xpert MTB/RIF



## Effectiveness of an Integrated Real-Time PCR Method for Detection of the *Mycobacterium tuberculosis* Complex in Smear-Negative Extrapulmonary Samples in an Area of Low Tuberculosis Prevalence

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Early extrapulmonary tuberculosis (EPTB) diagnosis is particularly difficult. Among 108 smear-negative extrapulmonary samples showing a positive culture for *Mycobacterium tuberculosis* complex (43 body fluids and 65 nonliquid specimens), 63 (58.3%) were positive with the Xpert MTB/RIF assay (GX). GX sensitivity was quite low for samples from sterile locations (especially for pleural fluids: 26.9%) but high for some nonliquid samples, like abscess aspirates (76.5%). In summary, GX may be a useful tool to be considered for EPTB diagnosis.

# PCR a Tiempo Real - Xpert MTB/RIF



TABLE 1 Results of Xpert MTB/RIF according to the source and MTBC culture of the samples

Clinical specimen	No. of specimens with <sup>a</sup> :						Total no. of specimens	Sensitivity	Specificity			
	Positive MTBC culture		Negative MTBC culture									
	GX+	GX-	GX+	GX-	GX IND							
Sterile fluids												
Pleural fluid	7	19	0	5	0	31	40.5%	100%				
Cerebrospinal fluid	2	0	0	12	0	14						
Joint fluid	5	2	0	0	0	7						
Ascitic fluid	0	1	0	2	0	3						
Pericardial fluid	1	0	0	2	0	3						
Nonsterile fluids												
Gastric aspirate	2	1	0	3	2 <sup>b</sup>	8	66.7%	100%				
Urine	2	1	0	1	0	4						
Lymph nodes	24	10	0	4	0	38	70.6%	100%				
Abscess aspirates												
Knee abscess	1	0	0	0	0	1	76.5%	100%				
Cervical abscess	3	2	0	1	0	6						
Skin abscess	3	1	0	0	0	4						
Osteitis pus	4	1	0	0	0	5						
Empyema	2	0	0	1	0	3						
Tissues												
Pelvic biopsy	0	0	0	1	0	1	41.7%	100%				
Testicular biopsy	1	0	0	0	0	1						
Colon biopsy	0	0	0	2	0	2						
Vertebral disc biopsy	0	0	0	1	0	1						
Spondylodiscitis puncture	0	0	0	1	0	1						
Pericardial biopsy	0	0	0	1	0	1						
Bone biopsy	1	2	0	0	0	3						
Bone marrow biopsy	0	0	0	1	0	1						
Synovial biopsy	0	2	0	0	0	2						
Mediastinal tissue	0	1	0	0	0	1						
Skin biopsy	1	1	0	1	0	3						
Larynx biopsy	0	1	0	0	0	1						
Costal cartilage biopsy	2	0	0	0	0	2						
Stool	2	0	0	0	0	2	100%	100%				
Total	63	45	0	39	2	149	58.3%	100%				

<sup>a</sup> MTBC, *Mycobacterium tuberculosis* complex; GX, Xpert MTB/RIF; GX IND, Xpert MTB/RIF indeterminate result.

<sup>b</sup> The two invalid GX results were not included in the calculation of the specificity.

# Usefulness of an Integrated Real-Time PCR Method for the Detection of *Mycobacterium tuberculosis* complex on Paraffin-Embedded Human Tissues

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25-28 April 2015

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## INTRODUCTION

- One of the principles of tuberculosis control is rapid and accurate diagnosis of the disease in order to allow prompt initiation of antimicrobial therapy and to prevent transmission.
- Direct detection of DNA (clinical specimens) by real time-PCR have emerged as useful tools for rapid diagnosis of tuberculosis and rifampicin resistance. In December 2010, WHO first recommended the use of the Xpert MTB/RIF assay for pulmonary tuberculosis in adult patients. In late 2013, WHO expanded its recommendations on the use of Xpert MTB/RIF to include the diagnosis of tuberculosis in children and some forms of extrapulmonary tuberculosis.
- However, fresh clinical samples for microbiological studies are not always available.

