Interpretation of new ELISA Tests in the Immunology Laboratory

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Tuberculosis (TB)

Infectious communicable disease

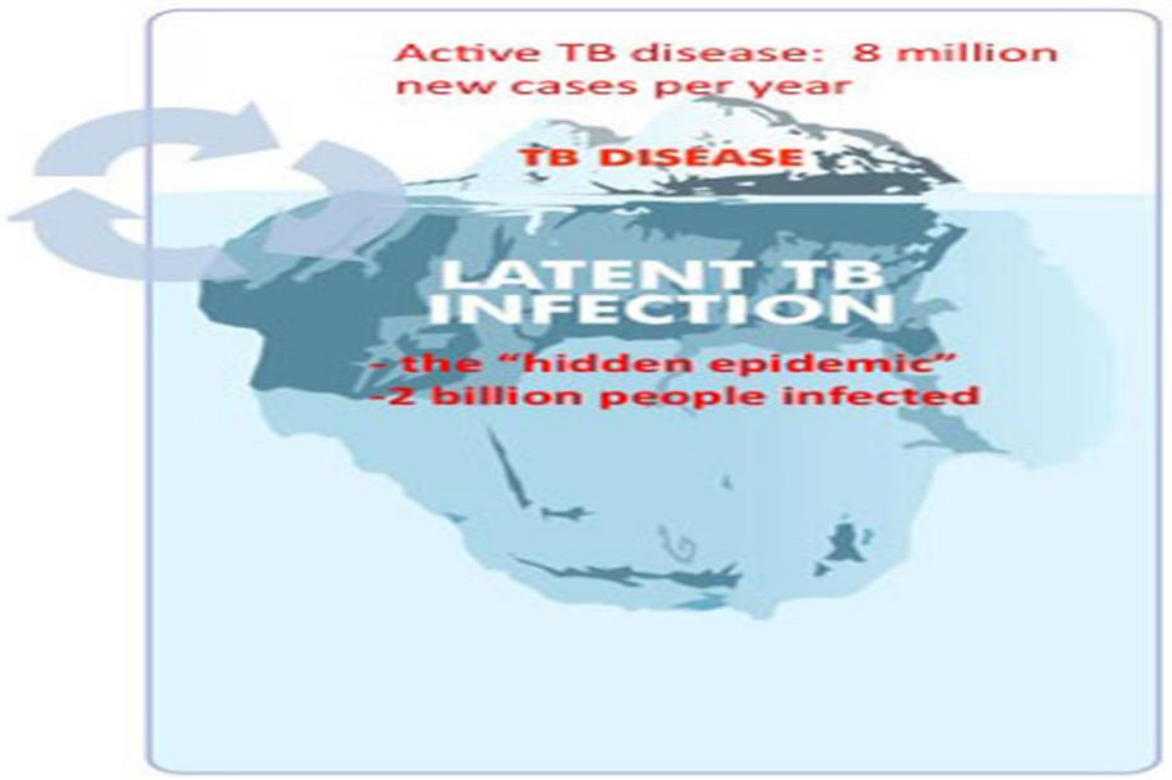
Caused by Mycobacterium tuberculosis complex organisms

- M. tuberculosis
- M. bovis
- M. africanum
- M. canetti
- M. microti

Affects different parts of the body:

- Lungs – Pulmonary TB (>70% of all TB cases)
- Other organs – extrapulmonary TB

Latent TB-The Hidden Epidemic



Testing for Latent TB Infection (LTBI)

The Tuberculin Skin Test (TST) or Mantoux test was first used over 100 years ago as a means of diagnosing both active and latent TB infections. It involves the injection of a purified protein derivative (PPD) under the skin of forearm. If positive a delayed type hypersensitivity, or wheal and flare reaction will occur under the skin

Interferon gamma release assays (IGRAs) are in vitro immunologic diagnostic tests used to identify MTB infection. The IGRA assays work by exposing PBMCs to MTB antigens. These antigens are processed and presented to the T cells present in the sample. If the patient has been previously exposed to TB infection, memory T helper 1 cells (Th1) will become activated and produce IFN-γ; which can be subsequently detected by ELISA.

