Whole blood IFN-γ assay for detecting TB in children

Connell *et al*[1] uses the QuantiFERON-TB Gold (QFT-Gold) test for tuberculosis (TB) infection, but it contains methodological inaccuracies. Unfortunately, the interpretation of the test by Connell *et al* was not that recommended by the manufacturer. The authors arbitrarily introduced a restriction on the nil control of the QFT-Gold test to <1 IU/ml interferon (IFN)-γ, falsely generating a number of "failed" tests. As a result, the data presented by Connell *et al* significantly overestimate the number of invalid test results and may be misleading to readers.

The authors also present results that attempt to correlate increasing IFN-γ responses to mitogen (up to 80 IU/ml) with age by extrapolating data beyond the range of the QFT-Gold ELISA. In addition, Connell *et al* have attempted to compare TB antigen induced IFN-γ levels with disease state. This assumption may or may not be accurate but, as stated in the QFT-Gold package insert, IFN-γ levels above 15 IU/ml cannot be relied upon as they are extrapolations outside the operational range of the instrumentation. All diagnoses of TB infection and identification of low immune function are within the linear range of the test. As such, the observations of Connell *et al* of an age associated mitogen response and a correlation between IFN-γ levels and TB disease cannot be made from this study.

Connell *et al* further express concerns on tuberculin skin test (TST) positive—even strongly TST positive—individuals who are QFT-Gold negative. This reflects a common misconception that the TST is a definitive diagnostic rather than an indicator of increased risk of TB infection. Large scale studies show that, for most populations, progression to active TB is low, even for large TST responders, and far below the often quoted 5–10%.[2–4] Because progression rate is an indicator of the true infection rate,[5] it can be concluded that many—if not most—TST positive individuals are not truly infected, independent of TST size.[5 6] Stratified TST interpretation guidelines, however, merge independent history risks (TB contact, birth country, HIV status, etc) with the diagnostic risk of size of the TST response into a simplistic positive/negative outcome, whereas appropriate interpretation may be increased risk/lower risk. In contrast, the high specificity of QFT-Gold allows a more definitive result of positive/negative, independent of risk and, as a more accurate test, we would expect it to disagree with many TST positive results, especially in BCG vaccinated individuals.

The bias of stratified interpretation of the TST emerges in Connell *et al*'s examination of TST responses, classifying children with known TB contacts as "infected" at >5 mm TST (independent of BCG), but using 10 mm or 15 mm (depending on BCG) to define infection in those without known contact. Superficially there seem to be more "TST positive" children (60%) in the known contact group and more "TST uninfected" children in the lower contact groups, reinforcing conventional wisdom. Yet, in reality, this distribution reflects little deviation from the total population of Connell *et al*, where 60% of all subjects are TST >5 mm; the high TST positivity rate in the "contacts" represents little more than a random sample of the study population at this cut off. The reverse applies for "unknown contacts" using a 10 mm or 15 mm reaction (fig 1).

Any new test must disagree with the TST to have prognostic value. Reasons for discordance are many and beyond this letter, but are not limited to small TST reactions. The level of disagreement varies with the true TB risk and TST size, and the TST and QFT-Gold tests are highly concordant in populations of high TB risk,[8] in the absence of BCG,[9] or serial conversion.[10] Sensitivity can be seen in detecting TB infection leading to disease in HIV infected individuals[11] and in numerous studies correlating QFT-Gold positives to exposure. Indeed, Connell *et al* detected all nine active TB cases and, in a separate study, two more active cases where the TST was negative.[12] As most QFT-Gold positive children were in the known contact group, this indicates sensitivity and specificity. High specificity of using RD-1 antigens with effector T cell detection directs QFT-Gold to detecting ongoing *Mycobacterium tuberculosis* infection which, at 24% of the children tested, is alarming enough, particularly as the reaction was highly associated with active disease and risk of exposure. While persons with QFT-Gold positive responses do progress to active TB disease,[11 13] Connell *et al* are correct in noting it is the prediction of future active disease that will decide accuracy.

