Quantification of Interferon Production for Investigating TB Exposure

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The NPHL has recently evaluated an FDA approved in-vitro test called the QuantiFERON® -TB Gold (QFT-G) assay (Cellistis Limited, Carnegie, Victoria, Australia) as an aid in investigating both latent and active *Mycobacterium tuberculosis (Mtb)* infections. One of the major uses is as a screen for tuberculosis in people who have been immunized with the attenuated BCG vaccine, since prior vaccination does not cause the QFT-G test to become positive.

In the assay, peripheral blood is tested for the production of interferon by *Mtb* stimulated lymphocytes. Two *Mtb* antigens are added to heparinized peripheral blood containing antigen presenting cells and T- lymphocytes. T-lymphocytes that were previously exposed to *Mtb,* produce interferon-γ (IFN-γ) in response to the addition of antigens. The quantity of IFN-γ produced is detected using a single-step enzyme-linked immunosorbant assay (ELISA).

In unvaccinated individuals without *Mtb* infection, clinical trials have shown a specificity of 99.8% for the QFT-G test as compared to 99.1% for the tuberculin skin test (TST). In culture-confirmed infected individuals, *Mtb* QFT-G testing demonstrated a sensitivity of 91.3%, compared to 79.2% with TST, while in BCG-vaccinated individuals, the specificities were 98.1% and 68.1%, respectively for QFT-G and TST. Studies in children and immunosuppressed patients, to include HIV-positive individuals, are currently on-going as the test is not approved to evaluate these groups. Studies showed that indeterminate test results were common (21%) among immunocompromised patients with negative TST results (1).

The QFT-G test was evaluated at the NPHL using peripheral blood from 15 TST positive individuals who were clinically negative for *Mtb* disease (all negative by chest x-ray), 16 TST-negative individuals, and six BCG-vaccinated individuals. Thirteen of the 15 TST-positive individuals without tuberculosis had a negative QFT-G test (two individuals tested positive). All 16 TST-negative cases and 5 of the 6 BCG-vaccinated individuals (one tested as indeterminate) were negative with the QFT-G assay. These data suggest the QFT-G assay could be used to reduce the number of individuals treated for a false-positive TST results.

The QFT-G test is appropriate to screen individuals who have received BCG vaccination and to evaluate health care workers who test positive by the TST. Cost savings are expected from a decreased need for treatment of skin-test positive individuals who test negative with the QFT-G assay. Additionally, repeat skin-testing should be limited in individuals who have consistently positive TSTs.

Specimen collection consists of two-5 ml green-top heparinized tubes (sodium heparin or lithium heparin). Other anticoagulants (EDTA, citrate dextrose) interfere with the assay and should not be used. The blood must be incubated with the test antigens within 12 hours after collection while the lymphocytes are viable. After the blood is incubated with antigens for 16-24 hours, the plasma is harvested at which time the processed specimens may be frozen prior to testing. A test is considered positive for an IFN-γ response to either antigen that is significantly above the non-stimulated IFN-γ level.

Questions regarding this new test may be directed to either Steven Hinrichs, M.D. (402-559-7203) or Jodi Garrett (402-552-3235). Further information can be found at www.cellestis.com and on the Centers for Disease Control and Prevention's website (www.cdc.gov).

References:

1. *Guidelines for Using the QuantiFERON®-TB Gold Test for Detecting Mycobacterium tuberculosis Infection, United States*, Morbidity and Mortality Weekly Report (MMWR), Recommendations and Reports, December 16th, 2005/54(RR15); 49-55 (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5415a4.htm).