## OBJECTIVES

- The aim of the study was to evaluate the effectiveness of an integrated and automated nucleic acid amplification system (Cepheid GeneXpert MTB/RIF®; GX) for direct detection of *Mycobacterium tuberculosis* complex (MTBC) in paraffin-embedded clinical specimens.

## MATERIAL AND METHODS

### Clinical specimens

- A total of 18 paraffin-embedded clinical specimens (each per patient) were collected in the Hospital Universitari de Bellvitge (Barcelona, Spain): 9 cervical or mediastinal lymph nodes, 2 liver biopsies, 2 renal biopsies, 1 lung biopsy, 1 bone biopsy, 1 bone marrow biopsy, 1 testicular biopsy, and 1 synovial biopsy.

## MATERIAL AND METHODS

### Deparaffinization of samples

- Six to twelve sections (5-10 µm thick) from each paraffin-embedded tissue block was performed by a simple and easy protocol with a commercial deparaffinization solution (Qiagen®).



In a microcentrifuge tube:

- Sections (6-12)
- Deparaffinization solution (320 µl)
- Incubation (56°C for 3 min)
- Buffer ATL (180 µl)
- Vortex and centrifugation
- Proteinase K (20 µl)
- Incubation (56°C ≥ 1 h; Lysed)
- Incubation (90°C for 1 h)
- Centrifugation
- Use the lower and clear phase

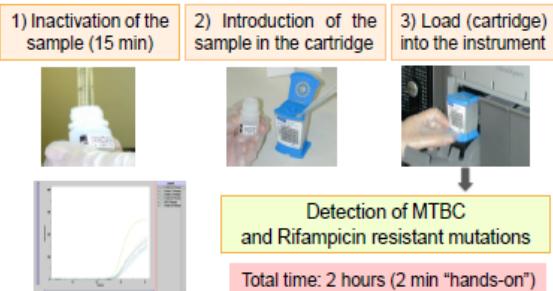
Total time: 2 h 15 min



Final working volume: 180 µl

### DNA detection of MTBC

- rpoB* gene of MTBC was detected by a fully integrated real-time PCR platform (GX) according to the manufacturer's instructions.



## Diagnosis of TB

- Diagnosis of tuberculosis disease was made by clinical, radiological, microbiological and histopathological data.

## RESULTS

- A total of 11 patients (55.6%) had definite tuberculosis diagnosis. Of these patients, 7 (63.6%) were males. Lymph nodes were more involved than others specimens (6/11). Acidfast bacilli were seen in 5 (45.5%) paraffin-embedded samples: 2 lymph nodes, 1 renal, 1 testicular, and 1 bone marrow biopsy.
- The GX was positive for MTBC detection in 8 cases (clinical extrapulmonary specimens; 5 were smear-positive) from 11 tuberculosis patients.
- The sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) of GX were as follows:

Method	SE	SP	PPV	NPV
GX	72.7 %	100%	100%	70%

- The Rifampicin resistance was not detected in any sample.

## CONCLUSIONS

- The GX system showed a good sensitivity and excellent specificity for direct detection of MTBC in paraffin-embedded clinical specimens. Only a previous rapid, easy and low cost deparaffinization method is required.
- This fact would have allowed an early treatment and contact tracing when fresh biopsy samples are not available for microbiological analyses.