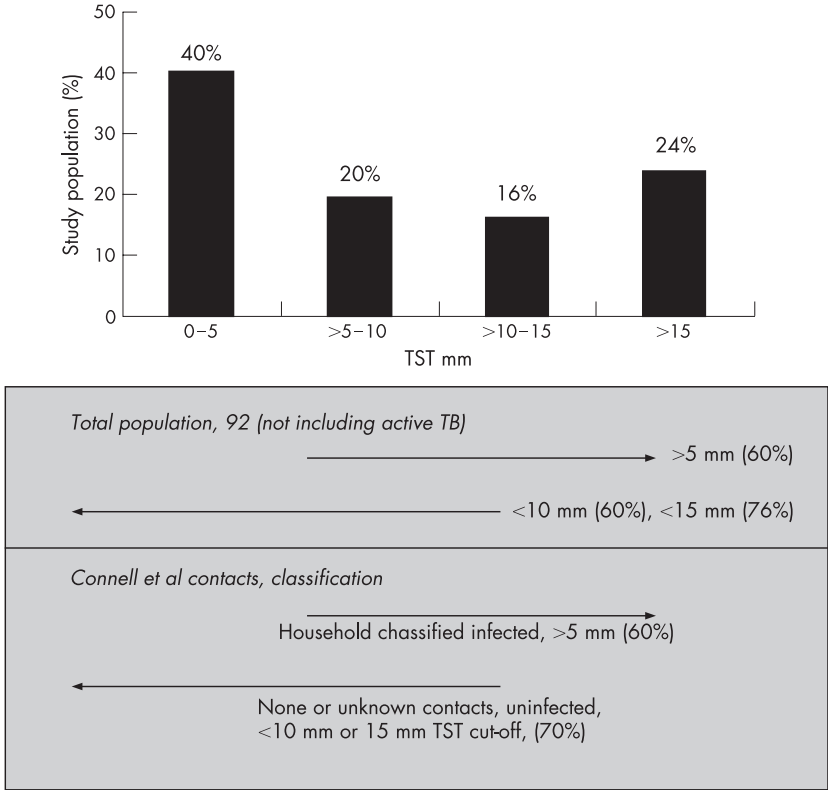

Figure 1 High and low risk groups in the study by Connell *et al*[1] compared with the total study population.

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Authors' reply

We stand by the results and conclusions of our study.[1] Radford and colleagues on behalf of Cellestis Ltd (who manufacture the assay used in our study) misinterpret the aim of the study, fail to mention the extent of the company's involvement in discussions about interpretation of high background levels, and misunderstand the importance of the identification and treatment of children with latent TB infection.

Dr Radford's main complaint—that we falsely generated indeterminate test results by deviating from the manufacturer's recommendations—is surprising. Far from being ''arbitrarily introduced'', the decision to categorise tests with a high nil (negative) control above 1.0 IU/ml interferon (IFN)-γ as ''indeterminate'' was made by the state Mycobacterium Reference Laboratory after discussion with Cellestis Ltd. The results for the children in our study comprised a fraction of the thousands of QFT-G tests reported by this laboratory, which undertook the assays in accordance with the manufacturer's instructions using interpretation guidelines that were valid at the time. The assay's package insert from June 2003 states that ''*under normal circumstances the nil control will not generate IFN-γ levels above 1.0 IU/ml*''. After confirmation from Cellestis Ltd that nil controls above 1.0 IU/ml ''should not occur'', the laboratory reported these assays as indeterminate.

A high nil control is problematic as a negative value may be produced after correction (table 1). Moreover, there are many potential explanations for high background IFN-γ levels that might interfere with the integrity of the assay, and a large body of literature supports exercising caution in interpreting immunological tests with high negative controls. There have been no published studies investigating this phenomenon or its effect on the validity of the QFT-G assay.

The wisdom of reporting assays with unexplained high background levels of IFN-γ as indeterminate is corroborated by the inclusion of an even lower level (0.7 IU/ml) in criteria for defining an indeterminate result in guidelines recently produced by the CDC[2] and subsequently included in the latest US QFT-G package insert.[3] Using these criteria (established after our study was undertaken), only one of 12 children with an indeterminate assay resulting from a high nil control has an interpretable result (table 1). Therefore, overall, 16 (rather than 17) of 101 children still have indeterminate results, and our conclusion that a significant proportion of assays fail when used in routine practice remains unchanged.

We disagree with the assertion that discrepant results from tuberculin skin tests (TST) and the IFN-γ release assay are unimportant because only a small proportion of individuals with latent infection progress to TB disease. Aside from undermining the routine clinical practice of testing those at high risk, this claim ignores the fact that progression from latent TB infection is higher in children with up to 50% of infants and 15% of older children developing active disease within 2 years.[4] Despite its limitations, the TST predicts progression to active TB disease[5–7] whereas the predictive value of a positive QFT-G in children remains unknown.