QFT-Plus Product Format

Overview of new product format — a fully QIAGEN product

ELISA

ELISA remains largely the same

- Same procedure
- New labeling
- New instructions for use
- New plate layout (4 wells per patient)
- New QFT-Plus analysis software

Easy transition for the lab

Tubes

Same phlebotomy practice and handling process

- Custom QFT-Plus tubes
  - Nil – same grey
  - TB1 – green, CD4 only
  - TB2 – yellow: ***CD4 and new CD8!***
  - Mitogen – same purple
- Option for standard lithium heparin tube

Flexible sample collection, still allows remote incubation

Interpretation

QuantiFERON-TB Gold Plus (QFT-Plus)

Interpretation of QFT-Plus results (1)

Nil (IU/ml)	TB1 minus Nil or TB2 minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-Plus Result	Report/Interpretation
≤8.0	≥0.35 and ≥ 25% of Nil	Any	Positive†	*M. tuberculosis* infection likely
≤8.0	<0.35	≥0.5	Negative	*M. tuberculosis* infection NOT likely
≤8.0	≥0.35 and <25% of Nil	≥ 0.5	Negative	*M. tuberculosis* infection NOT likely
≤8.0	< 0.35	<0.5	Indeterminate‡	Results are indeterminate for TB-Antigen responsiveness
≤8.0	≥0.35 and <25% of Nil	<0.5	Indeterminate‡	Results are indeterminate for TB-Antigen responsiveness
>8.0§	Any	Any	Indeterminate‡	Results are indeterminate for TB-Antigen responsiveness

* Responses to the Mitogen positive control (and occasionally TB Antigens) can be commonly outside the range of the microplate reader. This has no impact on test results.

† Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QFT-Plus ELISA. If repeat testing of one or both replicates is positive, the individual should be considered test positive.

‡ Refer to the "Troubleshooting" section for possible causes.

§ In clinical studies, less than 0.25% of subjects had IFN-γ levels of >8.0 IU/ml for the Nil value.

- Cutoffs have not changed from QFT
- Positive results by TB1, TB2, or both are considered positive

1. QuantiFERON-TB Gold Plus Package Insert. October 2014. 1083163EN.

Alzheimer's Biomarkers (ALZH)

Alzheimer's disease (AD) is characterized by degeneration of neurons and their synapses, together with the formation of plaques and neurofibrillary tangles in the hippocampus and other cortical areas of the brain.

AD is a slow progressive disorder, the degenerative process is thought to precede the clinical symptoms by 20 to 30 years.

Diagnosis of AD is a combination clinical assessment, MRI and PET scans as well as CSF biomarkers.

The 3 CSF Biomarkers tested in FUJIREBIO Kit

Total Tau protein (T-Tau)- Neuronal degeneration

Phosphorylated Tau (pTau)- Hyperphosphorylation of Tau leads to the tangle formations

β-amyloid protein (Aβ42)- Deposited in senile plaques

T-Tau

A protein found in neuronal axons of the brain.

Increased levels have been shown in states of rapid neuronal degeneration.

Up to a 300% increase in values can be seen in AD.

Sensitivity is 85-90%.

P-Tau

In AD Tau is hyper-phosphorylated, leading to a tendancy for tau to clump and form tangles.

In AD patients P-Tau is increased moderately to markedly.

Sensitivity is around 80%

β-amyloid

Is the major component of plaques in brain of AD patients, levels correlated in the CSF.

Aβ42 is the peptide measured in the kit. It is the free unbound isoform.

Markedly reduced in AD patients. (50% of normal)

Sensitivity is 85-90%.

Summary

Several studies have shown that CSF biomarkers are positive indicators very early in the clinical course of AD, e.g mild cognitive impairment (MCI)

Studies have shown that using the three biomarkers in combination lead to 95% of MCI cases being detected.

Some studies have shown that pre-clinical cases of AD shows reduction of β-amyloid.

sCD163 (M130) Assay Development

Despite advances in the understanding and management of small vessel vasculitis, a sensitive and specific biomarker which can track disease activity, inform treatment decisions and predict outcome is lacking.

CD163, a scavenger receptor protein expressed exclusively on myeloid antigen presenting cells, is upregulated in inflammatory states. Its soluble form in urine - sCD163 - is being investigated as a biomarker for active ANCA associated vasculitis.

sCD163 (Urine Vs Serum)

In human renal tissue, the degree of glomerular CD163 expression was tightly correlated with the level observed in urine but not serum.

ROC curve analysis using a 95% CI for differentiation of active renal vasculitis from remission defined an optimum cut-off of 0.33ng/mL/mmol creatinine.

Applying this to a validation cohort which included 155 patients with SVV allowed differentiation of active renal vasculitis with a sensitivity of 83%, specificity of 98% and positive likelihood ratio of 21.9.

Summary/Results so Far

Predominantly seen in renal vasculitis, not non-renal vasculitis.

Urinary sCD163 associates very tightly with active renal vasculitis. A positive result can therefore be used to guide treatment for renal flare in place of renal biopsy.