NORTH CAROLINA GUIDELINES FOR THE USE OF INTERFERON GAMMA RELEASE ASSAYS (IGRAS) IN TUBERCULOSIS DIAGNOSIS

Background

Interferon gamma release assays (IGRAs) are relatively new tests for tuberculosis (TB) infection. These tests measure the patient's immune response (interferon gamma release) after stimulation of white blood cells in a test tube with 2-3 relatively TB-specific antigens. In contrast to the tuberculin skin test, which requires two separate visits for placement and reading, IGRAs offer the possibility of testing for TB infection with a single blood draw at a single visit. In addition to the logistical advantage of requiring a single visit, IGRAs may have other advantages over the tuberculin skin test. The antigens used for the IGRA tests are not present in the Bacille Calmette-Guerin vaccine (BCG), so false positive tests due to BCG are unlikely to occur. The IGRA antigens are also not present in most nontuberculous mycobacteria, so false positive tests due to nontuberculous mycobacterial exposure or infection are less likely to occur with IGRAs than with tuberculin skin testing.

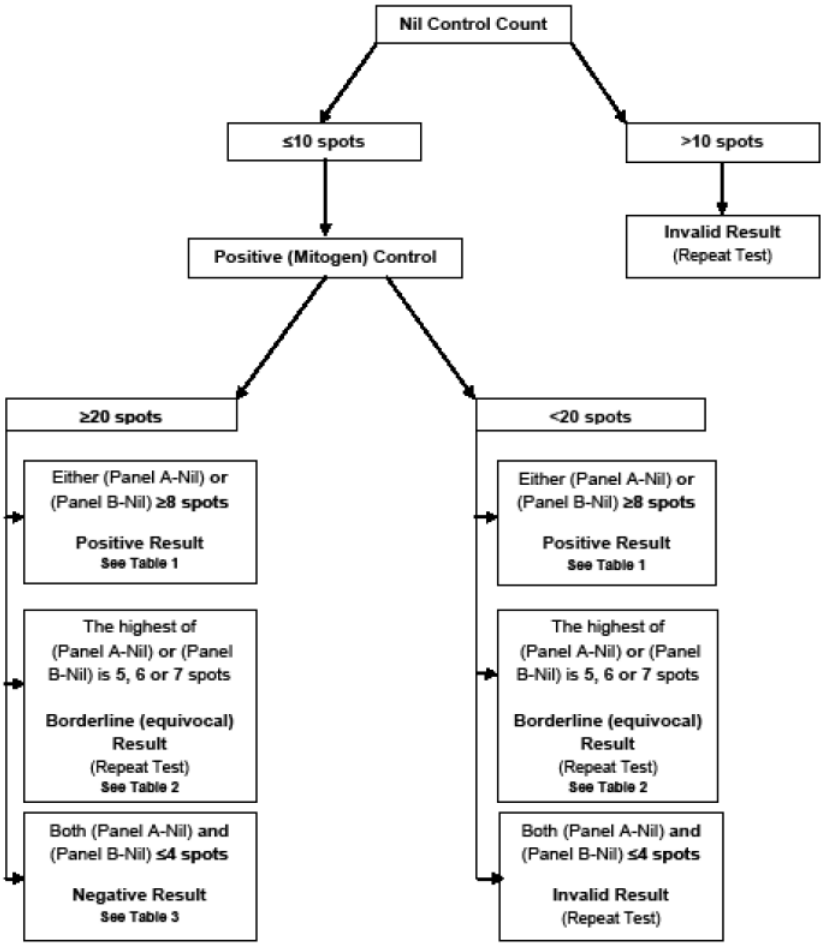
Despite these potential advantages, the IGRAs are imperfect tests for TB diagnosis. There is a large body of data demonstrating the association between a positive tuberculin skin test and the risk to develop active tuberculosis, but the data demonstrating a similar association between a positive IGRA and risk for active tuberculosis are much more limited. Additionally, every study comparing the two available IGRA tests has demonstrated a significant proportion of discordant results, the significance of which is unknown. This discordance means that the IGRAs are not interchangeable; switching among the IGRAs (or for that matter, between an IGRA and tuberculin skin testing) in the setting of serial testing may result in false "conversions" caused by discordance between the different tests used (as opposed to new TB infection). Furthermore, the cost of an IGRA is significantly greater than the cost of a tuberculin skin test.

