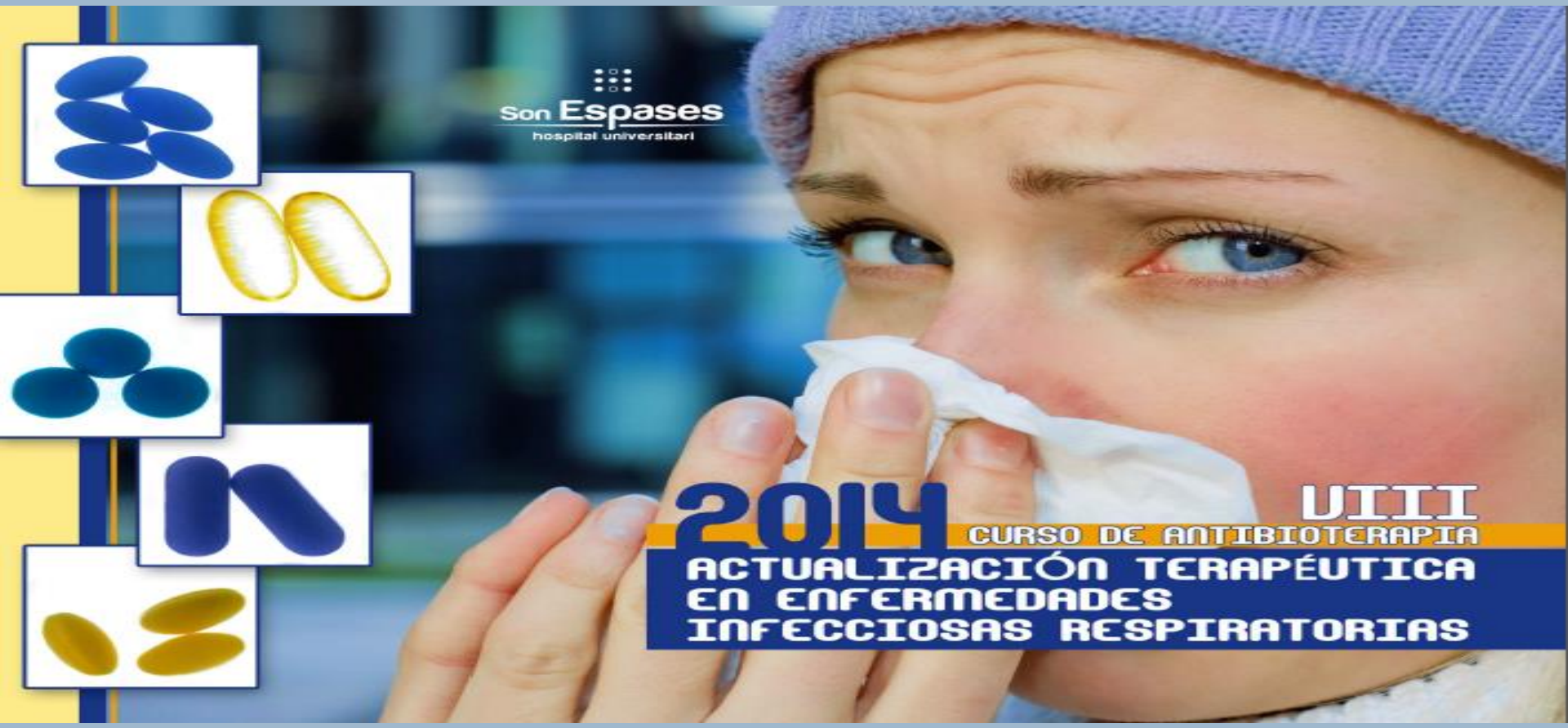



**ROSA RUBIO CASINO**

**Servicio de Microbiología HUSE**

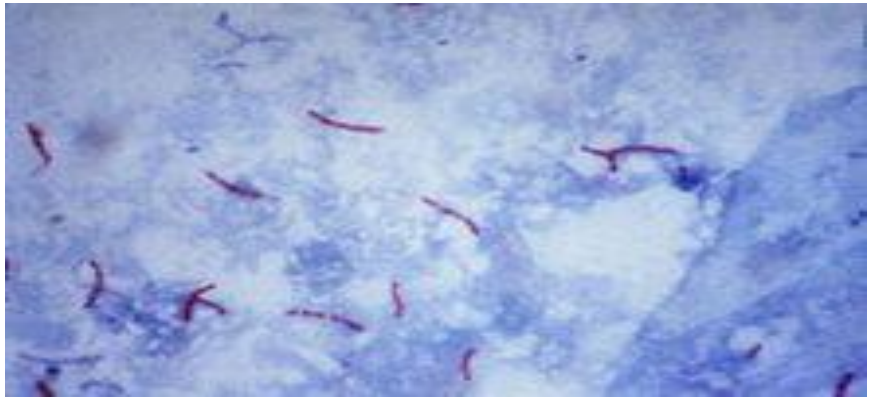
# WHO GLOBAL REPORT 2013

8,6 MILLONES DE TB ACTIVA

1,3 MILLONES DE MUERTES

35% infradiagnosticados

# Métodos directos- tinciones



ZIEHL-NEELSEN

**Ventajas:**

Simples
Bajo coste
Rápidas
Altamente específicos

AURAMINA

**Inconvenientes:**

Personal entrenado
Baja sensibilidad: 50-60%

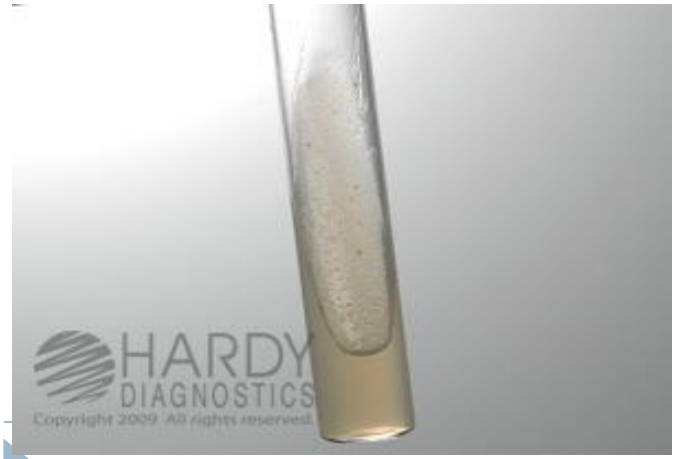
→ Método de cribado

# Métodos directos- cultivo (gold standard)

- Medios sólidos

MÉTODO LENTO:
8 SEMANAS

Lowenstein- Jensen
