Since the first study on IFN-γ release assay (IGRA) serial testing for new latent tuberculosis infection, published over a decade ago (1), a large number of studies, in high as well as low tuberculosis (TB) incidence settings, have shown high rates of IGRA conversions and reversions (2). Although IGRA converters have been shown to have a higher risk of active TB than those who remain test negative on repeat testing (3, 4), the prognosis of those with uncertain or unstable conversions has remained a puzzle. In this issue of the Journal, Nemes and colleagues (pp. 638–648) provide some long-awaited data on the prognosis of uncertain conversions with the QuantiFERON-TB Gold In-Tube assay (QFT; Cellestis/Qiagen, Carnegie, Australia) (5).

Soon after QFT was approved by the U.S. Food and Drug Administration (FDA) in 2005 and endorsed by the Centers for Disease Control and Prevention as a replacement for the tuberculin skin test (TST) for baseline and serial testing (6), many North American health systems adopted QFT for annual screening of low-risk healthcare workers. QFT was an attractive choice because of its logistical advantages and the expectation that it would prove more sensitive and specific than TST. Contrary to expectations, soon after its implementation, studies began to report unusually high conversion rates with QFT in low-risk healthcare workers compared with concurrent or historical TST rates (2). The uncertainty of conversions was rooted in the observation that the majority of converters would revert upon repeat testing and that most conversions had a low positive concurrent or historical TST rates (2). The uncertainly of conversions with the QuantiFERON-TB Gold In-Tube assay (QFT; Cellestis/Qiagen, Carnegie, Australia) (5).

As shown in Table 1, sources of IGRA variability can be broadly classified as preanalytical (i.e., blood collection and handling), analytical (i.e., laboratory testing), postanalytical (i.e., laboratory reporting), manufacturing (i.e., defective reagents), and immunological (i.e., boosting and modulation) (10). These sources can either increase or decrease IFN-γ responses and cause false-positive and false-negative results, respectively.

Whereas some sources of variability are systematic and predictable, and therefore can be mitigated with assay standardization as previously proposed and exemplified by Nemes and colleagues in their study, other sources of variability are random and therefore cannot be avoided (10). Assuming that systematic sources of variability can be completely eliminated through standardization, the existence of random sources demands creation of a zone of uncertainty (also known as a borderline zone) around the assay cutoff to account for uncontrollable sources of error and interpret the borderline result as uncertain-positive to indicate that such result could be a true-positive or a false-positive. This indeed is the case with the other commercial, FDA-approved IGRA, the T-SPOT.TB assay (Oxford Immunotec, UK), which includes a borderline zone as part of its interpretation. It is puzzling why the FDA did not demand a similar borderline zone for the QFT assay, including the recently FDA-approved version called QuantiFERON-TB Gold-Plus.

Because the QFT assay does not include a borderline zone, researchers and laboratory implementers were forced to develop their own borderline zones to make sense of variations in test results. So far, they have either arbitrarily defined a borderline range or experimentally defined it based on a single source of random variability. For example, Metcalfe and colleagues showed that the ELISA performed to measure IFN-γ can randomly fluctuate QFT results by as much as ±0.24 IU/ml when the initial result is between 0.25 and 0.80 IU/ml (11).

For the first time, Nemes and colleagues provide clinical evidence on the prognosis of uncertain QFT conversions in a South African cohort study. By categorizing individuals undergoing short-term serial testing as stringent nonconverters (i.e., IFN-γ level <0.2 IU/ml at Day 0 and Day 360), stringent converters (i.e., changed from <0.2 IU/ml at Day 0...
to >0.7 IU/ml at Day 360), and uncertain converters (i.e., <0.35 IU/ml at Day 0 and ≥0.35 IU/ml at Day 360, with at least one result within 0.2–0.7 IU/ml), they showed that stringent converters but not uncertain converters had a significantly higher TB incidence rate than stringent nonconverters (stringent nonconverters vs. stringent converters, 0.16 vs. 1.60 cases/100 person years, \( P = 0.0003 \); stringent nonconverters vs. uncertain converters, 0.16 vs. 0.66 cases/100 person-years, \( P = 0.229 \)). This relationship remained true when uncertain conversion was defined as a change in IFN-\( \gamma \) response from <0.2 IU/ml at Day 0 to 0.2–0.7 IU/ml at Day 360.

As one would expect, uncertain converters did have a higher TB incidence rate than stringent nonconverters, indicating that some individuals with borderline conversions had true infections and others had false conversions, most likely due to sources of variability. This study is in alignment with prior studies showing that higher QFT responses are both more stable (i.e., less likely to revert) (7, 8) and more predictive of active TB (3, 4).

What are the implications for clinical practice? The study by Nemes and colleagues, along with other studies on serial IGRA testing (2), as well as studies on reproducibility of IGRAs (10), collectively provide us a sound basis to revise existing guidelines on serial IGRA testing (12). Given the extensive evidence base, we must advocate for a more nuanced interpretation of IGRA results, especially in the context of serial testing and in low-risk individuals. Simplistic definitions of IGRA conversions, such as a change from negative to positive, are not defensible in light of current evidence. We also hope that Qiagen, the manufacturer of QFT, will formally introduce a borderline zone to ensure better interpretation of the assay.

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References