GenoType® Product Series Mycobacteria

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HAIN LIFESCIENCE

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Tuberculosis

Tuberculosis (TB) is a chronic, granulomatous disease which is caused by the members of the *Mycobacterium tuberculosis* complex. With 8.6 million new cases each year, TB is one of the world's most common severe infectious diseases and a significant problem both in the developing countries and to an increasing extent in the industrial nations. Approximately 2 million deaths per year are tuberculosis-related. A single patient with an open TB may infect 10-15 other people each year. Due to the increasing number of AIDS cases and immunosuppressive treatments, there is also a continual increase in non-tuberculous mycobacterial infections.

Four parameters are of crucial importance for the containment of TB:

- early diagnosis
- prevention of the spread of the disease
- effective treatment with tuberculostatics
- prevention of the development of drug-resistance

A quick and reliable differentiation of the pathogen is required to effectively combat the diseases caused by mycobacteria. An early diagnosis is essential, as multidrug-resistant mycobacteria have been shown to increase rapidly. For TB germs this trend has reached alarming proportions e.g. in some regions of Eastern Europe, but in Germany too the resistance pattern must be taken into consideration when planning treatment.

Mycobacteria

Mycobacteria are immobile, obligate aerobic, acid-fast bacilli. They are gram-positive with a high genomic G+C content (59-66%). The genus *Mycobacterium* comprises more than 120 different species which are assigned to the *M. tuberculosis* complex and the so-called non-tuberculous mycobacteria.

M. tuberculosis complex

The *M. tuberculosis* complex includes the closely related organisms *M. tuberculosis*, *M. bovis* (subspecies *bovis* and *caprae*), *M. bovis* BCG (Bacille Calmette-Guérin), *M. africanum* (subspecies I and II), *M. microti*, and *M. canettii*. These, with the exception of *M. bovis* BCG, are considered to cause TB in humans and animals. Despite their close genetic similarity, these organisms differ considerably with regard to epidemiology, pathogenicity, and their host spectrum. *M. tuberculosis* is considered to be the principal cause of TB in humans, and in Germany, for example, it is responsible for 95% of TB infections. The TB germs are spread through droplet infection, and crowded and poor hygienic conditions aid the spread of the disease. The pathogen settles in the lung where it may induce TB or persist for many years. *M. africanum* comprises a heterogeneous group of strains occurring mainly in Africa and is considered to be a human TB pathogen. Subtypes I and II are distinguished by their geographical origin. For *M. bovis*, resistance to pyrazinamide (PZA) serves as a criterion to distinguish the subspecies *M. bovis ssp. bovis*, the cause of bovine tuberculosis (PZA-resistant) and *M. bovis ssp. caprae* (PZA-sensitive). The BCG vaccine strain, used for around 70 years as a live vaccine for TB prophylactic immunization, was developed from *M. bovis* as an apathogenic variant. The use of the BCG vaccine as protection against the disease having fatal effects is in fact undisputed but the most widespread form of the disease, pulmonary tuberculosis in adults, cannot be prevented. Due to consistently low TB incidences and a corresponding risk-benefit analysis, the BCG vaccination is no longer recommended in Germany by the standing committee for vaccinations at the Robert-Koch-Institute (STIFKO) since March 1998. The BCG strain, however, is used in the treatment of malignant tumours (e.g. bladder cancer). *M. microti* mainly infects rodents; the human pathogen *M. canettii* has so far only rarely been isolated.

Non-tuberculous Mycobacteria

In addition to the representatives of the *M. tuberculosis* complex the non-tuberculous mycobacteria can also cause mycobacterioses in humans which are mostly chronic. Synonyms are "Mycobacteria Other Than Tuberculosis" (MOTT), "Non-Tuberculous Mycobacteria" (NTM) or "Atypical Mycobacteria". This heterogenic group is spread worldwide. Being classic opportunists, they predominantly infect patients already suffering from pulmonary diseases or immunodeficiency; however, the number of mycobacterioses is also increasing among immunocompetent persons. Therefore the *M. avium* complex is one of the most common pathogens in immunosuppressed patients, while *M. kansasii*, *M. malmoense* and *M. xenopi* are predominant in immunocompetent people. The clinical symptoms of mycobacterioses are varied, ranging from pulmonary infections through granulomatous infections of the skin to lymphangitides. Due to their distinctive resistances to tuberculostatics, the treatment of MOTT infections is extremely difficult; a reliable species differentiation and also a specific resistance analysis are consequently of great importance.

