



Cellestis Inc
28005 North Smyth Drive
Valencia CA 91355
Ph: 661 295 4627
800 519 4627
Fax: 661 295 4625
quantiferon@cellestis.com
www.cellestis.com

Colonel Jeffrey D. Gunzenhauser
Preventive Medicine Staff Officer
Office of the Surgeon General, US Army
5109 Leesburg Pike
Falls Church VA 22041-3258

July 16, 2002

Dear Colonel Gunzenhauser,

RE: QuantiFERON-TB and AFEB Report (15-1a) 00-4 dated 12 May 2000

Following the approval on November 29 2001 of the QuantiFERON-TB test by the FDA for use in immunocompetent adults, and with the CDC guidelines shortly to be released, we have prepared a response to the AFEB memo noted above. In the meeting described in the May 2000 memo, the Armed Forces Epidemiology Board (AFEB) considered tuberculosis management in the Armed Forces in general and considered the requirements of a new test for tuberculosis infection.

The response by Cellestis detailed below considers those aspects of the report dealing with the requirements of a new tuberculosis diagnostic and the specific questions relating to QuantiFERON-TB. We have not addressed those questions pertaining to the efficacy of therapy for latent tuberculosis infection (LTBI), as this question is not within our remit to answer, and not pertinent to the proposal that QuantiFERON-TB be adopted by the Armed Forces. You will find attached, however, a report prepared by Dr M.Rene Howell of the Johns Hopkins University addressing a number of the issues raised with respect to the nature of personnel to be tested, and variations between service branches in diagnostic practices. A précis presentation of this report was presented at AFEB. Beyond the report of Dr Howell our answers relate specifically to Sections 3 e and f of the AFEB report.

You will note that the Howell report was prepared for CSL limited prior to acquisition of the technology by Cellestis and was not to our knowledge submitted to AFEB or JPMPG. As it is

two years old several cost estimates are less than current pricing, both with respect to the salary rates – I note E-3 pay rates are now nearly 20% higher -and also with respect to costs of materials. Nevertheless the survey of practices we believe is likely to reflect current practices, the general costs assumptions remain valid, and the benefits of QuantiFERON-TB both in tuberculosis detection and cost are clear in a number of settings. We would draw attention to instances where there is a failure to read the TST and the need for a repeated TST in several settings, all of which contribute greatly to costs.

The current list price for QuantiFERON-TB is USD\$10 per test for reagents. If adopted by the US military it is expected we shall negotiate an agreed military purchase price depending on expected volume.

AFEB committee believed QuantiFERON-TB to show promise for Armed Forces use due to the quantitative and objective nature, the single subject visit, and the reduction in false-positive reactions due to Non-Tuberculosis Mycobacterial sensitization. The FDA improvements to the diagnostic cut-offs significantly address specific issues raised by both AFEB and contributors to the Howell report with respect to immunological performance of QuantiFERON-TB. We would also draw attention to the paper of Mazurek et al in JAMA (Appendix 1) which describes the effect of BCG, sensitization to Non-tuberculosis Mycobacteria, and TST digit preference (the tendency to round to diagnostic cut-offs) , as further explanation of immunological differences between the TST and QuantiFERON-TB.

Finally, we would draw attention to the fact that the interferon release system is recognized as a standard for TB diagnosis in cattle worldwide, and is approved by the USDA and European authorities. In cattle the gold standard of kill, necropsy and tissue culture is available to determine TB infection status, and this has validated the accuracy of interferon release testing in cattle, the best model available for human tuberculosis.

Detailed Responses

3 e What are the priorities for research

- 1) The whole blood cell tuberculosis assay holds great promise as an alternative method for tuberculosis screening of military personnel. While the incidence of active tuberculosis is low, it has the potential to rise in the future due to peacetime operations in high risk settings, and the increasing number of foreign-borne persons who will be entering military service.***

This situation has not changed and has possibly increased since the events of September 11. Afghanistan, for instance, has possibly the highest active TB rates in the world.

3 e

- 2) At present, this assay is not licensed for use in the United States, and there are a number of questions which should be considered before it could be considered for general use in military populations. Observations at Great Lakes suggest that this***

test could significantly increase the number of recruits found to be infected, greatly increasing the need for effective preventive therapy.

The QuantiFERON-TB (QFT) test has now been approved by the FDA following a PMA application, with unanimous agreement by the panel review. The CDC is drafting guidelines for its use. The draft CDC guidelines, as presented by the CDC in a series of consultative meetings, suggest specific utility in lower risk populations, employment screening specifically including military screening, and also use in situations where serial testing may be used.