Huevo entero, patata, glicerol, sales biliares y verde malaquita
Siembra de muestras
2-3 meses a 37°C

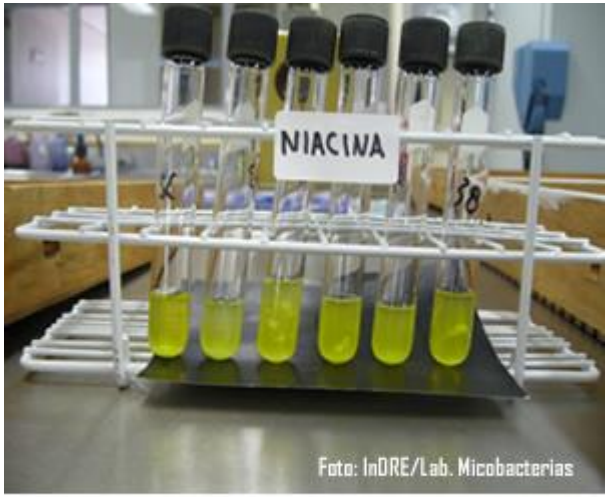



Middlebrook
Agar, albúmina, glicerol, caseína y otros ácidos grasos.
Aislamiento de cultivos mixtos
Menor caducidad.

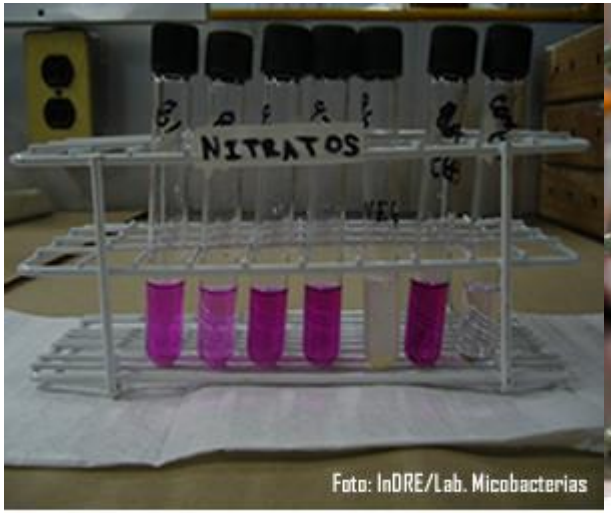
# Identificación bioquímica

1 mes más desde cultivo +

| | |
|---|---|
| Temperatura de crecimiento | 37 °C |
| Niacina | Positiva |
| Catalasa 68 °C | Negativa |
| Nitratos | Positiva |
| Tween 80 | Negativa |




Prueba de niacina. El color amarillo confirma la presencia de M. *tuberculosis* o M. *bovis*




