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CHEST Recent Advances in Chest Medicine

# Recent Advances in Testing for Latent TB

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**After more than a century of relying on skin testing for the diagnosis of latent TB infection, clinicians now have access to blood-based diagnostics in the form of interferon γ release assays (IGRAs). These tests are generally associated with higher sensitivity and specificity for diagnosis of latent TB infection. This article reviews the indications for testing and treatment of latent TB infection in the overall context of a TB control program and describes how IGRAs might be used in specific clinical settings and populations, including people having close contact with an active case of TB, the foreign born, and health-care workers.** ***CHEST 2010; 138(6):1456–1463***

**Abbreviations:** BCG = bacille Calmette-Guérin; CDC = Centers for Disease Control and Prevention; ELISPOT = enzyme-linked immunospot; IFN = interferon; IGRA = interferon γ release assay; QFT = quantiferon; TST = tuberculin skin test

Exposure to a person with infectious TB can result in one of three outcomes: the innate immune system can clear the infection immediately, leaving no evidence of exposure to TB; *Mycobacterium tuberculosis* organisms can begin to proliferate immediately, causing so-called primary TB; or the growth of organisms can be controlled or contained but not stopped by the host patient's immune response, setting up a state of latent infection. In this state, viable organisms are contained intracellularly within macrophages and within granulomas.[1] People with latent TB are at risk for active TB disease. Lifetime risk of active TB in patients with latent infection, as detected by means of a tuberculin skin test (TST), is estimated to be in the 5% to 10% range, with roughly one-half of that risk occurring in the first year or two after latent infection develops. In addition, the risk of development of reactivation is greater in people with medical conditions associated with immunosuppression, such as infection with HIV or treatment with drugs such as corticosteroids or tumor necrosis factor antagonists.[2]

In the United States, treatment of latent infection is a key component of the overall public health plan for controlling TB.[3] Cases of active TB in the United States have fallen steadily since 1992, and the overall rate of TB in this country now stands at 4.3/100,000, the lowest prevalence that has been recorded here.[4] The sharp decline in TB in the United States over the last several years has largely come about because of efforts aimed at diagnosing and treating patients with active disease, thereby reducing transmission and secondary cases. However, if TB is to be eliminated in this country (a stated goal of the US Public Health Service), there is no question that efforts in treating latent TB must be strengthened.[5] A recent study estimates that there is a reservoir of at least 20 million people in this country with latent TB infection, and without treatment, a large number of them will progress to active disease.[6] Furthermore, the majority of cases of active TB in this country now occur in people born outside the United States, and treating latent infection in recent immigrants from high-prevalence countries is the only way to prevent cases of active disease from developing.

For the past several years, the US Centers for Disease Control and Prevention (CDC) has emphasized

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targeted testing for latent TB, and European public health authorities have taken a similar approach[7,8]: Most people never need to be tested because they are at very little risk of having latent infection or, if they have latent infection, are at low risk for active TB. Thus, from a public health standpoint, the only people who should be tested for latent infection are those who would be treated if they were in fact found to have latent infection.[9] The list of such people is fairly circumscribed. It includes recent (within 5 years) immigrants to the United States from high-prevalence countries; people who are significantly immunosuppressed, including those with HIV infection and people being treated with tumor necrosis factor-α inhibitors, prolonged high doses of corticosteroids, or agents with similar effects on T-cell function; close contacts of people with active TB; and people with evidence of recent (within 2 years) infection with *M tuberculosis*, such as health-care workers who are tested annually and who have a newly positive test for latent infection. In addition, others with latent infection and medical conditions that increase the risk of reactivation, such as those with poorly controlled diabetes mellitus or end-stage renal disease, should also be considered for treatment. In this sense, a decision to test for latent TB infection is a priori a decision to treat if the test is positive.

## Tests for Latent TB Infection

The TST was first developed by Charles Mantoux in 1908,[10] and with relatively little modification, it has become the standard means of assessing the presence of TB infection ever since. In this test, purified protein derivative, a mixture of hundreds of antigens released by *M tuberculosis* grown in culture, is injected intradermally, and 48 to 72 h later the site of injection is inspected for induration indicative of prior TB exposure. A positive TST is an example of a type-4, delayed-type hypersensitivity reaction: Antigens injected intradermally are taken up by professional antigen-presenting cells in the skin and presented on their surface. If there has been prior exposure to TB, memory T cells bind the antigens and stimulate an inflammatory response characterized by the local secretion of various cytokines, most importantly interferon (IFN)-γ, and the characteristic induration results.[11] Chances that a TST will have a false-negative result are increased when a patient is immunosuppressed for any one of a variety of reasons.

