INDIRECT DIAGNOSIS OF THE INFECTION WITH MYCOBACTERIUM TUBERCULOSIS BY MEANS OF THE IMMUNOENZYMATIC TECHNIQUE

Antonela Chesca$^{1,2}$, Oana Ticau$^{2}$, Raluca Pirau$^{3}$, Sorina Anamaria Chesca$^{1}$

$^1$TRANSILVANIA UNIVERSITY OF BRASOV, FACULTY OF MEDICINE; $^2$HOSPITAL OF PNEUMOLOGY, BRASOV, $^3$CLINIC EMERGENCY COUNTY HOSPITAL OF BRASOV, ROMANIA

Summary
This study aims at emphasizing the possibility of establishing the indirect diagnosis of the infection with Mycobacterium tuberculosis, by means of current laboratory techniques. Within this context, the immunoenzymatic technique ELISA (Enzyme-Linked Immunosorbent Assay) used in the accomplishment of the QuantiFERON – TB Gold (In-Tube Method) test, represents a method used in the indirect diagnosis of tuberculosis. The objectives of the study focused on emphasizing the utility of using this test, in the indirect diagnosis of the infection with Mycobacterium tuberculosis. From this point of view, the study reveals that the results of the laboratory test are based on the immunoenzymatic technique and on the laboratory data of the Clinical Hospital of Pulmonology and Phtisiology Brasov of 2010.

Key words: Mycobacterium tuberculosis, laboratory, Immunoenzymatic technique, diagnosis

anto_chesca@yahoo.com

Introduction
Due to the correct and rapid diagnosis of the infection with Mycobacterium tuberculosis, we aim at using modern and efficient methods (Pineat, 2000; Wolinsky, 1988). From this point of view, the goal of this study is to emphasize the utility of certain medical techniques, in the screening of the infection with Mycobacterium tuberculosis (Bruins et.al., 1995). In this context, the study refers to the use of laboratory techniques that enable the indirect diagnosis of the tuberculosis (Streeton et. al., 1998). For this purpose, there was used a test that is based on the immunoenzymatic technique called ELISA (Enzyme-Linked Immunosorbent Assay), referred to as QuantiFERON-TB Gold (In-Tube Method) (Pottumarthy et.al., 1999).

The laboratory immunoenzymatic technique, QuantiFERON-TB Gold represents an indirect test for the infection with Mycobacterium tuberculosis, including the active form of the disease, but not exclusively for diagnosis purposes (Diel et.al., 2008).

The importance of this technique consists in the fact that it enables the quantitative determination of the $\gamma$-interferon that is released in the plasma as a consequence of the interaction between the peptic antigens that stimulates the mycobacterium proteins and the T lymphocytes of the individuals that are infected by the organisms of the Mycobacterium tuberculosis complex.

Material and methods
Within the study, there were taken into account the results of the QuantiFERON – TB Gold (In-Tube Method) test, as a consequence of carrying on the investigation during 2010. This method is used for diagnosing purposes, as much as possible, currently within the Tuberculosis Clinic of the Hospital of Pulmonology and Phtisiology of Brasov, by the modern devices of the clinical laboratory.
The work technique includes a series of stages whose succession and term must be strictly observed.

Samples taking and processing in order to fulfill the QuantiFERON-TB Gold test assumes the preservation of the samples in special vacutainers: Control Nil (Grey cover and white ring), Antigen TB (Red cover and white ring), Control Mitogen (Mauve cover and white ring).

An essential condition in correctly carrying on the investigation is to take all the plasma and reactive samples, at the ambient temperature, 22°C ± 5°C, 60 minutes before the use.

Taking into account the number of patients to which we add the 4 double standards, we may approximate the necessary number of tapes.

The necessary time for carrying on the test is appreciated according to the length of the two basic working stages.

The first stage consists in the incubation at 37°C of the three tubes with the blood taken from the patient. This stage takes between 16 and 24 hours.

After the incubation of the tubes at 37°C, the procedure of taking the plasma is facilitated by the centrifugation of the tubes for 15 minutes at 2000 up to 3000 centrifugal rotations (g).

The second stage refers to the effective work of the ELISA plaque.

The ELISA plaque enables the investigations for 29 patients, the length of this stage being estimated at approximately 3-4 hours.

Results and discussions
The study presents the results of the QuantiFERON – TB Gold (In-Tube Method) test, carried on in 2010, within the Tuberculosis Clinic within the Hospital of Pulmonology and Phthisiology in Brașov.

The working method consists in four double standard tests (Figure 1).

The working technique includes the use of an ELISA plaque, presented at the beginning of the procedure (Figure 2) and after the finalization of its completion with the plasma tests and standards (Figure 3).

The percentage estimation of the positive, negative and undetermined results, by means of the immunoenzymatic test may be expressed as percentages. Expressed in this manner, we may better notice the predominant percentage of the negative tests, in comparison to the percentage of the positive tests and to the extremely reduced percentage of the tests whose values could not be objectively expressed, determining the need to take other blood samples and to test again that patient (Figure 4).

The values of the indirect immunoenzymatic laboratory test carried on, in 2010, can be expressed on sexes, by digital expression, in a table. As this, we may better notice a sensitive difference in the favor of the females testing, in comparison to the males testing (Figure 5).

As a screening method of the infection with the Koch bacillus and for the diagnosis purpose, the results of the laboratory results of the QuantiFERON – TB Gold test must be correlated with the epidemiologic aspects of every person, with the clinical, radiological and laboratory examination.
Figure 3. ELISA Plaque with plasma samples and standards

Figure 4. Results of the immunoenzymatic laboratory test carried on in 2010 Expressed as percentages

<table>
<thead>
<tr>
<th>Result</th>
<th>No of tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>190</td>
</tr>
<tr>
<td>Positive</td>
<td>103</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>9</td>
</tr>
<tr>
<td>Total no. tests</td>
<td>302</td>
</tr>
</tbody>
</table>

Fig. 5 Results of the immunoenzymatic laboratory test carried on in 2010 Expressed on genders

Results of the test carried on males | Results of the test carried on females
147                              | 155

Conclusions

The QuantiFERON – TB Gold (In-Tube Method) test, which uses the ELISA (Enzyme-Linked Immunosorbent Assay) immunoenzymatic technique, is considered a highly sensitive laboratory method.

The immunoenzymatic technique on which it is based the QuantiFERON – TB Gold laboratory test represents one of the methods internationally used in diagnosing the infection with *Mycobacterium tuberculosis*.

The corroboration of the results of the test with the clinical, specialty paraclinical data and with the results of the imaging investigations enables the highly accurate establishment of the tuberculosis diagnosis.

References


