Guidelines & Recommendation’s for Using Blood Assays

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**GUIDELINES AND RECOMMENDATIONS**

**FOR USING BLOOD ASSAYS FOR
*Mycobacterium tuberculosis* (BAMTs)**

Before 2001, the tuberculin skin test (TST) was the only practical and commercially available immunologic test for *Mycobacterium tuberculosis* infection approved in the United States. Blood assay for M. tuberculosis (BAMT) is a general term to refer to recently developed in vitro diagnostic tests that assess for the presence of infection with *M. tuberculosis*. This term includes, but is not limited to, interferon-gamma (IFN-γ) release assays (IGRAs).

Since 2001, several IGRAs have been approved by FDA. In the United States, the currently available tests are the QuantiFERON®-TB Plus test (QFT-Plus) and the T-SPOT*.TB* test
(T-SPOT). The following recommendations are from updated guidelines for using IGRAs in the June 25, 2010 MMWR: (Note that CDC guidelines describe the use of IGRAs instead of the more inclusive BAMT.)

**KEY POINTS FOR USING BAMTs**

* A BAMT may be used in place of (but not in addition to) a TST in all situations in which CDC recommends tuberculin skin testing as an aid in diagnosing *M. tuberculosis* infection
* A BAMT is preferred for testing persons from groups that historically have low rates of returning to have TSTs read. For example, use of a BAMT might increase test completion rates for homeless persons and drug-users.
* A BAMT is preferred for testing persons who have received BCG (as a vaccine or for cancer therapy).
* A TST is preferred for testing children aged less than 5 years.
* Two-step testing is not required for BAMTS, because IGRA testing does not boost subsequent test results.
* Neither a BAMT nor TST can distinguish LTBI from active tuberculosis.
* As with TSTs, a negative BAMT result does not exclude LTBI or active TB disease

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**Recommendations for Use of IGRAs**

**General Recommendations for Use of IGRAs**

* TSTs and IGRAs (QFT-Plus, and T-SPOT) should be used as aids in diagnosing infection with *M. tuberculosis*. These tests may be used for surveillance purposes or to identify persons likely to benefit from treatment, including persons who are or will be at increased risk for *M. tuberculosis* infection (Box 1, below) or for progression to active tuberculosis if infected (Box 2, below).
* IGRAs should be performed and interpreted according to established protocols using FDA-approved test formats. They should be performed in compliance with Clinical Laboratory Improvement Amendment (CLIA) standards.
* Both the standard qualitative test interpretation and the quantitative assay measurements should be reported together with the criteria used for test interpretation. This will permit more refined assessment of results and promote understanding of the tests.
* Arrangement for IGRA testing should be made prior to blood collection to ensure that the blood specimen is collected in the proper tubes, and that testing can be performed within the required timeframe.
* Prior to implementing IGRAs, each institution and tuberculosis-control program should evaluate the availability, overall cost, and benefits of IGRAs for their own setting. In addition, programs should consider the characteristics of the population to be tested.
* As with the TST, IGRAs generally should not be used for testing persons who have a low risk for both infection and progression to active tuberculosis if infected (except for those likely to be at increased risk in the future). Screening such persons diverts resources from higher priority activities and increases the number of false-positive results. Even with a test specificity approaching 99%, when the prevalence of *M. tuberculosis* infection is ≤1%, the majority of positive results will be false positives. If persons at low risk for both infection and progression are to be tested, selection of the test with the greatest specificity will minimize false-positive results, reduce unnecessary evaluation and treatment, and minimize the potential for adverse events from unnecessary treatment.

**Test Selection**

* Selection of the most suitable test or combination of tests for detection of *M. tuberculosis* infection should be made on the basis of the reasons and the context for testing, test availability, and overall cost effectiveness of testing. Results of studies examining sensitivity, specificity, and agreement for IGRAs and TST vary with respect to which test is better. Although data on the accuracy of IGRAs and their ability to predict subsequent active tuberculosis are limited, to date, no major deficiencies have been reported in studies involving various populations. As use of these tests increases, greater understanding of their value and limitations will be gained.
* An IGRA may be used in place of (but not in addition to) a TST in all situations in which CDC recommends tuberculin skin testing as an aid in diagnosing *M. tuberculosis* infection, with preferences and special considerations noted below. Despite the indication of a preference in these instances, use of the alternative test (FDA-approved IGRA or TST) is acceptable medical and public health practice.