Although the TST has been useful tool for more than a century, it has several biologic and operational limitations.[12] The intradermal injection must be administered properly, the patient must return for the TST reading, and the measurement of the induration must be done correctly. A substantial percentage of the time, one or more of these things does not happen,[13-17] and as a result no meaningful or interpretable result, or a false one, is obtained 10% to 50% of the time.

Biologic limitations occur through confounding of results because of prior bacille Calmette-Guérin (BCG) vaccination or exposure to non-TB mycobacteria.[18] The BCG vaccination is administered to 80 to 100 million people per year in the world, usually within the first week of life. This vaccine is derived from *Mycobacterium bovis*, a *Mycobacterium* that is closely related genetically to *M tuberculosis,* and many of the antigens from *M tuberculosis* that are found in purified protein derivative are also expressed by *M bovis* and the vaccines strains of BCG. Thus, there may be false-positive TST results in patients with prior BCG vaccination. Exactly how often this happens is difficult to know with certainty because there is no true gold standard for diagnosis of latent TB infection. (This is a major impediment to a rigorous evaluation of new tests.) It is true that most people who receive the BCG vaccination within the first few days of life but who never have exposure to TB will become TST negative as adults.[19] Partly for this reason, the CDC recommends that the TST be interpreted without reference to prior BCG vaccination. However, it is also undeniably the case that the BCG vaccination can confound TST results in many people, particularly when two-step testing is used, as is the case with health-care workers who undergo annual testing. Overall, it is estimated by two recent, comprehensive, and separate reviews and metaanalyses by Menzies et al[20] and Diel et al [21] that the sensitivity of using the TST for detection of infection with *M tuberculosis* is just >70% and the specificity is roughly 66%.

## Newer Tests for TB Infection: IFN-γ Release Assays

Developments in basic immunology and microbiology have allowed the development of blood tests that are theoretically capable of replacing the TST.[11] There are two commercially produced interferon γ release assays (IGRAs) now licensed for use in the United States and in many other countries: QuantiFERON (Cellestis, Inc; Brisbane, Australia) and TSPOT.TB (Oxford Immunotec, Inc; Oxford, England). These tests share the same fundamental approach to detection of TB infection, though with slightly different platforms. Both tests use antigens that are expressed by *M tuberculosis* but not by BCG strains of *M bovis* to stimulate the production of IFN-γ from peripheral blood mononuclear cells. Both

assays require a phlebotomy to obtain a blood sample that is centrifuged so that the peripheral blood mononuclear cells can be stimulated with antigen, and both require incubation after antigen stimulation before IFN-γ production is measured. Both tests employ a mitogen-induced positive control. The quantiferon (QFT) assay measures IFN by enzyme-linked immunosorbant assay, and results are expressed in IU of IFN/mm$^3$. The TSPOT.TB assay uses an enzyme-linked immunospot (ELISPOT) technique, and results are expressed as the number of cells (spots) in the peripheral blood mononuclear cell fraction that are producing IFN-γ. Results from both tests are available 12 to 18 h after a blood sample is obtained and are reported as positive, negative, or indeterminate/borderline, using cutoff values specified by the US Food and Drug Administration, although quantitative results could theoretically be reported as well. Large clinical experience indicates that borderline or indeterminate results, usually the result of a technical problem in the laboratory or failure of the positive control to stimulate IFN-γ production, occur only about 2% to 4% of the time in good laboratories, so that meaningful results are obtained $>96\%$ of the time with both tests.[22]

There are many operational advantages to the IGRAs: There is no need for a return visit by the patient to obtain a result from the test, and quality control in the laboratory ensures that a meaningful result will be obtained nearly all the time. In contrast, the TST is associated with a high percentage of nonmeaningful or false results due to operational issues alone. Many patients will never return for a TST reading, and errors in the intradermal injection technique and in measuring the amount of induration can result in many false-negative and false-positive tests. For these operational reasons alone, 20% to 40% of TSTs probably fail to provide a meaningful result. The chief operational limitation posed by the IGRAs is their cost, which is substantially more than a TST. However, most studies suggest that IGRAs can be cost effective when used in the context of a large TB control program.[23-29]

