

PRACTICAL HANDBOOK FOR THE PHENOTYPIC AND GENOTYPIC IDENTIFICATION OF MYCOBACTERIA

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FOREWORD

*"The LORD God formed every beast of the field, and every fowl of the air; and brought [them] unto Adam to see what he would call them: and **whatsoever Adam called every living creature, that [was] the name thereof.**"*

(Genesis, chapter 2, Verse 19)

One cannot write a handbook on the identification of microorganisms without referring to taxonomy. Taxonomy, from the Greek words "taxis" (arrangement) and "nomas" (law), is the science of biological classification. Its purpose is to provide useful ways for identifying and comparing organisms. Classification is the arrangement of organisms into groups (taxa); nomenclature refers to the assignment of names to taxonomic groups.

Throughout the ages, man has given names to living organisms, and that tradition goes back to the very early times, as shown in the very first pages of the Bible.

Once the names given to "taxa", the characters making it possible to identify them must be described. The choice of these characters is not fixed forever; it can change in the course of time; names too! Taxonomy is thus a dynamic science, in constant evolution, which must be regularly revised and adapted by taking the most recent discoveries into account.

The taxonomy of mycobacteria started with the description of the genus *Mycobacterium* by Lehmann and Neumann in 1896.

As mentioned by Tortoli (2003), "two major periods may be distinguished in procaryotic taxonomy, one characterized by the utilization of phenotypic studies and one characterized by a focus on genotypic characteristics". The phenotypic period started at the end of the 19th century and, despite their declining importance, phenotypic tests still play a role at present. The genotypic period started one century later, during the last decade of the 20th century, and continues to the present.

It is impossible to refer to the taxonomy of mycobacteria without mentioning the paramount role played by the International Working Group on Mycobacterial Taxonomy (IWGMT) and its founder, Dr. L.G. Wayne. The IWGMT was organized in 1967 by L.G. Wayne, together with a group of researchers working in the field of mycobacteriology. A series of cooperative studies was undertaken, and the principles of numerical taxonomy were used to incorporate the data from the different laboratories into a unified taxonomic scheme. The group published several important articles based on phenotypic data. The results of all these cooperative studies clearly helped to consolidate the phenotypic taxonomy of mycobacteria (Wayne et al.,

1971, 1981, 1983, 1989, 1991) and to identify highly reproducible techniques for the identification of the most frequently isolated mycobacterial species in medical laboratories (Wayne et al., 1974, 1976).

However, identification of mycobacteria by phenotypic tests alone may result in erroneous or incomplete identification because of the unavailability of a sufficient number of discriminative tests to identify isolates. Genotypic approaches to mycobacterial taxonomy offered a good alternative to phenotypic taxonomy. Genotypic taxonomy started with DNA-DNA hybridization methods and, later, with the use of comparative sequence analysis of 16S rRNA. This analysis has offered a good alternative to phenotypic taxonomy and confirmed the validity of phenotype-based identifications. Most species identified by phenotypic methods were indeed validated by genotypic methods. However, some mycobacterial species such as *M. marinum* and *M. ulcerans* that are clearly differentiated by phenotypic tests cannot be separated by sequence analysis of their 16S rRNA. Moreover, these two species are responsible for different diseases with different clinical manifestations.

As correctly pointed out by Wayne et al. (1996), although 16S rRNA sequence analyses are clearly very valuable for establishing evolutionary pathways, it is difficult to specify to what extent two sequences must be different to decide that two strains must be placed in two different species. Wayne et al. (1996) suggest that "sequence differences in the 16S rRNA molecules of more than 10 nucleotides probably indicate that organisms should be separated at the species level". These findings are derived from a cooperative study from the IWGMT that performed semantide and chemotaxonomic analyses on some problematic phenotypic clusters of slowly growing mycobacteria. Semantides are large information-bearing molecules that can be used to determine the evolutionary relationships among organisms. Three classes of semantides have been defined according to their role in the cell: primary semantides (DNA), secondary semantides (RNAs) and tertiary semantides (proteins). These semantides, together with some chemotaxonomic features such as fatty acid and mycolic acid patterns, were used in the study of Wayne et al. (1996). The data obtained from this study permitted the evaluation of the role of each technique in classification and identification of mycobacteria. DNA-DNA hybridization still remains the gold standard to establish whether two strains belong to the same species, because it reflects the affinity of the whole genome. In the last study of the IWGMT, the authors conclude that "we should continue to rely on polyphasic taxonomy to provide satisfactory resolution of members of the genus *Mycobacterium*".

We all know how it may be dangerous "to put all one's eggs in one basket"! Therefore, polyphasic taxonomy based on the use of a wide range of phenotypic and genotypic tests should always be used to identify mycobacterial species and to describe new species. Minimal standards should be recommended for the description of new mycobacterial species. In 1992, we proposed minimal standards for the *Mycobacterium* genus and for description of new slowly growing *Mycobacterium* species (Vincent Lévy- Frébault and Portaels, 1992). These standards should be updated by including the recent genetic techniques and by expanding to all mycobacteria, including the rapidly growing mycobacteria.

Since the development of genetic techniques, the description of an increasing number of new species has taken place. In his extensive review on the impact of genotypic studies on mycobacterial taxonomy, Tortoli (2003) reminds us that 42 new mycobacterial species had been officially recognized in the last 14 years preceding the publication of his review article; thus about half of all mycobacterial species have been described recently, and the list grows longer every day!

Although the second chapter of *Genesis* authorized us to give names to living organisms, it may be wise to slow down this tendency to describe new species, as these species are sometimes described on the basis of too few criteria to allow differentiation from known species. Sometimes, it happens also that these new species are represented by only one or two isolates.

Mycobacteria can be found everywhere in nature, and at all latitudes (Portaels, 1995). During our 30 years' experience of isolation of mycobacteria from the environment in Africa, America, Europe and Australia, we have obtained thousands of mycobacterial cultures, and, among them, about 10% of mycobacterial isolates that had never been described before! enough to describe new species wearing the names of all our friends and relatives! However, let us keep our feet firmly on the ground! The taxonomy of mycobacteria should first aim at the identification of the mycobacteria that are the most frequently found in medical and veterinary laboratories. This does not exclude mycobacteria of the environment since as will be shown in chapter 2 of this handbook, our regular contacts with those mycobacteria can have a considerable impact on man and animals.

This handbook is written in that perspective. Its objectives are listed in the introduction. It is a practical handbook that combines both the phenotypic and genotypic identification of mycobacteria. The primary aim is to identify in the most simple and correct ways the mycobacteria that are the most frequently found in medical and veterinary laboratories. The handbook results from the sharing of knowledge and experience by several Latin Amer-

ican and European scientists, who have always enjoyed working as a team, with a constructive critical mind and the uncanny ability to share. It is also a fact that all our collaborative activities were based on the sharing of the human values that are required in any successful partnership, i.e. trust and mutual respect. Not surprisingly, the sharing of those values resulted in deep and rewarding friendships.

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