Evaluation of a new kit for the detection of interferon-gamma production for the immunological diagnosis of latent tuberculosis (IGRA-ELISA test)

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Introduction
The diagnosis of latent tuberculosis (TL) is based on a set of criteria, clinical, radiological, bacteriological and immunological. The frequency of Mycobacterium tuberculosis (MTB) specific effector T cells can be revealed by taking advantage of their ability to secrete interferon-gamma (IFN-g) upon re-stimulation. IGRA (IFN-g release assay) assays quantify the IFN-g produced after brief in vitro stimulation with specific MTB antigens.

Goal
The objective of this study was the comparative evaluation of 2 IGRA-ELISA tests. The Qiagen QuantiFERON®-TB Gold Plus kit (QFT®-Plus) contains 2 tubes coated with antigenic peptides associated with the MTB complex (ESAT-6 and CFP-10) capable of stimulating CD4 + T cells (TB1 tube) or T cells. CD4 + and T CD8 + (TB2 tube). This kit was compared with a new test (TB-Feron®) developed by the company SD Biosensor (Orgentec), containing a single tube of peptide antigens from the MTB complex (ESAT-6, CFP-10 and TB7.7). .

Material and methods
Heparinized blood from 64 patients was allocated to the test tubes. The pre-analytical steps (samples in coated test tubes, incubation at 37 ° C for 16 to 24h, centrifugation) are similar for the 2 kits studied. The quantitative results are obtained by subtracting the value obtained in the negative control (without stimulation) from the value obtained after stimulation by the antigens. For the 2 kits, we considered the result as positive when the IFN-g produced was greater than 0.35 IU / ml, thus following the recommendations of the 2 suppliers.

Left
The QFT®-Plus ELISA is composed of:
-A positive control (purple tube containing a mitogen)
-A negative control (gray tube)
A TB1 tube containing a mixture of antigens associated with the MTB complex (ESAT-6 and CFP-10) capable of stimulating CD4 + T lymphocytes (green tube)
- A TB2 tube containing a mixture of antigens associated with the MTB complex (ESAT-6 and CFP-10) capable of stimulating CD4 + T lymphocytes and CD8 + T (yellow tube). This test is automated on a DS2.

Right
The TB-Feron® ELISA test is composed of:
-A positive control (purple tube containing a mitogen)
-A negative control (gray tube)
-A TB Ag (antigens) tube containing a mixture of antigens associated with the MTB complex (ESAT-6, CFP-10 and TB7.7) capable of stimulating T lymphocytes (red tube). This test is automated on a GEMINI.

Result

A: Comparison of the quantitative results obtained (in IU / ml) with the 2 kits - A Pearson correlation
Of the 64 patients for whom latent tuberculosis screening was prescribed, 14 had a positive result with the QFT®-Plus kit (IFN-g product greater than 0.35 IU / ml). Using the same threshold with the TB-Feron® kit, satisfactory agreement is obtained in the qualitative interpretation of these assays (McNemar test, $p = 1$). A very good correlation is obtained between the quantitative results obtained with the TB1 and TB2 tubes of the QFT®-Plus kit and the TB Ag TB-Feron® tube ($r = 0.9607$, $p < 0.001$ and $r = 0.9638$, $p < 0.001$, respectively)

B: Reproducibility of the TB-Feron® kit
We tested the reproducibility of the TB-Feron® kit by assaying the production of IFN-g on the GEMNI in 2 independent series, with the same batch of reagents, for the same sample.
For 12 patients analyzed, we obtained a good correlation with a coefficient of 0.9974 ($p < 0.001$, A Pearson correlation).

C: Robustness of the TB-Feron® kit
The supplier recommends keeping the reaction tubes at 4 ° C. Since these tubes are kept in clinical departments, it would be preferable to store them at room temperature (RT). We measured the production of IFN-g from tubes stored at 4 ° C and TA for 29 patients. The correlation obtained shows that the preservation of the tubes at RT is possible.

Conclusion
The criticality of the pre-analytical steps of the IGRA tests is similar for both kits. With an easy grip on GEMINI, similar working conditions and a very good correlation with the QFT®-Plus kit, the TB-Feron® kit meets the criteria required for its routine use in screening for latent TB.