The presumed biologic advantages of the IGRAs have been addressed somewhat indirectly by research studies in this field. As noted previously, the lack of a gold standard for diagnosis of TB infection prevents a simple head-to-head comparison of the IGRAs and the TST. In light of this, the most efficient and meaningful assessment of these tests would involve assembling relevant cohorts of people (eg, contacts, immunosuppressed people, health-care workers), testing them for TB infection, and observing them over time, untreated, to determine which assay most accurately predicts in which people active TB will develop. Because active TB will never develop in most people with latent infection, such a study design would answer the most important question: What is the most accurate way to identify the people with latent infection in whom TB will actually develop? However, the logistical and ethical issues raised by such a study design are complex, and no such large study has yet been done.

In the absence of a direct evaluation of the IGRAs, most studies have taken similar approaches to assessing the operating characteristics of these tests. Sensitivity is generally estimated by comparing the tests in patients with active, culture-proven TB, though with the caveat that the state of one's immune system in the setting of active disease may very well be different than in the setting of latent infection. Specificity is generally estimated by comparing results of the various tests with the prevalence of characteristics felt to be associated with a high likelihood of TB infection, such as contact with a person with active TB.[20]

Systematic, separate reviews of the operating characteristics of the IGRAs and the TST by Menzies et al[20] and Diel et[21] al have yielded estimates of sensitivity and specificity of roughly 71% and 66% for TST, with much higher estimates for IGRAs. The second-generation QFT-Gold has been estimated to have a sensitivity and specificity of 76% and 97%, respectively, while the third-generation QFT In-tube assay, now in wide use, has a better sensitivity, probably about 85%, when used in developed countries. The TSPOT.TB test has a sensitivity estimated to be about 88% to 90% and a specificity of roughly 88% to 92%.

The effects of the superior operating characteristics of the IGRA tests can be seen in the following example: In the United States, latent TB is present in just under 20% of the non-US-born population, according to a recent study. If 1,000,000 of those people were tested with a TST and the sensitivity and specificity were as stated previously, there would be 140,000 true-positive test results, but 280,000 false-positive test results (Fig 1). The positive-predictive value of the TST would be only 33%, and the negative-predictive value would be 89%. Overall test accuracy (the number of all true-positive and true-negative test results divided by the total number of test results) would be only 66%. On the other hand, if a test had a sensitivity and specificity of 84% and 99% respectively, as the IGRA tests have, and the same 1,000,000 people were tested, there would be 168,000 true-positive test results and only 8,000 false-positive results (Fig 2). The positive predictive value of the test would be 95%, and the negative-predictive value would be 96%; overall test accuracy would be 96%.

The reduction in the number of false-positive tests for latent TB is far from trivial. Fewer patients will require physician examination, chest radiography,

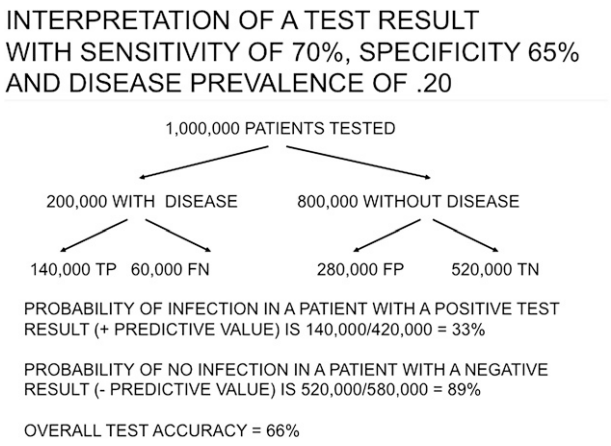


FIGURE 1. Interpretation of a test result with sensitivity of 70%, specificity of 65%, and disease prevalence of 0.20. FN = false-negative test results; FP = false-positive test results; TN = true-negative test results; TP = true-positive test results.

and preventive therapy with isoniazid. This will lead to substantial cost savings that should make IGRAs cost effective in most settings. Further, the number of people who need monitoring of liver function and in whom clinical (and occasionally fatal) drug-induced hepatitis develops will be sharply reduced.

