A Tuberculosis Guide for Specialist Physicians

2003
International Union Against Tuberculosis and Lung Disease
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2003

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This publication was made possible thanks to the support of the United States Centers for Disease Control and Prevention (CDC)
Dedication

To my parents, for their unconditional support, humanity, honesty, hard work, and sacrifice. Their teachings and tolerance have been the best guides in my life.

Acknowledgements

First, I especially wish to express my most sincere gratitude to Drs. Paula Fujiwara (USA) and Dr. Victorino Farga (Chile), professors and friends, whose exhaustive review of this Guide has greatly improved it in terms of content and clarity. I also wish to thank:

Dr. Nils E. Billo, Director of the International Union Against Tuberculosis and Lung Disease, for his unconditional support of my daily work and for his constant encouragement in the preparation of this book.

Dr. Raúl Díaz, an excellent professional and friend, for his constant help and excellent editing activities in relation to the Guide.

Dr. Rodolfo Rodríguez (Cuba), Regional Advisor for Tuberculosis (Pan American Health Organization/World Health Organization), who was largely responsible for the improvements in tuberculosis control throughout Latin America in the past six years. My thanks for his constant support of the Tuberculosis Updating Courses for Specialist Physicians, which have helped to ensure improved tuberculosis control in Latin America. The development of these courses has generated the material presented in the present Guide.

My colleagues in the Pneumology Service of “Dr. Negrín” University Hospital in Gran Canaria, and particularly Dr. Pedro Cabrera-Navarro, for his understanding and constant support and stimulus in my assistential and operative activities.

To the following colleagues, friends, and companions with whom long hours have been spent discussing tuberculosis, and whose teachings and publications have formed the basis of the information presented in this publication: Drs. José Alcaide (Barcelona), Nieves Altet (Barcelona), Vicente Ausina (Barcelona), María José Báguena (Valencia), César Bonilla (Peru), María Isolina Campos (Las Palmas de Gran Canaria), Manuel Casal (Córdoba), Joán Caylá (Barcelona), Donald Enarson (Canada), Antonio Lobo (Jerez-Cádiz), Pilar López-Facal (Las Palmas de Gran Canaria), José María Manterola (Barcelona), Juán Ruiz Manzano (Barcelona), Pere de March
(Barcelona), Carlos Martín (Zaragoza), Juan Domingo Palmero (Argentina), María José Pena (Las Palmas de Gran Canaria), José Luís Pérez-Arellano (Las Palmas de Gran Canaria), José María Pina (Barcelona), Rafael Rey (Madrid), Miguel Angel Salazar (Mexico), Jesús Sauret (Barcelona), and Rafael Vidal (Barcelona).
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>ADA</td>
<td>adenosine deaminase</td>
</tr>
<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
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<tr>
<td>ARI</td>
<td>annual rate of infection</td>
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<tr>
<td>Ak</td>
<td>amikacin</td>
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<tr>
<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>BCG</td>
<td>bacille Calmette-Guérin</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>Cfz</td>
<td>clofazimine</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>maximum concentration</td>
</tr>
<tr>
<td>Cp</td>
<td>capreomycin</td>
</tr>
<tr>
<td>Cs</td>
<td>cyclosorine</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>DOTS</td>
<td>Directly Observed Therapy, Short Course</td>
</tr>
<tr>
<td>E</td>
<td>ethambutol</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>ETH</td>
<td>ethionamide</td>
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<tr>
<td>H</td>
<td>isoniazid</td>
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<tr>
<td>HAART</td>
<td>highly active antiretroviral therapy</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency syndrome</td>
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<tr>
<td>IUATLD</td>
<td>International Union Against Tuberculosis and Lung Disease</td>
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<tr>
<td>Kn</td>
<td>kanamycin</td>
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<tr>
<td>LCR</td>
<td>ligase chain reaction</td>
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<tr>
<td>LTBI</td>
<td>latent tuberculous infection</td>
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<tr>
<td>MDR</td>
<td>multidrug resistance</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>NTP</td>
<td>National Tuberculosis Control Programme</td>
</tr>
<tr>
<td>Ofl</td>
<td>ofloxacin</td>
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<tr>
<td>PAHO</td>
<td>Pan American Health Organization</td>
</tr>
<tr>
<td>PAS</td>
<td>para-aminosalicylic acid</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PPD</td>
<td>purified protein derivative</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
</tr>
<tr>
<td>R</td>
<td>rifampicin</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>S</td>
<td>streptomycin</td>
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</table>
SGOT  serum glutamic oxaloacetic transaminase
SGPT  serum glutamic pyruvate transaminase
T    thiacetazone
TB   tuberculosis
TLC  thin-layer chromatography
LTBI latent tuberculous infection
$T_{\text{max}}$  time to maximum concentration
TU   tuberculin unit
UN   United Nations
UNICEF United Nations Children’s Fund
UV   ultraviolet
WHO  World Health Organization
Z    pyrazinamide
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The need for cooperation between tuberculosis control programmes and specialist physicians

Chapter summary
At the start of the new millennium, tuberculosis (TB) remains the most important infectious disease in the world despite efforts in the past decade to bring the problem under control. This dire situation led the World Health Organization in 1993 to declare TB a worldwide health emergency and to recommend the intensification of combined efforts against the disease, in the form of the Directly Observed Therapy, Short Course (DOTS) strategy. However, the implementation of this strategy faces an important series of limitations that, while similar in many parts of the world, differ among regions or countries. For instance, in low-income countries, where 65% of all TB cases are found, the main limitations are a deficient health care infrastructure and extreme poverty. In contrast, in middle-income countries, where 30% of TB cases are found, the problems are more of an organisational nature, such as the lack of cooperation and coordination among the different health care structures in the country, as well as the strong presence of a private health care system, which gives specialist physicians and health care groups little incentive to follow the guidelines of a National Tuberculosis Control Programme (NTP). In these countries—the majority of which have numerous universities, medical schools, and health care institutions—it is important to ensure that these specialist physicians (many of whom are in private practice) become involved with the programme, particularly since they may offer certain expertise. To this effect, it is necessary to: 1) identify and address the reasons for the lack of integration of these professionals into the NTPs; 2) define the level of participation of these professionals and the contributions they may make to the NTPs; and 3) work with this group of professionals, particularly to improve the efficacy of the programme. It is the purpose of this Guide to help achieve these aims.

Introduction
At the start of the new millennium, tuberculosis (TB) is still the most important infectious disease in the world despite efforts in the past decade to bring the problem under control. The discouraging statistics on mortality and mor-
bidity associated with a disease for which there has been a treatment for over 40 years and that has been preventable for several decades suggest that efforts to control TB have not been optimal. In 1999, the World Health Organization (WHO) estimated that there were 8,400,000 new cases of TB worldwide—a figure very similar to that reported in 1993, when TB was declared a global health emergency and subsequent recommendations to intensify collaborative efforts against the disease led to the development of the Directly Observed Therapy, Short Course (DOTS) strategy. The use of these measures has increased steadily, and as early as 1999 it was reported that 23% of all recorded cases of positive smear microscopy had been studied and treated according to DOTS guidelines. Moreover, the DOTS strategy has begun to be implemented, albeit in various degrees, in 127 countries (60%) throughout the world, including the 22 countries with the highest prevalence of TB (an estimated 80% of all new cases).

The five basic measures of the DOTS programme involve the implementation of a series of simple but strict strategies at the most peripheral levels of health care (see Chapter 12). However, the implementation of this strategy faces a series of obstacles that, although similar in many parts of the world, differ by region and country. For example, in low-income countries, where 65% of all cases of TB are found, the main limitations for implementing the DOTS strategy are an inadequate health care infrastructure and extreme poverty. In such cases, additional resource allocations are necessary if the strategy is to be successful. On the other hand, in middle-income countries, where approximately 30% of all TB cases are found, the problems are more of an organisational nature: the lack of adequate cooperation and coordination between the different health care structures in the country, and the strong presence of a private health care system with specialist physicians and health care groups that are little inclined to follow the strict protocols of the programme. In these countries, the majority of which have numerous universities, medical schools, and health care institutions, it is important to ensure that specialist physicians (many of whom are in private practice) become involved with the programme, possibly by serving in a capacity that takes advantage of their expertise.

Thus, even though TB control is improving worldwide, there are concerns affecting certain middle-income countries, which will become a problem in many poorer countries in the near future. One priority is to ensure that physicians in private practice (mostly specialist physicians) become involved in the National Tuberculosis Control Programmes (NTPs). The present Guide will examine the reasons for the lack of integration of specialist physicians in these NTPs, and suggest ways of improving their participation.
Extent of the problem of tuberculosis in middle-income countries

In the past decade, most of the efforts to control TB have been in low-income countries, where the prevalence of the disease has been by far the highest. However, despite the slow improvement in TB control worldwide, 30% of all cases are reported in middle-income countries. In 1997, 58 countries comprised the middle-income group—from Lesotho at the lower end to Greece at the upper end. According to WHO data published in 1999, these countries reported a total of 945,335 cases of TB in 1997, or 28.3% of the total number of new cases reported that year to the WHO. This percentage of new TB cases was similar (at around 28%) in 1998 and 1999. Of the 22 countries with the largest TB burden in 1997, seven were middle-income nations: Indonesia, the Philippines, South Africa, Russia, Brazil, Burma, and Peru. These seven countries accounted for 1,395,000 new cases in 1997, equivalent to 22% of cases from the 22 countries with the greatest TB problems in the world (6,361,000 cases in 1997) or 19% of the global total in that year (7,963,000 cases). Again, these figures were similar in 1998 and 1999. In North and South America—with the exception of the United States, Canada, Haiti, Nicaragua, and Honduras (the last three countries classified as low income)—the remaining middle-income countries reported a total of 214,342 new TB cases in 1997 (6.36% of the world’s total). In most of these middle-income countries, the existing health care infrastructure is adequate, and there are economical means of implementing the basic intervention strategies. The problem often lies in the above-mentioned organisational concerns, such as the management and integration of university and specialist physician groups, which have been overlooked in the intervention actions carried out to date.

Reasons for the lack of integration of specialist physicians in tuberculosis control programmes

TB is an infectious disease. Each case not only affects a certain individual, but also places the community at risk of infection. For this reason, measures are also needed to control the transmission of the disease. The best way to arrest TB transmission is to establish a diagnosis as early as possible, and particularly to ensure complete cure. However, although treatment may be relatively easy, cure is difficult; therapy is prolonged (6-8 months), lasting
for several months after the symptoms are gone. Thus, it is essential that patients strictly adhere to the prescribed treatment until cure is complete. Public health authorities in each country must assume this responsibility of monitoring patient compliance in order to ensure cure.

The implementation of a series of basic measures to control TB in the community is the objective of all NTPs. Since TB can affect all strata of society, an NTP must include all health care centres, including those at the most peripheral levels, where facilities are limited and personnel may lack specific skills. This means that the protocols must be very elementary in order to facilitate their implementation even when resources are severely limited. These basic measures—detection of respiratory symptoms, performance of serial smear microscopy in suspected cases, implementation of a single therapeutic scheme, follow-up of therapy until complete cure is achieved, and use of an elementary information/registry system—must be followed by all health care personnel, regardless of their level of training. However, it has been observed that the more specialised the staff, the more likely the lack of adherence to the NTP guidelines, and consequently the more difficult it becomes to ensure cooperation with the NTP. In short, the most qualified segment of the health care system, consisting largely of specialist physicians, has become a genuine obstacle in the course of NTPs in many low- and middle-income countries.

This lack of integration of specialist physicians can be attributed to two reasons: the characteristics of this professional group and the programme guidelines. Specialist physicians—particularly pneumologists, internists, infectious disease specialists, and paediatricians—who often possess solid training in the pathogenesis, diagnosis, and treatment of TB, are very reluctant to accept the strict and simplistic action plans, which were formulated as such to ensure that they could be carried out with the minimum of resources by individuals with limited training. These specialists must be made aware that although the knowledge of the pathogenesis, diagnosis, and treatment of the disease may be complex, the actual measures required for effective TB control are much simpler. In low- and middle-income countries, 70% of all diagnosed cases involve a positive smear microscopy, a rate that is higher in poorer countries with a worse epidemiological situation. All patients can be treated with perfectly standardised regimens that are well tolerated and easy to implement, even in the most peripheral areas. Smear microscopy is extremely simple and inexpensive to perform in any setting. For this reason, most patients with TB, including almost all of the most contagious cases, can be treated in any setting, as long as there is access to a conventional microscope and the basic antituberculous drugs are available.
The lack of specialist integration in these programmes is also partly related to the fact that NTPs have not been overly concerned about including this group of professionals in their activities. Indeed, the potential usefulness of contributions by specialists has been overlooked. Many specialist physicians are unaware of the NTP norms manual, often because the managerial body of the NTP did not contact them. Consequently, an opportunity for dissemination of the basic guidelines of the programme to this important group of physicians is being lost.

The role of specialist physicians in an NTP

Specialist physicians can play a fundamental role in many aspects of an NTP, particularly at the higher levels. All Central Units of an NTP should have a pneumologist or a specialist in a clinical area closely related to TB, an internist, an infectious disease specialist, or a paediatrician. This professional can work either full or part time for the programme, serving as its clinical authority. The work of the specialist would be fundamental to the preparation of the NTP norms manual, the training of personnel at all levels of the health care system, and the planning of health study programmes in universities and medical schools. The specialist would also serve as the chief authority for all cases involving diagnostic and treatment complications, particularly with regards to patients infected with multidrug-resistant strains. The specialist may be called upon to procure second-line drugs (these substances being costly and difficult to obtain) and to ensure that these drugs are dispensed responsibly throughout the country.

An important requirement is the establishment of a Central Unit Advisory Commission for the NTP, serving in a consulting capacity, which should meet every 1 to 3 months according to the phase and needs of the programme. The Advisory Commission should preferably include a member of each of the medical specialties related to TB, including a pneumologist, a microbiologist, an infectious disease specialist, an internist, a paediatrician, an epidemiologist, a nurse and/or auxiliary nursing staff member, and a social worker. This structure serves to ensure increased representation. Where possible, each of these individuals should be chosen by the respective scientific societies or professional colleges. This Advisory Commission will have a central role in preparing the NTP norms manual, designing the training model for health care workers in the country, planning the teaching curriculum in medical schools and universities, and preparing the action guidelines.
All regional units of the NTP should also have a clinical specialist, even if working only part time or as a consultant. This professional will be the regional specialist for the programme and will be in charge of coordinating the actions of the different specialist physicians in relation to diagnostic difficulties (e.g., performance of punctures, bronchoscopy, pleural biopsies) and treatment problems (e.g., drug intolerances, treatments in special situations, re-treatment). The regional specialist will also be involved in training personnel at the regional and peripheral levels. This specialist will preferably work in the hospital setting, where the more difficult cases are seen. This physician must therefore be completely familiar with the norms of the NTP, the performance of the different available diagnostic techniques, and the potential therapeutic options in situations of treatment difficulty. The integration of these specialist physicians in the actions of the NTP at the regional level is therefore critical.

At the peripheral level, the NTP does not require the intervention of specialist physicians, at least in the institutional sector. As has been commented above, the main functions at the peripheral level include the diagnosis of cases with positive smear microscopy and the supervision of treatment. Such activities can easily be carried out by less qualified personnel. Nevertheless, if specialist physicians are found working at the peripheral level, they must adhere to the NTP guidelines regarding the principal functions described above. The same applies to specialist physicians working in the private sector, who preferably should refer diagnosed cases to the NTP, which, in addition to providing free medication and diagnostic evaluations, can also ensure close monitoring of patients for proper adherence to therapy. In order to prevent the private practitioner from losing influence on the patient, he or she may conduct the required clinical controls throughout the course of therapy. Here again, problems are frequently found among specialist physicians working at the peripheral level, owing to a lack of adherence to the NTP guidelines.
Chapter 2 - Importance of the role of specialist physicians and their integration in the strategies of a tuberculosis control programme

Chapter summary
Having acknowledged the important role of specialist physicians in the activities contemplated by National Tuberculosis Control Programmes (NTPs), and particularly the obstacle such professionals may represent if they are not successfully integrated, attention should now focus on the need to implement concrete strategies for incorporating these specialist physicians in the work of an NTP. This task involves the development of specific training plans adapted to their level of expertise.