Currently two IGRAs are approved for use in the United States by the Food and Drug Administration. The Quantiferon Gold in-tube® is an enzyme-linked immunosorbent assay-based test. The test measures the concentration of interferon gamma in whole blood in 3 separate tubes: a nil tube (negative control), a tube containing 3 TB antigens (ESAT-6, CFP-10, and TB7.7), and a tube containing phytohemagglutinin (a mitogen used as a positive control). Blood is drawn from the patient directly into each tube (about 1 ml of blood each), and the tubes must then be shaken vigorously. The tubes must be placed into an incubator at 37 C within 14 hours of the blood draw, and are then incubated for 16-24 hours. After incubation, a machine is used to measure the concentration of interferon gamma in each tube. The criteria for test interpretation are listed below (from the package insert, available at www.cellestis.com).

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB Gold IT Result	Report / Interpretation
≤ 8.0	≥ 0.35 and ≥ 25% of Nil value	Any	**Positive**[2]	*M. tuberculosis* infection likely
	< 0.35 OR ≥ 0.35 and < 25% of Nil value	≥ 0.5	**Negative**	*M. tuberculosis* infection NOT likely
		< 0.5	**Indeterminate**[3]	Results are indeterminate for TB Antigen responsiveness
> 8.0[4]	Any	Any		

The T-SPOT.TB® is an enzyme-linked immunospot test. The test measures the number of spots on a plate containing 4 different antigens: nil (negative control), 2 TB antigens (ESAT-6 and CFP-10, also called Panel A and Panel B), and phytohemagglutinin (positive control). Each spot theoretically represents a white blood cell that is secreting interferon gamma. Blood is drawn from the patient (8 mL for adults, 4 mL for children 2-9 years old, and 2 mL for children under 2 years) and then must be processed in the laboratory within 8 hours. The white blood cells are separated from the rest of the blood, and a standard number of white cells is placed into each plate. The cells are incubated with the antigens for 16-20 hours, and then further steps are used to develop the spots for each plate. The criteria for test interpretation are outlined in the diagram below (from the package insert at http://www.oxfordimmunotec.com/Technical_Documents_North_America).

Figure 3: *Algorithm Flow Diagram*



A brief comparison of the tuberculin skin test and the two IGRAs follows in the Table.

	Tuberculin skin test	Quantiferon Gold in-tube	T-SPOT.TB
Number of visits required	2*	1	1
Time frame to get blood to lab	N/A	<14 hours (can be incubated in portable incubator)	<8 hours
Need for additional processing of blood before incubation	N/A	No	Yes
Result format	mm of induration	Concentration of	Number of spots on

		interferon gamma (IU/mL)	a plate
Reliability among observers	+/-	++	++
Potential as a "send out" test to distant labs	N/A	Yes	Not at present
Available in NC as of 5/2009	Yes	Yes	No
Cost to health department or healthcare facility	Reagent inexpensive, labor somewhat more	Moderately expensive	Unknown, probably will be similar to Quantiferon
Cross-reacts with BCG, nontuberculous mycobacteria	Yes (mostly an issue in foreign-born populations)	No	No

* 4 visits may be necessary if 2-step testing is performed

Guidelines for use of IGRAs in North Carolina

Medicine is constantly changing, and IGRAs are a very active area of research. **Clinical judgment based on the latest scientific evidence, with emphasis on how a given test will affect patient management, should always be used in deciding to order any diagnostic test and in interpreting the results.** The guidelines that follow are a consensus statement by the North Carolina Tuberculosis Control Program staff and the North Carolina Tuberculosis Medical Advisory Committee, designed to assist providers in determining when IGRAs may be useful in clinical practice. At the time of this writing, only Quantiferon Gold in-tube® is available to clinicians in North Carolina, but this may change with time.