Prueba de reducción de nitratos


Prueba de catalasa

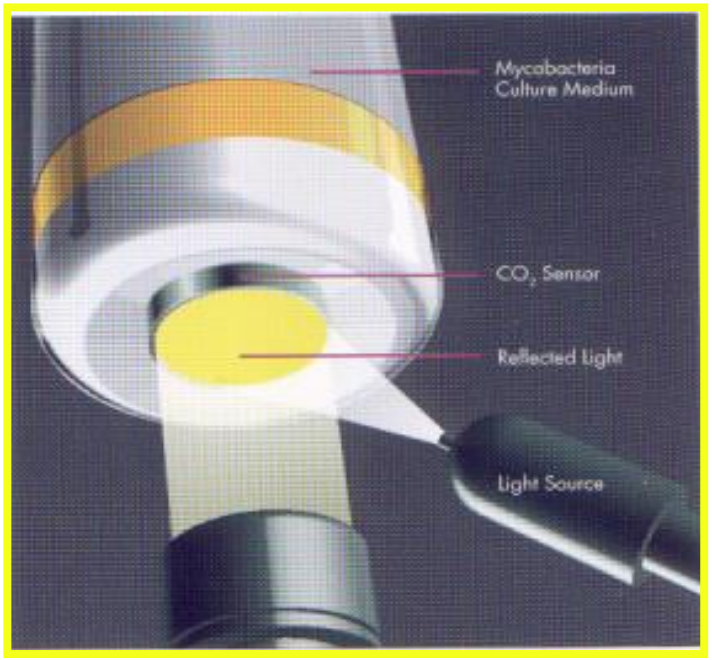
# Métodos directos- cultivo

## Medios líquidos

MÁS RÁPIDO: DIAS

CONTAMINACIÓN

- MB/ BactAlert 3d. 1995



# Métodos directos- cultivo

## Medios líquidos

- MGIT 960 BD


# Differences in time to detection and recovery of *Mycobacterium* spp. between the MGIT 960 and the BacT/ALERT MB Automated Culture Systems

Nicole Parrish*, Kim Dionne, Amy Sweeney, Annie Hedgepeth, Karen Carroll

*The Johns Hopkins Medical Institutions, Baltimore, MD 21287, USA*

Received 16 July 2008; accepted 20 November 2008

**Abstract**

*Mycobacterium* sp. recovery and time to detection were compared in the MGIT 960 and BacT/ALERT MB automated broth culture systems. The MGIT 960 demonstrated shorter time to detection (13.5 versus 25.2 days) and greater sensitivity (100% versus 66.6%) for recovery of the *Mycobacterium tuberculosis* complex than the BacT/ALERT MB system.



Table 3
Time to detection comparison between the MGIT 960 versus BacT/ALERT MB in bottles spiked with known concentrations of *M. tuberculosis* (H37Rv)

| MTB (CFU/mL) | Time to detection (days) | |
|---|---|---|
| | MGIT 960 | BacT/ALERT MB |
| $1 \times 10^4$ | 6.50 | 8.25 |
| $1 \times 10^3$ | 7.90 | 10.25 |
| $1 \times 10^2$ | 9.70 | 12.2 |
| $1 \times 10^1$ | 10.85 | 13.3 |

The colony-forming units per milliliter was verified using traditional plate counts on Middlebrook 7H11 agar.

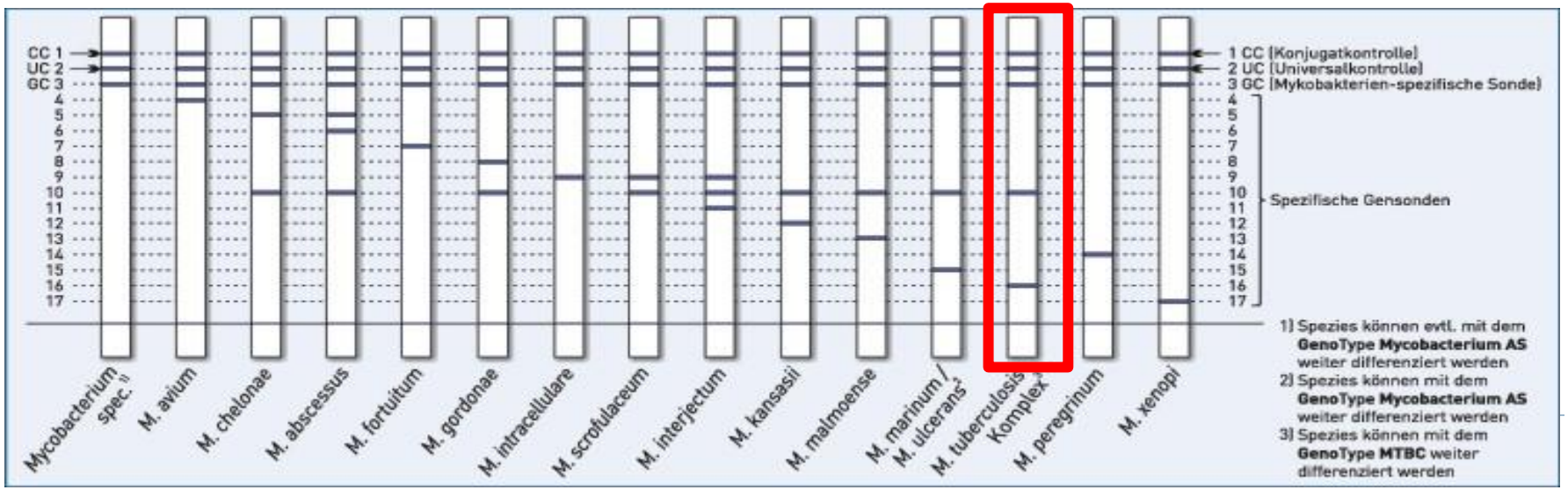
# Métodos directos: detección antigénica

## Hibridación con sondas ADN complementarias

## *AccuProbe® M. tuberculosis* complex

- Referente actual en la identificación rutinaria de *M. tuberculosis* complex
- A partir de cultivo +
- Técnica de hibridación genética
- Luminómetro.

