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Title: Improved performances of the Quantiferon-TB in tube assay with novel mycobacterium tuberculosis specific antigenic peptides

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Body: Background: Interferon-gamma release assays (IGRA) are representing the present standard for the diagnosis of TB infection. IGRAs present a variable sensitivity in different population and test conditions and excellent specificity. Aim: To evaluate the usefulness of novel Mycobacterium tuberculosis (MTB) antigens to improve the performance of the QuantiFERON-TB Gold in tube (QFT) test. Patients and methods: Six novel HLA-class II promiscuous, multiepitopic, MTB-specific peptides, from 4 MTB proteins were selected from 88 MTB-specific peptides. The ability of the pooled peptides, added at the concentration of 1 mg/ml each to the QFT antigen tube, to improve QFT performance was assessed in 205 healthy unexposed controls and 31 microbiologically confirmed, active TB patients. Results: Two out of 205 healthy controls were QFT-positive. Of the 203 negative QFT tests and all still resulted negative after addition of the novel peptide pool. Of the 31 TB patients, 27 were QFT-positive and 30 turned QFT-positive after adding the novel peptide pool ($p>0.05$). In addition, addition of the novel peptide pool resulted in an increased IFN-γ release [QFT: 6.12+1.15 IFN-γ IU; QFT plus novel peptides 7.02+1.74 IFN-γ IU (paired t-test, $p<0.05$)]. Conclusions: The addition of novel MTB-specific peptide antigens to the QFT test increases sensitivity and IFN-γ release, while maintaining very high specificity.