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PEARLS OF LABORATORY MEDICINE

Interferon Gamma Release Assays (IGRAs)

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CCTC Clinical Chemistry Trainee Council

Tuberculosis (TB)

- Caused by *Mycobacterium tuberculosis*
- Leading cause of infectious disease mortality globally
- Two forms:
  - Latent TB infection (LTBI)
  - Active TB disease
- Detection and treatment of LTBI is critical for TB control

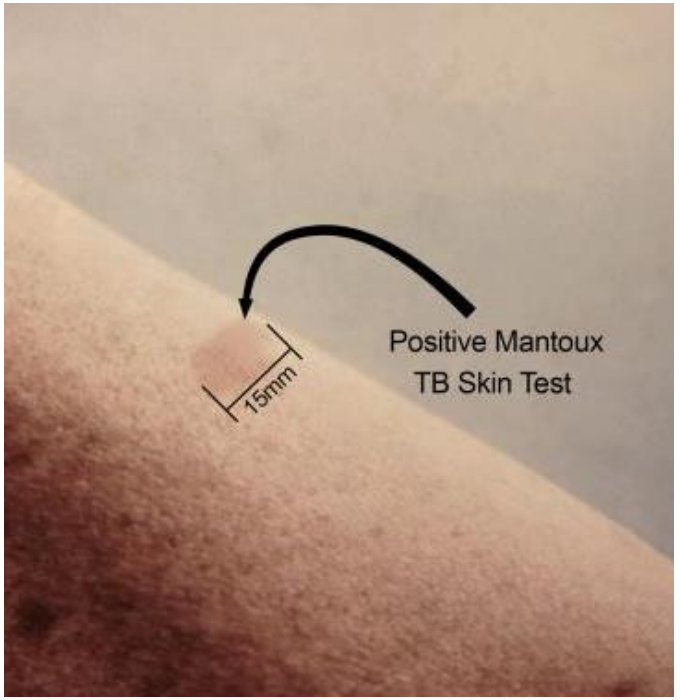
Computer-rendered image of *Mycobacterium tuberculosis*
Image: James Archer, CDC Public Health Image Library (PHIL)

Tests for TB Infection

- Two types of tests available to detect TB infection
  - Tuberculin skin test (TST)
  - Interferon gamma release assays (IGRAs)
- Both are indirect tests for *M. tuberculosis* that measure cellular immune response to mycobacterial protein antigens
- If testing positive, active TB must be ruled out (no gold standard diagnostic test for LTBI)

Tuberculin Skin Test (TST)

- Before 2001, the only commercially available immunologic test for TB infection in the United States (US)
- In the US, performed by the Mantoux method
- Limitations:
  - False positive results may occur due to BCG vaccination or nontuberculous mycobacterium (NTM) infection
  - Patients must return to a healthcare provider for test reading
  - Inter- and intrareader variability



Photograph of positive tuberculin skin test.
Image credit: David Kopanoff, CDC Public Health Image Library (PHIL)

Interferon Gamma Release Assays (IGRAs)

- Developed to overcome TST limitations
- *In vitro* blood tests
- Recommended by CDC as a *diagnostic aid* for TB infection
- First FDA-approved in 2001
- Two available types in the US:
  - QuantiFERON Gold Plus (Qiagen)
  - T-SPOT.*TB*® (Oxford Immunotec Ltd)

Basis of IGRAs

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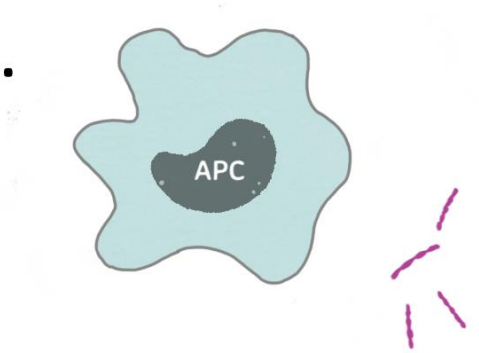