2-4 horas



# Métodos directos: detección antigénica

## Inmunocromatografía: BD MGIT® TBc identification test

15 minutos


- Técnica de Inmunocromatografía
- Id. De *Mycobaterium tuberculosis* complex
- Proteína MPT64
- A partir de cultivo +
- Sin aparataje

# Métodos directos: PCR

## Xpert ® MTB/RIF

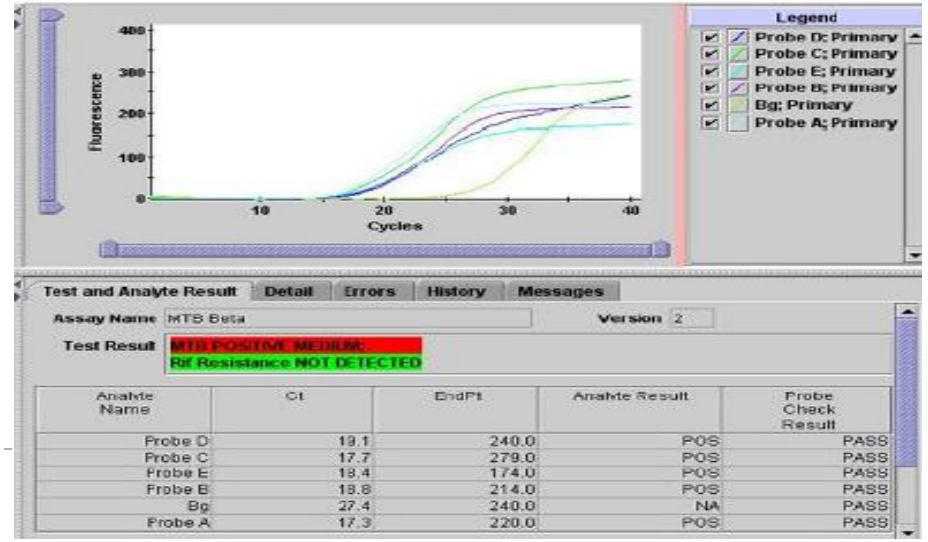
< 2 horas

Detección de *M. tuberculosis* complex y resistencia a Rifampicina

Muestra directa sin procesar

Límite de detección: 5 copias DNA o 131 UFC/ml en esputo. (comparación tinción 10,000 UFC/ml)

Resistencia a rifampicina: gen *rpoB*.



Cochrane Database Syst Rev. 2014 Jan 21;1:CD009593. doi: 10.1002/14651858.CD009593.pub3.

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

Steingart KR[1], Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N.

WHO Xpert. MTB/RIF Policy Update 2013).

Table 1. Xpert MTB/RIF assay for detection of TB and rifampicin resistance

| Type of analysis (Number of studies, participants) | Median pooledsensitivity (95% credible interval) | Median pooledspecificity (95% credible interval) | Median predicted sensitivity (95% credible interval) | Median predicted specificity (95% credible interval) |
|---|---|---|---|---|
| Xpert MTB/RIF used as an initial test for TB detection replacing microscopy (22, 8998) | 89% (85, 92) | 99% (98, 99) | 89% (63, 97) | 99% (90, 100) |

Sensibilidad baciloscopia: 65%; Sensibilidad GXPERT 89% (24% más)

Table 2. Impact of covariates on heterogeneity of Xpert MTB/RIF sensitivity and specificity, TB detection

| Covariate (Number of studies) | | Median pooled sensitivity (95% credible interval) | Median pooled specificity (95% credible interval) |
|---|---|---|---|
| **Smear status** | | | |
| Smear + (21) | | 98% (97, 99) | *** |
| Smear - (21) | | 67% (60, 74) | 99% (98, 99) |

| HIV status | | Median pooled sensitivity (95% credible interval) | Median pooled specificity (95% credible interval) |
|---|---|---|---|
| HIV- (7) | | 86% (76, 92) | 99% (98, 100) |
| HIV+ (7) | | 79% (70, 86) | 98% (96, 99) |
| Difference (HIV- minus HIV+) | | 7% (-5, 18) | 1% (-1, 3) |
| P (HIV- > HIV+) | | 0.90 | 0.85 |
| **Covariate (number of studies)** | **Within smear positive** | **Within smear negative** | |
| | **Median pooled sensitivity (95% credible interval)** | **Median pooled sensitivity (95% credible interval)** | **Median pooled specificity (95% credible interval)** |
| **HIV status** | | | |
| HIV- | *** | *** | *** |
| HIV+ (4) | 97% (90, 99)** | 61% (40, 81)** | 99% (97, 100)# |