Overall, the operational and biologic advantages of IGRAs should lead to their widespread adoption and the replacement of the TST in nearly all settings. The CDC has issued guidelines that state that IGRAs can be used in all situations in which the TST is currently used.[30] Several large public health systems, such as the New York City Department of Health and Mental Hygiene and the San Francisco Department of Health, have replaced the TST with IGRAs in nearly all circumstances.[29] In many other countries, including Canada and the United Kingdom, national guidelines suggest using IGRAs only to confirm a positive TST. These recommendations are made mainly on the basis of cost, however.

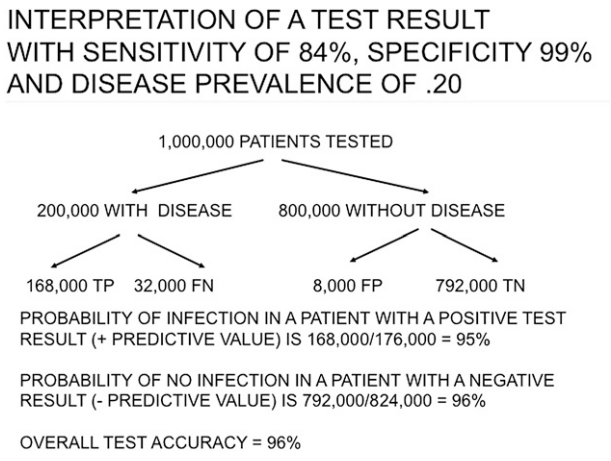


FIGURE 2. Interpretation of a test result with sensitivity of 90%, specificity of 95%, and disease prevalence of 0.20. See Figure 1 legend for expansion of the abbreviations.

## USE OF IGRAS IN SPECIFIC SITUATIONS AND POPULATIONS

### *Foreign-Born People*

As noted previously, there is now great emphasis on targeted testing of particular populations for latent TB infection, rather than widespread testing in unselected populations. One such targeted population is foreign-born people from high-prevalence countries because people in this group now account for more than half of all cases of active TB in the United States. How do IGRAs perform in these people? Because many, if not most, of these people have received the BCG vaccination, one would predict that IGRAs would be vastly superior to the TST in this population, and this in fact seems to be the case.

Kang and colleagues[31] in South Korea conducted a study using the TST and the QFT-Gold assay in four groups of people in that wealthy, industrialized country, in which most people have received the BCG vaccination. They studied people with no history of TB exposure, casual contact with a person with active TB, close contact with a person with active TB, and patients with active TB themselves. The TST was positive in 51%, 60%, 71%, and 78% of these people, respectively. The QFT yielded a positive result in 4%, 10%, 44%, and 81% of people, respectively. Thus, the QFT results showed much greater discrimination than the TST results among various epidemiologic risk categories for TB infection. In addition, the QFT performed as well or better in patients with active TB.

Brodie and colleagues[32] compared the TST with the TSPOT.TB test in a largely immigrant population with a high rate of prior BCG vaccination in New York City. They found that overall the odds that a positive TSPOT.TB result was associated with a high epidemiologic likelihood of having TB infection (assessed in this study as being a close contact of a person with active TB) was 2.9, whereas the odds of the TST being associated with an epidemiologic likelihood of true TB infection was 0.5. Thus, not only was the OR lower for the TST, it was on the other side of 1, meaning that the TST had, if anything, an inverse correlation with the epidemiologic risk of TB infection. In countries such as the United States, with a low prevalence overall of active TB, there is no question that IGRAs are superior in testing for latent infection in foreign-born people with a history of prior BCG vaccination.

### *Contacts*

People who have contact with patients with active TB receive very high priority for testing for latent infection because people in this group are at high risk for active TB. The San Francisco Department of Health started using IGRAs routinely for contact investigations in 2005, and now many health departments

in the United States have adopted IGRAs as screening tests for contact investigations. A variety of reasons were responsible for the somewhat slow adaptation of the tests in clinical practice. The requirement for a phlebotomy has been an issue for some public health workers who believed it required more skill than TST placement. Some public health authorities initially also had concerns about using a test that might be less sensitive and could potentially miss some cases of true infection or even possibly some people with active disease, though nearly all evidence from low-prevalence countries indicates that these tests are as sensitive and are likely more sensitive than the TST. Cost has been another consideration for public health departments whose budgets are strapped.

