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UNDER SECRETARY FOR HEALTH'S INFORMATION LETTER

USE OF INTERFERON-GAMMA RELEASE ASSAYS AS AN AID FOR DIAGNOSING *MYCOBACTERIUM TUBERCULOSIS*

1. Purpose. This Information Letter discusses the use of interferon-gamma release assays (IGRAs) as aids for diagnosing *Mycobacterium tuberculosis* (MTB) infection, including active tuberculosis (TB), as well as latent *M. tuberculosis* infection (LTBI).

2. Background

a. Infection with MTB is a significant problem worldwide as well as in the United States (U.S.). In 2009, the Centers for Disease Control and Prevention (CDC) reported 11,545 cases of active MTB infection from the fifty U.S. states and the District of Columbia. Of those cases, 41 percent were individuals born in the United States. In 1999-2000 data from the National Health and Nutrition Examination Survey estimated there were 9.5 to 14.7 million persons with LTBI in the United States. In addition to mycobacterial culture of specimens, there are currently two general modalities to test for tuberculosis infection: tuberculin skin tests (TSTs) and IGRAs. Both methods evaluate T-cell mediated immunity in response to proteins derived from MTB.

b. TSTs are performed by injecting 5 tuberculin units (0.1 milliliter) of purified protein derivative (PPD) intradermally on the inner surface of the forearm (the Mantoux method). The appropriate medical follow-up is to read the skin test result 48-72 hours after injection. Ensuring the patient's return to have the PPD read may be problematic. False-negative skin tests occur in at least 20 percent of individuals with active MTB infection and are more frequent in immunocompromised people, such as: recipients of organ transplants, those with Human Immunodeficiency Virus (HIV) infection, and those on high-dose steroid therapy. False-positive TSTs may be caused by non-TB mycobacterial infections since PPD has cross reactivity with antigenic proteins from non-TB mycobacterial strains and proteins used in Bacille Calmette-Guerin (BCG) vaccine or cancer therapies.

c. IGRAs are blood tests that were developed to eliminate reader error, overcome issues with noncompliance and limit test cross-reactivity with other mycobacterial species which are all problems with TSTs. IGRAs measure the amount of interferon-gamma released by T-cells in response to stimulation by antigens more specific to MTB. The antigens include early secreting antigenic target 6 (ESAT-6), culture filtrate protein 10 (CFP-10), and Rv2654 antigen (TB7.7).

These antigens are more specific for MTB complex organisms (e.g., *M. tuberculosis*, *M. bovis*, *M. africanum*) than PPD and are not shared with BCG. In studies involving culture filtrates, MTB complex organisms share cross-reactivity of ESAT-6 with *M. marinum*, *M. kansasii*, and *M. szulgai* and share cross-reactivity of CFP-10 with *M. marinum*, *M. kansasii*, and *M. leprae*; however, the antigen TB7.7 is highly specific for MTB.

3. <u>Interferon-Gamma Release Assays</u>: The two commercially available IGRAs that have approval from the Food and Drug Administration are:

a. **QuantiFERON-TB® Gold in Tube (QFT-GIT)** (Cellestis Limited). QFT-GIT was developed to overcome the time constraints involved in the process of its predecessor, QuantiFERON®-TB Gold, with respect to the specimens requiring incubation within a few hours of collection. In the QFT-GIT test, whole blood is first collected into three separate tubes: the negative (nil) control tube, the MTB antigen tube, and the positive (mitogen) control tube. The whole blood begins to incubate with the reagents upon collection. The antigen tube contains amino acid sequences of ESAT-6, CFP-10, and TB7.7 that stimulate the release of interferon-gamma from T-cells. The negative control tube contains heparin alone and the positive control tube holds heparin, dextrose, and phytohemagglutinin. After 16-24 hours of incubation the interferon-gamma concentration is determined by an enzyme linked immunosorbent assay (ELISA). The tuberculosis response is calculated by QFT-GIT Analysis Software by subtracting the interferon-gamma concentration in the negative control tube from the interferon-gamma concentration in the MTB antigen and positive control tubes respectively. ***NOTE:*** *Interpretation criteria for QFT-GIT are available at:* *http://www.cellestis.com/IRM/Company/ShowPage.aspx?CPID=1171*.

b. **T-Spot®.TB** (Oxford Immunotec Limited, Oxford, United Kingdom). Peripheral blood mononuclear cells are placed in wells precoated with media containing antibodies to interferon-gamma. Four wells are used for each patient sample. The positive control well contains phytohemagglutinin and the negative control well contains media alone without antigen. The two other test wells contain ESAT-6 and CFP-10 respectively. The test uses an enzyme-linked immunospot (ELISPOT) methodology to detect increases in the number of cells that secrete interferon-gamma (represented as spots in each well) after stimulation with antigen. TB response is determined by the number of spots in the antigen wells compared to the number of spots in the negative control well. ***NOTE:*** *Interpretation criteria for T-Spot®.TB are available at:* *http://www.oxfordimmunotec.com/USpageInsert*.