It is important to recognise that these professionals have long been excluded from the training strategies of NTPs, strategies that are key to ensuring the future and sustainability of a tuberculosis (TB) control plan. However, this group of highly qualified professionals cannot be dealt with using the same simple training terms as those designed for personnel working at the peripheral levels. Owing to the complexity of elaborating a training strategy specifically for specialist physicians, and the difficulty of finding qualified personnel to implement the strategy, NTPs have largely neglected the need to integrate and train specialist physicians.

Specialised physicians with extensive knowledge in the clinical practice associated with TB, and with sufficient operational knowledge regarding control of the disease in the community, should carry out the training of this important professional sector. In this way, specialist physicians can gain both up-to-date information on the subject and practical knowledge that can be applied to the specific problems in their respective countries. Training should be an interactive process in which specialists are free to voice their opinions. Through active dialogue, agreements can be reached regarding the activities of this professional sector, whose role is to support the endeavours of the NTP.

The above reasoning process led the International Union Against Tuberculosis and Lung Disease and the Pan American Health Organization in 1997 to identify specialist training as one of the priorities in Latin America. A series of intensive 25-hour training modules were thus designed, lasting 3 to 5 days, which have since been implemented in many countries in the region. These measures have resulted in a fundamental change in the level of participation of specialist physicians in the NTPs. The methodology of these modules is described in the present chapter.
Rationale for the operational applicability of current knowledge on tuberculosis

As with all areas of knowledge, it is important to be able to understand what information can be effectively applied in real life and what part is for reasoning or hypothesizing. In the field of tuberculosis (TB), it is important to be able to determine what information about the disease is relevant to, and useful in, the control of infection in the community.

TB is most likely the disease that has given rise to the most publications and scientific studies in the history of medicine. However, the great majority of such publications come from wealthier, industrialised countries where TB is not a major problem, and consequently their research findings often have little relevance in those parts of the world where TB is a genuine problem. Such literature, which is read mostly by specialist physicians, may lead their readers to adopt diagnostic and therapeutic attitudes that are different from those suggested by the National Tuberculosis Control Programmes (NTPs). Therefore, these physicians tend not to follow or contribute to the programme’s information and registry system.

It should be noted that the main problem of controlling TB has little to do with having profound knowledge of the disease. As explained in Chapter 1, the present focus is on curing patients diagnosed by smear microscopy and detecting as many cases as possible with the least possible delay. The success of such efforts depends on the availability of drugs, the identification of most respiratory symptomatic cases with smear microscopy evaluation, and treatment that is supervised by health care personnel.

Provided sufficient medication is available, with acceptable distribution of smear microscopy laboratories—concerns of the NTP that are not a problem in most middle-income countries—the objective of effective TB control can be achieved by decentralising diagnosis and treatment routes. Only by taking diagnosis and treatment as close to the periphery of the health care system as possible can the objectives of case detection and cure rates be met. This is why the protocols must be as elementary as possible, since many patients will not be cared for by nurses or clinical auxiliary personnel.

TB control at the peripheral level requires the availability of personnel who know that any person with a cough and/or expectoration for more than 2 weeks should be subjected to serial smear microscopy, and that all individuals with a diagnosis of TB must be treated and followed for adherence to treatment until cure is complete. Any deviation from these norms will jeopardise the goals of a TB control plan.
Specialist physicians must be made aware that they should avoid under-utilisation of their expertise when dealing with the diagnosis and treatment of cases with positive smear microscopy, unless these patients have complications or pose diagnostic or management difficulties. The hospitalisation of uncomplicated TB cases as a result of management by specialist physicians is a common problem in many countries, one that is associated with increased costs and losses in efficacy. Often, once the patients have been discharged, they encounter poor access to smear microscopy laboratories and difficulties in the direct supervision of their treatment. However, specialists may and should play an active role in TB control in their countries, although, as was explained in Chapter 1, such participation should take place at higher levels. Furthermore, their responsibilities must be approved by the NTP management body, which often comprises epidemiologists and health care specialists with a sound understanding of the measures required to control TB in the community.

Although the points discussed above seem reasonable, very little effort has been made to ensure that they are understood and accepted by specialist physicians. Frequently, specialist physicians do not even know what such disease control programmes are, or why the NTP defines guidelines that seem so basic to them. Much effort is therefore required to educate these physicians so as to facilitate their participation in the plans contemplated by an NTP.

The need for specific training among specialist physicians

One of the fundamental tasks of all NTPs is continuous supervision and training. This is the only way to ensure that the personnel participating in the plan are well trained and that their skills remain up-to-date. Much of the aid supplied by rich countries to nations with fewer resources is for the training of personnel. However, when the managing bodies of NTPs develop plans for the training of health care personnel, their efforts almost exclusively focus on the peripheral levels of medical care, which is a reasonable action considering that these are the areas where the main activities of the TB control plan take place. The regional or national director of the programme may undertake training of personnel at the peripheral levels. However, the more specialised professionals have almost always been excluded from the instruction process, based on the false assumption that training in their case is much more difficult.
It is true that in the case of specialists it is not always possible to implement the standard training measures of simply teaching the basic rules of the Directly Observed Therapy, Short Course (DOTS) strategy, since such messages appear so simple to specialist physicians that they tend to ignore them. Also, the NTP management bodies often do not have the resources to train these professionals, a situation that results in a lack of consensus between specialists and the NTP about standard interventions in TB control. If this pool of professionals is small in size (as in low-income countries), they will have little influence on the results obtained by the NTP, whereas if there is a reasonable number of specialist physicians, some of whom working at the middle or peripheral levels of the health care system (as is often the case in middle-income countries), then their influence will be greater and more likely to affect the results of the programme. This group of specialist physicians therefore needs to undergo specific instructional measures to ensure that their knowledge is current and applicable to the problems in their respective countries.

Such specific training is all the more important considering that most specialists, such as pneumologists, internists, and paediatricians, from low-and middle-income countries do not undergo their postgraduate/specialist training in their own countries, but do so in developed countries where the health care and economic conditions are most likely very different. Consequently, the training of such specialists may not be applicable in their native countries where completely different approaches to addressing the problem of TB are often required.

**Experience of the IUATLD in the specific training of specialist physicians**

Having recognised the importance of these specialists in the actions contemplated by an NTP, and the obstacles they may inadvertently pose, the training of these professionals should be considered a priority in many low- and middle-income countries. For this reason, and because the great majority of Latin American countries meet the expressed requirements, in 1997 the International Union Against Tuberculosis and Lung Disease (IUATLD) and the Pan American Health Organization (PAHO) identified specialist training as one of the priorities in the region. It was concluded that the best way to engage this group of physicians would be to provide high-quality, specific training conducted in close cooperation with the Central Units of the NTP and the different scientific societies in each country. A series of intensive
25-hour training modules were thus designed to be conducted over the course of 3 to 5 days, with the length of the course depending on the availability of the physicians, since many physicians are in private practice and have tight schedules.

These courses or modules, titled “Importance of the actions of specialist physicians and their adequate integration in the strategies of an NTP”, have been progressively adopted in the different countries throughout the region. The target audience comprises a select group of pneumologists, specialists in infectious disease, internists, paediatricians, and other specialists, as well as supervisors of the Central Units of the NTP. The course enrolment is deliberately kept small so as to facilitate interaction between instructor and trainee.

The topics covered during these training sessions are presented in Table 1. Information on each topic is updated and supported by sound scientific evidence, and sections that have practical application are detailed. There is special emphasis on the role of specialist physicians, so as to elicit their maximum participation and to ensure their best possible integration in the actions of the NTP. For each topic, 60% of the time is dedicated to providing up-to-date information on the subject, whereas another 30% is dedicated to discussing what part of the information has practical application in the field. The remaining 10% of the time is used to reach specific agreements among the participants on courses of action regarding each of the topics. As a special rule, the next topic is not dealt with until the entire group agrees on the issues presented on each subject and signs off on agreements on lines of action. These courses are conducted by specialist physicians with extensive knowledge on the clinical practice of TB and who have been adequately instructed about the practical control of the disease in the community.

If the course takes place over 3 days, as is often the case, then the first day is dedicated to the epidemiology and pathogenesis of the disease; the second day to the diagnosis, detection, and treatment of cases, including resistance to antituberculous drugs; and the third day to TB control, NTP management, and other relevant topics, such as TB in children, extrapulmonary TB, and mycobacteriosis. In addition, each participant receives extensive literature, including the most relevant studies on the topics covered in the course. The literature is in Spanish, since the courses are given in Spanish-speaking countries. The reading package comprises at least 70 articles classified by topic, together with the most relevant TB guides published by the IUATLD and WHO/PAHO. At the end of each course, a set of collaboration agreements is drawn up to facilitate the integration of these specialist
physicians in the activities of the programme. These agreements are in compliance with the NTP rules, and it is hoped that the participants will follow them.

A total of 21 courses have been conducted up until late 2002: five in Mexico, three in El Salvador, two in Honduras, two in Guatemala, two in Nicaragua, one in the Dominican Republic, two in Peru, one in Bolivia, one in Costa Rica, and two in Ecuador. In all these countries there has been a marked improvement in the integration of specialist physicians in the respective NTPs—although this improvement is somewhat difficult to quantify with objective parameters since it is based only on the observations of the national supervisors of the control programmes. Still, these supervisors have been very satisfied with the training model, and almost complete integration of these specialists in the NTP has been achieved in all the participating countries.

The activities described here should be viewed as the first specific intervention relating to TB control for this group of specialist physicians. Such interventions can be further improved in the future.

Table 1. General programme of the training courses for specialised health care professionals of the IUATLD

| 2. | Rationale for the course. Role of the specialist in the activities of the NTP. Action levels of the specialist in the context of an NTP. |
| 3. | The natural history of TB. |
| 4. | Etiologic agent and transmission epidemiology. Factors associated with transmission. |
| 5. | Epidemiology of TB. Epidemic wave phenomena in TB. Principal TB evaluation indicators. |
| 6. | Assessment of the state of TB infection in the community. Utility of, and difficulties associated with, tuberculin evaluations. |
| 7. | Current situation of TB in Latin America and the rest of the world. |
| 10. | Tuberculin testing. Standardisation and interpretation of results. |
| 11. | Clinical manifestations of TB. Symptoms and signs according to location. |
| 12. | Radiology and other imaging techniques in the diagnosis of TB. |
14. New diagnostic methods for TB.
Applicability in low- and middle-income countries.

15. Treatment of TB. Bacterial populations.
Rationale of the initial treatment scheme.
Special situations in TB therapy.

16. First- and second-line antituberculous drugs.
Mechanisms of action and side effects.

17. Basic concepts for planning re-treatment.
Re-treatment regimens.

18. Side effects of antituberculous drugs.
Adverse reactions to antituberculous drugs.
Role of the specialist.

Basis and genetic markers of resistance.

20. Epidemiology and the current situation of drug resistance worldwide.
Strategies for avoiding the development of drug resistance.

21. Extrapulmonary TB.
Clinical and epidemiological importance.
Role of the specialist.

22. TB in childhood.
Differences with respect to adult TB.

23. Diseases caused by environmental mycobacteria.
Epidemiology, pathogenesis, diagnostic methods, and therapeutic regimens.

24. Basic measures for TB control in the community.

25. Strategies for increasing cure rates.

26. Strategies for increasing case detection.
Development of a national TB laboratory network.
Quality control.

27. Measures for preventing TB.

28. Chemoprophylaxis.

29. BCG vaccination.

30. Importance of training, supervision, and evaluation.

31. Elaboration and management of a registry and information system within the context of an NTP.

32. Drug provision with adequate quality control.

33. Health education and community participation.

34. Elaboration of an infrastructure for adequate NTP functioning.
Role of specialised care.

35. Conclusions of the course.
Collaboration agreements.
Development of possible work projects integrated within the NTP.

BCG = bacille Calmette-Guérin; DOTS = Directly Observed Therapy, Short Course; HIV = human immunodeficiency virus; IUATLD = International Union Against Tuberculosis and Lung Disease; NTP = National Tuberculosis Control Programme; TB = tuberculosis.
Chapter 3 - A brief history of tuberculosis

Chapter summary
Much has been speculated about the origin of tuberculosis (TB) in humans. TB is considered by many to be one of the oldest diseases affecting humans. However, despite this age-old relationship, there has been a considerable lack of knowledge about its pathogenesis until very recently. This chapter speculates about the possible origin of Mycobacterium tuberculosis and describes the efforts made throughout history to combat the disease. The only known measures for controlling this endemic disease are improving the existing socioeconomic conditions (which can lead to an annual risk reduction in infection of 4-6%) and using effective pharmacotherapeutic measures (which is associated with an additional 7-9% in risk reduction).

Speculations on the origin of Mycobacterium tuberculosis

Tuberculosis (TB) is one of the oldest diseases known to affect humans. The causal microorganism is one of the best examples of how the selection process allows the survival of a species that has been able to adapt to change and adverse conditions. Thus, although Mycobacterium tuberculosis is estimated to have existed for 15,300 to 20,400 years, based on its infrequent loss of nucleotide diversity and its mutation capacity, it is increasingly accepted that the species evolved from other more primitive microorganisms belonging to the same genus, Mycobacterium. It is reasonable to assume that if the majority of organisms belonging to this genus naturally inhabit water and soil, the genus probably originated in a similar environment. Different species have emerged in the course of history, and environmental pressures have conditioned changes in their evolution. Some species of Mycobacterium (e.g., M. ulcerans) are estimated to be 150 million years old, suggesting that the genus is far older than that of the primates, including Homo sapiens. It would not be unreasonable to suppose that at some time in the course of evolution, some mycobacterial species, as a result of natural selection, could have established their reservoir in animals. This may have given rise to an early precursor of M. bovis, which is considered by many to be the oldest of the species forming part of the “M. tuberculosis complex”, which includes M. tuberculosis, M. bovis, M. africanum, and M. microti. Based on this widely accepted theory, a next step in the evolution of Mycobacterium would
be the infection of humans by *M. bovis*, which coincides with the domestication of cattle. It is at this point where *M. tuberculosis* may have emerged as a human pathogen. Indeed, scientific evidence has shown that in the last few thousand years the bacterium has maintained its ability to adapt to hostile environments, as did its predecessors. Thus, in the last 100 to 150 years *M. tuberculosis* has gradually flourished in the most vulnerable areas of the planet, where poor living conditions aid the organism’s survival and transmission and limited economic resources hinder efforts to combat the disease. Indeed, at the start of the new millennium TB remains the most important infectious disease in the world, and *M. tuberculosis* is the pathogen responsible for the largest number of deaths, with rates that compete with those of human immunodeficiency virus and the agent that causes malaria. Furthermore, although TB can be cured and controlled, it has not been eliminated in many industrialised countries.

**History of tuberculosis and the struggle to combat the disease**

Despite the fact that TB is a disease of antiquity and it is probably one of the illnesses most dealt with in the literature, there has been surprisingly little sound knowledge of the disease through the course of history, which has not helped contemporary efforts to combat the illness. From the time of Hippocrates (c. 460-377 BC) up until the nineteenth century, the infectious nature of the disease was not even acknowledged; rather, TB was considered a hereditary disorder. However, air—a common vehicle for the transmission of live germs—was included among the interpretations of the possible origin of the disease. For this reason, the dietary regimen proposed by Hippocrates and Galen (c. 130-200 AD) remained the basis of treatment up until the Renaissance. This practice changed very little in the seventeenth century, the sole difference being the recommendation of physical exercise and the use (as with other diseases) of new medicinal substances introduced in Europe at the time, such as quinine, coffee, tea, cocoa, and even tobacco. Such lack of understanding partly explains why humankind has been unable to defend itself against this terrible illness for the most of history—the only option being to fall ill and ultimately die.

Only towards the latter half of the nineteenth century did the infectious nature of TB become apparent, as a result of the studies by Villemin (1865) and, particularly, Robert Koch (1843-1910). Koch was the first to suggest the possibility of controlling this endemic disease, with the presentation of
the results of his research (in 1882) that showed that TB was a contagious disease. He not only isolated the bacterium, which was latter named after him (Koch’s bacillus), from the sputum of infected patients, but also proposed that the principal measure for controlling the disease in the community would be to isolate affected patients. This suggestion paved the way for the “sanatorium” era of TB, during which prolonged confinement of patients in sanatoriums was believed to be the only effective way to cure TB and control its transmission.