The test approved by the FDA has different diagnostic cut-off levels to that originally considered by AFEB (see Appendix 2, Clinicians Guide to QuantiFERON-TB). These changes were suggested by the FDA and FDA review panel to allow much greater specificity in low risk populations. The introduction of a single stratification level parallels the method used for the TST, although only two stratification levels (at risk and low risk) are used for the QuantiFERON-TB test. The effects of these changes are clearly seen in clinical trials results as reported in Section 10 of the Product Insert (Appendix 3). In low risk populations specificity is increased to 98% with minimum loss of sensitivity, similar to the TST, and QFT positive rates in high risk groups are also similar to the TST. Thus there is no *a priori* reason that preventive treatment rates may increase with military use of QFT, and use of QFT can maintain similar rates of preventive therapy .

In practice the use of QFT as an initial screen can be used to lower LTBI treatment rates considerably for the low risk populations that form the bulk of military screening for TB, with minimal effect on TB detection rates. Within lower risk populations neither the TST nor the QFT test can be considered to have high positive predictive accuracy due to the low numbers of truly positive infected individuals compared to the number of false positive reactors. Current practice, however, is to treat for LTBI on the basis of a positive TST, which leads to a large number of truly uninfected individuals receiving an unnecessary nine months of LTBI treatment with concomitant monitoring, follow-up, side-effects and personnel limitations.

To overcome this issue the use of QFT has been proposed as a preferred initial test for LTBI in low-risk populations. This provides an initial test for infection without affecting the immune system of the individual, unlike the TST that by its very nature is immunoreactive and compromises repeat testing either with TST or QFT. Prior to LTBI treatment initiation initial testing with QFT in low risk populations can be followed by confirmatory testing with the TST on the few per cent of QFT positive individuals, as QFT testing does not prejudice a later TST result. This practice will allow much more accurate identification of individuals before initiation of lengthy and tedious treatment regimen, greatly reducing the number on preventive therapy.

The risks of such a strategy are very low compared to the benefits. Studies in TB infected individuals and CDC studies in populations with a high incidence of LTBI show that a minimum of 2 in 3 individuals positive to the QFT are also positive to TST and vice versa (Mazurek et al, 2001). Furthermore, as QFT obtains results from nearly 100% of individuals with one visit, it thus finds individuals who would have failed to have a TST result due to failure to return or poor reading practices. Any risk of missing a case of infection, therefore, lies in the two possible discordant groups, which are;

QFT negative, TST positive:

The CDC study of Mazurek et al (2001) showed that 15% of these individuals were NTM reactive, that they also had much higher levels of BCG vaccination (6 X odds ratio), and they were associated with the TST reading problem of digit preference (rounding to the nearest critical diagnostic cut-off). This suggests only a small number of the sub-group are truly infected. Further evidence of this is seen in animal studies noted below.

QFT positive, TST negative:

No prospective studies exist on this group of individuals. Within the CDC study the only factor associated with this discordant group was individual clinical site variations, indicating TST reading variation. Although animal data indicates such persons are likely to be infected, these individuals are currently being missed by TST anyway so there is no possible change in sensitivity from current practices due to this source of error. Treatment on QFT alone would eliminate this issue.

Extensive studies and practice in bovine tuberculosis, the closest animal parallel to human tuberculosis, indicate that IFN- γ positive, skin-test negative animals are likely to be truly infected and that very few animals that are skin-test positive IFN- γ negative are infected. These studies have shown that whole blood IFN- γ method is more sensitive and accurate in assessing TB infection and that in discrepancies with skin testing the IFN- γ method is considerably more likely to provide an accurate diagnosis of TB infection (Wood and Jones, 2001). Cattle can be killed and tissue cultured to assess TB infection; as this practice is unavailable to human populations there is no gold standard for LTBI, but there is no reason to believe this finding of superior accuracy will not also apply to QFT use in humans. Whole blood IFN- γ testing is USDA approved for bovine and primate TB testing.

The net effect of initial QFT screening in the military should be both beneficial to TB control and reduce costs by significant *reduction* of the amount of preventive therapy. The logistic benefit of QFT in not requiring a second subject visit will greatly improve the capture of results in the first phase, particularly in those groups where arranging a TST reading by properly trained medical personnel may be problematic. This will improve the overall pickup of LTBI. Follow-up and confirmatory TST can then be carefully focussed on QFT positive cases. Treatment of only double QFT/TST positive cases will reduce unwarranted treatment due to TST false positives attributable to non-tuberculosis mycobacteria, which may be as high as 50% in low risk populations (NTM; see Von Reyn et al, 2001) and also due to other sources of TST inaccuracy, with associated cost reductions in both treatment and monitoring. In low risk groups it will provide strong credibility for treatment programs for those found positive to both QFT and then TST, increasing compliance.

- *Studies should be done to determine compliance with preventive therapy among recruits and skin test converters. This should include addressing risk factors for noncompliance*
- *A cost effectiveness study of directly observed preventive therapy should be undertaken, including alternative dosing regimens*

LTBI therapy compliance and treatment methods within the military are beyond the remit of Cellestis. Nevertheless, for the reasons noted above we consider adoption of QFT can contribute to improved compliance. Directly observed therapy for a nine month period has obvious cost implications, and reducing the incidence of such therapy due to sequential testing will reduce costs.