4. <u>Comparison of IGRAs to TST:</u>

a. Neither IGRAs nor TSTs can distinguish active TB from latent LTBI.

b. The "gold standard" in diagnosing active TB remains clinical examination combined with an acid fast smear and culture of tissue samples. There is; however, no "gold standard" test for the diagnosis of LTBI.

c. Multiple studies have evaluated sensitivity and specificity of QFT-GIT and T-Spot®.TB in comparison with TST with variable results. These studies are difficult to compare because of the

different study populations, test methods, and interpretation criteria. The study populations may significantly differ from the Department of Veterans Affairs (VA) population, thereby altering the test performance among Veterans.

(1) Based on the results of published studies, pooled sensitivities for TST, T-Spot®.TB, and QFT-GIT were 95 percent, 91 percent, and 84 percent respectively.

(2) QFT-GIT and T-Spot®.TB use more specific antigens for *M. tuberculosis* than TST, and therefore, are expected to be more specific. In studies of persons unlikely to have TB pooled specificities for QFT-GIT, T-Spot®.TB, and TST were 99 percent, 88 percent, and 85 percent respectively.

d. Several analyses have tried to determine cost-effectiveness of IGRAs as a substitute to TSTs with differing results. The direct and indirect costs of IGRAs as an alternative to TSTs in VA were specifically studied (see subpar. 6a) and it was found that the use of IGRAs leads to better clinical outcomes and lower costs than TSTs. The involved costs; however, represent a shift from health care providers (with TSTs) to the laboratory (with IGRAs).

5. Conclusions

a. IGRAs are cost-effective tests that can be used in place of TSTs in all situations in which the CDC recommends TST; including contact investigations, testing during pregnancy, and screening health care workers.

b. Further head-to-head comparison studies need to be performed to determine if one IGRA is more sensitive or specific than another. Preliminary data suggests that T-Spot®.TB may be more sensitive than QFT-GIT, while QFT-GIT may be more specific than T-Spot®.TB.

c. IGRAs are preferred to TSTs in people with a history of BCG exposure and in those who potentially will not return for follow-up for reading of the tuberculin test.

d. Routine testing with both methods is generally not recommended. However, administering tests both may be helpful in certain situations, such as clarifying the diagnosis of active TB in a person who has a positive TST who was previously given BCG or when confirmation of a positive test would improve compliance with treatment. TSTs may boost the IGRA response but studies examining that effect have had conflicting results. Definitive data does not exist to determine the specific timing of IGRAs after PPD injection.

e. The sensitivities of IGRAs are not adequate to exclude the diagnosis of TB in patients with clinical signs and symptoms of the disease.

f. So far studies have not shown a significant difference in the proportion of positive QFT-GIT results compared to positive TST results in HIV-infected persons, but limited data are available regarding IGRA use in HIV- infected and other immunocompromised persons.

g. IGRAs can have conversions and reversions during serial testing (such as a surveillance program for health care workers) especially for values close to the assay's "cut off" value. Some studies have identified high false-positive rates with the use of IGRAs due to this "conversion" phenomenon. The T-Spot®. TB assay attempts to address this issue by defining a "Borderline" range, but additional studies need to be performed to define optimum cutoffs for conversions to maximize the performance of IGRAs in serial testing.

h. The use of IGRAs can negate both the "booster" effect potentially seen in serial testing as well as the role of two-step testing with TSTs.

i. Prior to implementing IGRAs, it is suggested that individual facilities determine test availability and overall costs and benefits of testing. It is recommended that consideration be given to having specific days to collect and process the IGRA samples to ensure appropriate collection procedures, adequate laboratory support and accurate test results.

j. Given the development and increasing use of IGRAs, it may be feasible to re-evaluate the selection of tests for the diagnosis of active as well as latent tuberculosis.

6. References

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7. Inquiries. Questions regarding this Information Letter may be addressed to the Infectious Diseases Service (513) 475-6398.

Robert A. Petzel, M.D.
Under Secretary for Health

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