Results: Overall agreement between IFN-gamma assay and TST was 89.3% (kappa = 0.052). In LRG, agreement between the two tests was 52.6% (95% confidence interval, 44—60%) with κ values of 0.019. In HRG agreement between the two tests was 63.2% (95% confidence interval, 42—84%) with κ values of 0.28.

Conclusion: In conclusion, the IFN-gamma assay showed acceptable results for determining latent M. tuberculosis infection in vaccinated population. Although TST and IFN-gamma assay appear comparable, they have different performance and operational characteristics; therefore, the decision to select one test over the other will depend on the population, purpose of testing, and resource availability.

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48.003

Real-Time PCR for the Detection of Fluoroquinolone Resistance in *Mycobacterium Tuberculosis*

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Background: A Fluoroquinolone (FQN) is the drug of first choice for the treatment of multi-drug resistant tuberculosis (MDR TB) but the FQN-resistance rate in Vietnam is unknown. A rapid and effective test to detect FQN-resistance before treatment is urgently needed. FQNs inhibit the function of the DNA gyrase, encoded by the *gyrAB* genes of *Mycobacterium tuberculosis* and mutations in the Quinolone Resistance Determining Region (QRDR) of *gyrA* have been shown to account for 60—70% of FQN-resistant isolates. A Real-time-PCR (RT-PCR) test was developed to detect the most common mutations in this region.

Methods: gyrA sequencing data from 40 FQN-sensitive and 42 FQN-resistant (ofloxacin 2 μg/ml) clinical isolates was used to develop the RT-PCR test. Three Locked-Nucleic-Acid probes were used to detect mutations at codons 90, 91 or 94 which accounted for 97% of the FQN-related mutations in QRDR of *gyrA*. A set of 131 consecutive isolates from retreatment patients from Pham Ngoc Thach Hospital, resistant to either Isoniazid or Rifampin, was used to evaluate the RT-PCR and estimate the resistance rate.

Results: Sequencing data showed that all 40 FQN-sensitive isolates were wild-type in the QRDR. Among 42 FQN-resistant isolates, 10 were wild-type, 20 carried single mutations and, surprisingly, 12 were heterogeneous containing both wild-type and mutated populations. Of these 12, 4 contained a mutation at 2 resistance-associated alleles. The RT-PCR test identified all wild-type and single mutation isolates correctly and 12/16 mutations in heterogeneous isolates. Five percent of isolates (7/131) from retreatment

Conclusion: This RT PCR assay is a quick, relatively simple and cheap test to screen for FQN-resistance with 100% specificity. FQN resistance is estimated at > 5% in retreatment patients in Southern Vietnam. Studies to determine other FQN-resistance mechanisms are vital to improve molecular diagnosis and treatment.

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48.004

Differential B-Cell responses are induced by Mycobacterium tuberculosis Ag85A synthetic peptides in two populations from Venezuela

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Background: The aim of the present study was to assess progress made in the diagnosis of pulmonary tuberculosis when evaluating B-cell responses to 16 Ag85A synthetic peptides, the recombinant antigen 85 (rAg85) and the non-recombinant PPD antigen.

Methods: The B-cell responses of tuberculosis patients and healthy individuals were evaluated by an IgG-ELISA. A total of 120 individuals were included in this study. Patient groups were conformed of 20 Warao indigenous (WP) and 20 Creole non-indigenous (CP), whilst healthy control groups were composed by 40 Warao indigenous (WC) and 40 Creole non-indigenous (CC). Both control groups included 20 positive and 20 negative individuals for the tuberculin skin test (TST). Association of positive tests for each antigen, defined with receiver operator characteristics (ROC) analysis, was assessed for each population.

Results: Different patterns of the B-cell responses were displayed by each population. The anti-29878 IgG method reached highest sensitivity of 95.0% (negative predictive value (NPV) = 94.4) within the Warao population, but was lowly specific, 42.5%, (positive predictive value (PPV) = 45.2), compared to highest specificity showed by the anti-29879 IgG method (100.0%, PPV = 100). Regarding the Creole population, anti-11006 IgG showed highest sensitivity of 95.0% (NPV = 90) but was lowly specific (22.5%, PPV = 38). Anti-10998 IgG was found to be the most specific (100.0%, PPV = 100), followed by the anti-PPD IgG method (90.0%, PPV = 66.7). These findings indicate that population-to-population heterogeneity of peptide antigen recognition, rather than recognition of particular antigens, is a characteristic feature of antibody responses in these two populations. Furthermore, responses to anti-29879 IgG and anti-10998 IgG were associated to inactive TB.

Conclusion: Ag85A peptides were more specific than sensitive, showing that these peptides' high specificity does not stimulate primed T cells in TST+ individuals, suggesting that