**Situations in Which an IGRA Is Preferred But a TST Is Acceptable**

* An IGRA is preferred for testing persons from groups that historically have low rates of returning to have TSTs read. For example, use of an IGRA might increase test completion rates for homeless persons and drug-users. The use of IGRAs for such persons can increase test completion rates, so control efforts can focus on those most likely to benefit from further evaluation and treatment.
* An IGRA is preferred for testing persons who have received BCG (as a vaccine or for cancer therapy). Use of IGRAs in this population is expected to increase diagnostic specificity and improve acceptance of treatment for LTBI.

**Situations in Which a TST Is Preferred But an IGRA Is Acceptable**

* A TST is preferred for testing children aged <5 years. Use of an IGRA in conjunction with TST has been advocated by some experts to increase diagnostic sensitivity in this age group. Recommendations regarding use of IGRAs in children have also been published by the American Academy of Pediatrics.

**Situations in Which Either a TST or an IGRA May Be Used Without Preference**

* An IGRA or a TST may be used without preference to test recent contacts of persons known or suspected to have active tuberculosis with special considerations for follow-up testing. IGRAs offer the possibility of detecting *M. tuberculosis* infection with greater specificity than with a TST. Also, unlike TSTs, IGRAs do not boost subsequent test results and can be completed following a single patient visit. However, data on the ability of IGRAs to predict subsequent active tuberculosis are limited. If IGRAs are to be used in contact investigations, negative results obtained prior to 8 weeks after the end of exposure typically should be confirmed by repeat testing 8--10 weeks after the end of exposure. This recommendation is similar to one used for TST, because data on the timing of IGRA conversion after a new infection are not currently available. Use of the same test format for repeat testing will minimize the number of conversions that occur as a result of test differences.
* An IGRA or a TST may be used without preference for periodic screening of persons who might have occupational exposure to *M. tuberculosis* (e.g., surveillance programs for health-care workers) with special considerations regarding conversions and reversions. For serial and periodic screening, IGRAs offer technical, logistic, and possible economic advantages compared with TSTs but also have potential disadvantages. Advantages include the ability to get results following a single visit. Two-step testing is not required for IGRAs, because IGRA testing does not boost subsequent test results. Disadvantages of IGRAs in this setting include a greater risk of test conversion due to false-positive IGRA results with follow-up testing of low-risk health-care workers who have tested negative at prior screening. CDC has published criteria for identifying conversions for TSTs and IGRAs. TST conversion is defined as a change from negative to positive with an increase of ≥10 mm in induration within 2 years. TST conversion is associated with an increased risk for active tuberculosis. An IGRA conversion is defined as a change from negative to positive within 2 years without any consideration of the magnitude of the change in TB Response. Using this lenient criterion to define IGRA conversion might produce more conversions than are observed with the more stringent criteria applied to TSTs. Furthermore, an association between an IGRA conversion and subsequent disease risk has not been demonstrated. The criteria for interpreting changes in an IGRA that identify new infections remain uncertain. CDC encourages institutions and programs in which IGRAs are used to publish their experiences, particularly in regard to rates of conversion, reversion, and progression to active tuberculosis over time.