Based on the above, and throughout the prolonged history of the disease, it can be seen that the human host defences were the only means to counter *M. tuberculosis*. In this confrontation between the microorganism and the immune defence system, the latter tended to prevail—as a result of which only a very small proportion of infected individuals eventually developed the disease. However, when the disease became more established, the prognosis became very bleak in most cases—with a mortality of more than 50% five years after the onset of the disease. In turn, 25% of infected patients died within 18 months. Cure was only achieved in 25% to 30% of cases; the rest remaining chronically ill while continuing to spread the disease throughout the community. This extremely poor prognosis led to the development of various treatment attempts, most of which were empirical in nature and which proved to be ineffective. For this reason, when reviewing the history of TB therapy, two major divisions should be established: treatment in the pre-pharmacotherapeutic era, and treatment in the period corresponding to the last 50 years during which effective cure became possible.

In the eighteenth century, treatment recommendations included moving to the countryside and partaking in moderate activities. There was still special attention to diet, with medication reserved for the initial or “inflammatory” stage of the disease. Thus, during the initial phase, treatment involved bleeding, antiemetic agents, and a light diet, whereas treatment in the “ulcerative” phase of the disease involved balsamic products, expectorants, and opium.

In the early part of the nineteenth century, the practice of bleeding became more common, after the “irritative” doctrine developed by Brousais, who introduced the use of leeches as therapy for TB in the first third of that century. Some were opposed to this practice, including Laënnec, on the grounds that bleeding neither prevented the formation of tubercles nor eliminated them once they had developed. The debate over what constituted appropriate treatment continued over the subsequent years, during which the notion of the disease being associated with “impure air” regained popularity.
Thus, climate, exercise, and diet were again regarded as fundamental to TB therapy. Accordingly, patients were sent to places where they could exercise outdoors while observing a “proper” diet and medication regimen, all under strict medical supervision.

In this way, sanatoriums for patients with TB were created and became the standard treatment in all rich countries during the second half of the nineteenth century and the first half of the twentieth century. Indeed, the level of health care in a country could be determined by the number of sanatoriums it possessed. This emphasis on sanatoriums was reinforced by physiologists at the time, who considered the disease to be a consequence of the inability of the heart to drive blood through the lungs, which supposedly favoured the growth of tubercles. For this reason, sanatoriums were constructed at high altitudes, where a reduction in atmospheric pressure was believed to increase cardiac function and, consequently, improve pulmonary circulation. These theories had epidemiological support, which suggested that communities living at high altitudes were less likely to suffer from TB.

What, however, were the actual treatment success rates in these sanatoriums? Possibly the best records are those published by Sabourin in 1913, which were based on the results of 20 years of experience with 1,200 patients treated in the sanatorium in Durtol, France. The rate of complete healing was 39%, a figure very similar to that observed when TB is allowed to evolve on its own—thus questioning the efficacy of sanatoriums. However, the rate of healing reached 71% among patients in the early stages of the disease, as compared with 7.5% among the rest of patients. This difference clearly pointed to the importance of an early diagnosis and treatment, which are the same considerations in TB control today.

Another important period in the history of TB management involved the use of different surgical procedures to heal the disease. As early as the second century, Galen pointed out that the main difficulty in healing lung ulcerations in TB was the impossibility of keeping the lung at rest because of continuous breathing movements. Isolated observations indicated that when a lung collapsed spontaneously during the course of the disease, the disease proved easier to heal. Consequently, it was proposed to collapse the lung to allow it to rest and thus aid healing. This theory led to the development of surgical procedures such as chondrotomy of the first rib; thoracoplasty (removal of ribs to ensure pulmonary collapse); resection surgery; sectioning of the phrenic nerve to achieve diaphragmatic paralysis; scalenotomy (sectioning of the scalene muscles inserting in the first rib); extrapleural pneumolysis (separation of the lung and both pleural layers); filling the
extrapleural space with substances such as abdominal fat, paraffin, air, polyethylene sponges, Lucite pellets, ox spleen capsules, or wax; and, especially, pneumothorax induction. The last approach marked the beginning of the surgical era in the treatment of TB and was the most widely used methodology from the late nineteenth century to well into the mid-twentieth century.

There have been no adequate studies allowing an in-depth evaluation of the impact of these invasive techniques on patient healing. What is clear is that they were associated with high morbidity and mortality. Moreover, general anaesthesia was in its infancy at the time, and most of these procedures were carried out using local anaesthesia. The healing rate probably did not exceed 40%—this being the figure recorded for thoracoplasty up until 1927, with an associated mortality of 16%. As with the non-surgical treatments, this rate of healing was only slightly higher than that associated with untreated disease.

Therapy for TB changed dramatically with the introduction of antibiotics for the management of infectious diseases. In fact, each newly introduced antibiotic was tested against TB—the main health care problem in the world at the time. Sulphanilamide was the first sulpha drug (in 1938) to be used against TB. However, this agent was found to be ineffective in humans, although it had an inhibitory effect on TB in guinea pigs. A similar lack of efficacy was observed with more complex sulpha agents such as promanide (1943) and penicillin, which Alexander Fleming began to use in clinical practice in 1941. All this changed with the introduction of streptomycin by Waksman and Schatz in 1943, which has been used against TB since 1944. Since then, other effective antituberculous agents have been developed, leading to TB finally becoming a treatable disease in the mid-1950s.

**History of tuberculosis control**

It should be pointed out that even before these advances in TB treatment, TB had started to come under control in the richer countries without the adoption of any specific control measures. In effect, improvements in the socioeconomic conditions in developed countries since the eighteenth century had started to have a slight effect on curbing the spread of the disease, with a reduction in the annual mortality rate of 4% to 6%.

It has not been possible to demonstrate whether patient isolation in sanatoriums had an impact on TB control or whether it contributed to the annual reduction in disease mortality attributed to improved living conditions. In
reality, few patients had access to such centres and there was a lack of effective therapy, which supports the suggestion that sanatoriums did not have an important epidemiological effect on the evolution of TB. Likewise, the surgical procedures developed to treat TB most likely had little impact on the disease. Such treatments may have benefited a select few; most patients continued to spread the disease in the community for a long time. Thus, it is likely that such procedures afforded little more than what was offered by the improvement in socioeconomic conditions of the time.

The discovery of streptomycin and the beginning of the chemotherapeutic era of TB management was undoubtedly the most important advance in the struggle against the disease, not only for the individual, but also for the community. By adopting these measures alone, countries were able to lower the annual risk of infection by 7% to 9%. Furthermore, by combining chemotherapy with improved socioeconomic conditions, steady annual reductions in infection risk of 12% to 14% could be achieved. This has been the situation in the majority of developed countries in the past 30 to 40 years. In contrast, poorer regions have not seen these improvements, and consequently the gap between developed and developing countries in relation to the disease has widened.

**Recommended reading**

Chapter 4 - Epidemiology of Tuberculosis

Chapter summary
The study of the epidemiology of tuberculosis (TB) should comprise two major parts: the epidemiological chain of transmission, and the detailed analysis of the state of the epidemic in the world and the factors influencing it. The epidemiological transmission of TB requires a causal agent (the *M. tuberculosis* complex), a reservoir or host (infected healthy and/or diseased human) capable of infecting others (diseased human), a transmission mechanism (airborne), and a susceptible host.

With regards to the state of the epidemic and its evolution, three parameters—mortality, morbidity, and infection—may be quantified by following the new cases (i.e., the incidence of the disease) reported in the population every year. The World Health Organization estimates that there were 8,417,000 new cases in 1999 (global rate 141/100,000), of which 3,724,000 corresponded to smear-positive carriers (rate 62/100,000). Eighty percent of all these cases were from 23 countries.

It is clear that a series of factors or interventions are able to influence the course of this endemic disease. Of these factors, only improvement in socioeconomic conditions (affording an annual reduction in infection risk of 4-6%) and adequately administered anti-TB therapy (offering an additional annual risk reduction of 7-9%) have been shown to halt the progression of TB. In contrast, poverty, HIV, massive immigration from highly endemic zones, the non-existence or ineffective establishment of National Tuberculosis Control Programmes, and demographic growth increase the prevalence of TB in many parts of the world.

The epidemiological chain

Section summary
*M. tuberculosis* is a bacillary-shaped (i.e., rod-like) microorganism and a strict aerobe. Its growth depends on the presence of oxygen and the surrounding pH level. It is highly resistant to cold, freezing, and drying, and is very sensitive to heat, sunlight, and ultraviolet radiation. Under adverse metabolic conditions, the bacterium enters a latent or dormant state, and multiplication can be postponed from days to many years. These characteristics have clearly helped the microorganism to survive in humans.
Humans (either healthy infected or diseased) are the main reservoir or host for *M. tuberculosis*, whereas cattle are the main reservoir for *M. bovis* and other animals serve this function for the *M. tuberculosis* complex. The source of infection is almost exclusively represented by the diseased human. In turn, air is the most important route of transmission, and is responsible for almost all cases of infection. In this context, pulmonary TB patients who are smear positive, along with those who cough and those who are not receiving treatment, are the most contagious. A number of conditions increase the likelihood of clinically manifesting the disease in the event of infection. These risk factors are often associated with some form of immune deficiency.

As with most infectious diseases, the tuberculosis (TB) epidemiological chain of transmission requires the existence of: 1) a causal agent capable of bringing about the disease; 2) a reservoir or source of infection where the microorganism is found; 3) a mechanism of transmission; and 4) a susceptible host.

**Causal agent**

Taxonomically, the causal agents for TB belong to the order *Actinomycetales* and the family *Mycobacteriaceae*. TB is caused by one of the four microorganisms comprising the so-called *M. tuberculosis* complex: *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*. From a health care perspective, TB produced by *M. tuberculosis* is the most important aetiology of TB because it is by far the most common. TB caused by *M. bovis* is less frequent in industrialised countries, owing to the existing control of TB in livestock and to the widespread pasteurisation of milk. However, *M. bovis* remains an important problem in developing countries. *M. africanum* is in turn responsible for a smaller number of TB cases in Africa, owing possibly to its lesser virulence. Finally, infection due to *M. microti* (the causal agent for TB in rodents) has recently been described in humans, mainly immunosuppressed subjects. The family *Mycobacteriaceae* includes more than 90 additional microorganisms that are found mainly in the environment. They exhibit little pathogenicity, although some can cause disease, particularly in situations of host immunodeficiency. These microorganisms are best described as environmental mycobacteria, or mycobacteria other than *M. tuberculosis*.

Although a single microorganism can cause TB from the moment it gains access to the host, it behaves as a polyvalent germ in the course of its growth. This is because the metabolism of the pathogen is dependent on
the variations in oxygen partial pressure and the pH of the infected organ system.

The chemical structure of *M. tuberculosis* comprises proteins, carbohydrates, vitamins belonging to the B complex, and minerals such as phosphorus, magnesium, and calcium. The protein component is the fundamental substrate responsible for delayed hypersensitivity reactions, and rapidly induces the so-called tuberculin reaction. Although this microorganism lacks a cerulean capsule, it contains a considerable amount of mainly complex lipids. Of these, mycolic acid is the most characteristic and is considered to be responsible for the staining properties of the bacterium, which are shared by other species such as the *Nocardia* and can change according to the age of the microorganism. The lipid-rich wall of the bacterium is also responsible for a number of its biological characteristics, such as resistance to macrophage action and drying. The isolated lipid component is capable of inducing the same host responses as the whole microorganism, including the formation of epithelioid cells and, occasionally, caseation. *M. tuberculosis* is unable to produce toxins, as a result of which it lacks primary toxicity. Nevertheless, it possesses a very important and complex antigenic component that is responsible for variation in virulence and pathogenic capacity features. This topic will be described in greater detail in Chapter 5.

Another important characteristic of the bacterium is its very slow rate of division (60-fold slower than that of *Staphylococcus*), which explains the lack of specificity in the clinical presentation and the very slow development of the disease. Such slow proliferation also explains why it is not necessary to administer the medication several times a day. The growth of *M. tuberculosis* is conditioned by the presence of oxygen and the surrounding pH level. There also appears to be interdependence between the anatomical distribution of the disease and the oxygen tension available in the area. For example, in TB of the upper lung lobes, where the comparatively lesser blood flow and ventilation induces an increase in alveolar oxygen pressure, the disease tends to spread towards these regions from the post-primary seeding sites. The ideal conditions for proliferation of the bacterium comprise a pH of 7.40 and an oxygen partial pressure of 100 to 140 mm Hg. Nevertheless, even under these conditions multiplication is very slow, occurring once every 14 to 24 hours.

When *M. tuberculosis* does not encounter a favourable environment (i.e., low oxygen pressures and low pH levels), it enters a dormant state, and multiplication can be postponed from days to many years. This latent state is also responsible for the maintenance of the disease.
Lastly, *M. tuberculosis* is a bacillary-shaped (i.e., rod-like) organism. It is highly resistant to cold, freezing, and drying, and very sensitive to heat, sunlight, and ultraviolet radiation.

### Source of infection

Humans (healthy and infected or diseased) are the fundamental reservoir or host for *M. tuberculosis*. Healthy infected individuals are one of the main factors that contribute to the perpetuation of TB, since they do not show any signs or symptoms of the disease. For example, a healthy infected population can act as silent carriers of the tubercle bacilli until their death, with only a number of them at some point developing tuberculous disease, especially if they have acquired some form of immune deficiency.

Although humans are the main reservoir for *M. tuberculosis*, cattle are the principal reservoir for *M. bovis*, and practically all animals (including monkeys, dogs, and cats) can also serve as reservoirs for the *M. tuberculosis* complex. However, while the great majority of these animals, particularly pets, can suffer from the disease, they are unable to transmit the illness because their size allows them to carry only relative small bacillary populations.

Only when non-diseased, infected humans develop tuberculous disease do they act as a source of infection. However, their ability to infect others depends on the location and stage of the disease. The most infectious presentations correspond to pulmonary TB, where the host capacity to spread bacteria is greatest. Among such individuals, the potential for contagion is greatest among those with the highest bacterial loads. Such patients show cavities on chest radiographs or have positive smear microscopy results.

### Mechanism of transmission

The airborne route is responsible for almost all cases of TB transmission. When speaking, singing, laughing, sneezing, and especially coughing, the infected patient expels microdroplets into the air, which contain the mycobacteria. Although the largest microdroplets (> 10 µm in diameter) also contain the largest number of bacteria, they tend to be deposited in the upper airways because of their greater weight, and thus possess a lower potential for infection. Aerosolised droplets measuring 5 to 10 µm reach the more proximal portions of the upper airways of the new host, where the conditions are not optimal for multiplication. However, the microdroplets that measure 1 to 5 µm in diameter and that are formed from the larger droplets as a result
of condensation after losing part of their water content typically contain 1 to 5 bacilli per microdroplet. Microdroplets are highly infectious since they can be deposited within the alveolar spaces. A minimum of 10 to 200 of such microdroplets can cause infection. The ideal site of deposition for these microorganisms in the new host is the best-ventilated region of the lungs, i.e., the subpleural zone of the lower lobes. It is in this distal portion of the lungs, with the high oxygen partial pressure, that conditions are ideal for multiplication. Initially, the macrophages, followed by the lymphocytes, migrate towards this region, and in the majority of cases are able to arrest microbial multiplication. However, when this initial defence mechanism is impaired, primary TB develops.

There are also other less frequent transmission mechanisms, such as the digestive route, where infection occurs via the pharyngeal or intestinal lymphatics owing to the consumption of beef infected with *M. bovis*. This route is also common in infections associated with *M. avium* in AIDS patients. Other means of transmission include the urogenital route, through urine and sexual transmission; the mucocutaneous route; inoculation; and placental transmission, particularly in cases of maternal miliary TB where the organism ultimately crosses the placental barrier (200-300 cases have been described in the literature to date). This last route of transmission gives rise to what is known as congenital TB.