Despite Dr Radford's comments, stratified interpretation of the TST is well established and accepted, and has been used in studies of the performance of QFT-G in adults to which Cellestis Ltd have not objected. Moreover, this strategy is used worldwide and is recommended by the American Academy of Pediatrics (AAP), the American Thoracic Society (ATS), the Infectious Disease Society of American (IDSA), and the Centers for Disease Control and Prevention (CDC).[5 8] Analysis using a non-stratified approach in any case has no influence on the outcome of our study.

The figure produced by Dr Radford is erroneous as a wrong denominator has been used. In fact, 24 (80%) of the 30 children with a household TB contact were TST positive, as shown in table 1 of our original paper. This proportion is significantly higher than the 55 children (60%) with a TST result >5 mm in the total population of 92 ($p<0.05$; χ^2 test). The fact that children with a TB contact history were more frequently TST positive supports the suggestion that the negative QFT-G tests in some children with latent TB identified by TST were false negatives.

Dr Radford offers no suggestion on how to manage the 31 children out of 42 at high risk of TB infection with a positive TST in whom the QFT-G result was negative or indeterminate. As detailed in our paper, there is evidence to suggest that many of these children had latent TB infection and we therefore treated them accordingly.

We are unable to comment on Dr Radford's interpretation of the study by Richeldi *et al*[9] which, for the most part, seems to refer to unpublished data and have little relevance to our study.

The TST is imperfect and, although we agree that a better test must necessarily give discrepant results, it does not follow that disagreement between the two tests when QFT-G is negative can automatically be attributed to a false positive TST. We highlighted examples where this was unlikely to be the case. Any false negative TST results in our study imply false negative QFT-G as there were no QFT-G positive/TST negative children. Our conclusion that, for the diagnosis of latent TB infection in children, there is poor agreement between QFT-G and TST, and that the assay may have lower sensitivity than TST in this setting, is valid. Similar findings have recently been reported by Ferrara *et al*.[10]

We were excited by the prospect of using QFT-G to guide the treatment of children at high risk of TB. However, our *independent* study revealed a disappointingly high failure rate in routine practice in children. More importantly, it suggests that a significant proportion of children with latent TB infection have a negative QFT-G assay and that it is premature to consider replacing TST in clinical practice.[2 11]

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Table 1 Results of IFN-γ release assays from the 12 patients out of 17 with tests categorised as ''failed'', in which the test failed as a result of a high nil control

Patient	Age (years)	Nil control (IU/ml IFN-γ)	ESAT-6 (IU/ml IFN-γ)	ESAT-6 – Nil (IU/ml IFN-γ)	CFP-10 (IU/ml IFN-γ)	CFP-10 – Nil (IU/ml IFN-γ)	Criteria 1*	Criteria 2*
1	14	23.48†	24.29†	**0.81**	24.23†	**0.75**	Indeterminate	Indeterminate
2	13	22.63†	36.27†	**13.64**	32.44†	**9.81**	Indeterminate	Indeterminate
3	6	19.74†	20.23†	**0.49**	20.25†	**0.51**	Indeterminate	Indeterminate
4	13	11.16	11	**−0.16**	10.95	**−0.21**	Indeterminate	Indeterminate
5	16	9.02	8.75	**−0.27**	9.51	**0.49**	Indeterminate	Indeterminate
6	8	8.02	7.17	**−0.85**	8.17	**0.15**	Indeterminate	Indeterminate
7	8	2.51	2.64	**0.13**	1.82	**−0.69**	Indeterminate	Indeterminate
8	2	2.19	2.48	**0.29**	2.54	**0.35**	Indeterminate	Indeterminate
9	12	1.58	3.01	**1.43**	2.32	**0.74**	Indeterminate	Positive
10	14	1.52	1.81	**0.29**	2.17	**0.65**	Indeterminate	Indeterminate
11	9	1.24	1.31	**0.07**	1.31	**0.07**	Indeterminate	Indeterminate
12	6	1.13	1.11	**−0.02**	1.05	**−0.08**	Indeterminate	Indeterminate

*Criteria 1: Current guidelines at the time of our study (see text). Criteria 2: Modified guidelines from manufacturer US package insert and CDC[2] which state that a test may be interpreted as positive when the background level of IFN-γ is above 0.7 IU/ml only if the response to the corrected ESAT-6 or CFP-10 result is at least 50% greater than the background level; otherwise the result is indeterminate.
†IFN-γ levels above 15 IU/ml cannot be measured accurately.