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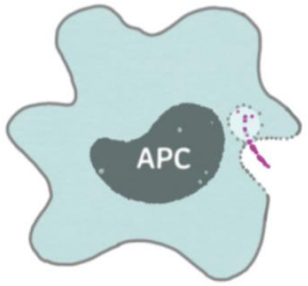

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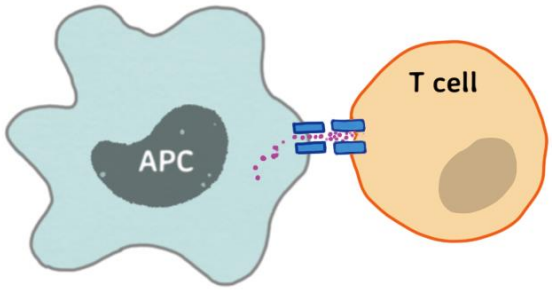

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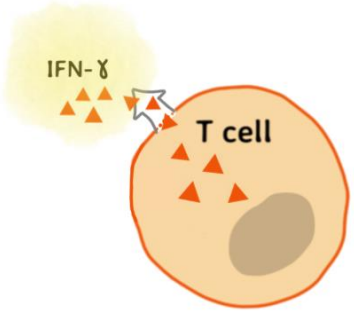
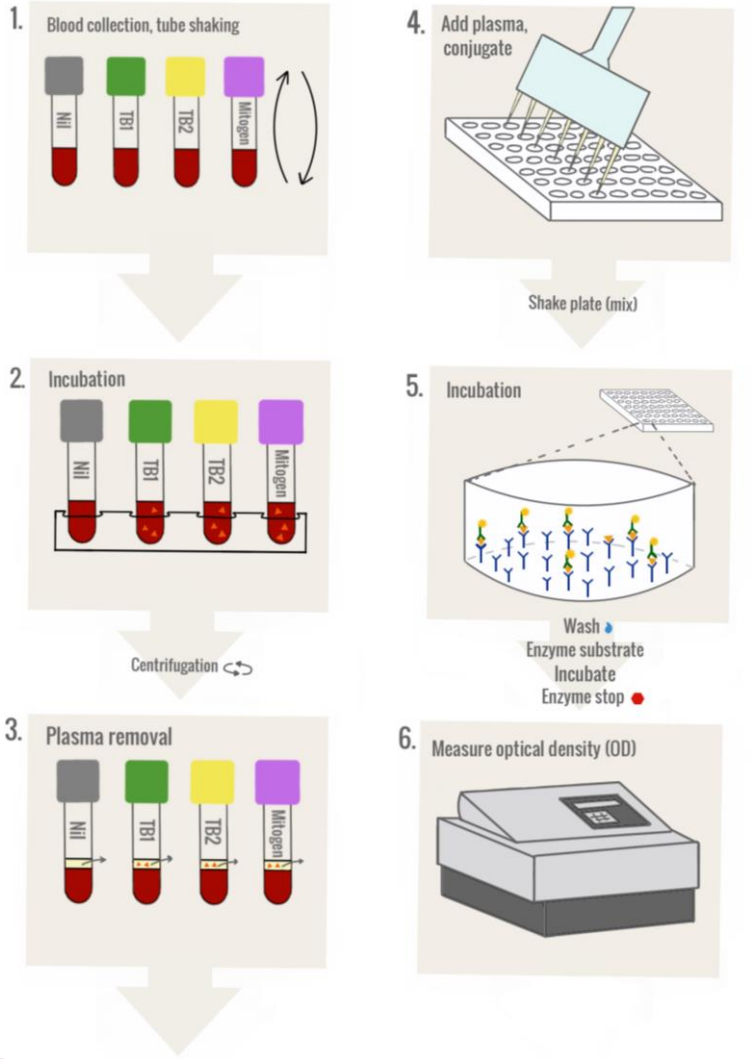