Muestras procesadas > no procesadas
Muestras frescas > congeladas
Casos TB (> 30%) > casos TB (<30%)
No diferencias entre países con alta o baja prevalencia

**Table 1. Xpert MTB/RIF assay for detection of TB and rifampicin resistance**

| Type of analysis (Number of studies, participants) | Median pooledsensitivity (95% credible interval) | Median pooledspecificity (95% credible interval) | Median predicted sensitivity (95% credible interval) | Median predicted specificity (95% credible interval) |
|---|---|---|---|---|
| Xpert MTB/RIF used as an initial test for TB detection replacing microscopy (22, 8998) | 89% (85, 92) | 99% (98, 99) | 89% (63, 97) | 99% (90, 100) |
| Xpert MTB/RIF used as an add-on test for TB detection following a negative smear microscopy result (21, 6950) | 67% (60, 74) | 99% (98, 99) | 67% (42, 85) | 99% (89, 100) |
| Xpert MTB/RIF used as an initial test for rifampicin resistance detection replacing conventional DST as the initial test * | 95% (90, 97) | 98% (97, 99) | 95% (80, 99) | 98% (94, 100) |

* For rifampicin resistance detection, pooled sensitivity and specificity estimates were determined separately by univariate analyses. Pooled sensitivity, number of studies = 17 (555 participants); pooled specificity, number of studies = 24 (2411 participants).

- Limitaciones: electricidad, control de temperatura y calibración anual.

Global Laboratory Initiative 2010:

- Laboratorios nivel intermedio: microscopia, método molecular rápido, cultivo y DST.
- Laboratorios nivel periférico: microscopia → laboratorios de Alto nivel.

2 estrategias:

1. Sustituir las tinciones
2. Hacer en aquellos casos de tinción negativa

Problema económico

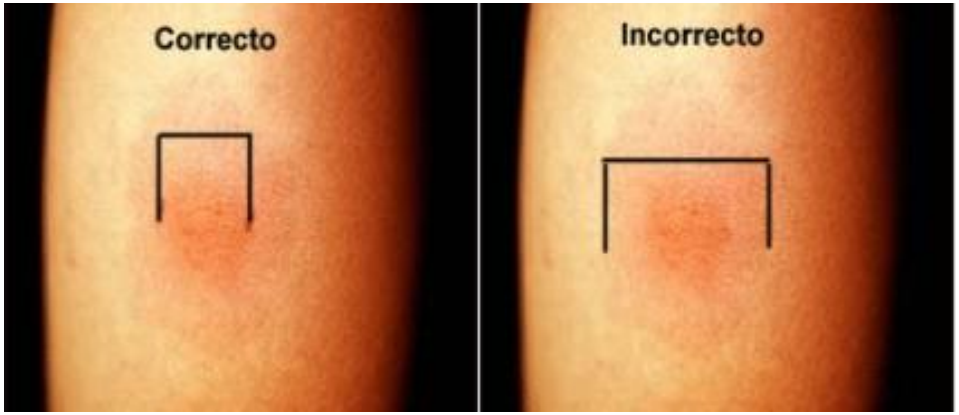
# Métodos indirectos

- Miden la inmunidad celular
- No distinguen entre LTBI y TB activa




# Métodos indirectos: tuberculina

- >10 mm
- Especificidad: 97% en no BCG, 60% en BCG




Falsos positivos:

- BCG vacunación
- NTM infecciones

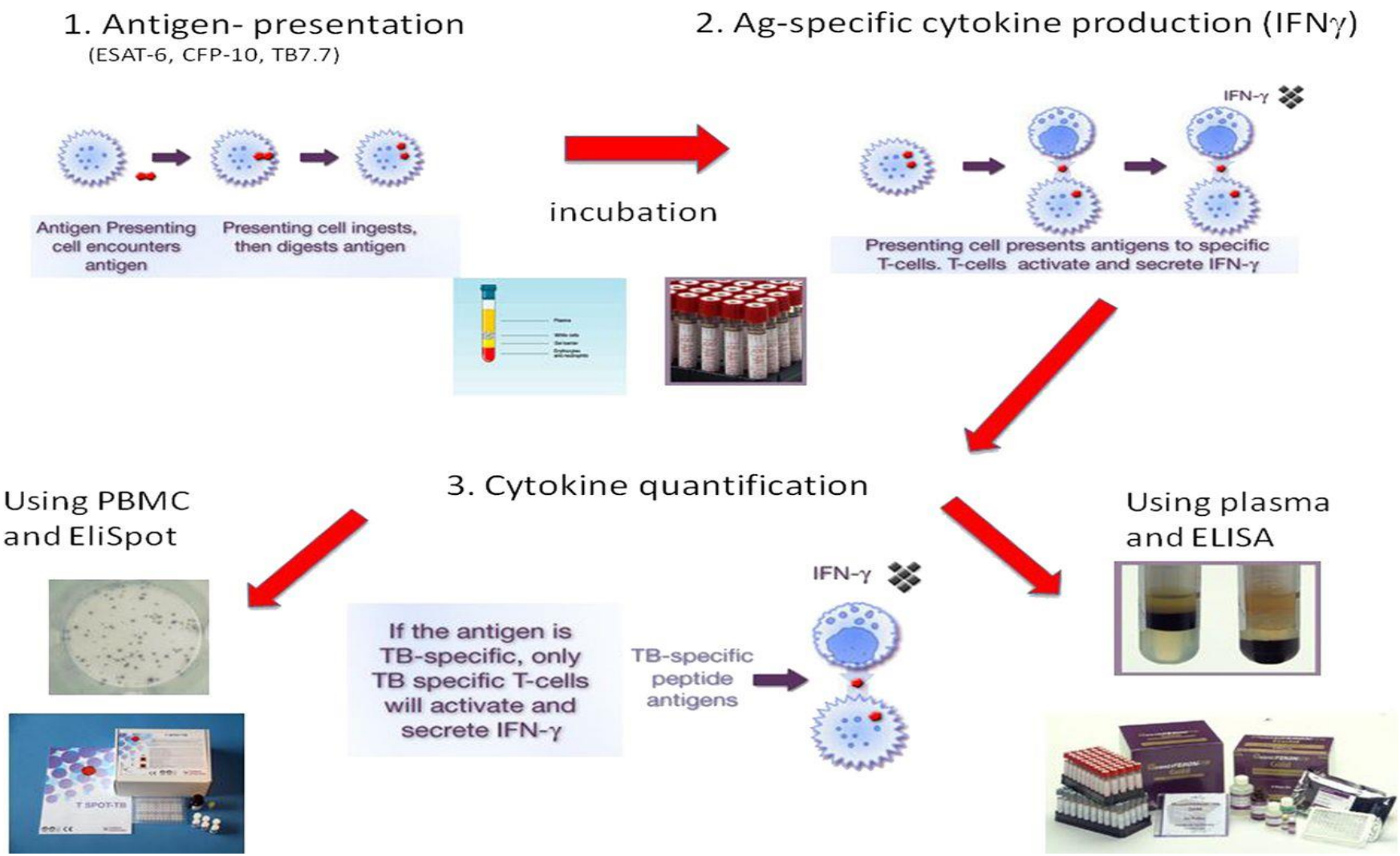
Falsos negativos:

- Inmunosupresión
- Variabilidad del test y su interpretación

Problemas:

- Reproducibilidad
- Variabilidad lectura
- Hipersensibilidad a TB o boosting
- Conversiones
- Reversiones