Reporting of IGRA results

Reporting of IGRA results as "Positive," "Negative," or "Indeterminate" is clinically useful, but may be suboptimal in certain circumstances. Both clinically available IGRAs have a certain amount of test-retest variability ("wobble" — for example see Pai M et al., American Journal of Respiratory and Critical Care Medicine 2006 174: 349). Particularly when results are near the threshold for a positive test, repeat testing has a significant probability of producing a result on the other side of the threshold. This problem is of particular concern in the setting of repeat testing, when a patient may test negative at just below the threshold on one occasion, and then test positive at just above the threshold on another occasion solely due to inter-test variability.

Recommendations: To assist with test interpretation, the NC Tuberculosis Control Program recommends that laboratories report the following information:

Quantiferon Gold in-tube®

- Nil tube interferon gamma concentration (IU/mL)
- TB antigen tube interferon gamma concentration (IU/mL)

- TB-Nil value (difference, IU/mL)
- Criteria for a positive value (i.e. difference of ≥35 IU/mL and >25% of nil)
- Interpretation ("Positive," "Negative," "Indeterminate")

T-SPOT.TB®
- Nil plate spots (number)
- TB antigen plate spots (number, both plates)
- Highest difference (TB antigen-nil)
- Criteria for a positive value (i.e. difference of ≥8 spots)
- Interpretation ("Positive," "Negative," "Indeterminate")

Management of indeterminate results

Both IGRAs may yield indeterminate results, either due to high Nil background or an inadequate interferon gamma response to mitogen. Indeterminate results are more common in the setting of immunosuppression, but can also occur in apparently immunocompetent hosts. However, a significant proportion of persons with indeterminate results on one occasion will have a non-indeterminate result on repeat testing. Similarly, while an indeterminate result is associated with an anergic response to the tuberculin skin test, not all persons with an indeterminate response from an IGRA will have anergy in response to the tuberculin skin test. In most studies, the T-SPOT.TB® produces a smaller proportion of indeterminate results than the Quantiferon Gold in-tube®.

Recommendations:
- If an indeterminate result is obtained from IGRA testing, repeating the same IGRA test should be considered.
- If an indeterminate result is obtained from one IGRA test (outside the setting of serial testing) performing a different IGRA test (if available) or a tuberculin skin test can be considered

Diagnosis of active tuberculosis

Like the tuberculin skin test, IGRAs are at best imperfect tools in the diagnosis of active TB. A recent meta-analysis estimated that the sensitivity of the tuberculin skin test among persons with active tuberculosis was 77%, the sensitivity of the Quantiferon Gold in-tube® was 70%, and the sensitivity of the T-SPOT.TB® was 90% (Pai M et al., Annals of Internal Medicine 2008; 149: 177). IGRAs will therefore be falsely negative in a significant proportion of persons with active TB. Also, IGRAs cannot discriminate between latent TB infection and active TB disease in a given patient. However, one study did demonstrate that IGRAs were useful in distinguishing children with TB cervical lymphadenitis from children with nontuberculous mycobacterial lymphadenitis (Clin Infect Dis. 2007; 45(3):322) in a low-incidence area.

Recommendations:
- Like the tuberculin skin test, IGRAs should not be relied upon to make or disprove the diagnosis of active TB in adults.

- IGRAs may be used in children as part of diagnostic algorithms for TB diagnosis, keeping in mind their imperfect test characteristics.
- Microbiologic diagnosis (culture) is the gold standard and should be aggressively pursued in both adults and children.

