# Diagnosis of tuberculosis: Update on interferon-gamma release assays in clinical practice

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9,15 million of people suffer from tuberculosis worldwide

BUT

**the RESERVOIR of infected people is by far more important**:

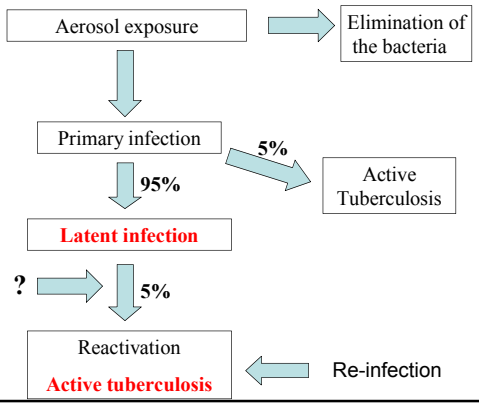
**1/3 of the world population infected:~** 2 milliards of individuals

**One new person is infected every second**

**8 – 10 millions of people are infected each year**

→ Development of evidence-based policies on TB diagnosis is urgently needed for effective control of the global TB epidemic

## Tuberculosis : natural evolution




## Importance of diagnostic

**Latent TB**

Identification and treatment of subjects at risk to develop active TB

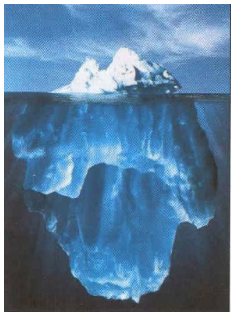
- RECENT infections (< 2 yrs)
- BEFORE starting immunosuppressive treatment
- Subjects with persistant bacterial multiplication ?

**Active TB**

Early treatment

- to diminish morbidity and mortality
- to diminish transmission

## *Mycobacterium tuberculosis* Infection



**Active TB**: 5-10%

**Latent TB**: 90-95 %

- **Active infection:** bacterial replication
  maintained at a subclinical level
- **Quiescent infection**: controlled infection with persistance of a few NON REPLICATING bacteria
- **Infection eliminated**
  with persistant sensitised T lymphocytes
  = acquired immunity

**Infection eliminated**
- without T lymphocyte sensitisation
  = innate immunity

*Adapted from Young D., Trends in Microbiology, 2009*

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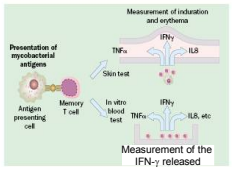
**The acquired immune response against *M. tuberculosis* is characterized by an important synthesis of IFN-γ in response to mycobacterial antigens**
→ *development of immunological tests for the diagnosis of M. tub. infection*

Initial LUNG infection → 8 – 9 days → The bacteria reach the LYMPH NODES:
Naive T cells → Effector T cells

18-20 days

*circulation*




*Pai M et al. Lancet Infect Dis 2004, 4: 761-76*

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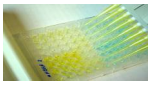
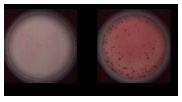
Detection of this specific IFN-γ synthesis can be done by different techniques

***ELISA***



**QuantiFERON**

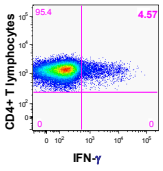
***ELISPOT***



**T-SPOT.TB**

***FLOW CYTOMETRY***

HBHA-stimulated

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**QuantiFERON-TB Gold assay**

- Evolution of the test:
  - 2001 (USA): QuantiFERON-TB assay : PPD from *Mycobacterium tuberculosis*
  - 2004 (USA): QFT-TB Gold:
    *early secretory antigenic target-6 (ESAT-6)*
    *culture filtrate protein (CFP-10)*
    antigens NOT encoded by the genome of BCG
    by the genome of most nontuberculous mycobacteria
    *exceptions: M. kansansii, M. marinum, M. flavescens, M. szulgai*
  - 2005 (Europe): QFT-TB G In-Tube (QFT-G IT):
    *early secretory antigenic target-6 (ESAT-6)*
    *culture filtrate protein (CFP-10)*
    *TB7.7 peptide*

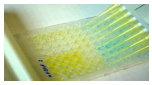
## QuantiFERON-TB Gold assay

*Methodology*

Blood: 1 mL 1 mL 1 mL

37°C 16-24 hrs

Plasma collection



Mitogen: + control

Nil: - control

TB antigens

POS: IFN-γ (TB-nil) ≥ 0.35 IU/ml

INDETERMINATE:
mitogen < 0.5 U/ml
background response > 8.0 U/ml

## QuantiFERON-TB Gold assay

*Advantages / TST*

- Very high specificity
- does not boost responses
- does not require a 2nd clinical contact
- results theoretically available within 24 hrs