The potential of a patient to infect others depends on the following factors:
1. The extent of disease. Patients with positive smear microscopy and those presenting radiographic evidence of pulmonary cavitations are regarded as highly infectious.
2. The severity and frequency of coughing. Infected patients who cough frequently are more contagious, since the microdroplets expelled are smaller.
3. The quality and volume of the respiratory secretions. Scantly viscous sputum often constitutes an ideal aerosol vehicle, and is therefore more contagious.
4. The antituberculous chemotherapy provided. Patients who receive such therapy are 50 times less infective than those who do not receive therapy. It is generally accepted that a patient is no longer infective after 2 weeks of treatment, although confirmation is not possible until smear microscopy results are negative.
5. The characteristics of exposure: i) the concentration of bacilli in the atmosphere, with the greatest transmission potential being found in small, closed rooms where the smear microscopy-positive TB patient spends
many hours; ii) room ventilation, with fewer bacilli being found in the air of well-ventilated rooms; and iii) the duration of exposure to the infectious TB patient, where the risk of transmission increases with close and prolonged contact.

**The susceptible host: risk factors**

Children under the age of 5 years and elderly subjects older than 65 to 70 years are more vulnerable to TB, partly because immunity is slightly reduced at these ages. It is not known, however, why children between the ages of 6 and 14 years are less susceptible.

Worldwide, TB affects men more often than it does women (60-70%). This higher prevalence has been attributed to differences in social habits, although an increasing number of studies are suggesting a slight genetic predisposition on the part of women.

Not all individuals are at the same risk of developing TB disease once infected. Several risk factors have been identified that are known to facilitate the development of the disease. These factors imply a greater or lesser degree of immune deficiency, with up to a 1000-fold increase in the risk of developing TB as compared with normal, immune-competent individuals. These factors and the relative risk of developing active TB versus the normal population are shown in Table 2.

*Table 2. Risk factors associated with developing TB disease. Relative risk with respect to the normal population.*

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV infection</td>
<td>50-100</td>
</tr>
<tr>
<td>Jejunocaecal shunt</td>
<td>27-63</td>
</tr>
<tr>
<td>Solid tumours</td>
<td>1-36</td>
</tr>
<tr>
<td>Silicosis</td>
<td>8-34</td>
</tr>
<tr>
<td>Head and neck neoplasms</td>
<td>16</td>
</tr>
<tr>
<td>Haemodialysis</td>
<td>10-15</td>
</tr>
<tr>
<td>Haematological neoplasms</td>
<td>4-15</td>
</tr>
<tr>
<td>Fibrotic lesions</td>
<td>2-14</td>
</tr>
<tr>
<td>Immunosuppressive drugs</td>
<td>2-12</td>
</tr>
<tr>
<td>Haemophilia</td>
<td>9</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>5</td>
</tr>
<tr>
<td>Low body weight</td>
<td>2-4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2-4</td>
</tr>
<tr>
<td>Heavy smoking</td>
<td>2-4</td>
</tr>
<tr>
<td>Normal population</td>
<td>1</td>
</tr>
</tbody>
</table>
Recommended reading


Analytical parameters of tuberculosis

<table>
<thead>
<tr>
<th>Section summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>The magnitude and temporal course of TB in a given community can be quantified by three parameters: infection, morbidity, and mortality. Anti-TB treatment introduced on a large scale in the community rapidly reduces mortality. However, mortality is not a good parameter for assessing the magnitude and temporal course of the disease. The best way to assess the TB trend in a community is to follow the new cases detected in the population on a yearly basis, particularly among those involving positive smear microscopy findings. The most interesting parameters of infection are the annual rate of infection (ARI) and the prevalence of infected individuals. The incidence is calculated by the so-called ARI, which describes the percentage of the population that will be infected or reinfected in the course of a year, and is based on a mathematical formula requiring knowledge of the prevalence of tuberculous disease in the community at a given age. Calculation of this parameter therefore depends on the results of community-based tuberculin test surveys (primarily tuberculin skin test conversion rates), which often vary and are associated with another set of difficulties. Estimations of the incidence of the disease derived from ARI results should be interpreted with caution. A much more useful approach is to observe its trends at different time periods.</td>
</tr>
</tbody>
</table>

The extent and time course of TB in a given community can be quantified by three parameters: infection, morbidity, and mortality. Since the introduction of
anti-TB therapy, mortality ceased being a parameter by which the evolution of this disease could be followed, since few patients in treatment tended to die of the disease. Hence, although treatment introduced on a large scale in the community rapidly reduces mortality, it does not allow a good assessment of the evolution of the disease. Other measures to ensure complete patient compliance with therapy are necessary. For this reason, when speaking of TB, treatment (which depends on the use of good therapeutic regimens) is not the same as cure (which depends on strict patient compliance).

Following the introduction of anti-TB agents in the community, TB can only be accurately quantified by the corresponding morbidity parameters: incidence and prevalence. The best way to assess trends in TB (i.e., disease incidence) in the community is to follow the new cases detected in the population on a yearly basis. This parameter is dependent on accurate notification of cases, and thus on the type of information system used for case reporting by the professionals in charge of diagnosis. Since diagnosis at the peripheral levels of the health care network are based on smear microscopy (discussed in Chapters 7 and 12), the best means of following the evolution of the disease is to register the number of cases and the annual incidence of patients who have positive smear microscopy results. With TB, the incidence rates (new annual cases) and prevalence (cumulative number of cases) are expressed as the number of cases per 100,000 persons.

Despite the above considerations, it should be noted that tuberculous infection reflects the past and present TB burden in a given region and facilitates future prognosis by identifying infected individuals (reservoirs for disease). The most interesting parameters of infection are the incidence or annual rate of infection (ARI) and the prevalence of infected persons. The incidence is calculated by the ARI, which describes the percentage of the population that will be infected or reinfected in the course of a year, and is based on a mathematical formula requiring knowledge of the prevalence of tuberculous disease in the community at a given age. Despite all that has been written about ARI, many factors influence its determination and results. The information derived from this parameter must therefore be evaluated with great caution. A much more valid approach is to observe TB trends in different periods. Utilisation of ARI is not advised until a National Tuberculosis Control Programme has been established and functions effectively, and several years of reliable data on disease incidence, cure rates, and default rates, as well as information on certain basic case detection parameters (see Chapter 12), become available. Thus, estimations of the incidence of the disease and of other TB parameters based on ARI should be avoided.
Recommended reading


The current situation of tuberculosis in the world

Section summary

TB remains the most important infectious disease worldwide. The current global situation of TB reflects the enormous economical and social differences among countries, as can be seen from the fact that 95% of all cases of TB and 98% of all deaths caused by TB are from low-income countries. In 1999, a total of 3,689,822 new cases were reported to the World Health Organization (WHO), although the true figure is estimated to be about 8,417,000 cases (a global rate of 141/100,000), of which 3,724,000 corresponded to smear-positive microscopy cases (62/100,000). In that same year it was estimated that 80% of the TB burden in the world was confined to 23 countries, which accordingly have received priority rating from the WHO.

As has been mentioned earlier, TB remains the most important infectious disease in the world at the start of the new millennium. This ancient endemic disease, which has affected humans for thousands of years, may not only be the worst plague ever suffered by humanity, but today still escapes epidemiological control. Indeed, the global statistics on affected patients and deaths due to TB continue to rise every year. However, in the face of this bleak situation, most industrialised nations consider the disease to have been overcome, and efforts against TB have decreased as a result. In reality, the current situation of TB in the world reflects the enormous economic and social differences among countries. TB will remain a global problem until the disease disappears entirely from the planet.
In 1999, a total of 3,689,822 new cases of TB were reported to the World Health Organization (WHO), which estimated that this figure actually represented less than half of the actual cases in the world. In the great majority of countries, particularly those with low or middle incomes, case under-detection or under-reporting exceeds 50%, a fact that led the WHO to estimate that in 1999 there was a total of 8,417,000 new cases of TB (a global rate of 141/100,000), of which 3,724,000 corresponded to smear-positive cases (62/100,000). The distribution of these TB cases varies considerably from one region to another (Figure 1). Sixty-three percent of the total case notifications in 1999 were from Asia (41% in Southeast Asia and 22% in the Western Pacific), followed by 17% in sub-Saharan Africa, despite the fact that this was the region of greatest incidence. The Americas in turn reported 6% of the cases recorded for that year, whereas Europe reported 10% and the Mediterranean 4%.

The distribution of TB by countries also differs within the same region. Thus, for a number of years it has been estimated that 80% of the global TB burden is concentrated in 23 countries (Table 3), which have been assigned priority consideration by the WHO. These are the 23 countries with the largest TB figures in absolute terms (i.e., not in cases per 100,000 inhabitants). The fact that they are highly populated also plays a role. Heading this list is India, with an estimated 1,847,000 new cases in 1999. This may be the country with the worst TB problem, and where, historically, the worst approach to antituberculous therapy—private practice—has been adopted.

It is worrisome to analyse the factors that have led to the present dire situation, especially since TB has been a treatable disease for over 40 years and the scientific rationale for its control in the community has been established for the past 30 years. Even more disheartening is the existence of enormous inequalities among different parts of the world. TB remains fully out of control in the great majority of poor countries but has been on the decline in the developed world for over a century. Such a disparate situation reflects the existence of a series of factors that contribute to the progressive spread of this disease or its sustained yearly reductions.
Figure 1. Estimated worldwide distribution of TB cases. Source: WHO, 1999.

<table>
<thead>
<tr>
<th>Country</th>
<th>Population*</th>
<th>No. of cases</th>
<th>Rate per 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. India</td>
<td>998</td>
<td>1,847,000</td>
<td>185</td>
</tr>
<tr>
<td>2. China</td>
<td>1,266.8</td>
<td>1,300,000</td>
<td>103</td>
</tr>
<tr>
<td>3. Indonesia</td>
<td>209.2</td>
<td>590,000</td>
<td>282</td>
</tr>
<tr>
<td>4. Nigeria</td>
<td>108.9</td>
<td>327,000</td>
<td>301</td>
</tr>
<tr>
<td>5. Bangladesh</td>
<td>126.9</td>
<td>306,000</td>
<td>241</td>
</tr>
<tr>
<td>6. Pakistan</td>
<td>152.3</td>
<td>269,000</td>
<td>177</td>
</tr>
<tr>
<td>7. The Philippines</td>
<td>74.4</td>
<td>234,000</td>
<td>314</td>
</tr>
<tr>
<td>8. Ethiopia</td>
<td>61</td>
<td>228,000</td>
<td>373</td>
</tr>
<tr>
<td>9. South Africa</td>
<td>39.9</td>
<td>197,000</td>
<td>495</td>
</tr>
<tr>
<td>10. Russia</td>
<td>147.1</td>
<td>181,000</td>
<td>123</td>
</tr>
<tr>
<td>11. Dem. Rep. Congo</td>
<td>50.3</td>
<td>151,000</td>
<td>301</td>
</tr>
<tr>
<td>12. Vietnam</td>
<td>78.7</td>
<td>149,000</td>
<td>189</td>
</tr>
<tr>
<td>13. Kenya</td>
<td>29.5</td>
<td>123,000</td>
<td>417</td>
</tr>
<tr>
<td>14. Brazil</td>
<td>167.9</td>
<td>118,000</td>
<td>70</td>
</tr>
<tr>
<td>15. Tanzania</td>
<td>32.7</td>
<td>112,000</td>
<td>340</td>
</tr>
<tr>
<td>16. Thailand</td>
<td>60.8</td>
<td>86,000</td>
<td>141</td>
</tr>
<tr>
<td>17. Mozambique</td>
<td>19.2</td>
<td>79,000</td>
<td>407</td>
</tr>
<tr>
<td>18. Burma</td>
<td>45</td>
<td>76,000</td>
<td>169</td>
</tr>
<tr>
<td>19. Uganda</td>
<td>21.1</td>
<td>72,000</td>
<td>343</td>
</tr>
<tr>
<td>20. Afghanistan</td>
<td>21.9</td>
<td>71,000</td>
<td>425</td>
</tr>
<tr>
<td>21. Zimbabwe</td>
<td>11.5</td>
<td>65,000</td>
<td>562</td>
</tr>
<tr>
<td>22. Cambodia</td>
<td>10.9</td>
<td>61,000</td>
<td>560</td>
</tr>
<tr>
<td>23. Peru</td>
<td>25.2</td>
<td>58,000</td>
<td>228</td>
</tr>
<tr>
<td>Total: 23 countries</td>
<td>3,760</td>
<td>6,700,000</td>
<td>178</td>
</tr>
<tr>
<td>World total</td>
<td>5,975</td>
<td>8,417,000</td>
<td>141</td>
</tr>
</tbody>
</table>

* Population expressed as millions of inhabitants

Recommended reading

Causes for the increase in tuberculosis in the world

Section summary
Poverty and the growing inequalities in the distribution of wealth have always been historical allies of TB, and remain responsible for the extremely bleak situation of the disease worldwide. Moreover, over the past 20 years the HIV pandemic has affected TB (particularly in the poorer countries) by further straining the already deficient health systems in the developing world, a situation that will certainly continue to worsen in the coming decades. These three factors—poverty, HIV infection, and *M. tuberculosis*—together with uncontrolled population growth in the poorer parts of the world, operate freely in the most vulnerable parts of the planet, with extremely dire consequences for the future. In comparison, migration, albeit a problem for wealthier countries, probably has little effect on the global figures of the disease, although extensive internal migration does make TB control more difficult.

The fight against poverty is extremely complex and depends on a series of globalisation strategies. In turn, the struggle against HIV infection presently appears futile in the poorest regions of the world. This is why the only remaining possibility is to implement TB control strategies as a way of combating the disease.

Poor or no application of tuberculosis control programmes

Early detection and cure of TB cases constitute the basis for control of the disease in the community. While over the past 40 years developed nations have employed effective TB control programmes for the early detection and cure of most cases, developing countries have achieved little in the struggle against the disease and continue to have large numbers of infectious cases in the community that elude detection and treatment. This variability in exposure to a very different risk of TB infection explains why 80% of infected persons in the developed world are over the age of 50 years, whereas 75% of infected individuals in developing countries are below that age. This difference in the distribution of infected subjects is influencing the added problem posed by HIV infection. The mistakes of the past in relation to antituberculous measures are now exacting a high price in most countries, particularly with the appearance of the HIV epidemic.

The extremely serious global problem of TB led the WHO in April 1993 to declare the disease a global health emergency, and to recommend the coordination of efforts against the illness—the so-called Directly Observed Therapy, Short Course (DOTS) strategy, which comprises five
components: 1) the political commitment on the part of governments to solve the problem of TB; 2) diagnosis by smear microscopy accessible to the entire population; 3) directly observed or supervised treatment (at least in the first phase); 4) the guaranteed and regular supply of drugs; and 5) the implementation of an adequate registry and information system. This strategy is relatively simple to implement, provided the political will exists to do so. This represents the only possible line of action against the factors influencing the increase in the prevalence of TB. The fight against poverty itself is unfortunately extremely complex. In turn, the struggle against HIV infection presently appears hopeless in the poorest regions of the world. However, implementation of TB control strategies is a possibility. Although the spread of such measures has been constant, in late 1999 it was acknowledged that only 23% of all reported smear-positive cases have been studied and treated following strict guidelines, and that the DOTS strategy has been initiated to some degree or other in 127 countries (60%). Figure 2 shows that many parts of the world still do not use the DOTS strategy, or do so in only a very small percentage of the population.

Poverty and the widening gap between the rich and the poor

The improvements in living conditions achieved in the past 150 years in industrialised countries remain unattainable to the great majority in the poorest parts of the world. In these areas, extreme poverty continues to be the main ally of TB. The distribution of world wealth (Figure 3), based on per capita income reported by the World Bank in 1998 and by the categories of rich, middle income, and low income, shows that the distribution of wealth coincides greatly with the global distribution of TB. The minor variations between the two maps are attributable to the fact that the range corresponding to middle-income level is very broad (US$786-9655). However, if the group of middle-income countries is further divided into two subgroups (Figure 4)—poorest (US$786-3125) and richest (US$3126-9655)—the similarities between TB case distribution and wealth distribution are even more pronounced, confirming that poverty, the historical partner of TB, remains the main factor influencing the serious situation of the disease in the world today.

However, economics not only affects the poorer nations but also the lower-income groups in richer countries. Studies conducted in the United States, Canada, and Western Europe have shown increased rates of TB in families with the lowest incomes, particularly among those who live below
Figure 2. Worldwide distribution of the use of the Directly Observed Therapy, Short Course (DOTS) strategy. Source: WHO, 1999. DDR = percentage of patients with TB subjected to DOTS; TS = percentage treatment success.
Figure 3. Per capita income distribution in the world. Source: World Bank, 1998.
Figure 4. Per capita income distribution in middle-income countries. Source: World Bank, 1998.
the poverty threshold. These studies also show that there is a level of income above which no further reduction in TB rates is recorded—this level corresponds to families with fewer children and secured access to food and shelter. This relation between socioeconomic level in wealthier countries and TB reflects the extreme sensitivity of TB as a parameter of development, inequality, and poverty.