Targeted testing for latent TB infection in immunocompetent adults and older children
A large body of epidemiologic evidence associates an elevated risk to develop TB disease with a positive tuberculin skin test. No such evidence exists for any IGRA test at this time outside of a few studies of persons who were contacts to infectious TB cases. While IGRAs are clearly more specific for TB infection than the tuberculin skin test, some investigators believe that the tuberculin skin test may be more sensitive for remote TB infection. The use of IGRAs for targeted TB testing may therefore result in fewer positive tests (and thus a lower number of persons offered latent TB treatment), with the possibility of missing some persons who truly have latent TB. The present costs of either IGRA from a public health department perspective are significantly greater than the cost of a tuberculin skin test, and routine use of IGRAs for targeted testing would divert scarce resources away from higher-priority activities.

Recommendations:
- The tuberculin skin test is preferred for routine targeted testing for latent TB infection in immunocompetent adults and children 5 years and older in the public health setting
- IGRAs are acceptable alternatives for targeted testing for latent TB infection in immunocompetent adults and children 5 years and older, and may be preferred in some settings (see below "*Screening for latent TB infection at sites where tuberculin skin testing is not frequently performed*")
- If serial testing (healthcare workers, residents of long-term care facilities) is planned, see specific recommendations below.

Contact investigations
IGRAs have been studied in a number of contact investigation settings, and a positive IGRA result generally correlates more closely with the extent of TB exposure than a positive tuberculin skin test. Limited data suggest that IGRAs predict subsequent progression to active TB disease after TB exposure at least as well as tuberculin skin testing (Diel R. et al., American Journal of Respiratory and Critical Care Medicine 2006; 174: 349 and Bakir M et al, Annals of Internal Medicine 2008 149: 777). The primary advantages of IGRAs over the tuberculin skin test are the availability of a result with one patient encounter (as opposed to two with the tuberculin skin test) and the lack of IGRA cross-reactivity with BCG and most nontuberculous mycobacteria. The cost of IGRAs, however, limits their general use in the public health setting.

Recommendations:
- IGRAs can be used for adults and children 5 years and older in contact investigations in place of the tuberculin skin test
- The tuberculin skin is preferred for children <5 years old who are contacts to a case of active TB. T-SPOT.TB (currently not available in NC) can be used for contacts <5 years in place of the tuberculin skin test. **Note: Neither the tuberculin skin test nor**

IGRAs are particularly sensitive for TB infection in children under 6 months of age. Per NC guidelines, children under 6 months old who are close contacts to an infectious TB case should be given presumptive treatment for latent TB infections regardless of skin test/IGRA results.

- The use of IGRAs should be particularly considered in populations with suboptimal healthcare access/utilization (e.g. homeless, substance abusers, migrant workers) who are unlikely to return for tuberculin skin test reading
- Where possible, IGRA use in contact investigations should be paired with opt-out testing for the human immunodeficiency virus to identify close contacts who would be candidates for latent TB treatment regardless of IGRA result
- The same test (IGRA or TST) should be used for initial and repeat (8-week post-exposure) testing of contacts.

Serial testing in healthcare workers

The use of IGRAs in serial testing of healthcare workers has not been extensively studied. However, the problem of test "wobble" has become apparent in early studies (Pai M et al., American Journal of Respiratory and Critical Care Medicine 2006 174: 349); that is, persons whose results are close to the cutoff for a positive result on one occasion have a high likelihood to have a different test interpretation if retested. The cost of serial testing of healthcare workers with IGRAs may be a significant additional burden on infection control programs that should be assessed in the context of the entire program. However, IGRAs may increase acceptance of latent TB treatment among healthcare workers who test positive, especially in those workers who have received BCG vaccine (Sahni R et al., Infection Control and Hospital Epidemiology 2009; 30: 197).