*BUT….*

- sensitivity ? *Difficult to evaluate…*
- reproducibility over time ?
- risk to develop active TB in patients with a positive results ?
- requires lab equipment (incubation within 16 hrs – Elisa)
- requires blood sampling (3 mL)
- Expensive (25 E)

## QuantiFERON-TB Gold assay

SENSITIVITY

- evaluation hampered by the lack of a gold standard for the diagnosis of LTBI
- test results are compared to those of the TST in high- and low-risk populations
  - Active TB / Contacts of active TB
  - Low TB incidence countries / No contact
- QFT-G does not discriminate between active and latent TB
- DIAGNOSIS OF ACTIVE TB: QFT-G IT is NOT recommended:
  false negative results (low cellular immune responses)
  and
  false positive results (latent infection)

## QuantiFERON-TB Gold assay

- DIAGNOSIS of LATENT TB:
  - The main advantage of QTF over TST is its ability to overcome false positive skin tests:
    - in BCG-vaccinated individuals
    - in patients who may be infected with non-TB mycobacteria

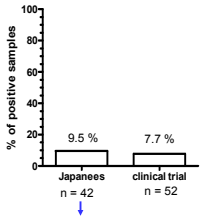
    ⇨ Recommanded use of IGRAs only to confirm a positive TST result in contacts with a low probability of acquired TB (Canada, UK, Germany)
  - Diagnostic value in immunocompromised patients incompletely assessed: numerous indeterminate results
  - Different studies suggest a lower sensitivity of QFT-G IT / TST for past infection:

    ⇨ probably not suitable for the detection of latent TB before starting an immunosuppressive treatment

## QuantiFERON-TB Gold assay

SPECIFICITY

**Healthy « non-infected » subjects**



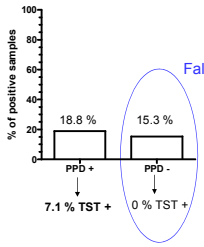

- TST 13 mm – BCG – TB contact
- TST 15 mm – BCG – TB contact

**False + (4.8 %)**

- TST doubtfull – BCG – NO TB contact
- TST neg - - NO TB contact

**Old patients (≥ 70 yrs)**

n = 85
**TST 7.1 % +**
**QFT +** n=29 ; 34.1 %




False + ?

*A. Jamai et al.*

## ALTERNATIVE TESTS

**T-SPOT *TB***

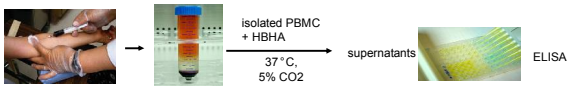
- detects the NUMBER of IFN-γ-secreting cells
- after stimulation with TB antigens: ESAT-6 and CFP-10
- somewhat lower specificity / QFT (92 vs 97%)
- slightly better sensitivity / QFT (88 vs 76 %)
  and fewer indeterminate results in the immunocompromised host

## ALTERNATIVE TESTS

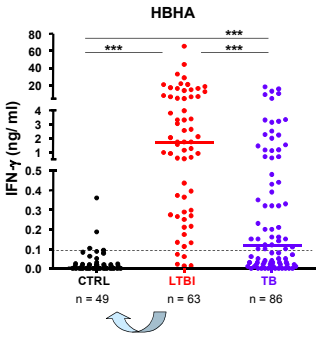
HBHA IFN-γ-release assay

- the Heparin-Binding HemAgglutinin
  Methylated protein expressed at the surface of the members
  from the *Mycobacterium tuberculosis* complex

- Interferon-gamma release assay (IGRA) for the diagnosis of latent TB




## IGRA in response to HBHA




LTBI defined by
-TST conversion (n=52)
-Clin hist and TST +(n=11)

**HBHA = latency antigen**

**Sensitivity: 92.06 %**
**Specificity: 93.88 %**

*Hougardy JM et al. PLoS ONE, 2007*

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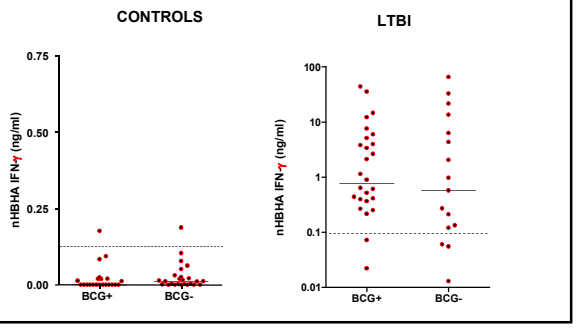
## DETECTION OF LATENT TB