Using this association between socioeconomic level and TB, the future global distribution of TB can be estimated. In effect, the future course of the disease can be charted by examining the annual United Nations (UN) report on the distribution of wealth in the world, which emphasises the increasing gap between rich and poor countries.

The impact of HIV infection

Undoubtedly, HIV infection has greatly complicated the problem of TB control in the world. In fact, it is difficult to imagine a microorganism better suited than HIV to function as an ally of *M. tuberculosis*. The virus selectively destroys or alters the function of those immune cells that defend the host against Koch’s bacillus. In short, TB, the oldest of infectious diseases, and HIV, the most recent pandemic to affect the human species, have combined their pathogenic effects to become the primary cause of death in many parts of the world. It is estimated that substantial areas of the poorest countries will lose their youngest populations in the coming decades.

Towards the end of 1999, the WHO estimated that 33.6 million people worldwide had HIV infection/AIDS and that this disease had already caused 16.3 million deaths. The number of AIDS cases continues to grow each year. In 1999, there was already an estimated 5.6 million new cases of HIV infection. The best indication of the devastating spread of this disease is the fact that in 1999 a total of 2.6 million people were estimated to have died of AIDS, causing HIV to surpass *M. tuberculosis* as the infectious agent responsible for the largest number of deaths in the world. In the same way as with TB, the world distribution of AIDS is very heterogeneous, with 95% of all cases being located in the poorest parts of the globe. Both pathogens have therefore gradually spread to the most vulnerable parts of the planet. At the end of 1999 it was estimated that 70% of all cases of HIV infection (23.3 million individuals) were from sub-Saharan Africa, 20% were from Southeast Asia and the Western Pacific (6.6 million cases), and 5% were from Latin America and the Caribbean (1.6 million). The number of new
infections and deaths had a similar distribution, as did the location of patients with dual HIV-TB infection. Indeed, the distribution map for HIV infection can be superimposed on the distribution map for TB infection and disease, as well as poverty. Thus, poverty, HIV infection, and *M. tuberculosis* operate freely in the most vulnerable parts of the planet, with extremely dire consequences for the future. According to the predictions of the Population Division of the UN, the life expectancy of the populations in the nine African countries with the highest prevalence of HIV infection will be reduced by an average of 16 years between 2010 and 2015—a further obstacle to development in these countries, where poverty can be expected to increase with the decimation of an economically active population.

The situation, however, is completely different in the industrialised world, where only 5% of all HIV infection/AIDS cases are found, together with 5% of the cases of HIV-TB infection. Because most developed countries had implemented appropriate antituberculous measures during the past few decades, 80% of persons with *M. tuberculosis* infection are over the age of 50 years, whereas 85% to 90% of all HIV-infected persons are under this age. In other words, the two groups with TB or HIV infection are unlikely to overlap, as a result of which the impact of HIV on TB has been (and can be expected to remain) limited. In contrast, the developing countries are characterised by large numbers of individuals from the same age group (ages 20-49 years) who are infected with both pathogens—a situation that will undoubtedly worsen in the coming decades.

Moreover, since 1996, highly active antiretroviral therapy (HAART) has been available for the treatment of HIV infection. HAART, which comprises the combination of three or more antiviral drugs, including a protease inhibitor, affords marked and sustained increments in peripheral blood CD4+ lymphocyte counts, together with reductions in the plasma HIV viral load to undetectable levels. These drugs, however, are very expensive, and their use has largely been limited to industrialised countries. Here again, the countries with the greatest HIV problem are not benefiting from these treatments.

**Massive immigration from tuberculosis endemic zones**

The industrialised nations that have combated TB effectively in the past 40 to 50 years made the initial mistake of believing that the struggle against the disease ended at their own frontiers, and as a result failed to help poorer countries overcome the disease. Now, because of the massive migratory
movements due to extreme poverty in much of the world, and the ease of long-distance travel, industrialised nations are paying a high price for their past neglect and witnessing a substantial increase in their TB rates resulting from the arrival of infected immigrants from regions where TB is still endemic. In their countries of destination, these immigrants reproduce the same endemic situation found in their original countries. This situation persists for two to three generations, since immigrants from developing countries tend to live in relatively closed communities, with their own people and common cultural values, under very similar living conditions. Moreover, the great majority live in marginalised communities, which have limited access to health care services and which are less likely to be tested for TB.

Immigration has been one of the main reasons why TB rates have not only failed to decrease but have even increased in the past decade in many industrialised nations. Because TB rates are higher among immigrants in many developed countries, specific antituberculous strategies have been developed, including the systematic examination of all immigrants upon entering a country. There has also been an increase in funds donated to poorer countries in the last 5 to 10 years, with the aim of solving the problem at its origin.

Another consideration is the existence of internal migration in low- and middle-income countries. Such internal migration has grown considerably in the last two decades, owing to factors such as drought, famine, insecurity, internal conflicts, and terrorism. As a result, the population characteristics in many low-income countries are changing very rapidly, from a rural to a predominantly urban population. These large-scale internal migratory phenomena lead to the development of urban settlements that lack the minimum required health infrastructure, with extensive crowding and poverty that assist the spread of \textit{M. tuberculosis}. Moreover, it may be years before health services are available in these poverty zones, making it very difficult to combat TB in these areas.

\textbf{The demographic explosion}

Population growth is leading to an increase in the absolute number of TB cases in the poorest parts of the world. The demographic explosion in the Third World has led to increased crowding and poverty, which in turn has facilitated the transmission of \textit{M. tuberculosis} and led to an increase in the number of TB cases. It has been estimated that the world population will double within the next 30 years, fundamentally as a result of population
growth in the developing (and poorest) countries. An analysis of the predictions by the WHO for Africa for the period 1990-2000 reveals that a percentage of the new cases of TB can be attributed to this population explosion. This fact becomes all the more important when one considers that high population and low income characterise the 23 countries that account for 80% of all TB cases in the world.

**Recommended reading**

Measures for reducing tuberculosis in the community

Section summary
Of all known measures for controlling TB, only improvement in the existing socioeconomic conditions (leading to a 4-6% reduction in infection risk) and adequate chemotherapy (affording an additional annual risk reduction of 7-9%) have been able to arrest the evolution of the disease. The rest of measures adopted to date have had little impact on TB in the community, including chemoprophylaxis and bacille Calmette-Guérin vaccination.

Improvement in socioeconomic conditions
Mortality due to TB has been steadily decreasing in the developed countries since the late eighteenth century, almost a full century before TB was identified as an infectious disease and *M. tuberculosis* was first discovered. Consequently, the disease had begun to come under control in richer countries without the adoption of any specific control measures. The improvements in the socioeconomic conditions in developed countries from the mid-eighteenth century had already started to have a slight effect on the disease, with a sustained decrease in associated mortality and morbidity. It is now accepted that once an optimum level of development has been achieved, the resulting reduction in crowding and poverty has an important effect on TB disease burden. By reducing crowded living conditions, each source of bacterial transmission is no longer able to generate sufficient new infected cases to ensure a new infectious smear-positive TB patient. In this sense, each TB patient would have to infect 20 people, of whom 20% (2 subjects) would ultimately develop disease with positive smear microscopy and the remaining without. This is why just the reduction in the number of people living in a home can have a significant effect on the transmission dynamics of TB. Moreover, extreme poverty results in malnutrition, which is another risk factor for developing the disease. It has been calculated that improved socioeconomic conditions ultimately lead to a sustained 4% to 6% annual drop in infection risk. Hence, if adequate living conditions could be guaranteed worldwide, TB could potentially be eradicated without the need for any medical intervention.

Adequate chemotherapy with high cure rates
After the discovery of streptomycin in 1943, other drugs such as para-aminosalicylic acid and isoniazid soon followed in its wake, along with the scien-
tific reasoning required to define the first therapeutic regimen capable of curing TB. This progress led many to believe that definitive control of the disease in the community was at hand. Indeed, in developed nations a steadily decreasing trend in TB-related mortality could be observed from the late eighteenth century, with a marked acceleration beginning in the 1950s and coinciding with the introduction of effective treatment regimens. None of the control measures have been as effective as chemotherapy in modifying the natural decline in TB rates, which, when administered correctly and with good patient compliance, can decrease rates by 7% to 9%. In sum, chemotherapy is the only certain method for shortening the transmission chain, by quickly curing diseased individuals and preventing them from infecting others. However, as has been mentioned, successful treatment in TB requires patient compliance to guarantee cure. Thus, ensuring compliance to appropriate treatment is the main challenge in order to attain high cure rates and have an impact on tuberculosis control.

Chemoprophylaxis of infected individuals at high risk of developing tuberculosis

Although chemoprophylaxis is regarded the primary tool for eradicating TB in the United States, its impact has been very limited in countries where the measure has been implemented on a large scale. As will be analysed in detail in a later chapter, the efficacy of this measure depends on three major factors: 1) the risk group selected for administration of preventive therapy; 2) the efficacy of the prescribed regimen; and 3) patient adherence to therapy. The first and, particularly, last of these factors greatly influence the results of preventive therapy. As a result, before deciding to implement this strategy in the community, mechanisms should be established to ensure compliance. On the other hand, the results obtained cannot be compared with those afforded by standard therapy, for while the latter directly treats the sources of infection (i.e., a diseased patient), preventive chemoprophylaxis only acts on the reservoir (and most individuals do not go on to develop the disease). Chemoprophylaxis has only been able to reduce TB rates by less than 1% per year, and only when correctly implemented.

BCG vaccination

Although the best measure for ensuring the eradication of an infectious disease is mass vaccination, the lack of efficacy shown by bacille Calmette-Guérin (BCG) vaccination in many parts of the world has effected practi-
cally no change in the epidemiology of TB. Moreover, if the vaccine were able to protect effectively against TB, the beneficial effect would mainly be in children under the age of 5 years who practically do not transmit the disease (and of whom 95% show negative smear microscopy).

**Recommended reading**

Chapter 5 - Pathogenesis of tuberculosis: infection and disease

Chapter summary
Alveolar macrophages are the key cells in the host immune response to tuberculosis (TB). After successfully phagocytosing the bacteria, alveolar macrophages process the antigens of *M. tuberculosis* and present them to the specific T lymphocytes. Before a cellular immune response is elicited (within 4-8 weeks), the bacteria grow unhindered, and are therefore able to enter the bloodstream and spread to other parts of the body, mainly the apical regions of the lungs, but also to any other organs.

Ninety percent of all people have the tubercle bacilli under control in a latent state throughout their lifetime, as a result of their immune defence system. Five percent develop progressive primary TB, while another 5% develop the disease in late stages of life (i.e., reactivation TB). This situation changes drastically in the case of patients with HIV infection, of whom 50% to 60% who are also infected with *M. tuberculosis* will develop active TB in the course of their lifetime.

Based on the above considerations, all TB-infected individuals are potential patients.

Tuberculosis (TB) is the example of the interaction between an exogenous agent and the host immune defence system. It may be estimated that while 1,900 million people throughout the world are infected with *M. tuberculosis* (representing an enormous reservoir that contributes to perpetuation of this disease), only 8 million actually suffer from the disease each year. This situation is explained by the fact that the human defence mechanism is highly effective and can overcome the disease in most cases.

The chemical characterisation of the structural components of *M. tuberculosis* has facilitated the investigation of the biological properties of the pathogen. The protein and peptide components are known to be responsible for host cellular immune response stimulation and for delayed hypersensitivity reactions, whereas polysaccharide elements such as the arabinomannans, while able to induce a humoral defence response, possess immunosuppressive properties. In turn, certain glycopeptides (e.g., cord factor or sulpholipids) modify macrophage function. Cord factor is a potent chemoattractant that induces granuloma formation, whereas sulpholipids inhibit phagosome-lysosome fusion.
Understanding of the pathogenesis of TB is largely attributable to research in animal models. Although many models have been developed that illustrate different aspects of the disease, the contributions of Lurie should be noted, as well as those of Dannenberg and Lefford. Lurie used syngenic rabbits that were sensitive or resistant to TB infection. In his model, three phases of tuberculous infection were defined based on bacterial counts in the lungs: a first phase of bacterial destruction (3-7 days); a second “symbiotic” phase involving exponential growth of the bacterial population; and a third phase of immune control of the infection. The resistant and sensitive rabbit strains differed only in relation to the first phase. Resistant animals were able to destroy the bacteria faster and more effectively than the sensitive rabbit strains. The studies carried out by Lurie and Dannenberg highlighted the importance of the natural host defences against Koch’s bacillus, and the added role played by immune response. In comparison, Lefford emphasised the importance of an acquired immune response using rat models. Lefford and colleagues infected rats with an avirulent strain of \textit{M. tuberculosis}. After a few days, they reinjected the same animals with a virulent strain of the same microorganism. They found that the rats had acquired resistance to infection, and that this resistance was of a cellular nature, since the lymphocytes obtained by lymphatic drainage protected those animals that had not been in contact with the bacterium. Thus, these experimental studies clearly demonstrated that the antituberculous host defence involves the participation of both natural and acquired mechanisms.

Still, the results from animal models should be interpreted with caution, since immunologic characteristics differ with mammalian species. For example, Rook reported a markedly different bactericidal macrophage response in murine (mouse) and human macrophages to the administration of interferon \(\gamma\) and vitamin \(D_{3}\).

Based on the above considerations, the most widely accepted pathogenic mechanism of TB infection and disease clearly distinguishes between tuberculous primary infection and the post-primary infection phenomena—each of which has its own clinical manifestations.

**Primary infection**

Primary infection refers to the general biological phenomena that take place when an individual comes into contact with the tubercle bacillus for the first time. During primary infection, 95% of all affected individuals remain asymptomatic or present with only minimal clinical manifestations similar
to those seen with the common cold. Only 5% develop manifest disease. Tuberculin skin test conversion usually occurs in these individuals. This phenomenon typically takes place in childhood, as a result of which primary infection is often associated with childhood TB. Nevertheless, primary infection can occur at any time in life, and is the result of inhalation of bacteria-loaded particles by an individual who has not been previously exposed to the microorganism. Because of the weight of these particles, some of them tend to sediment and are therefore not infective. Other airborne particles, known as Pflüger droplets, with a diameter of 5 to 10 µm, either sediment or are cleared by the defence mechanisms of the airways. However, upon condensation of these droplet nuclei and the loss of part of their water content, smaller particles measuring 1 to 5 µm are formed, containing approximately three tubercle bacilli each; these droplets are infective. The defence mechanisms of the upper airways (i.e., cough reflex, mucociliary system) non-specifically prevent particles measuring over 5 µm from reaching the lung parenchyma. Under infective conditions, however, some particles measuring 1 to 5 µm reach the distal airways and are deposited in the alveoli. It is believed that at least 10 to 200 of such microdroplets must reach the alveoli in order for infection to take place. The preferential zone of arrival is the best-ventilated part of the lungs, corresponding to the subpleural region of the inferior lobes.

Upon arrival in the alveolar region, the bacteria encounter three types of cells that potentially oppose infection: the alveolar macrophages within the alveolar lumen, the natural killer cells, and the γδ T lymphocytes. In mice, exposure to aerosols containing mycobacterial antigens, or immunisation with *M. tuberculosis*, gives rise to a marked increase in the presence of this cell line in the lungs and regional lymph nodes. On the other hand, γδ T lymphocytes are able to recognise certain epitopes of *M. tuberculosis*. Studies involving tuberculous patients, however, have for the most part been unable to confirm this cell line increment in either the blood or granulomas of infected patients. It should be noted though that all of these studies have been conducted outside the context of primary infection, which makes extrapolation of results difficult.

In humans, alveolar macrophages are considered to be the key type of cell involved in the initial interaction with the tubercle bacillus. Some important characteristics of alveolar macrophages should be pointed out. First, these cells originate in the bone marrow and reach the alveoli after coming into contact with the systemic circulation. As a result, different systemic and local factors can influence their functional characteristics. For example, HIV
is able to infect these alveolar macrophages and thereby increase host sensitivity to tuberculous infection. Second, the antigen-presenting capacity of alveolar macrophages is low in humans, as compared with in other animal species. Third, alveolar macrophages are cells that live in an oxygen-rich environment, as a result of which their free oxygen radical production potential is theoretically great. However, and probably to avoid toxicity due to these radicals, alveolar macrophages lack myeloperoxidase (although they do generate superoxide radicals). Fourth, alveolar macrophages contain abundant lysosomal enzymes.