Recommendations:

- IGRAs should not generally be used for serial testing of healthcare workers until more data become available
- If serial testing of healthcare workers (or other persons at risk of TB infection) is performed, the same test should be consistently used in the same person over time. Switching among the tuberculin skin test, Quantiferon Gold in-tube®, and T-SPOT.TB® may result in false conversions due solely to test discordance.
- IGRAs may be considered as confirmatory tests for healthcare workers at low risk for progression to TB disease as a tool to increase acceptance of latent TB treatment (particularly among BCG-vaccinated healthcare workers)

Screening of young children (<5 years) for latent TB infection

Limited data exist demonstrating the utility of IGRAs in young children. At present, the T-SPOT.TB® has more extensive data justifying use in young children (e.g. Bakir M et al, Annals of Internal Medicine 2008 149: 777), and reported rates of indeterminate results for T-SPOT.TB® are generally lower in young children than for Quantiferon Gold in-tube® (e.g. Bergamini BM et al., Pediatrics 2009; 123: e419). However, not all studies support the superiority of T-SPOT.TB® in children (e.g. Kampmann B et al., European Respiratory Journal 2009; epub).

Recommendations:
- IGRAs should not generally be used for screening children <5 years old for latent TB infection
- IGRAs should not generally be used as confirmatory tests after a positive tuberculin skin test among children <5 years old

Screening for latent TB infection at sites where tuberculin skin testing is not frequently performed

One of the chief deficiencies of the tuberculin skin test is the poor inter-reader reliability when performed by inexperienced healthcare workers. In settings where tuberculin skin tests are not frequently performed, IGRAs may provide a more reliable test result than the tuberculin skin test.

Recommendation:
- IGRAs should be considered instead of the tuberculin skin test for latent TB screening (adults and children 5 years and older) at sites where tuberculin skin testing is not frequently performed
- If latent TB screening of children <5 years old is indicated, referral to a site where tuberculin skin testing is frequently performed (e.g. public health clinic) may be appropriate if the referring site does not frequently perform tuberculin skin testing

Screening for latent TB infection in immunocompromised populations

An increasing body of data has evaluated the performance of IGRAs in immunocompromised populations, including persons infected with the human immunodeficiency virus and persons on treatment for rheumatic disease. Similar to the tuberculin skin test, IGRAs are less sensitive for active tuberculosis in immunocompromised persons than in immunocompetent persons (e.g. Raby E et al. PLoS ONE 2008 3: e2489). Immunocompromised persons may have both false-negative IGRA results and indeterminate IGRA results in the setting of TB disease. The T-SPOT.TB® seems less likely to give an indeterminate result in some immunocompromised populations than the Quantiferon Gold in-tube® (e.g. Ferrara G et al, Lancet 2006 367: 1328), but the clinical importance of this finding is unclear.

Recommendations:
- The tuberculin skin test is still the first-line test for screening immunocompromised populations for latent TB infection
- If the patient is unlikely or unwilling to return for reading of the tuberculin skin test, an IGRA may be considered
- A negative IGRA in an immunocompromised individual does not exclude either latent infection or active disease.

<u>Conclusions</u>

IGRAs represent a significant new development in TB diagnostics, but also a challenge for a healthcare system with limited resources. Targeted use of IGRAs in the public health setting may provide significant benefits. These guidelines should be interpreted in

the context of local expertise and resources, and will evolve as scientific knowledge of IGRAs continues to evolve. As always, clinical judgment, accompanied by expert consultation where appropriate, is vital in the use and interpretation of any new test.

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Quick reference table for recommended use of IGRAs. "+" indicates that the test is recommended for use in a given setting.

Population	TB skin test	IGRA	Comment
Suspect adult active TB			Culture diagnosis best
Suspect child active TB		May be helpful	Culture diagnosis best
Targeted testing for latent TB infection	+	Acceptable, not preferred	TB skin test preferred in most settings
Contact investigation	+	+	IGRA best in high-risk individuals unlikely to return for read, especially paired with HIV testing
Serial testing (healthcare workers, others)	+		May be helpful as "confirmation" of a positive skin test in otherwise low-risk, BCG-vaccinated persons unlikely to take latent TB treatment otherwise
Children < 5 years old	+		May be helpful in BCG vaccinated child < 5 who has not lived in endemic country and who has no documented TB exposure
Immunocompromised patients (latent TB screening)	+		
Screening of low-risk adults and older children (5+) at sites where tuberculin skin testing is not commonly performed		+	