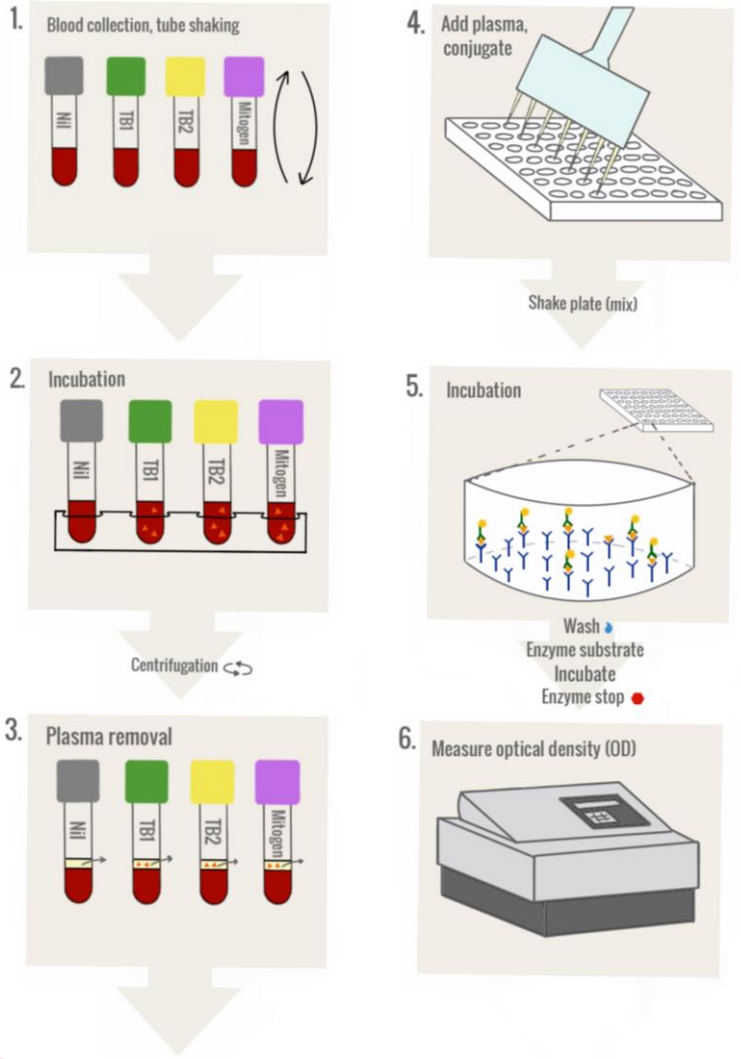
IGRAs: Basic Procedure

1. Blood collection from patient
2. Exposure of T cells in blood to *M. tuberculosis*-derived antigens (ESAT-6, CFP10)
3. Measurement of IFN-gamma
4. Patient sample results compared with positive and negative control values