**Situations in Which Testing with Both an IGRA and a TST May Be Considered**

* Although routine testing with both a TST and an IGRA is not generally recommended, results from both tests might be useful when the initial test (TST or IGRA) is negative in the following situations: 1) when the risk for infection, the risk for progression, and the risk for a poor outcome are increased (e.g., when persons with HIV infection or children aged <5 years are at increased risk for *M. tuberculosis* infection) or 2) when clinical suspicion exists for active tuberculosis (such as in persons with symptoms, signs, and/or radiographic evidence suggestive of active tuberculosis) and confirmation of *M. tuberculosis* infection is desired. In such patients with an initial test that is negative, taking a positive result from a second test as evidence of infection increases detection sensitivity. However, multiple negative results from any combination of these tests cannot exclude *M. tuberculosis* infection.
* Using both a TST and an IGRA also might be useful when the initial test is positive in the following situations: 1) when additional evidence of infection is required to encourage compliance (e.g., in foreign-born health-care workers who believe their positive TST result is attributable to BCG) or 2) in healthy persons who have a low risk for both infection and progression. In the first situation, a positive IGRA might prompt greater acceptance of treatment for LTBI as compared with a positive TST alone. In the latter situation, requiring a positive result from the second test as evidence of infection increases the likelihood that the test result reflects infection. For the second situation, an alternative is to assume, without additional testing, that the initial result is a false positive or that the risk for disease does not warrant additional evaluation or treatment, regardless of test results. Steps should be taken to minimize unnecessary and misleading testing of persons at low risk.
* Repeating an IGRA or performing a TST might be useful when the initial IGRA result is indeterminate, borderline, or invalid and a reason for testing persists. A second test also might be useful when assay measurements from the initial test are unusual, such as when the Nil value is higher than typical for the population being tested (e.g., IFN-γ concentration for Nil by QFT-G or QFT-GIT >0.7 IU/mL for most of the U.S. populations), the Nil value is appreciably greater than the value obtained with *M. tuberculosis* antigen stimulation (e.g. when IFN-γ concentration for Nil by QFT-G is 0.35 IU/mL greater than the concentration obtained with either ESAT-6 or CFP-10 stimulation, or when the number of spots for Nil by T-SPOT is four spots greater than the number with either ESAT-6 or CFP-10 stimulation), or the Mitogen value is lower than is expected for the population being tested (e.g., the Mitogen Response by QFT-G or
QFT-GIT is <0.5 IU/mL, or the number of spots in the mitogen well by T-SPOT is <20). If an IGRA is to be repeated, a new blood sample should be used. In such situations, repeat testing with another blood sample usually provides interpretable results.

**Medical Management After Testing**

* Diagnoses of *M. tuberculosis* infection and decisions about medical or public health management should not be based on IGRA or TST results alone, but should include consideration of epidemiologic and medical history as well as other clinical information.
* Persons with a positive TST or IGRA result should be evaluated for the likelihood of *M tuberculosis* infection, for risks for progression to active tuberculosis if infected, and for symptoms and signs of active tuberculosis. If risks, symptoms, or signs are present, additional evaluation is indicated to determine if the person has LTBI or active tuberculosis.
* A diagnosis of LTBI requires that active tuberculosis be excluded by medical evaluation, which should include taking a medical history and a physical examination to check for suggestive symptoms and signs, a chest radiograph, and, when indicated, testing of sputum or other clinical samples for the presence of *M. tuberculosis*. Neither an IGRA nor TST can distinguish LTBI from active tuberculosis.
* In persons who have symptoms, signs, or radiographic evidence of active tuberculosis or who are at increased risk for progression to active tuberculosis if infected, a positive result with either an IGRA or TST should be taken as evidence of *M. tuberculosis* infection. However, negative IGRA or TST results are not sufficient to exclude infection in these persons, especially in those at increased risk for a poor outcome if disease develops, and clinical judgment dictates when and if further diagnostic evaluation and treatment are indicated.
* In healthy persons who have a low likelihood both of *M. tuberculosis* infection and of progression to active tuberculosis if infected, a single positive IGRA or TST result should not be taken as reliable evidence of *M. tuberculosis* infection. Because of the low probability of infection, a false-positive result is more likely. In such situations, the likelihood of *M. tuberculosis* infection and of disease progression should be reassessed, and the initial test results should be confirmed. Repeat testing, with either the initial test or a different test, may be considered on a case-by-case basis. For such persons, an alternative is to assume, without additional testing, that the initial result is a false positive.
* In persons with discordant test results (i.e., one positive and the other negative), decisions about medical or public health management require individualized judgment in assessing the quality and magnitude of each test result (e.g., size of induration and presence of blistering for a TST; and the TB Response, Nil, and Mitogen values for an IGRA), the probability of infection, the risk for disease if infected, and the risk for a poor outcome if disease occurs.
* Taking a positive result from either of two tests as evidence of infection is reasonable when 1) clinical suspicion exists for active tuberculosis (e.g., in persons with symptoms, signs, and/or radiographic evidence of active tuberculosis) or 2) the risks for infection, progression, and a poor outcome are increased (e.g., when persons with HIV infection or children aged <5 years are at increased risk for *M. tuberculosis* infection).
* For healthy persons who have a low risk for both infection and progression, discounting an isolated positive result as a false positive is reasonable. This will increase detection specificity and decrease unnecessary treatment.
* For persons who have received BCG and who are not at increased risk for a poor outcome if infected (Box 2, below), TST reactions of <15 mm in size may reasonably be discounted as false positives when an IGRA is clearly negative.
* In other situations, inadequate evidence exists on which to base recommendations for dealing with discordant results. However, in the absence of convincing evidence of infection, diagnostic decisions may reasonably be deferred unless an increased risk exists for progression if infected and/or a high risk exists for a poor outcome if disease develops.”