Several studies using IGRAs in contact investigations have now been reported. A large contact investigation in the Netherlands (a country with a low prevalence for active TB) for a person with smear-positive pulmonary TB who worked in a supermarket included 20,000 people.[33] An analysis of a random sample of this group showed that greater contact to the person with a source case was associated with a positive IGRA but was not associated with a positive TST. The authors of this study believed that the test was useful because it identified people with recent exposure. The blood-based IGRA tests had a lower sensitivity than the TST, but this potentially was because the IGRAs might have identified those with recent exposures, while a positive TST might reflect both recent and distant exposure. The authors of this study believed that the test was highly advantageous to use because the population that was evaluated for latent TB infection had a high rate of BCG vaccination.

Other studies have shown similar results, suggesting that more time spent close to the person with the source case or identification as a close contact is associated with a positive IGRA. In the study by Ewer and colleagues,[34] a contact investigation was done in a school where the time spent in close proximity to the person with the source case was carefully determined for all children in the school. All children were tested with a TST (in this case, the Heaf test, a version of the TST used in Great Britain) and an ELISPOT assay for IFN-γ production. The ELISPOT results, but not the TST results, correlated well with the amount of time that contacts had spent in close proximity to the person with the source case. The only thing that predicted a positive TST was a prior BCG vaccination.

The majority of people with a negative IGRA after an exposure to active TB do not have TB infection and do not require further testing. However, the immune reaction to TB can take weeks to develop so that like the recommendations for TST testing, the IGRA should be repeated 8 weeks (postwindow) after the last exposure to the active source case to rule out infection.

One approach used by public health systems in Canada and the United Kingdom has been to continue the use of the TST as an initial screening test, and only people with a positive result are given an IGRA test. This approach has not been accepted in the United States. Some experts have voiced concerns that a TST could boost the immune system and lead to a false-positive IGRA test. However, a recent study demonstrated that if the IGRA is performed within 3 days of the TST, no boosting occurred.[35,36]

A limitation of tests for latent TB infection is that they do not accurately predict which patients will progress to active TB disease. Active disease will develop in only about 5% to 10% of contacts infected with TB, but currently there is no way to distinguish these patients. There is some evidence that IGRAs are very useful in identifying people with infection after a recent contact and exposure to a person with a source case, but may not identify people with remote infections.[37] This is theoretically an advantage of the IGRAs because people with recent infection are at the highest risk for the disease and therefore need evaluation for possible anti-TB therapy. There is some evidence that the IGRAs will have a higher predictive value than the TST for identifying those contacts who will progress to active TB after an exposure. In one important study, Diel and colleagues[38] observed a cohort of close contacts who progressed to active TB over a 2-year period. Many of the contacts refused isoniazid preventive therapy, creating an interesting and valuable natural history experiment. Contacts were tested with both a TST and a QTF-gold-in-tube assay. Six contacts out of 41 identified as infected by means of a positive QFT assay progressed to active TB during the follow-up period, whereas only five out of 219 identified as infected with a TST progressed, and the QFT assay missed none of the positives picked up by the TST. Thus, the rate of progression to active TB was much higher (14.6% vs 2.3%) among people with a positive IGRA than with a positive TST. If this is borne out by future studies, it will prove a tremendous advantage for IGRAs over the TST.

### *People Infected With HIV*

TB is the leading opportunistic infection to cause death in people infected with HIV, and treatment of latent TB with isoniazid in people infected with HIV significantly reduces the risk of active TB. Patients infected with HIV and latent TB receiving antiretroviral therapy still have a significantly increased risk of progression to active disease compared with people who are not infected with HIV. A sensitive and accurate test to readily identify latent TB in people with HIV infection would greatly assist in the primary goal in

TB control of identifying and preventing the further spread of the disease.