Diagnosis of Mycobacterioses

The diagnosis of mycobacterioses is carried out in compliance with DIN regulations. After preparation of a direct microscopy, the primary samples are decontaminated in order to remove any interfering accompanying flora. Subsequently, a further microscopic preparation is analyzed and two solid media and one liquid medium are inoculated. The microscopic analysis of smears on acid-fast bacilli is indeed a quick but also very insensitive and not very specific method: as it is impossible to distinguish between either living or dead bacteria or between tuberculosis bacteria and MOTT, a culture report is always required. A positive result using solid culture can be expected after about 3-4 weeks, a negative assessment however is only available after incubation of 8 weeks. The sensitivity limit of the solid culture is 10^2 bacteria/ml. By using liquid culture systems, in addition to increasing sensitivity (10 bacteria/ml), the detection time can also be reduced. Therefore a positive result is already available after 1-2 weeks, and safe exclusion is possible after an incubation time of 6 weeks. However, since a morphological evaluation is not possible from liquid culture, only molecular genetic systems offer quick identification and differentiation.

Direct detection

With DNA- or RNA-based techniques it is possible to detect specific fragments of the mycobacterial genome. This involves multiplication of short nucleic acid sequences of the mycobacterial genome using enzymatic methods and the subsequent detection of the amplified fragments. Therefore in the event of grounds for suspicion and microscopic negative sputum and also in the case of persons at risk and severe symptoms, rapid detection directly from patient specimens is possible with the aid of NAT (Nucleic Acid Amplification Technology).

Differentiation

In addition to the discrimination of TB pathogens requiring obligate treatment from ubiquitous mycobacteria, species differentiation is also of crucial importance for the selection of adequate antibiotics, as drug resistance differs considerably between species. Conventional biochemical methods are time-consuming and require trained staff. Molecular genetic mycobacterial detection in conjunction with culture data is therefore the method of choice.

Resistance testing

The treatment of TB involves an uninterrupted administration of antibiotics for 6 months. If the treatment plan is not observed consistently or the treatment is discontinued prematurely, drug resistance will be developed. For some time an increase has been observed in the occurrence of mycobacterial strains resistant to one or more antibiotics. In particular multiresistant mycobacteria ("MDR", multidrug-resistant) represent a considerable risk. They are resistant to the most important tuberculostatics isoniazide and rifampicin. Their eradication requires a significantly more extended and comprehensive treatment (approx. 2 years) with correspondingly greater health risks for the patients and higher costs. For this purpose alternative antibiotics are available and after a fast diagnosis it is possible to change quickly to an effective antibiotic.