The initial interaction between *M. tuberculosis* and alveolar macrophages involves non-specific phagocytosis of the bacilli and their inclusion within phagocytic vacuoles. Considering that these alveolar macrophages have not been primed by lymphocytic cytokines and that various mycobacterial components inhibit the bactericidal systems of these cells, it is reasonable that bacterial growth predominates in this initial stage. Practically all bactericidal macrophage systems are inhibited by products derived from the mycobacteria. Thus, glycolipids inhibit phagosome-lysosome fusion, while other less well-known components alter lysosomal acid pH, thereby complicating enzyme action. Catalase, in turn, destroys hydrogen peroxide, and different mycobacterial components inhibit superoxide production. This phase concludes with destruction of the alveolar macrophages by proliferating intracellular bacilli. Natural resistance to the infection fundamentally occurs during this phase.

Tubercle bacilli products such as cord factor and the activation of other chemokine factors exert a potent chemical effect, attracting blood monocytes that ingest the released bacilli. At this point, a symbiotic relation is established in which the bacteria and young macrophages do not destroy each other. The monocytes have not been activated, and the bacteria are not toxic, at least on an acute basis. The tubercle bacilli increase exponentially in a similar manner, killing host cells and spreading locally. In the lung, intense alveolitis takes place at the expense of the young cells of the mononuclear phagocyte system.

The third essential phenomenon in this phase of the disease is mycobacterial spread systemically via the lymphatics towards the regional lymph nodes. In this region, the host immune response to tuberculous infection takes place. In some instances, this immune response is sufficient to arrest the progression of infection, although often times the bacilli escape towards the lymphatic duct and penetrate the pulmonary bloodstream, from where there is hematogenous spreading of the bacilli to the other organs. The main
metastatic or target zones of such bacterial dissemination are the highly irritated organs and tissues—the central nervous system, spongy bone, liver, kidneys, and genitals. In each of these zones, the arriving bacilli are phagocytosed by the local cells of the mononuclear phagocyte system.

In most cases, this period implies immunologic control of the infection as a result of two mechanisms: cell-mediated immunity and delayed hypersensitivity. From the bacteriological perspective, the consequence of this situation is an abrupt interruption of the bacterial growth curve in both resistant and susceptible individuals. Cellular immunity is not responsible for this growth arrest, since susceptible individuals have only a weak cell-mediated immune response and resistant subjects have not yet developed an effective immune reaction. Delayed hypersensitivity is the phenomenon responsible for the destruction of macrophages that contain intracytoplasmic bacteria, thereby forming a characteristic focus of caseous necrosis. Although the bacteria may survive within this necrotic focus for years, they are unable to reproduce due to the prevalent acidosis, the lack of oxygen, and the presence of inhibitory fatty acids. The principal factors influencing delayed hypersensitivity reactions are the cytotoxic T lymphocytes, although other factors such as cytokines (tumour necrosis factor α), oxygen reactive species, and nitrous oxide may also be involved. Such initial necrosis is therefore beneficial for control of the infection. However, delayed hypersensitivity must be "reinforced" by cell-mediated immunity, since susceptible hosts with weak immune responses are not only unable to control the infection but also produce granulomas with an increased caseous presence, probably due to the intervention of mycobacterial proteins.

Resistant animals, in the same way as immunocompetent humans, avoid tubercle bacilli spread from the tuberculous focus in a second phase, owing to the development of a potent cell-mediated immune response at the expense of the helper T lymphocytes, which activate the macrophage population. From the clinical perspective, immunocompetent individuals develop a balance between themselves and the mycobacteria, which persists throughout life until some predisposing event is able to reactivate the infectious focus. An indirect approach to demonstrate this immunologic phenomenon is represented by the tuberculin skin test. In immunocompromised individuals who are unable to control the infection, TB disease develops and the subjects do not usually show a positive tuberculin test. In addition to prior immunodeficiency, one factor that clearly influences the conversion from latent infection to disease is the age at which primary infection takes place. Conversion to disease is more frequent in the very young and very old. Adolescents and
early youth also show a tendency towards conversion to disease, although for reasons that remain unclear.

**Tuberculous reactivation**

Tuberculous reactivation is defined as the development of tuberculous disease in a patient who had already been infected with the tubercle bacillus in the past. Although there are well-documented cases of exogenous reinfection, it is currently accepted that most cases of infection are attributable to endogenous reactivation. However, this assumption may change in the coming years, in view of recent information appearing in the literature.

It has been calculated that only a minority of people infected with *M. tuberculosis* actually progress to active disease. In general terms, 90% of infected individuals can be expected to keep the tubercle bacilli in a latent state for life, owing to the intervention of the host immune defences. Five percent will develop progressive primary TB, and the remaining 5% will develop the disease in later stages of life, a condition known as reactivation or post-primary TB (Figure 5). This situation changes drastically in patients with HIV infection, of whom an estimated 50% to 60% who are also infected with *M. tuberculosis* will develop active TB in the course of their lifetime. Individuals presenting with risk factors for TB should be actively evaluated. The number of patients at risk of TB has increased in the course of history, and presently includes some populations that do not demonstrate immunodeficiency but that have other risk factors for TB (Chapter 4, Table 2).

In resistant individuals, the immune control of haematogenous seeding sites is dependent on local as well as systemic factors. Some systemic factors (e.g., HIV infection, corticotherapy, malnutrition) may account for tuberculous reactivation, although it is less clear how other local factors affect reactivation. It has been speculated that a decrease in interferon γ production and the intervention of arabinomanans may be involved in this reduction or in the generation of a specific suppressor response. One of the known important pathogenic phenomena of reactivation is caseum liquefaction. Although not all the factors implicated in such liquefaction are known, the phenomenon has been attributed to lysosomal enzymes released by the macrophages, and to a delayed hypersensitivity reaction to mycobacterial products. The immediate consequence of caseum liquefaction is the production of an excellent growth medium for the bacteria, which begin to multiply and release products similar to tuberculin that have great toxic potential. In the case of the
Figure 5. Risk of developing tuberculous disease in a person infected with *M. tuberculosis*. PPD = purified protein derivative.

Based on the above considerations, all TB-infected individuals should be considered patients, and the greatest protection against *M. tuberculosis* corresponds to a subject who has never been infected. The great problem with TB facing us today is that there is a very large number of infected individuals who are never free from the risk of the disease. These individuals, while potentially capable of developing the disease at any time in life, particularly if they develop some form of immunodeficiency, are expected to present with alerted memory lymphocytes in the event of past exposure to tubercle bacilli. Theoretically, this would confer relative protection against such possible exogenous reactivation. In this sense, there has been specula-
tion as to whether the ideal situation is instead represented by the infected subject, who would be more protected against future exogenous reinfections. However, such reasoning is mistaken, since the development of TB due to endogenous reactivation in infected individuals is much more likely than the development of the disease due to exogenous infection in non-infected subjects.

**Recommended reading**

Chapter 6 - Diagnosis of tuberculous infection: the tuberculin test

Chapter summary
The tuberculin test demonstrates the existence of a state of host hypersensitivity to the proteins of the tuberculous bacillus, most often acquired as a result of infection with *M. tuberculosis*, although hypersensitivity can also be induced by bacille Calmette-Guérin (BCG) vaccination or infection with environmental mycobacteria. The tuberculin skin test gives rise to an inflammatory reaction with an important dermal cellular infiltrate at the location of the tuberculin inoculation. This reaction is identified as a visible and palpable induration at the site of inoculation, and can be accompanied by swelling, erythema, and, sometimes, vesiculation, necrosis, and regional lymphadenitis.

The performance of the tuberculin skin test requires the use of 2 units of tuberculin purified protein derivative (PPD)-RT23 or 5 units of PPD-CD68, both doses being bioequivalent to 5 units of PPD-S, which is considered the international standard. The result is expressed in terms of millimetres of induration, taking into account that its interpretation is very complex and dependent on many variables that can influence not only reaction size but also the appearance of false-negative and false-positive readings, particularly in the case of BCG vaccination. The tuberculin test does not affect non-infected individuals, regardless of how often it is performed.

In low- and middle-income countries, because of the difficulty of preserving tuberculin at the most peripheral levels of the health care system, and because of large-scale BCG vaccination, this test is rendered practically unusable. Its use should therefore be restricted to centres where a paediatrician is available and to referral hospitals. In these settings, the indication for the tuberculin test is limited to children with suspected tuberculosis (TB) (disease, not infection), health care personnel (in an attempt to identify recent converters), and to very select cases of severe immune deficiency. The tuberculin test cannot be used as a discriminatory test for indicating preventive treatment or chemoprophylaxis. The test should not be repeated if there is evidence that the test had already been performed in the past and shown to be positive, regardless of the diameter of the induration. The second test might amplify the reading, giving rise to erroneous conclusions.

In those subjects in whom the skin test is performed, the positivity limit is 5 mm, based on the demonstrated high positive predictive value of this size in individuals at high risk of TB. These high-risk groups constitute the sole indication for the tuberculin skin test, since they represent the only individuals in whom performance of the test can lead to intervention (treatment or preventive chemotherapy).
The only method available for diagnosing tuberculous infection is the tuberculin skin test. Even today, this test remains a widely used technique, although it is one of the topics most frequently addressed in the history of medicine and which has generated much controversy. In view of its limitations, which will be described later in this chapter, the tuberculin test should be used much less frequently, particularly in low- and middle-income countries where there is a high prevalence of *M. tuberculosis* infection and where bacille Calmette-Guérin (BCG) vaccination is frequently administered at birth. Although the tuberculin test is an old technique, it has not been surpassed to date by any other method as a means of diagnosing tuberculous infection. There have been recent attempts to develop alternative tests capable of assessing tuberculous infection with greater specificity, of which the most promising may be the detection of the ESAT-6 antigen (secreted by T lymphocytes) based on an enzyme-linked immunosorbent assay for interferon γ (ELISPOT).

The history of the tuberculin skin test goes back to Robert Koch, who used tuberculin in his tireless quest for a vaccine against tuberculosis (TB). Koch had obtained a sample (“Koch’s lymph”) from tuberculous bacterial cultures, with the purpose of curing the disease. Unfortunately, the potential importance of his first conclusions presented at the 10th International Congress of Medicine in Berlin in 1890—that tuberculin could be used to detect a past or present tuberculous state—was not recognised and left a negative mark on his otherwise brilliant career. In 1905, von Pirquet (who coined the word “allergy” in reference to certain immune phenomena of TB) introduced a test based on the cutaneous reaction, while in 1908 Mantoux and Mussou developed the intradermoreaction, which remains the most widely used technique today.

**Pathogenic basis of the tuberculin test**

Specifically sensitised T lymphocytes proliferate in the regional lymph nodes of the host penetration site of *M. tuberculosis*. After 2 to 8 weeks, these lymphocytes enter the circulation where they remain for a prolonged period of time. The tuberculin test consists of the administration of tuberculin via the intradermal route. When tuberculin penetrates the skin, it is partially removed or cleared via the lymphatic system; the rest remains localised and is phagocytosed by the macrophages. This phenomenon produces an inflammatory reaction of mild-to-moderate intensity, with the involvement of polymorphonuclear cells and some mononuclear cells. In non-sensitised individuals, this inflammatory reaction soon disappears. In persons who have been sensitised
as a result of prior mycobacterial infection, the initial inflammatory response
intensifies, and lymphomonocytic perivascular infiltration develops as a result
of recruitment by the lymphokines secreted by the circulatory T lymphocytes
specifically sensitised to the bacterial antigens, which have recognised the
tuberculin injected into the dermis. This inflammatory reaction, which occurs
at the site on the skin where tuberculin was administered, gives rise to a visi-
ble and palpable induration (Figure 6) that can be accompanied by oedema
and erythema because of alteration of the permeability of the vessels located
within the inflammatory site. More intense reactions can show vesiculation
and necrosis with regional lymphadenitis and, occasionally, febrile syndrome.
The host response to tuberculin begins within 5 to 6 hours, usually peaking
in intensity after 48 to 72 hours and persisting for several days. This phenome-
non represents a cell-mediated delayed immune reaction.

However, from the early days of the tuberculin test, several limitations
were identified. Such problems became more relevant when the test began
to be used on a large scale. It became necessary to standardise the technique
(e.g., in terms of the tuberculin used, dosage, method of administration, and
reading and interpretation of the result), and to assess the limitations posed by
storage of the product and the possibility of false-positive and false-negative
results. These aspects greatly influence the use of the test and, particularly,
interpretation of results.

Figure 6. Tuberculin test performed with 2 units of purified protein derivative-RT23,
yielding a 22-mm induration after 72 hours.
Factors affecting the result of the test

Test standardisation

Tuberculin

Tuberculin is obtained from a sterilised and concentrated *M. tuberculosis* culture filtrate. The first tuberculins used (referred to as “old tuberculin”) contained impurities from the culture medium and mycobacterial development, and their composition varied from one batch to another, which posed a problem when comparing results. In 1934, Seibert prepared a purified protein derivative (PPD). Most of the components of this substance are low molecular weight proteins, and their multiplicity explains the incomplete specificity of PPD, since some of the allergenic constituents of the product, obtained from *M. tuberculosis* cultures, are also common to the other environmental mycobacteria. In 1951, this PPD was adopted by the World Health Organization (WHO) as the international standard, and the designation PPD-S was used. Consequently, all commercial PPD formulations must be standardised with respect to PPD-S. Such formulations are identified by a suffix following the initials “PPD”, such as PPD-RT21, PPD-RT23, and PPD-CT68. The formulation most widely used in the world, and recommended by the WHO, is PPD-RT23.

Dosage

The tuberculin dose must be administered in 0.1 ml of solvent, which is the volume to be injected into the dermis. Many studies were performed to define a PPD dose that allowed the maximum possible number of true reactive individuals (i.e., infected with *M. tuberculosis*) with the fewest possible false reactors. The ideal dosage was found to be 5 tuberculin units (TU) of PPD-S. Since the biological equivalence of PPD-RT23 is more than twice that of PPD-S, only 2 TU of PPD-RT23 are required (bioequivalent to 5 TU of PPD-S).

Method of administration

Charles Mantoux introduced and developed an intracutaneous method that remains common practice today. It is recommended both in view of its precision and sensitivity to 5 TU of PPD-S (or its bioequivalent doses of
other PPDs) when performing the skin test in individuals who are known to be infected with *M. tuberculosis* (bacteriologically confirmed diseased TB patients).

The tuberculin skin test is performed with a syringe graded in tenths of a millilitre (as in the case of insulin syringes), which has a short, bevelled needle (27G or 0.4/12). The syringe can be made of glass or, preferentially, disposable plastic. The injection site should be the anterior or posterior surface of the forearm, although any other skin region can also be used. It is advisable to perform the injection away from the veins, and for the skin to be free of lesions. Injection is performed beneath the skin, with the needle bevel positioned upwards (Figure 7). The confirmation that tuberculin is deposited intradermally is based on the appearance of a pale skin elevation (blister) at the injection site, which persists for some time after the inoculation.

![Figure 7. Photograph showing administration of the tuberculin skin test.](image)

**Reading of results**

Before the factors influencing test specificity became known, the results of the test were simply expressed as negative if no reaction was observed, or positive if a reaction was observed. Previous attempts were made to quantify
the reaction based on a cross-numbering system (e.g., +, ++). However, once the tuberculin dose was standardised and the formulation could be administered with considerable precision (owing to the Mantoux technique), the size of the reaction began to be assessed, particularly when it was seen that the induration diameter adopted a normal (Gaussian) distribution. At the same time, advances were being made in understanding the cellular infiltrative nature of the response found in tuberculin-sensitive individuals.

The result of the tuberculin skin test performed with 5 TU of PPD-S or its bioequivalent in other PPDs, based on the Mantoux technique, should always be expressed in millimetres of induration, and measured along the diameter transverse to the long axis of the forearm. The reading can be obtained 48 to 72 hours after performing the test, which is when induration is most apparent, although the reaction may remain without much variation for 4 to 7 days, followed by gradual weakening. This induration is visible, palpable, and measurable (Figures 6 and 7).