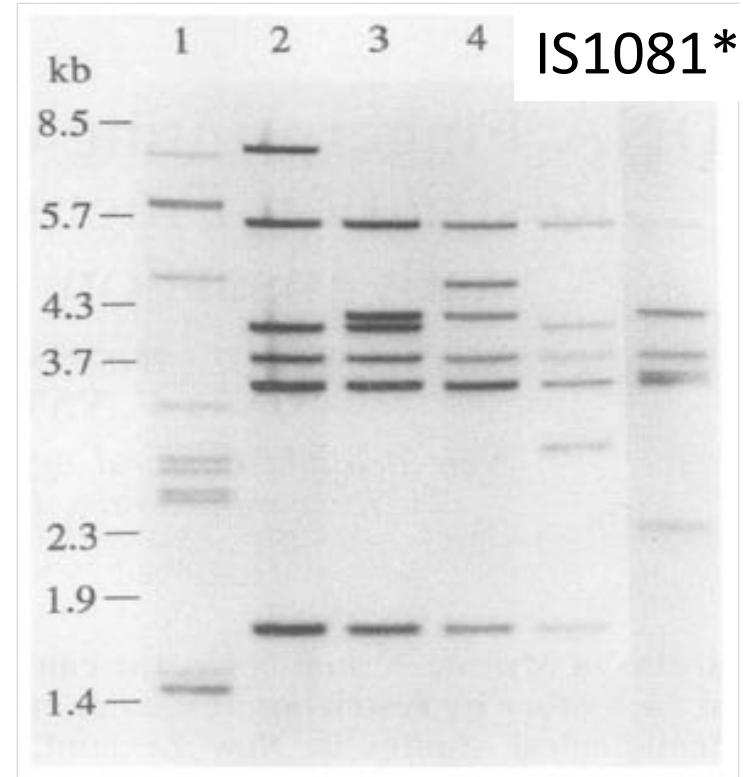
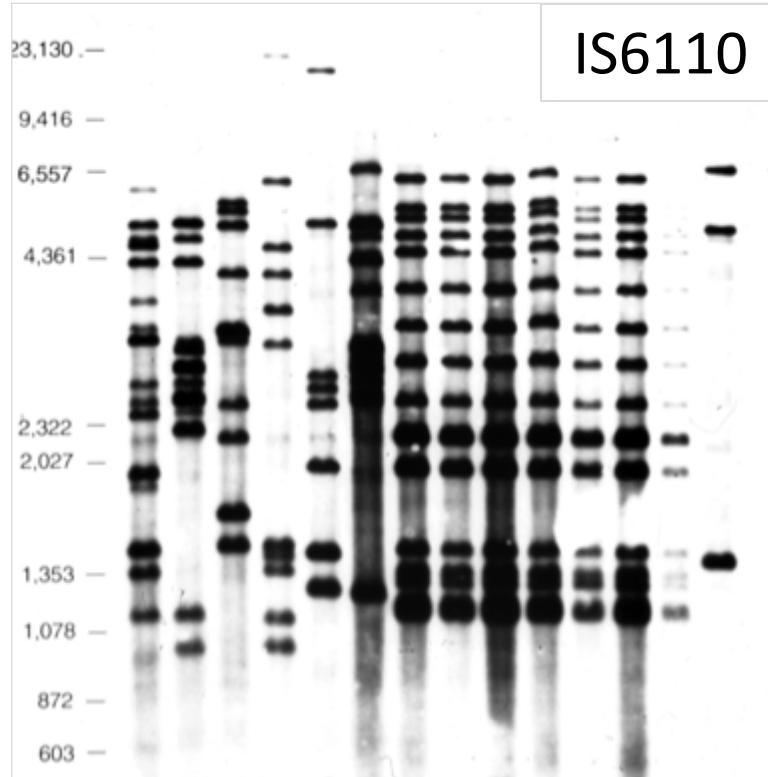
## Acknowledgements

- We are grateful to Cepheid for providing us the required reagents.

