DILUTING SAMPLES WITH HIGH QUANTIFERON-TB GOLD IN-TUBE RESULTS

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Dear Editor,

With interest we read the recent article of Veerapathran et al (1) about deriving cut-off values for conversions using reproducibility data of the QuantiFERON-TB Gold in-tube (QFT-GIT). Besides detailing the inter-assay variability at low levels of IFN-γ, the authors also suggest that IFN-γ values can be accurately estimated if not exceeding 15 IU/ml (1).

We would like to point out that interpretation of this upper limit of IFN-γ production should be made more carefully. This especially pertains to the use of quantitative results of QFT-GIT in the follow-up of patients, with values exceeding the highest concentration included in the standard. To investigate this matter and assess precisely IFN-γ values of undiluted samples exceeding 4 IU/mL in QFT-GIT, we repeated the assay (n=21) with undiluted and of 10-fold and 50-fold diluted plasma samples after stimulation with TB specific antigens. Samples with IFN-γ exceeding 10 IU/ml were tested also at 50× dilutions. The results, as summarized in Figures 1 and 2, demonstrate that in undiluted samples IFN-γ concentrations of more than 10 IU/ml underestimate the true IFN-γ concentration by a factor of about 10 (Figure 1). Upon comparison of 10-fold and 50-fold diluted samples (Figure 2), a good match was obtained up to a IFN-γ concentration of about 100 IU/ml. At such a high concentration, again diluting the sample further will raise the reliability of the finding. There are at least two explanations for this discrepancy. Firstly, the actual concentration in the undiluted sample could have been much higher than extrapolated from the standard curve, indicating that assuming linearity beyond the highest standard value is not justified. Secondly, a prozone effect could interfere with the accurate measurement of high IFN-γ concentrations. This phenomenon is explained by aggregation of IFN-γ molecules present at a high concentration, preventing access of the ELISA reagents to their binding sites, and leading to falsely low measurements (2).

The fact that at high concentrations the value of IFN-γ apparently is underestimated may not be an issue as far as the use of QFT-GIT for diagnosis of TB infection is concerned, since the cut-off value for positivity is well within the range of the used standard series. However, if the QFT-GIT is used quantitatively for clinical follow-up or for relating intensity of the response to other characteristics of disease, a problem does arise and outcome values should always be checked at various dilutions of the sample.
Figure 1.
Final IFN-γ concentration in undiluted and 10× diluted samples.

Figure 2.
Final IFN-γ concentration in 10× diluted and 50× diluted samples.

REFERENCES