The TST has significantly decreased sensitivity in people infected with HIV who have advanced immunodeficiency. The sensitivity of the TST is so low that recommendations from the CDC are to treat close contacts who are HIV infected despite the results of the TST. IGRAs are theoretically limited by the same inhibition of the immune system. Several studies have shown that IGRAs are less sensitive in patients who are immunocompromised compared with those who are not immunocompromised.[39] However some studies have shown that IGRAs (particularly the TBSPOT.TB assay) might be more sensitive than the TST in detecting latent TB infection in people who are HIV infected with severe immune suppression.[40-47] A study in South Africa demonstrated that patients with very low CD4 counts who had recently been treated for active TB had high rates of response using the TSPOT.TB test. This study suggested that the TSPOT.TB has utility even in a population that had a mean $CD4^+$ T-cell count of 98.[48] In a South African population with a high TB prevalence, the TST was compared with the T-SPOT.TB and the QTF-gold-in-tube tests. While a lower proportion of TST tests were positive in the subjects who were HIV infected, the blood-based IGRA tests were not affected by HIV status. This study suggested that the sensitivity of the IGRAs was not significantly impaired by HIV status.[49]

Finding a test with a high negative-predictive value would be important for TB control programs so that just those patients truly needing therapy for latent TB infection could receive it. A longitudinal study from Austria published in 2009 followed 830 patients infected with HIV for a median of 19 months. At baseline, eight patients had active TB, of whom seven had positive QFT results. Progression to active TB occurred in three additional patents among the remaining cohort, all with positive baseline QFT results; during the period of observation, active TB did not develop in any of the 738 patients with an initial negative test.[50] Overall, the QFT was positive in 10 of 11 (90.0%) patients in whom cases of TB were diagnosed in this study.

Both available IGRA tests have shown an increase in indeterminant results in patients with immune system compromise. In most cases, this is probably analogous to a finding of anergy in patients undergoing the TST. Currently, there are no recommendations on how to interpret repeatedly indeterminant results, though making a decision about treatment with isoniazid based on assessment of epidemiologic risk (eg, status as a close contact) seems sensible in these cases.

### *Other Populations of Interest*

Two other populations deserve mention: children and health-care workers. Most of the published data support the use of IGRAs in place of the TST in children, although there is still a paucity of data in children under the age of 2 years.[51] Cellular immune function is not as well developed in very young children, and some have expressed concern that any single test may be insensitive in this population.[52] Several relatively small studies suggest that IGRA performance in children generally is good and compares favorably with the TST.[53-56] Notably, the study by Bakir and colleagues[55] assessed the ability of the ELISPOT assay to predict the future development of active TB in children and adolescents who were contacts to people with active TB. The rate of active TB that developed was 21/1,000 person-years in children with a positive ELISPOT, which is higher than the rate of 17/1,000 person-years in children with a positive TST. There is little reason to think that the tests would be inferior to the TST in these children, and though some have counseled caution in adopting them, others have recommended their use more generally.[57,58]

The major issue in the use of IGRAs in health-care workers involved the variability of IGRA results over time, since these workers will generally be tested once a year. It is possible that small, inevitable fluctuations in IGRA results, say from 0.32 IU/$mm^3$ to 0.36 IU/$mm^3$ in a QFT result or from five spots to eight spots in a TSPOT.TB, could be mistakenly interpreted as conversions from negative (no latent infection present) to positive (latent infection present) when no true infection has occurred. Such a phenomenon has been reported in one study of household contacts, for example.[59] At present, most published data on the use of IGRAs in health-care workers seem reassuring, and many health systems have adopted these assays.[23,60-63] A large study of 2,500 health-care workers followed with the TST and both commercially available IGRAs every 6 months for 18 months is currently being conducted by the CDC-funded Tuberculosis Epidemiologic Studies Consortium, and the data will be extremely valuable in assessing the use of these tests in this population. It may well be that the definition of a conversion when using an IGRA may need to involve more than moving from one side of the cutoff value to the other. Perhaps a conversion will be defined as an absolute increase of a certain amount or some relative increase (eg, a twofold increase) over the baseline test results.

## Conclusions

IGRAs represent the first significant advance over the TST for the detection of TB infection in over 100 years. These tests offer major operational and

biologic advantages over the TST, and on a large scale, because of their greatly increased accuracy, their use could be cost saving and should dramatically reduce the number of patients treated inappropriately for latent TB infection. Cost savings may accrue despite the price of any given IGRA because of a greatly reduced need for physician evaluations and chest radiographs in people previously falsely identified as infected. In addition, fewer people will be started on isoniazid preventive therapy, and though isoniazid is inexpensive, the costs of monitoring in some patients and the cost of the occasional case of serious or life-threatening isoniazid hepatotoxicity can be substantial. These assumptions should be carefully analyzed in future studies. CDC guidelines recommend use of these tests widely, and this should be of great benefit both to individual patients and to national TB control efforts generally.

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