### nHBHA-IFN-γ release assay

### NO INFLUENCE OF A PREVIOUS BCG VACCINATION ( > 15 years)

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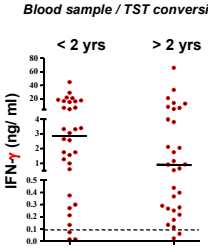
## DETECTION OF LATENT TB

### nHBHA-IFN-γ release assay

### DETECTION OF BOTH RECENT AND REMOTE INFECTION

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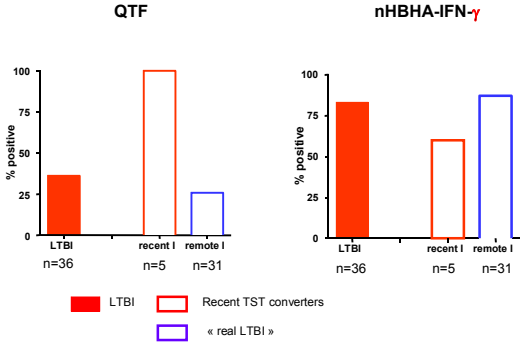
**The Quantiferon-TB Gold**
(peptides of ESAT-6, CFP-10, TB 7.7)

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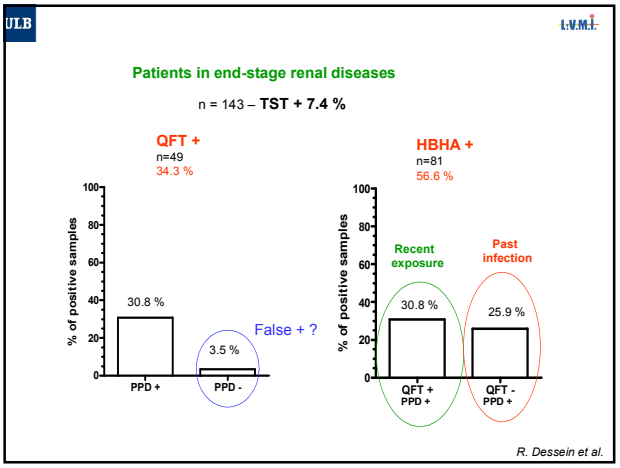
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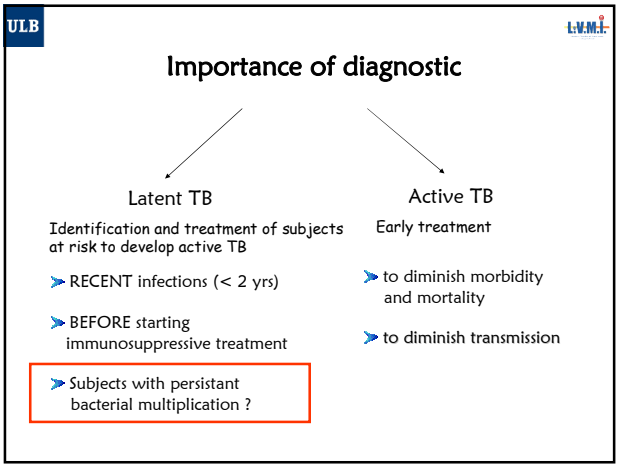
Patients in end-stage renal diseases
n = 143 – TST + 7.4 %
QFT +
n=49
34.3 %
HBHA +
n=81
56.6 %
% of positive samples
30.8 %
3.5 %
False + ?
PPD +
PPD -
Recent exposure
Past infection
30.8 %
25.9 %
QFT + PPD +
QFT - PPD +
R. Dessein et al.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Importance of diagnostic
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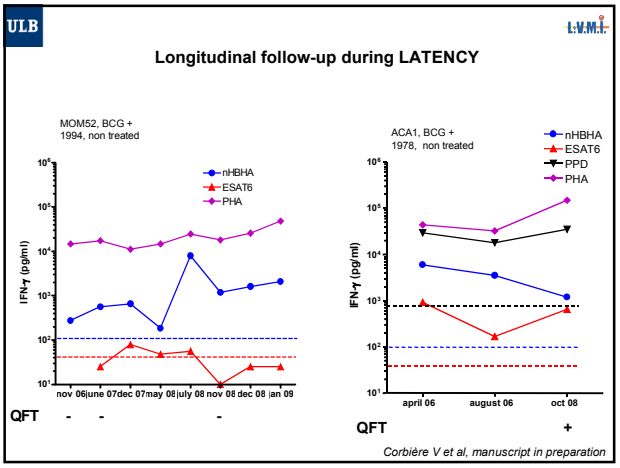
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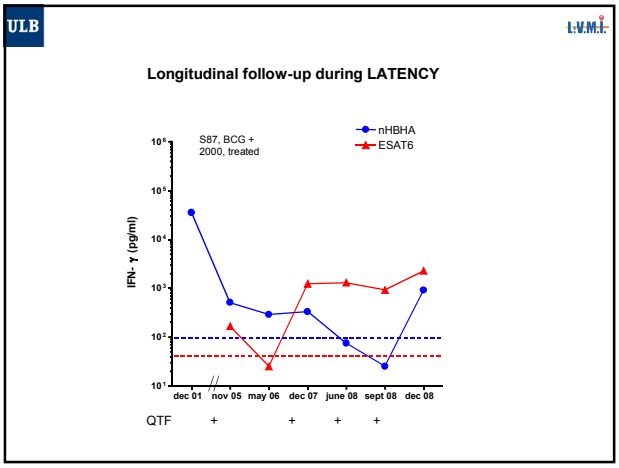
Longitudinal follow-up during LATENCY
MOM52, BCG +
1994, non treated
nHBHA
ESAT6
PHA
IFN-γ (pg/ml)
nov 06 june 07 dec 07 may 08 july 08 nov 08 dec 08 jan 09
QFT - - - -
ACA1, BCG +
1978, non treated
nHBHA
ESAT6
PPD
PHA
IFN-γ (pg/ml)
april 06 august 06 oct 08
QFT +
Corbière V et al, manuscript in preparation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Longitudinal follow-up during LATENCY
S87, BCG +
2000, treated
nHBHA
ESAT6
IFN- γ (pg/ml)
dec 01 nov 05 may 06 dec 07 june 08 sept 08 dec 08
QTF + + + +