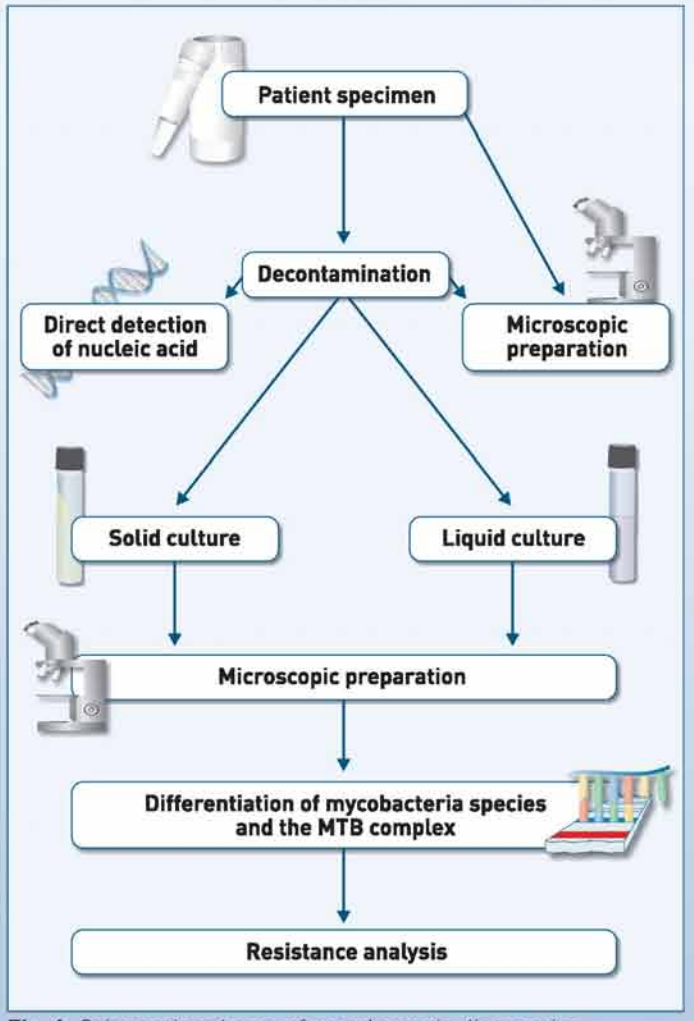

Fig. 1: Schematic scheme of mycobacteria diagnostics

Species	characteristics
M. abscessus	biochemical similarity to *M. chelonae*, *M. fortuitum*, *M. peregrinum*; predominantly in Europe, chronic pulmonary diseases, wound infections
M. africanum	MTB complex; causes tuberculosis in tropical Africa
M. alvei	similar to *M. peregrinum*; non-pathogenic environmental germ; fast-growing
M. asiaticum	related to *M. gordonae*; rarely causes pulmonary diseases; slow-growing, photochromogenic
M. avium	MAIS complex; ubiquitous environmental germ; pulmonary diseases, causes lymphadenitis in children; slow-growing
M. bovis BCG	MTB complex; tuberculosis vaccine strain, non-pathogenic
M. bovis ssp. bovis	MTB complex; causes tuberculosis in cattle and primates; pyrazinamide (PZA)-resistant
M. bovis ssp. caprae	MTB complex; PZA-sensitive
M. celatum	similar to *M. xenopi*; fast-growing
M. chelonae	causes cutaneous lesions, subcutaneous abscesses, rarely pulmonary tuberculosis, disseminated TB in patients with immune deficiency
M. fortuitum	see *M. chelonae*
M. gastri	can easily be mixed up with *M. avium* or *M. kansasii*; no clinical relevance
M. genavense	related to *M. simiae*; causes opportunist infections; slow-growing
M. goodie	related to *M. smegmatis* and the *M. fortuitum* complex; infection after bone fractures; fast-growing
M. gordonae	often contaminant in clinical samples; no clinical relevance
M. haemophilum	in patients with immune deficiency; causes cutaneous abscesses; slow-growing
M. heckeshornense	closely related to *M. xenopi*; human pathogen; slow-growing
M. immunogenum	similar to *M. chelonae* and *M. abscessus*; associated e.g. with catheter infections
M. interjectum	similar to *M. scrofulaceum* and *M. simiae*; in connection with chronic lymphadenitis; slow-growing
M. intermedium	closely related to *M. szulgai*; causes pulmonary diseases
M. intracellulare	MAIS complex; causes pulmonary diseases, disseminated tuberculous infections (in particular in connection with AIDS); slow-growing
M. kansasii	causes pulmonary diseases, lymphadenitis, disseminated TB in patients with immune deficiency; slow-growing
M. lentiflavum	related to *M. genavense*; initial isolation from a patient suffering from spondylodiscitis; slow-growing
M. malmoense	causes cervical lymphadenitis (particularly in children), pulmonary infections; slow-growing
M. mageritense	related to *M. fortuitum* and *M. peregrinum*; initially isolated from sputum; fast-growing
M. marinum	causes cutaneous lesions (swimming pool granuloma); slow-growing
M. microti	MTB complex; causes tuberculosis in rodents
M. mucogenicum	similar to *M. fortuitum*; causes post-traumatic wound infections and catheter sepsis; fast-growing
M. palustre	potentially pathogenic environmental germ; slow-growing; forms yellow, scotochromic colonies
M. peregrinum	similar to *M. fortuitum*; no significant clinical relevance; fast-growing
M. phlei	ubiquitous; non-pathogenic; fast-growing
M. scrofulaceum	MAIS complex; causes lymphadenitis (children), disseminated TB in patients with immune deficiency, scrofulosis; slow-growing
M. septicum	similar to *M. peregrinum*; pathogenicity is assumed
M. shimoidei	considered a pulmonary pathogen; slow-growing
M. simiae	clinical signs similar to *M. avium*; causes pulmonary TB (rarely); slow-growing
M. smegmatis	similar to *M. fortuitum*; may cause soft tissue infections; fast-growing
M. szulgai	closely related to *M. malmoense*; causes pulmonary TB, cervical adenitis; slow-growing
M. tuberculosis	causes tuberculosis in humans
M. ulcerans	closely related to *M. marinum*; causes skin ulcerations (tropics); slow-growing
M. xenopi	causes pulmonary diseases; slow-growing

Table 1: Relevant mycobacteria species

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