- ***Additional head to head comparisons of the TST versus the whole cell blood (sic) assay should be done.***

The FDA Summary of Safety and Efficacy (<http://www.fda.gov/cdrh/pdf/p010033.html>) provides details of the evidence presented from clinical studies conducted in the US that support the FDA decision to approve QuantiFERON-TB. These include analysis of studies performed by the CDC on 1218 low risk, high risk, and active and past-treated TB cases.

- ***Cohort studies of personnel should be undertaken to determine sequential behaviour of the whole blood assay, including reproducibility of results.***
- ***The cohort of personnel who are skin test negative, but whole cell assay positive should be followed to determine their risk for active tuberculosis.***
- ***Test reproducibility should be studied by splitting samples between laboratories to gauge consistency of results. Such studies should be done in the types of settings where screening will likely occur in the military.***

A summary of reproducibility, for individual sequential studies and between laboratories, is provided in the FDA Summary of Safety and Efficacy. Further information on cohort studies and for split samples is provided in Appendix 4.

There are practical constraints to the follow up of TST-/QFT+ subjects. Within the CDC studies 72 of the 944 subjects in the high risk group fell into this category, and were not followed up. For a TST+ result no more than 7 of 72 individuals would be expected to develop active tuberculosis in their lifetime, making the per annum incidence extremely low. The lifetime risk of active TB following TST-/QFT+ would need to be extremely high, much greater than the 5-10% estimate for a TST+ result, for meaningful data to be obtained within a reasonable time period from studies of any practical size.

Adequate reassurances of the efficacy of the QFT test in detecting true tuberculosis infection can be found in the good sensitivity of the test in active tuberculosis, its high specificity in low-risk populations, and its reasonable parallels with TST testing. As noted previously, data from bovine tuberculosis studies provides added reason to consider the QFT a more accurate system.

- ***Studies should be done to determine the feasibility of using whole blood assays given the 12 hour time constraint for the test to be run.***

The initial feasibility for that facility was demonstrated in the Great Lakes study conducted by WRAIR. The 12 hour constraint applies to the period in which the initial blood culture phase must be initiated. It is clear that, as there are situations where the TST limitations (minimum 48 hours, subject return required) there will be circumstances where logistics preclude use of QFT.

We would suggest that this be factored into any policy recommendation to allow Medical discretion depending upon circumstances.

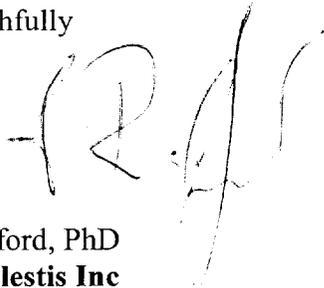
- ***Cost analysis is needed to determine the cost impact of switching to a whole blood cell assay and changing screening policies.***

Cost analysis attached as Appendix 5 (Howell Report) indicates that use of QFT in practice reduces the cost of TB testing, mainly by reducing the labor burden associated with the second reading when the overwhelming majority of personnel are negative for TB infection.

Further arguments for the adoption of QFT come from the savings outline where the use of QFT as a primary screen in low-risk individuals, followed by TST confirmation, will further reduce the cost burden of treatment by elimination of unnecessary therapy. Apart from direct therapy costs, this provides benefits by freeing such military personnel from restrictions on mobility and activity associated with LTBI treatment.

I trust the answers provided and the associated documents meet the questions as proposed by AFEB and that the document will meet any concerns of JPMPG. At Cellestis we remain available to discuss with you and other members of JPMPG any residual concerns they may have, and of course we are available to present to JPMPG should this be of assistance.

Yours faithfully

A handwritten signature in black ink, appearing to read 'TR', is written over a faint, large watermark of the same signature.

Tony Radford, PhD
CEO, Cellestis Inc

References

von Reyn CF, Horsburgh CR, Olivier KN, P. F. Barnes PF, R. Waddell R, Warren C, Tvaroha S, Jaeger AS, Lein AD, Alexander LN, Weber DJ, Tosteson ANA* Skin test reactions to *Mycobacterium tuberculosis* purified protein derivative and *Mycobacterium avium* sensitin among health care workers and medical students in the United States Int.J. Tuber. And Lung Dis,2001.: 5(12):1122–1128

Wood, PR and Jones SL. BOVIGAM: an in vitro cellular diagnostic test for bovine tuberculosis. Tuberculosis (Edinb). 2001;81(1-2):147-55.

Appendixes

- 1) Mazurek GH, et al , Comparison of a whole-blood interferon- γ assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. JAMA, 2001, 286: 174-1747
- 2) Clinicians Guide to QuantiFERON[®]-TB, November 2001
- 3) QuantiFERON-TB package insert 03200000H
- 4) QuantiFERON-TB performance characteristics (including Technical Note re reproducibility using predominantly negative samples)
- 5) Screening for *Mycobacterium tuberculosis* in the US Military: Considerations for a cost-effectiveness model (May 16, 2000)