# Métodos indirectos: interferón γ (IGRA)

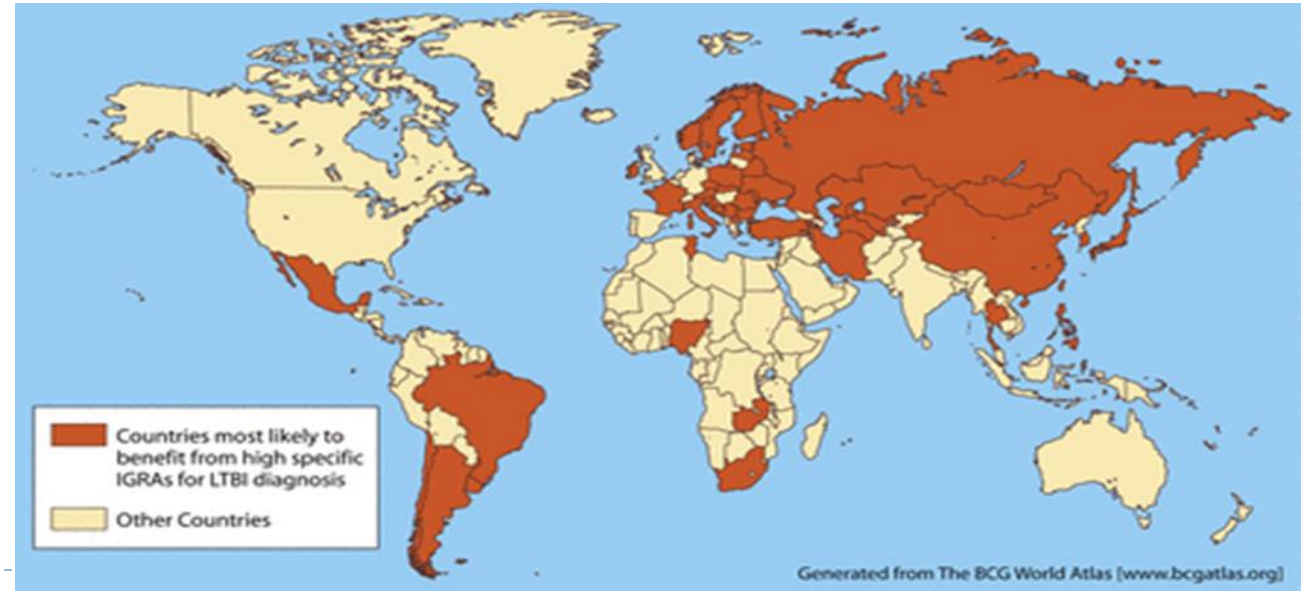



- **T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom**

**QuantiFERON-TB Gold In-Tube (QFT) assay (Cellestis/Qiagen,Carnegie,Aust.)**

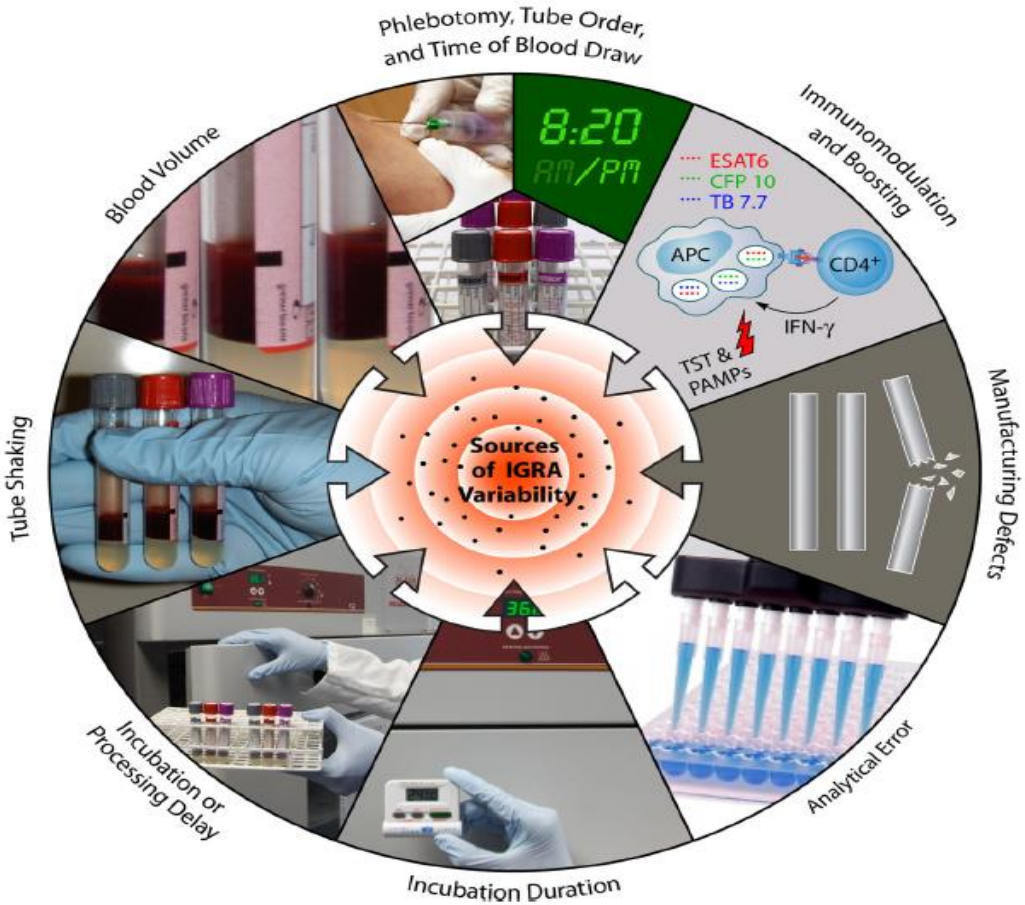
| Sensibilidad | | Especificidad | |
|---|---|---|---|
| Spot: 90% | QTF: 80% | Spot no vacunados 100% | QTF no vacunados 99% |
| | | Spot vacunados 93% | QTF vacunados 96% |

Especificidad no afectada por el estado de vacunación.