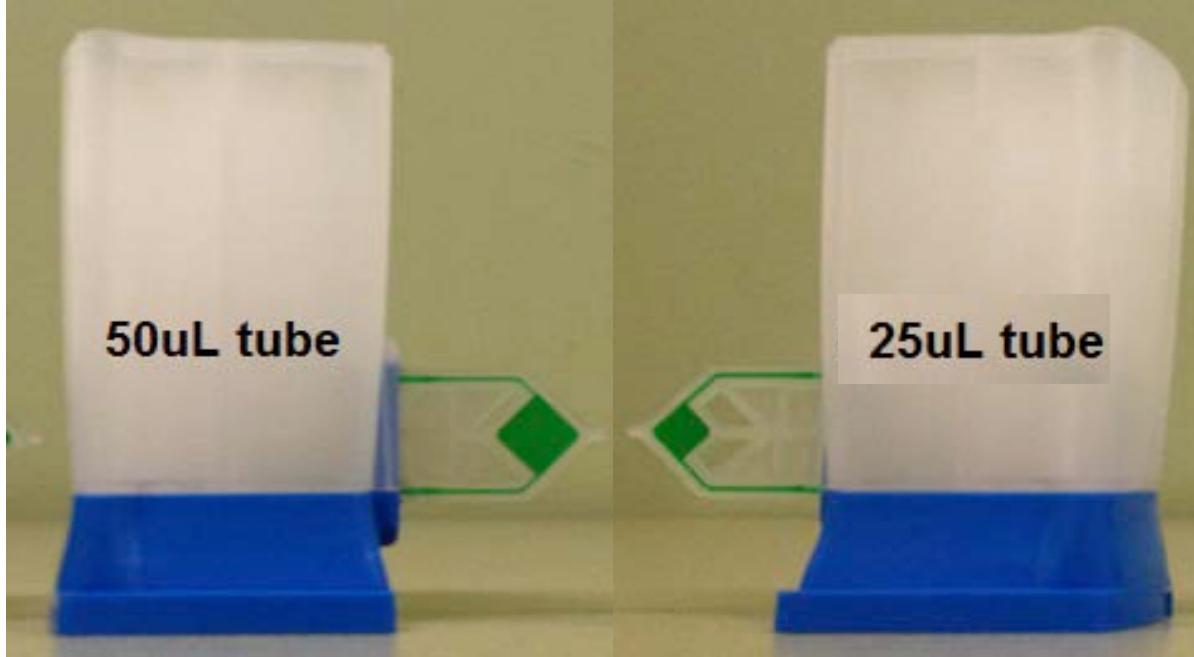
# GeneXpert® MTB/RIF Ultra



- Xpert MTB/RIF: Detects TB with a **single copy target (*rpoB* gene)**
- Ultra: Two additional **multi-copy targets (IS6110 & IS1081)** added



# *GeneXpert® MTB/RIF Ultra*



**Both 50uL and 25uL configurations will run on existing GeneXpert Modules**

# GeneXpert® MTB/RIF Ultra



*Design goal: Provide sensitivity equivalent to liquid culture*

- *Intended product attributes:*
  - Double detection of MTB-complex and rifampicin resistance
  - Use of big tube format (50mL) and improved microfluidics
  - Addition of multi-copy assay targets and melt curve analysis
  - Dual internal controls: Sample Processing and Probe Check
  - Fast time-to result: < 2 hours
  - Compatible with raw sputum and sputum sediment
- *Preliminary data shows improved sensitivity over current Xpert® MTB/RIF test<sup>1</sup>*

# GeneXpert® MTB/RIF Ultra

PLoS Med. 2017 Dec 14;14(12):e1002472. doi: 10.1371/journal.pmed.1002472. eCollection 2017 Dec.

## Estimated clinical impact of the Xpert MTB/RIF Ultra cartridge for diagnosis of pulmonary tuberculosis: A modeling study.

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### Author information

#### Abstract

**BACKGROUND:** The Xpert MTB/RIF (Xpert) assay offers rapid and accurate diagnosis of tuberculosis (TB) but still suffers from imperfect sensitivity. The newer Xpert MTB/RIF Ultra cartridge has shown improved sensitivity in recent field trials, but at the expense of reduced specificity. The clinical implications of switching from the existing Xpert cartridge to the Xpert Ultra cartridge in different populations remain uncertain.

**METHODS AND FINDINGS:** We developed a Markov microsimulation model of hypothetical cohorts of 100,000 individuals undergoing diagnostic sputum evaluation with Xpert for suspected pulmonary TB, in each of 3 emblematic settings: an HIV clinic in South Africa, a public TB center in India, and an adult primary care setting in China. In each setting, we used existing data to project likely diagnostic results, treatment decisions, and ultimate clinical outcomes, assuming use of the standard Xpert versus Xpert Ultra cartridge. Our primary outcomes were the projected number of additional unnecessary treatments generated, the projected number of TB deaths averted, and the projected number of unnecessary treatments generated per TB death averted, if standard Xpert were switched to Xpert Ultra. We also simulated alternative approaches to interpreting positive results of the Ultra cartridge's semi-quantitative trace call. Extensive sensitivity and uncertainty analyses were performed to evaluate the drivers and generalizability of projected results. In the Indian TB center setting, replacing the standard Xpert cartridge with the Xpert Ultra cartridge was projected to avert 0.5 TB deaths (95% uncertainty range [UR]: 0, 1.3) and generate 18 unnecessary treatments (95% UR: 10, 29) per 1,000 individuals evaluated—resulting in a median ratio of 38 incremental unnecessary treatments added by Ultra per incremental death averted by Ultra compared to outcomes using standard Xpert (95% UR: 12, indefinite upper bound). In the South African HIV care setting—where TB mortality rates are higher and Ultra's improved sensitivity has greater absolute benefit—this ratio improved to 7 unnecessary treatments per TB death averted (95% UR: 2, 43). By contrast, in the Chinese primary care setting, this ratio was much less favorable, at 372 unnecessary treatments per TB death averted (95% UR: 75, indefinite upper bound).

