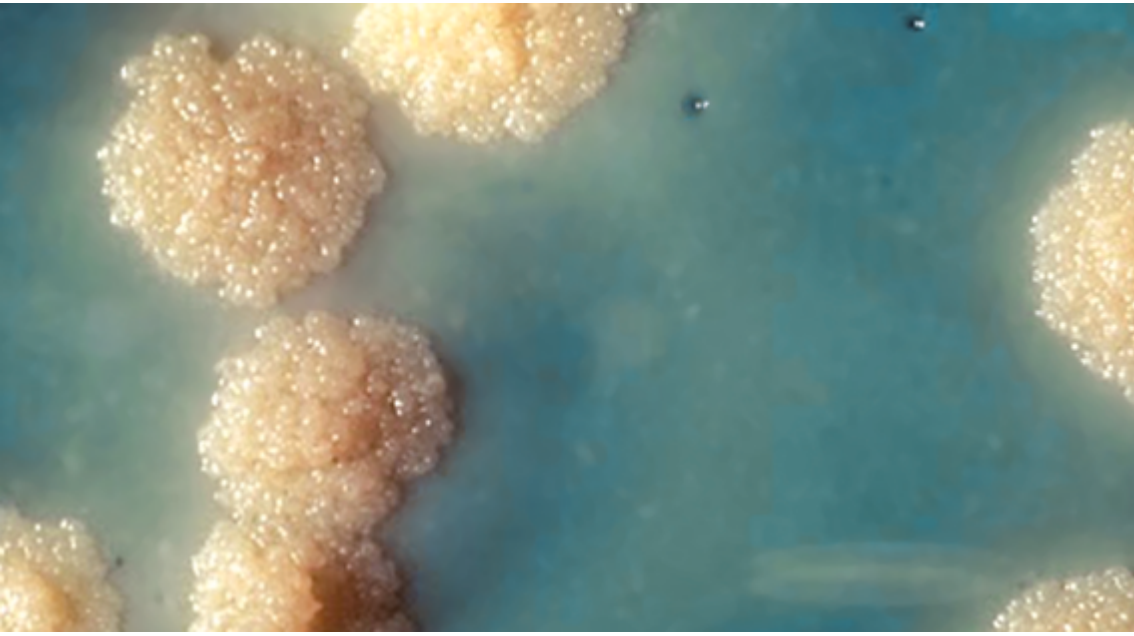
eurofins | Biomnis



Diagnostics of mycobacterial infections

Tuberculosis

Approximately ¼ of the world's population are latent tuberculosis carriers; this means that although these people carry the Koch bacillus, they do not become ill. They cannot transmit the bacterium, but they have approximately a 5% risk of developing active tuberculosis. Every year, 10 million people develop active tuberculosis and 1.4 million die as a result.Tuberculosis remains one of the ten main causes of mortality. Infected persons who are also HIV positive are at 20 to 30 times greater risk of developing active tuberculosis.

(Source: WHO Global Tuberculosis Report 2016)

Multidrug-resistant-tuberculosis (MDR-TB) (resistant to isoniazid and rifampicin) is a major health risk. The WHO estimates that there are **600,000** new cases with resistance to rifampicin, **490,000** of which are cases of multidrug-resistant tuberculosis.

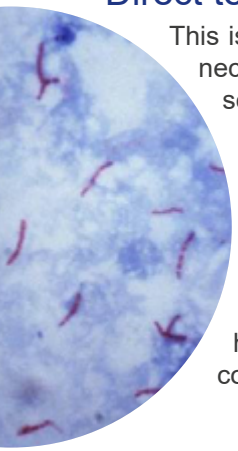
Atypical mycobacterial infections

These infections are caused by **non-tuberculous** (or atypical) **mycobacteria**. More than a hundred such bacteria have been described and for the most part, these are ubiquitous microbes present in the water or soil. A great many are opportunistic and give rise to nosocomial infections or infections in weakened patients.

Symptoms are varied (lung infections, adenitis, skin or subcutaneous disorders, generalised forms).

Tuberculosis treatments are normally inactive; an antibiogram can be carried out in some situations. Where possible, surgery is to be preferred. There is **no inter-human contamination** with these diseases.