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## HBHA-IGRA for the diagnosis of ACTIVE TB:

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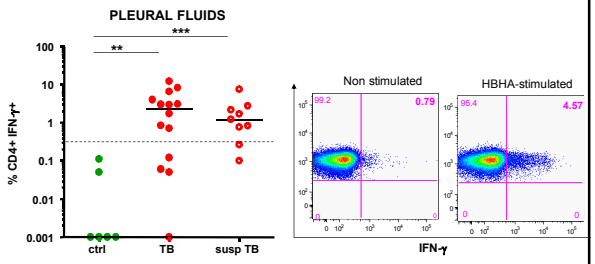
LOCAL IGRA




*Place S et al. Manuscript in preparation*

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## HBHA-IGRA for the diagnosis of ACTIVE TB:

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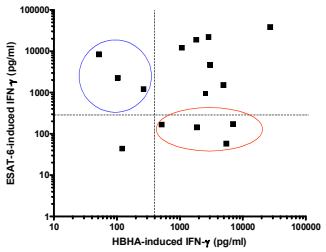
LOCAL IGRA on PLEURAL fluids

*Combining the IFN-γ response to 2 different mycobacterial antigens gives a higher sensitivity*

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## High local production of IFN-g in response to HBHA

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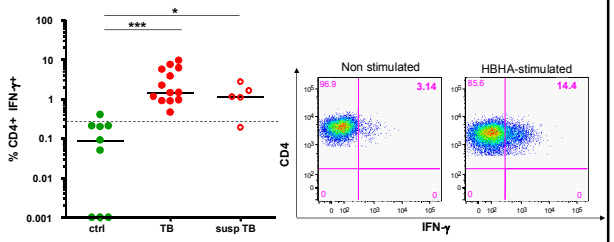
BRONCHO-ALVEOLAR LAVAGES

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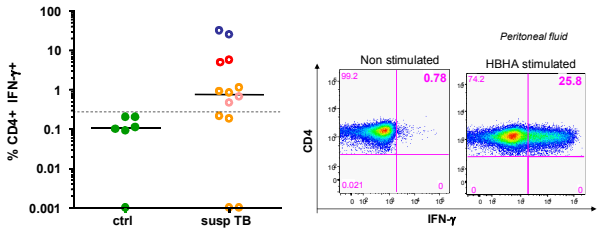
PERICARDIC – PERITONEAL – SYNOVIAL – CEREBROSPINAL FLUIDS



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**Diagnosis of tuberculosis:**
**Update on interferon-gamma release assays in clinical practice**

**CONCLUSIONS**

- IGRAs represent a major progress for the diagnosis of *M. tuberculosis* infection
- However, several progresses are still needed:
  - more data about the specificity and sensitivity
  - differential diagnosis between active and latent TB
  - marker of the risk to develop active TB
  - lower prize
- Today, IGRAs represent an usefull adjunct to the TST.
- Their result should always be interpreted within the clinical context


## THANKS !

L.V.M.I.

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