The procedure by which the induration is measured using a ballpoint pen is known as the “Sokal ballpoint pen method”. Its sensitivity is no better than that afforded by direct and careful palpation. In fact, the latter technique is better able to detect the point where the elevation of induration begins. Correct measurement of the diameter is important, since interpretation of the result is dependent on an accurate reading. The longitudinal diameter should not be measured, since it has not been assessed in any of the epidemiological studies on which knowledge of test interpretation is based. If there is no induration, the result should be registered as zero (0 mm). Although the term “negative” is often used when referring to such cases, it is better to use the designation “non-reactor”. Induration is often accompanied by erythema, which usually exceeds the limits of the induration, although only the diameter of the actual induration should be taken into account. If only erythema without induration is noted, the reading is 0 mm (i.e., non-reactor status). When the reaction is very intense, it tends to be accompanied by vesiculation, necrosis (Figure 8), and, occasionally, lymphangitis and satellite adenopathies. These observations should be duly recorded since they are highly specific of reaction due to *M. tuberculosis* infection.

The most frequent reason for erroneous tuberculin skin test readings is the interpretation of the test as being either positive or negative, instead of simply reading the result. As will be discussed below, the same result can be interpreted as positive or negative depending on many circumstances. Thus, a test report should include each of the steps expressed in this chapter.
on standardisation of the test. In other words, the report should include the type of tuberculin used, the dose administered, the administration technique, and the detected induration (in mm). An example of such a report would be: “Tuberculin skin test performed with 2 TU of PPD-RT23, revealing an induration of --- mm after 72 hours”.

![Image of a tuberculin reaction](image)

**Figure 8.** Tuberculin test performed with 2 TU of PPD-RT23, revealing a 30-mm induration after 72 hours, with vesiculation and necrosis.

**Tuberculin storage**

Tuberculin should be stored at 4°C to 8°C, since it loses activity over time outside this temperature range. This consideration is very important, particularly in many poor countries that have tropical climates. In such environments, a refrigerator and a continuous electrical power supply would be necessary. Moreover, tuberculin undergoes denaturalisation when exposed to sunlight. These aspects alone make it practically impossible to perform the test at the peripheral level in the great majority of low- and middle-income countries.
False-negative and false-positive readings

False-negative readings

Twenty-five percent of *M. tuberculosis*—infected individuals can yield a negative tuberculin test at the time of diagnosis. In many instances, individuals with active TB with a negative test result (anergy) represent atypical TB, although a negative result is more frequent in cases of severe and disseminated disease. Patients with TB disease who develop a negative result heal with adequate treatment, and the tuberculin test response undergoes positive conversion in over 50% of cases. The lack of cutaneous response to tuberculin has been attributed to a quantitative or qualitative depression of circulating T lymphocytes mediated by the action or intervention of suppressor cells (monocytes or macrophages). This effect is only observed in the area where tuberculin is applied (i.e., distant from the active TB site), with no inhibitory effect on adequate response in the area of inflammation. Extensive clinical and experimental evidence supports the fact that delayed sensitivity to PPD, while linked to cellular immune function, is actually differentiated from true and effective cellular immunity.

Other potential causes of false-negative responses to the tuberculin skin test are related to the type of tuberculin administered, its storage, and the technique used to perform the test. These factors are very important, for it has been estimated that inexperienced clinical auxiliary personnel perform the technique incorrectly and/or interpret the test result incorrectly in 75% of cases. Moreover, other conditions also affect cellular immunity and can lead to false-negative results. For instance, HIV-infected or AIDS patients, depending on the degree of host immune suppression, can have a negative result over 50% of the time. The factors that may give rise to false-negative results are detailed in Table 4.

On the other hand, it is necessary to remember that after infection, 2 to 12 weeks are required for the sensitised T lymphocytes to be able to recognise tuberculin deposited within the dermis. During this latency period, and although infection is present, there is no tuberculin test response. In newborns, tuberculin test positivity cannot be detected until after 8 to 12 weeks of life in some instances, and after 6 months in others. In turn, the tuberculin response capacity does not remain invariable in the course of a lifetime; in effect, it tends to weaken over time and can become undetectable in elderly individuals who were infected in youth. In this setting, in order to detect the so-called booster effect (which will be discussed in greater detail in the
following section), a new test is performed 7 to 10 days later, and the result of this second test is used to classify the subject as either a reactor or non-reactor.

**Table 4. Factors that may give rise to false-negative tuberculin test results**

1. Factors related to the person subjected to the test:
   - High fever of any origin
   - Malnutrition
   - Viral infection: HIV, measles, parotitis, varicella
   - Bacterial infection: TB, particularly severe forms and involving pleural location, typhoid fever, brucellosis, whooping cough, leprosy
   - Blastomycosis
   - Live viral vaccination. Measles (does not suppress response in first 48 hours after vaccination), poliomyelitis, parotitis, varicella, yellow fever
   - Oral antityphoid vaccination
   - Chronic renal failure
   - Leukaemia, lymphomas, Hodgkin’s disease
   - Sarcoidosis
   - Newborn infants or advanced age
   - Stress, surgery, burns, mental disorders
   - Immunosuppressors, corticoids
2. Factors related to the tuberculin used:
   - Inappropriate storage (exposure to heat or light)
   - Inappropriate dilutions
   - Chemical denaturalisation
   - Adsorption by the container (partially controlled by Tween 80 detergent)
3. Factors related to the method of administration:
   - Administration of too little antigen
   - Subcutaneous injection
   - Delay in administration after extracting the dose from the container
   - Injection too close to other antigens
4. Factors related to registry of the result:
   - Reader inexperience
   - Errors

**False-positive readings**

False-positive tuberculin test readings can occur due to multiple reasons, although the most important is the interpretation of a tuberculous infection when in fact the infection involves other environmental mycobacteria or the patient had previously received a BCG vaccination. Occasionally, the presence of a haematoma (bruise) or small abscess at the injection site can be interpreted as an induration when it is indeed secondary to injection-related trauma or another infection.
When infection by environmental mycobacteria is suspected, the ideal approach is to resort to a specific antigen of the microorganism in question, so as to allow dual testing. If the response to the antigen is greater than the response to PPD, the infection may be attributed to the environmental mycobacteria instead of to *M. tuberculosis*. However, this second test is very difficult to perform in practice (in view of the limited availability of specific antigens) and to interpret.

The BCG vaccination is a particularly important consideration, especially in low- and middle-income countries where BCG vaccination at birth constitutes one of the priorities of local health care policies. Adequate interpretation of the tuberculin skin test requires an evaluation of the vaccination history of the individual, with particular emphasis on the identification of the post-vaccination scar in the deltoid region or in some other skin zone. The post-vaccination scar is characteristically small and located on the same level as the surrounding skin; it has a pearly smooth appearance, and fine folds are formed on compression of the scar between the fingers. However, not all vaccinated individuals become reactors to tuberculin, and the indurations recorded tend to be smaller than in patients infected with *M. tuberculosis*. Moreover, hypersensitivity to tuberculin as a result of BCG vaccination decreases before hypersensitivity attributable to *M. tuberculosis* infection develops, although it is not possible to determine precisely when the reaction disappears, since some studies have shown that a substantial percentage of vaccinated individuals exhibit a positive tuberculin test (attributable to the vaccine) after as long as 25 years. Therefore, the tuberculin sensitivity induced by BCG vaccination may last indefinitely, and although sensitivity disappears in a substantial proportion of cases, in others it can persist and interfere with test interpretation even if the positivity limit is established at 15 mm.

For this reason, countries with BCG vaccination coverage from birth (i.e., practically all low- and middle-income countries) should not use the tuberculin skin test as a discriminatory diagnostic test for possible indication of preventive therapy. In these settings, the criteria of epidemiological antecedents and age should prevail. Accordingly, a child living with a person with TB and who exhibits smear-positive results should always receive preventive treatment, regardless of the tuberculin test result obtained.
Interpretation of results

Interpretation of results is perhaps the most complicated aspect of the tuberculin skin test. Efforts have been made to simplify this step, although often with little success.

The tuberculin test, like any other diagnostic test, has a sensitivity and specificity that varies according to where the positivity threshold or cut-off value is established. The closer the cut-off point to 5 mm, the greater the test sensitivity, and the test will be able to diagnose more cases of *M. tuberculosis* infection. However, this also increases the possibility of more false-positive readings—in other words, a loss of specificity. In contrast, when the cut-off is established at larger diameters, particularly 16 to 17 mm, which corresponds to the normal distribution of TB patients, sensitivity decreases and there is an increase in the number of false-negative readings as well as an increase in specificity. For this reason, when it is important to establish a diagnosis of *M. tuberculosis* infection because the patient has a high risk of developing TB, the cut-off point should be set at 5 mm to ensure maximum sensitivity, avoiding false-negative diagnoses that would prevent the patient from receiving treatment. It may be better to prescribe one preventive treatment too many (in the event of a false-positive case attributable to environmental mycobacteria because of use of such a low cut-off value in an individual at high risk of TB) than to do without treatment and run the risk of developing TB. In contrast, if there is no particular risk of developing TB, the cut-off value should be set at a point (e.g., 15 mm) that would be associated with the least possible number of false-positive diagnoses. This way, maximum specificity at the expense of sensitivity would be achieved, which in this setting would not have negative effects since the risk of disease would be sufficiently low to contraindicate preventive therapy.

The issue dealt with above is closely related to the positive predictive value (PPV) of the tuberculin skin test, which represents the probability that an individual with a positive test result is truly infected with *M. tuberculosis*. The PPV is what is important when interpreting a tuberculin test result, and it is directly related to the prevalence of the latent infection of *M. tuberculosis*. Consequently, in a population composed of contacts of infectious smear-positive TB patients, the prevalence of infected individuals is much greater than in the general population, since the cut-off value of 5 mm affords a PPV of 99%. The PPV of the tuberculin test has been calculated for different prevalences of true *M. tuberculosis* infection, according to whether the test specificity is 99% (the value estimated when no cross-reactions exist with
other environmental mycobacteria or with BCG vaccination) or 95% (when there is interference by environmental mycobacteria).

Thus, the cut-off point established in a certain geographical setting should take into account not only the existing situation in terms of infection by environmental mycobacteria, which may increase the possibility of false-positive results, but also in terms of the prevalence of TB in each population group studied. If the intention is to establish an exact limit for the entire population, without properly assessing the risk of TB in each group, it must be taken into account that the limit in question is different for each population (i.e., it is dependent on the prevalence of the infection), and that it changes in different communities over time because of changes in infection prevalences.

Consequently, it is practically impossible to impose a precise cut-off value for tuberculin skin testing, since it is influenced not only by the size of the reaction but also the corresponding PPV.

**Tuberculin test repetition. Tuberculin conversion. “Booster effect”**

Before addressing this section, it should be pointed out that the tuberculin test does not sensitize non-infected individuals, regardless of how often it is performed. In some instances, the test must be repeated due to the existence of a more-or-less sustained risk of TB infection, as with health care workers.

Tuberculin conversion is defined as a test response in an individual previously classified as a non-reactor. This diagnosis is extremely important if the time elapsed between the two tests is less than 2 years, since it implies that the individual had been infected during this period, and that infection is recent. The risk of developing the disease during this time is the highest and thus constitutes an absolute indication for preventive treatment. Tuberculin conversion consists of the detection of a tuberculin test result of over 5 mm in an individual previously unresponsive to tuberculin. This limit is not unanimously accepted; several well-respected scientific societies have instead established a conversion limit of 6 or 12 mm. The phenomenon implies the acquisition of tuberculous infection provided a booster effect has previously been rejected.

With time, the *M. tuberculosis*—infected individual weakens in his or her capacity to react to a tuberculin test, as the result of a loss of memory T lymphocyte capacity, giving rise to a negative test result. However, since the response capacity persists, the PPD used in the first tuberculin skin test...
can produce a stimulant or “booster effect”. Thus, a second test may yield a positive result because of this recovered memory phenomenon, and the individual may be classified as a tuberculin converter, when in fact he or she had been previously infected. Whenever present, the booster effect is not detectable until 7 days after the tuberculin test result is regarded as negative, and it may persist for years. Therefore, and in order to reject the possibility that a lack of tuberculin test reaction may be due to a weakening in response and not to the absence of infection, testing should be repeated 7 to 10 days later, accepting the outcome of the second test as the definitive result. If positive, it will help to avoid the false diagnosis of recent conversion. Since TB infection is generally acquired in infancy and youth, this weakening in response capacity to the tuberculin skin test is observed more often in elderly individuals. Therefore, in the industrialised world, a second test is advised in subjects over the age of 55 years who previously had negative tuberculin skin test results. However, although less frequently, such weakening response may also occur in non-elderly individuals. Thus, a booster effect should also be ruled out in certain high-risk groups, such as health care personnel.

The sensitivity to tuberculin due to BCG vaccination decreases at a faster rate than the sensitivity acquired as a result of natural infection with M. tuberculosis. Consequently, vaccinated individuals of any age who have a negative result should in theory be retested after 7 to 10 days to reject or detect a possible booster effect. This imposes serious limitations to the diagnosis of latent infection of contacts of active TB cases in low- and middle-income countries where BCG vaccination is carried out extensively.

Skin testing should not be repeated when there is indication that the test had already been performed in the past and was positive (regardless of the diameter of the induration). Indeed, a second test might result in a booster effect and amplify the reading, thereby giving rise to erroneous conclusions.

**Indications for tuberculin testing**

The tuberculin skin test, in the same way as any diagnostic test, should only be performed in individuals in whom the test result may lead to some therapeutic intervention. Only two intervention possibilities exist for TB: the treatment of active disease TB patients, and preventive treatment of infected individuals at high risk of developing TB disease. In helping to diagnose TB disease, the tuberculin test only offers a high PPV in children, with considerable lesser performance in patients with immune deficiencies. Regarding the possibility of preventive treatment, in the case that TB infection is detected,
such therapy is only indicated in groups at high risk of developing TB disease, in whom the PPV of the test is very high. In low- and middle-income countries, these high-risk groups are limited to those subjects who live with patients with confirmed smear-positive results, and to HIV-infected persons. However, among people living with smear-positive subjects, the tuberculin test is not indicated in view of the important interference represented by BCG vaccination. Thus, possible preventive management must be decided based on epidemiological risk factors. Likewise, in HIV-infected patients, the tuberculin test loses much of its usefulness, since immune deficiency is a frequent source of false-negative test results. Since low- and middle-income countries have a high prevalence of *M. tuberculosis* infection, preventive treatment would be indicated in these high-risk groups regardless of the tuberculin test results obtained. Obviously, this recommendation, which is accepted by the WHO, is questioned by some experts on the grounds that it is possible that a considerable number of anergic patients may not benefit from such preventive treatment, and that these patients have a significant chance of not being infected.

In view of the above, a tuberculin test would only be indicated in low- and middle-income countries in the following situations:

1. **Children with symptoms suggestive of TB.** Here, a tuberculin test offers a high PPV in diagnosing the disease. However, the test should not be used to diagnose infection or for managing contacts. Specialists in pediatrics should carry out the procedure.

2. **Severe immune deficiencies.** Use in support of the diagnosis of TB disease. These cases are to be dealt with in the reference hospital.

3. **Health care personnel,** particularly those beginning to work in health care. If a tuberculin test proves positive, it can be assumed that the previous infection can afford some protection against later exposure to *M. tuberculosis,* and no further actions would be required. However, in health care workers with an initial negative tuberculin test, periodic tuberculin screening is indicated (every 6-12 months, depending on the risk) to establish when a tuberculin test turns positive. At this point, recent conversion has occurred and preventive treatment would then be indicated.

**Positivity criteria and indications in low- and middle-income countries**

The above considerations imply that there is more than one limit to the tuberculin test, depending on the risk of TB—which greatly complicates its appli-
cation in the field, since people working at the peripheral level would then be required to make decisions based on the risk factors of each individual. In truth, the different cut-off points would only be applicable in the reference centres and to highly expert personnel, not at the peripheral level. Nevertheless, the tuberculin test should be used sensibly, taking care to avoid excessive, inappropriate use. As has been mentioned above, the test should only be used to make a clinical decision, or for epidemiological studies. The most important consideration is to define the clinical indications, since conducting tuberculin surveys is very complex and expert personnel should only design such studies. From the perspective of supporting a clinical decision, the test is only indicated in those persons in whom the result implies some intervention: treatment if TB disease is diagnosed, or preventive therapy, which is only recommended in high-risk groups. To simplify things, the indications for a tuberculin test should be restricted to groups at a high risk of developing TB. The positivity threshold for the test should be set at 5 mm, which affords a PPV of 99% in such groups. In this way, interpretation of the test is simplified and its utilisation is facilitated.