**Table 1. Interpretation of QFT-Plus test results**

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| Nil (IU/ml) TB1 minus Nil TB2 minus Nil Mitogen minus QFT-Plus Result Report/interpretation (IU/ml) (IU/ml) Nil (IU/ml)\*  |
| <8.0 | >0.35 and>25% of Nil | Any | Any | Positive† | M. *tuberculosis* infection likely |
| Any | >0.35 and>25% of Nil |
| <0.35 or>0.35 and<25% of Nil | <0.35 or>0.35 and<25% of Nil | >0.50 | Negative |  M. *tuberculosis*Infection NOT likely |
| <0.35 or>0.35 and<25% of Nil | <0.35 or>0.35 and<25% of Nil | <0.50 | Indeterminate‡ | Likelihood of M. *tuberculosis*Infection cannot bedetermined |
| >8.0§ | Any |

\* Responses to the Mitog en positive control (and occasionally TB Antigen) can be outside the range of the microplate

 reader. This has no impact on test results. Values >10 IU/ml are reported by the QFT-Plus software as >10 IU/ml.

† 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 test result

 is considered positive.

‡ Refer to “Troubleshooting Guide”, page 58 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.

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| **TABLE 2. Interpretation criteria for the T-SPOT.*TB* Test (T-****SPOT)** |
| **Interpretation** | **Nil\*** | **TB Response†** | **Mitogen§** |
| Positive¶ | ≤10 spots | ≥8 spots | Any |
| Borderline\*\* | ≤10 spots | 5, 6, or 7 spots | Any |
| Negative†† | ≤10 spots | ≤4 spots |  |
| Indeterminate\*\* | >10 spots | Any | Any |
| ≤10 spots | <5 spots | <20 spots |
| **Source:** Based on Oxford Immunotec Limited. T-SPOT.*TB* [Package insert]. Available at <http://www.oxfordimmunotec.com/USpageInsert> .\* The number of spots resulting from incubation of PBMCs in culture media without antigens. † The greater number of spots resulting from stimulation of peripheral blood mononuclear cells (PBMCs) with two separate cocktails of peptides representing early secretory antigenic target-6 (ESAT-6) or culture filtrate protein-10 (CFP-10) minus Nil.§ The number of spots resulting from stimulation of PBMCs with mitogen without adjustment for the number of spots resulting from incubation of PBMCs without antigens.¶ Interpretation indicating that *Mycobacterium tuberculosis* infection is likely.\*\* Interpretation indicating an uncertain likelihood of *M. tuberculosis* infection.†† Interpretation indicating that *M. tuberculosis* infection is not likely. |

References:

CDC. Recommendations and Reports. Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection. MMWR 2010; 59(No. RR-5)

<http://www.quantiferon.com/wp-content/uploads/2017/10/QFT-Plus-ELISA-IFU-L1095849-R02.pdf>