Clin. Microbiol. Rev. January 2014 vol. 27 no. 1 3-20

- Problema: la reproducibilidad ⟶ variabilidad 80%




doi: 10.1128/CMR.00034-13 .Clin. Microbiol. Rev. January 2014 vol. 27 no. 1 3-20

**TABLE 4** Comparison of TST and IGRA[a]

| Characteristic | Comments | |
|---|---|---|
| | TST | IGRA |
| Potential advantages or benefits | Simple, low-tech test | Requires fewer visits than TST for test completion (follow-up visits will be needed for both tests for IPT initiation) |
| | Can be done without a laboratory | Potential for boosting test response eliminated |
| | No equipment necessary | Results can be available within 24 to 48 h (but are likely to take longer if done in batches) |
| | Can be done by a trained health care worker even in remote locations | Does not have cross-reactivity with BCG |
| | Effect of BCG on TST results is minimal if vaccination is given at birth and not repeated | Has less cross-reactivity than TST with nontuberculous mycobacteria, though data are limited for low- and middle-income countries |
| | Longitudinal studies have demonstrated its predictive value, and systematic reviews of randomized trials show that isoniazid preventive therapy (IPT) is highly effective in those who are TST positive | |
| Risks or undesired effects | May give false-negative reactions due to infections, live virus vaccines, and other factors | Requires a blood draw (which may be challenging in some populations, including young children) |
| | May give false-positive results because of BCG vaccination and nontuberculous mycobacteria | Risk of exposure to blood-borne pathogens |
| | Requires an intradermal injection | Risk of adverse events with IGRA may be reduced compared to that with TST |
| | Can rarely cause adverse reactions (acute reactions, skin blistering, and ulceration) | Interpretation of serial IGRAs is complicated by frequent conversions and reversions and a lack of consensus on optimal thresholds |
| | Interpretation of serial TST is complicated by boosting, conversions, and reversions | Reproducibility is affected by several preanalytical and analytical factors as well as manufacturing defects |
| | Interpretation is affected by inter- and intrareader variation | |
| | Requires 48 to 72 h for a valid result | |
| Values and preferences | Patients may prefer to avoid visible skin reaction to TST | Patients may prefer to avoid blood draw (for cultural or technical reasons) |
| | Patients may prefer not to come back for repeat visit for reading the test result | Patients with prior BCG may not trust TST results and prefer IGRA |
| | Patients with prior BCG may not trust TST results and may be reluctant to accept IPT | |
| | Patients may self-read their TST results erroneously | |
| Resource implications | Less expensive than IGRAs (reagent cost is substantially less than IGRA kit costs), but personnel time costs will have to be factored, along with time and cost for 2 patient visits | Need to establish well-equipped laboratory, with electricity, that can perform ELISA or ELISPOT assay |
| | No laboratory required | Need to procure equipment and supplies for IGRA performance and quality assurance (IGRA reagents cost more than TST reagents) |
| | Need to establish a program with trained staff to administer and read TST results | Need for staff training, including blood-borne pathogen training |
| | Staff training is needed to minimize reading errors and variability (underreading, within- and between-reader variability, digit preference, etc.) | Need for cold chain for transport of kits and reagents and for their storage |
| | PPD must be stored at optimal temperatures | Need for careful handling (e.g., tube shaking) and processing of blood samples (incubation of samples within a specific time window) to ensure reproducibility of tests |
| | Only standardized PPD must be used | Availability of well-trained staff or staff to be trained |
| | | High likelihood of false-positive conversions during serial testing |

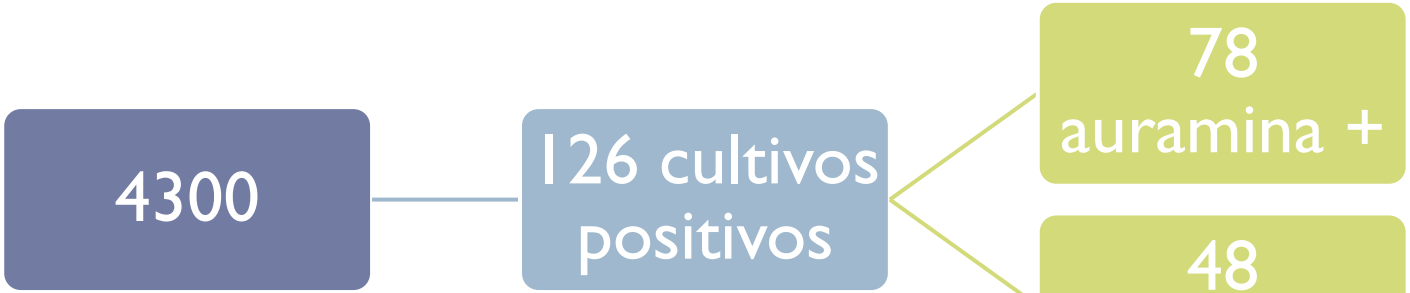
# Pacientes candidatos:

- Tratamiento con anti-TNF
- Trabajadores con TST dudosa
- Vacunados
- Inmunodeprimidos

# Nuestros datos. Servicio de microbiología HUSE

18 meses (junio 2012-diciembre 2013)

Muestras procesadas: 4300 de 2500 pacientes




SENSIBILIDAD BACILOSCOPIA:
78/126
62%

# Análisis GeneXPERT

83 muestras con baciloscopia, cultivo y PCR realizadas

27 casos positivos

| | BACILOSCOPIA | CULTIVO | GeneXPERT |
|---|---|---|---|
| Positivos TBC | 19 | 22 | 25 |
| Negativos TBC | 64 | 61 | 58 |
| SENSIBILIDAD | 66,6% | 81,4% | 92,6% |
| ESPECIFICIDAD | 98,2 % | 100% | 100% |
| VPP | 94,7% | 100% | 100% |
| VPN | 86% | 91,8% | 96,6% |

- 2 falsos negativos
- Ningún falso positivo
- No se detectó resistencia a rifampicina ni por antibiograma ni por PCR


GRACIAS POR SU ATENCIÓN.
1.00µm