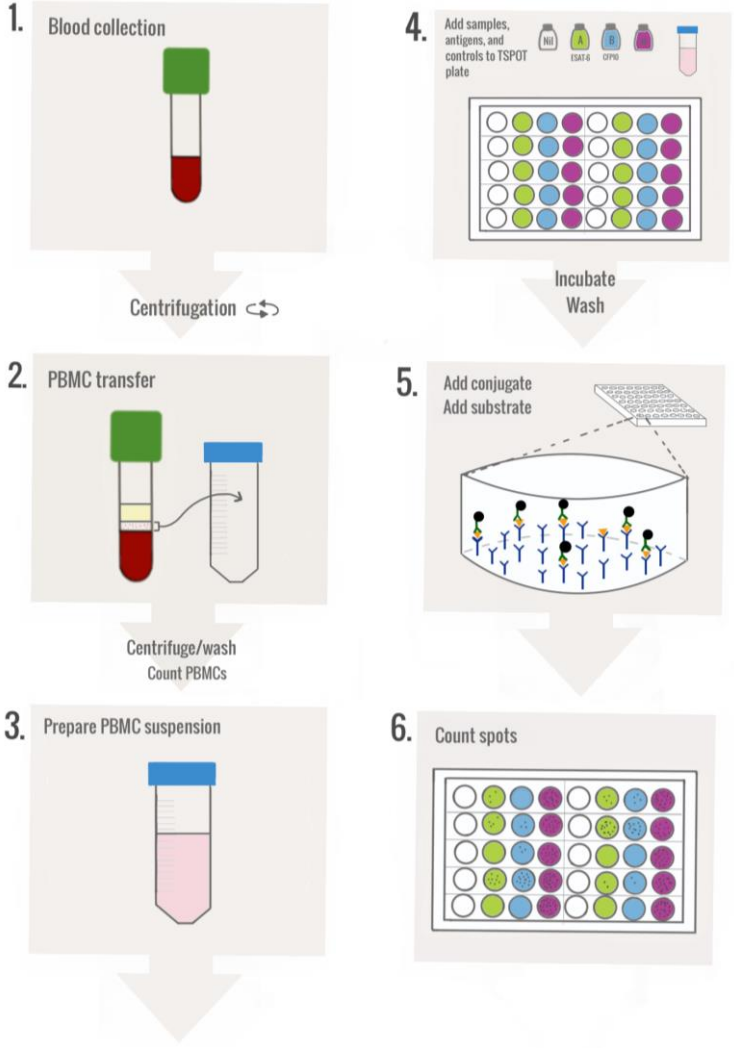
QuantiFERON Gold Plus



QuantiFERON Gold Plus



T-SPOT.*TB*®



IGRA Result Possibilities

QuantiFERON Gold Plus

- Positive
- Negative
- Indeterminate

T-SPOT.*TB*®

- Positive
- Negative
- Borderline

IGRA Performance Characteristics

Sensitivity 80-90%+

- most studies conducted among patients with *culture-confirmed* active TB (biased)
- lower among patients with immunosuppression (HIV, immunosuppressive therapy), children < 2 years old

Specificity *generally* 95%+ for LTBI in settings with low TB incidence

- studies conducted among low-risk individuals with no known risk factors

IGRA Strengths & Limitations

Strengths

- No cross-reactivity with BCG or *most* non-tuberculous mycobacteria
- Results in 24-48 hours
- Only one patient visit needed for result
- No boosting effect

Limitations

- Pre-analytic sources of variability
- Lower sensitivity in immunosuppressed, young children (↑ false negatives)
- Can't distinguish LTBI from active TB
- Cross-reactivity with *M. marinum, M. szulgai, M. flavescens,* and *M. kansasii*

CDC 2010 IGRA Testing Guidelines

https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm

IGRAs (and TSTs) should be used as *aids* in diagnosing *M. tuberculosis* infection; can be used for surveillance or to identify persons likely to benefit from treatment:

- people at increased risk for TB: contacts of active TB patients, people from areas w/ high incidence of TB, etc.
- people at increased risk for progression if infected: immunosuppressed patients, children < 5 yrs, etc.

IGRAs (and TSTs) should NOT be used for

- testing persons with low risk for infection and progression to active TB if infected.
- monitoring anti-TB treatment response

CDC 2010 IGRA Testing Guidelines (cont.)

https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm

IGRA preferred	TST preferred
• Groups with low rates of return for TST read (e.g. homeless persons, drug-users) • Persons who have received BCG	• Children aged < 5 years old
Either test acceptable	**Consider both tests**
• Recent contacts of active TB patients • Periodic screening of persons at risk for occupational exposure (e.g. HCWs)	• Initial test negative, but high suspicion for active TB, or risk for infection/progression increased • Initial test positive, but risk for infection/progression low

In Summary...

- Interferon gamma assays (IGRAs) are a diagnostic aid for TB infection
- Detect IFN-gamma release from T cells in response to TB-derived antigens
- Sensitivity and specificity generally high but can be lower among some patient groups (interpret results in context)
- Keep in mind strengths and limitations
- CDC guidelines for IGRA use: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm

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Disclosures/Potential Conflicts of Interest

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- **Patents:** No disclosures

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