## Introducción

La tuberculosis sigue siendo un problema importante en términos de epidemiología, diagnóstico clínico-microbiológico y tratamiento. El desarrollo de nuevas técnicas diagnósticas rápidas y sensibles (especialmente en muestras extrapulmonares). En este trabajo, se analizó una nueva técnica de RT-PCR para la detección del Complejo *Mycobacterium tuberculosis* (MTBC) en muestras clínicas extrapulmonares comparando la misma con métodos convencionales.

## Objetivo

Analizar una nueva técnica de RT-PCR para la detección del Complejo *Mycobacterium tuberculosis* (MTBC) en muestras clínicas extrapulmonares comparando la misma con métodos convencionales. Dicha técnica pertenece a la plataforma de PCR a tiempo real (*Multiplex assays-short® RT-PCR*) para la detección de ADN/ARN de patógenos de interés clínico (protagonizada mediante la patente europea EP15382637).

## Material y métodos

Entre Julio y Septiembre de 2016, se evaluaron 75 muestras extrapulmonares con cultivos positivos al MTBC y 19 con cultivo negativo. Los tipos de muestra extrapulmonares están mostrados en la Tabla 1. La extracción de ADN de las muestras se realizó a través de la plataforma Abbott m24sp (Abbott, Chicago IL). Para la detección del ADN procedente del MTBC se utilizó el kit de ensayo multiplex *TB-short® RT-PCR*, el cual presenta dos dianas de detección (*whi* y *pstS11*). La PCR a tiempo real se realizó en Abbot m2000rt (Abbott, Chicago, IL). Los resultados fueron analizados mediante SPSS v. 18.0.

## Resultados

Los porcentajes de comparación de la técnica *TB-short® RT-PCR* con los cultivos se muestran en la Tabla 1. La sensibilidad, especificidad, Valor Predictivo positivo (VPP) y Valor Predictivo Negativo (VPN) de la técnica fueron del 88,0%, 94,7%, 98,5% y 69,2%, respectivamente.

	MUESTRAS	<i>TB-short® RT-PCR</i>				SENSIBILIDAD
		POSITIVO	NEGATIVO	INHIBICIÓN	TOTAL	
Cultivos POSITIVOS	Abcesos/Adenopatía/Pus	19	3	0	22	86.4%
	Biopsia/Tejido	11	2	0	13	84.6%
	Frotis herida	4	0	0	4	100%
	L. estériles (LCR, L. pleural, L. Ascítico)	12	2	0	14	85.7%
	Oriña	16	1	0	17	94.1%
	Heces	4	0	1	5	80.0%
	TOTAL	66	8	1	75	88.0%
		POSITIVO	NEGATIVO	INHIBICIÓN	TOTAL	ESPECIFICIDAD
Cultivos NEGATIVOS	Abcesos/Adenopatía/Pus	0	4	0	4	100%
	Biopsia/Tejido	0	10	0	10	100%
	L. estériles (LCR, L. pleural, L. Ascítico)	0	1	0	1	100%
	Oriña	1	3	0	3	100%
	Heces	1	0	0	1	0.0%
	TOTAL	0	18	0	19	94.7%

## Conclusión

La técnica de PCR a tiempo real en formato multiplex, *TB-short® RT-PCR*, mostró una gran eficacia en la detección del MTBC en muestras clínicas extrapulmonares.

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