Direct testing



This is carried out after decontamination, concentration and fluidification (if necessary) of the sample, by means of specific staining: auramine during screeningand then Ziehl-Neelsen for confirmation.

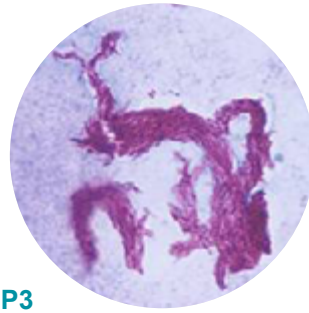
This test is not very sensitive (< 50%); it is non-specific and can determine whether AARB (Acid-Alcohol Resistant Bacilli) are present or not.

With results reported in less than 4 hours after receipt, it does, however, in the case of a positive result for the expectorate, allow the contagious patient to be isolated.

Culture

Initially performed on Jensen and Coletsos gel mediums, Jensen and Coletsos, the preference is now for a **liquid Middlebrook medium** and an **agar medium** (some strains can only be tested on one of the two). The liquid media are placed in a robot and bacterial growth is automatically monitored at hourly intervals, which means that a result can be available approximately one week earlier.

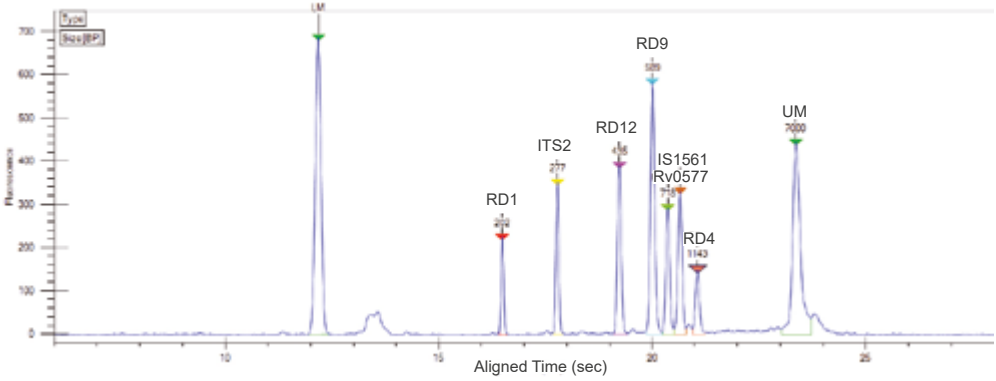
This is **the most sensitive test** available and is essential for the identification of mycobacteria and creation of antibiograms.



Identification of Mycobacterium tuberculosis complex

All technical handling of live strains must take place in a **P3 laboratory**, i.e. a laboratory with a biosecurity level 3 (cf. max. possible level of 4). After extracting the bacterial DNA from the culture, a multiplex PCR is performed to identify discriminant regions (regions of difference) between the mycobacteria of the tuberculosis complex (MTBC).

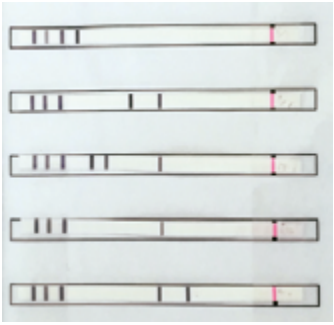
The multiplex PCR is used to sequence seven regions of the genome, whereby the presence or absence of amplicons allows the **identification of the MTBC**. The various amplicons are then analysed by the LabChip® robot, which electrophoretically separates the PCR products according to size.



The molecular typing methods (MIRU-VNTR, spoligotyping) are reserved for epidemiological studies and comparison between strains or patients, as well as the search for nosocomial or intra-laboratory contamination.

Identification of atypical or non-tuberculous mycobacteria

Atypical mycobacteria are also identified by means of **molecular tests**. After extraction of the DNA, a PCR is performed at the endpoint and then screening is performed by means of DNA-DNA hybridisation on a test strip. This method allows for the identification of the **fifteen or so most frequently encountered species of atypical mycobacteria**.



Strips 1 to 4	*Mycobacterium avium*
Strips 1, 2, 3, 8, 10	*Mycobacterium gordonae*
Strips 1, 2, 3, 5, 6, 10	*Mycobacterium abscessus*
Strips 1, 2, 3, 10	*Mycobacterium sp.*
Strips 1, 2, 3, 10, 12	*Mycobacterium kansasii*

Identification by mycobacterium CM genotype (Hein)

If the atypical mycobacterium concerned is one of the rarer types not included on the strips, sequencing of certain genes is performed: intergenic transcribed spacer (ITS), gene hsp65, gene rpoB and sometimes the gene coding for 16S ribosomal RNA (16S rRNA).

Antibiogram

For *M. tuberculosis*, the proportional method has been adapted to the liquid medium. With the Bactec® MGIT, results are generally obtained within approximately ten days for: **streptomycin, isoniazid, rifampicin, ethambutol and pyrazinamide**.

For atypical mycobacteria, the method used is the liquid medium **antibiogram on a plate**. No nomogram is available for many antibiotics and the antibiograms of atypical mycobacteria are above all useful for monitoring **the onset of resistance under treatment**.

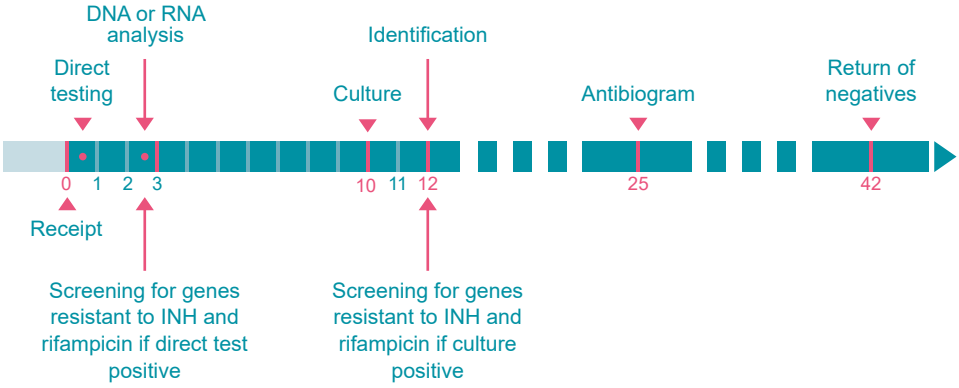
Direct detection of the genome of the mycobacterium tuberculosis complex and detection of resistance genes

A PCR can be performed directly in the sample to detect MTBC, enabling a reliable result to be obtained on the same day.

Its sensitivity is excellent if the direct testing is positive (99%) and approximately 80% for respiratory samples, if the direct testing is negative.

Direct PCR can also be used to find **rpoB gene mutations** and to therefore provide a valuable indication as to **sensitivity to rifampicin** of a mycobacterium tuberculosis complex.

Screening for genes resistant to rifampicin (rpoB genes) and isoniazid (InhA gene for low level resistance and KatG for high level resistance to INH), can also be carried out directly on samples if the result of the direct PCR is positive; in fact, if direct screening is negative, the sensitivity of this technique is very weak and it is therefore best to carry out this screening on the strain after culture.



QuantiFERON-TB Gold Plus

This blood test determines the capacity of the patient's lymphocytes to release interferon γ after stimulation by specific BK purified proteins (ESAT-6 and CFP-10).

Its sensitivity is 95% and **specificity 98%** in tuberculosis infection screening.

NB : it does not differentiate between latent and active tuberculosis. An antigen free negative control and a positive control with mitogen validate the result.

Indications:

1. Migrant children aged under 15 years old from an area where tuberculosis is highly endemic;
2. Patients with HIV (systematic screening included in the initial assessment of an HIV patient);
3. Before initiating anti-TNF treatment;
4. As a diagnostic aid for extra-pulmonary tuberculosis;
5. Employees with occupational exposure on recruitment;
6. For documented tuberculosis exposures : tuberculosis contact investigations.

To facilitate the collection and direction of your samples, we provide you with the following:

1. **A K4-INTGB Quantiferon sample kit** : to be ordered at Eurofins Biomnis before sampling.
2. **A K4P-INTGB** to be followed and returned to us once filled in.

International Division
E-mail: international@eurofins-biomnis.com

Eurofins Biomnis
17/19 avenue Tony Garnier
BP 7322 - 69357 LYON Cedex 07 - FRANCE
www.eurofins-biomnis.com

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