In summary, it is necessary to consider that low- and middle-income countries not only suffer from unreliable health care distribution and resources, which makes storing tuberculin in the peripheral centres almost impossible, but that these countries also practise extensive BCG vaccination, which complicates interpretation of the tuberculin test result. For these reasons, these countries should only indicate the tuberculin test in centres where children with suspected TB are seen and where there is a paediatrician, as well as in reference hospitals to help in the diagnosis of TB infection in patients with immune deficiencies. The test should be contraindicated at the peripheral level, and should not be used as a discriminatory test for indicating preventive treatment. As will be seen in the corresponding chapter, the indication for preventive treatment should be evaluated according to the risk of TB disease among the different population subgroups, without the need for a tuberculin skin test.

**Recommended reading**


Chapter 7 - Diagnosis of tuberculosis

Chapter summary
The diagnosis of tuberculosis (TB) should be based on a series of ancillary methods and confirmatory microbiological techniques. The accessory diagnostic methods are non-specific and include the clinical manifestations, radiological findings (highly sensitive), histopathology (more specific than the other approaches), and tuberculin testing (which contributes little in terms of diagnosis). Efforts must be made to ensure that the diagnosis can be confirmed by smear microscopy (which is accepted for the purpose of TB control programmes) and/or culture.

When faced with a possible case of tuberculous disease, it can be presumed that *M. tuberculosis* infection has already progressed to the point where a series of clinical manifestations have developed, compelling the host/patient to seek medical help. It is therefore necessary to be familiarised with the clinical picture of tuberculosis (TB) and with the necessary diagnostic techniques. It should be emphasised that among the different diagnostic techniques, the microbiological study of samples is by far the most important instrument and the only method available for establishing the disease with certainty. It is therefore reasonable to refer to microbiological study as the diagnostic method for TB, with the rest of techniques constituting ancillary diagnostic procedures. Each of these methods will be dealt with in this chapter. We begin though with the clinical manifestations of TB, since they constitute the initial medical consultation and play a role in the subsequent suspected diagnosis.

Clinical assessment

Section summary
TB lacks the symptoms, exploratory findings, and analytical/laboratory data that can clearly differentiate it from other respiratory diseases. In most instances, the onset of clinically manifest TB is insidious and not particularly alarming; as a result, months can go by before the diagnosis is established. This points to the importance of implementing complementary measures in the case of even the slightest clinical suspicion. Hence, the physician must be perfectly familiarised with the symptoms and signs suggestive of TB, since such knowledge can lead to increased suspicion of the disease and to an earlier diagnosis. Consequently,
clinical evaluation is very important in strategies designed to increase case detection.

TB can manifest as any sign or symptom in any part of the body. However, since respiratory TB is the most frequent presentation and is the form associated with the greatest potential for contagion, increased case detection and an earlier diagnosis prompt the suspicion of TB in any patient with cough and/or expectoration lasting more than 2 to 3 weeks. Such patients are referred to as symptomatic respiratory subjects. In all subjects presenting with signs and symptoms suggestive of TB, a series of pertinent tests are indicated to rule out the possibility of the disease. Serial sputum smear microscopy is the minimum but most important measure that should be included in the diagnosis of TB.

### Clinical symptoms

TB lacks specific clinical manifestations that allow it to be differentiated from other respiratory diseases. In most cases, the onset of clinically manifest TB is insidious and not particularly alarming; as a result, months can go by before the diagnosis is established. Thus, it is important to implement complementary measures in cases of even the slightest clinical suspicion. The physician must therefore be perfectly familiarised with the symptoms and signs suggestive of TB, since such knowledge can lead to increased suspicion of the disease and to an earlier diagnosis. Early diagnosis affords a double benefit: the affected individual undergoes earlier treatment, which results in fewer sequelae and improved survival, and the period during which the individual is able to spread the infection in the community is shortened. Ruling out the disease is an important measure for controlling this infectious condition.

As has already been commented, *M. tuberculosis* can spread to any part of the body from its initial phase of entry into the host. In short, TB can affect any organ or tissue. The most common location is the lungs—the primary entry site of the tubercle bacilli—which is implicated in 80% to 85% of all TB cases in immunocompetent individuals. The clinical manifestations of TB depend on the location of the disease, although in all cases the manifestations are vague and non-specific. Accordingly, it is possible to include TB in the differential diagnosis of any clinical syndrome, regardless of its location and presentation. In other words, any sign or symptom, in any location, may be suggestive of TB.

Apart from pulmonary involvement, the most common extrapulmonary locations of the disease are (in decreasing order): pleural, lymphatic, urogenital, osteoarticular, and meningeal—although as has been pointed out, any
organ or tissue can be affected. In immunocompetent patients, the frequency of presentation of extrapulmonary TB is no greater than 15% to 20%, with this figure increasing in situations of immune deficiency, as in the case of patients with AIDS, in whom extrapulmonary disease accounts for 50% to 60% of all TB cases.

In addition to the local manifestations, TB often produces general signs and symptoms, including febricula, intense perspiration, asthenia, anorexia, and weight loss—which are suggestive of a chronic infectious disease.

Primary infection is usually subclinical, or may involve symptoms as non-specific as cough and febricula. This is why the persistence of respiratory symptoms for more than 15 days in a child constitutes an indication for chest radiographs, particularly when accompanied by systemic or extrapulmonary manifestations such as anorexia, weight loss, and erythema nodosum.

In turn, the onset of adult or post-primary TB often includes cough, mucopurulent expectoration, nocturnal sweating, and easy fatigue. In some instances, the onset can be acute, in the form of high fever, chills, haemoptoic expectoration, or haemoptysis—a situation that tends to lead to earlier medical consultation and a comparatively lesser delay in establishing the diagnosis. A special type of onset is represented by TB pneumonia, involving a clinico-radiological syndrome similar to that seen in patients with bacterial pneumonia. Extensive pulmonary spread is associated with progressive dyspnoea and respiratory failure, which in severe cases can result in adult distress syndrome.

On the other hand, miliary TB, which always implies haematogenous spread of the disease and is therefore serious (although the patient may sometimes present with few symptoms), is mainly characterised by general signs and symptoms. In such situations, it is often necessary to establish a differential diagnosis with a fever of uncertain origin, particularly if during the initial period no miliary pattern is observed on the radiographs.

An essential consideration for optimum case detection is adequate diagnostic suspicion. It is therefore very important to define which patients constitute suspected TB cases, in order to reduce the study population and therefore increase the positive and negative predictive values of the diagnostic tests used.

Based on the above, and considering that pulmonary TB is the most frequent presentation and the form with the greatest contagion potential, efforts should focus on the detection of these cases. Since pulmonary TB is most often characterised by persistent cough and expectoration, increased
case detection and an earlier diagnosis call for the suspicion of TB in any patient with cough and/or expectoration lasting more than 2 to 3 weeks. Such patients are referred to as symptomatic respiratory subjects. These symptoms, in addition to being the most frequent manifestations of pulmonary TB, are also responsible for the greatest contagion potential (infectivity increases with the degree of coughing). In symptomatic respiratory patients, and in all subjects presenting with signs and symptoms suggestive of TB, a series of pertinent tests are indicated to rule out the disease.

One topic deserving special mention is TB associated with HIV infection. If such HIV-positive individuals have not yet developed immune deficiency, the TB symptoms tend to be similar to those observed in the rest of cases. However, in the immunosuppressed AIDS patient, the initial manifestations tend to be non-specific, with a predominance of systemic symptoms (e.g., nocturnal fever, asthenia, weight loss, peripheral adenopathies), a high likelihood of tuberculin test negativity, and a high incidence of extrapulmonary involvement. All AIDS patients should therefore be subjected to active screening to identify TB disease or infection.

Physical examination

The physical examination of the patient with TB disease is likewise lacking in specificity and often contributes very little to the diagnosis. In many instances, the patient appears to be healthy. Nevertheless, a systematic examination is always required, noting possible clues such as the following:

- Crackling rales in the infraclavicular space or in the interscapular-vertebral zone, in relation to exudative and cavitary lesions.
- Uni- or bilateral bronchial rales (rhonchus, subcrepitations) in cases of bronchogenic disease dissemination.
- In cases of pleural involvement: dull percussion, absence or reduction of vesicular murmur.
- Evidence of extrathoracic locations:
  - Erythema nodosum
  - Cervical and submaxillary fistulas and adenopathies, anal fistulas, osteoarticular involvement
- If the patient presents with dysphonia, an indirect laryngoscopic exploration is advised.
- If haematogenous spread is suspected, the central nervous system and ocular fundus should be explored.
General laboratory tests

The general laboratory study likewise offers no characteristic information, although it should always be carried out for diagnostic purposes and, in some instances, for patient follow-up during treatment. While very non-specific, the following should be noted:
– Moderate anaemia and hypoproteinaemia in long-evolving cases.
– An increased erythrocyte sedimentation rate, which usually does not exceed 50 to 60 mm in the first hour.
– Altered coagulation test results.
– Acute and febrile presentations can show leucocytosis with neutrophilia, although lymphocytosis is more common in the subacute and chronic forms of the disease.
– In some instances, before starting treatment, changes in liver enzyme levels can be detected (e.g., increases in transaminase and/or gamma-glutamyl transferase levels). These changes are often not attributable to liver infiltration but to the host toxic state or alcoholism.
– Some serious disseminated cases can present with hyponatraemia or hypochloraemia due to inappropriate antidiuretic hormone secretion.
– The presence of haematuria without colic pain and pyuria with negative urine culture suggest possible renal TB.

Tests indicated for patients with suspected tuberculosis

When dealing with a patient with suspected TB at the peripheral level of the health care network, a serial study of three sputum smears is always required. If the results prove positive, the diagnosis of TB is assumed and the patient is admitted for treatment into the National Tuberculosis Control Programme (NTP). In turn, the approach indicated in the event of negative smear studies depends on the available resources and diagnostic methods. The most rational decision for low- and middle-income countries is to recommend a broad-spectrum antibiotic to assess patient response. If the clinical manifestations persist after this treatment cycle, repeat serial smear microscopy is indicated, with culture of one of the samples in solid medium. Where available, this step should also include a chest radiograph. On the other hand, a tuberculin test would be indicated in children. Other diagnostic tests are not indicated except in very rare situations.

Figures 9 and 10 show the algorithms indicated for attempting to establish a diagnosis of TB in symptomatic respiratory patients.
Figure 9. Approach indicated in symptomatic respiratory patients: first medical consultation.

Figure 10. Approach indicated in symptomatic respiratory patients: second medical consultation (patient continues to cough). * If the radiographs are suggestive of TB, action should be planned according to the available resources. At the peripheral level, if there are no further available resources, TB treatment can be started under TB control programme conditions. However, if the patient is at a reference centre that has other diagnostic possibilities, sampling involving other techniques should be assessed before prescribing treatment, such as invasive procedures (e.g., bronchoscopy, biopsy).
**Recommended reading**


**Microbiological diagnosis**

**Section summary**

Sample collection and handling influences the sensitivity of the different microbiological techniques employed. Whenever possible, samples should be collected before starting chemotherapy and in open areas or well-ventilated rooms. The patient should be instructed on the correct way to collect sputum; if difficulties are encountered, induced sputum sampling can be attempted by clapping and/or a physiological saline aerosol, taking care not to discard any sample, even if saliva is involved. Bronchoscopy is not advised except in special instances. In children who fail to expectorate and who have been hospitalised, gastric lavage can be performed on 3 successive days. In the case of pulmonary TB, a serial study is required, collecting three sputum samples on 2 consecutive days. These samples should be clearly labelled and sent to the laboratory as quickly as possible, with refrigeration during the waiting period. All biopsy specimens should also be sent to the microbiology laboratory, without fixation and with a few drops of distilled water added to avoid drying. Formalin should not be used.

Despite the advances made in the past 20 years in relation to the microbiological techniques used for diagnosing TB, only a small portion of the global world population can benefit from them. The main means of diagnosis and treatment of TB in countries with low or middle incomes is smear microscopy with the Ziehl-Neelsen technique, due to its simplicity, rapidity, reproducibility, low cost, and effectiveness in detecting infectious cases. Its main inconvenience is its low sensitivity, which in turn is influenced by the severity of disease, sample quality, and time dedicated by the observer to read the smear microscopy result as negative. However, the specificity of the technique is very high (almost 100% in countries with average or high rates of endemic disease) and is only limited by false-positive readings attributable to other environmental mycobacteria.

The other basic technique in the diagnosis of TB is culture, which is the only method available that can establish a definitive diagnosis and which is
appropriate for evaluating patient follow-up and ensuring cure. Culture, moreover, has higher sensitivity than smear microscopy. However, the inconvenience posed by the long waiting time to obtain a result (at least 6-8 weeks), its increased cost, and complexity in performance and maintenance prevent its use at the most peripheral level of health care. Consequently, for practical purposes and under programme conditions, the diagnostic and follow-up technique of choice is smear microscopy, despite its limitations. The above considerations imply that the indication for culture is dependent on the endemic area involved, and on the available health care infrastructure and resources. Thus, while the general use of culture is recommended in industrialised countries, use of culture is much more restricted in poorer nations, and somewhat more common in middle-income countries where bacterial culture should be included in the diagnostic algorithm of cases with negative smear microscopy findings (Figure 10). Solid media cultures should be the only ones indicated for routine use in low- and middle-income countries.

With regards to identification of the different mycobacterial species, biochemical techniques can be used, as well as chromatography and genetic probes. However, only biochemical testing is indicated on a routine basis in countries with low or middle incomes, particularly because of the lower cost, even though these methods may be complicated to perform, are slow, and lack reproducibility. It should also be noted that that the importance of species identification is very relative, in comparison with the importance of smear microscopy and culture, since in countries with medium or high rates of TB, over 99% of the cases of positive smear microscopy are attributable to \( M. \) \textit{tuberculosis}. Indeed, cases involving environmental mycobacteria are so rare that only one laboratory with the capacity to identify mycobacteria species based on biochemical tests is needed per country.

In countries with low- or middle-income levels, the study of drug sensitivity should be carried out based on Canetti’s method of proportions, using Löwenstein-Jensen medium. The time to reading is 4 to 5 weeks, and the laboratory must inform the clinician of the amount of growth occurring in the media with antituberculous drugs, in comparison with the media without medication. Again, the indication for performing such tests depends on the available resources and on the endemic disease found in each region, although sensitivity assays should always be performed in cases of treatment failure, relapse, and treatment discontinuation, as well as to periodically monitor levels of resistance. These susceptibility tests, however, have limited use in poor countries, since the information they offer is obtained late (by 4-5 months), is not always reliable (with no good \textit{in vitro} and \textit{in vivo} correlation), and is based on a group of patients (i.e., re-treated patients) that is not considered a public health priority (priority is instead given to initial cases with smear-positive results).
The contribution of microbiology to the diagnosis of TB depends on the quality of the samples collected and the techniques employed. The present section reviews the basic protocols for collecting, transporting, and processing samples, followed by a review of the different techniques. In this section, only the conventional diagnostic techniques will be discussed, as these are the only options recommended on a routine basis in low- and middle-income countries. The remaining microbiological methods will be examined in Chapter 8. This latter set of techniques is not indicated for general use in low- and middle-income countries, except in very specific cases.

**Importance of sample collection and processing**

A series of norms have been established for the collection, storage, and shipment of samples. These guidelines must be followed consistently, since any deviation may affect the sensitivity of the different microbiological techniques used. The basic recommendations for the handling of samples can be summarised as follows:

1. Whenever possible, sampling should be carried out before starting chemotherapy.
2. Sampling is to be carried out in open areas or well-ventilated rooms, and away from other people.
3. Sputum and urine samples are to be shipped in clean, wide-lipped glass or plastic containers with airtight screw-on covers. Sterilisation is not necessary.
4. If the sample is obtained by direct methods (e.g., puncture of abscesses, cerebrospinal fluid, biopsy), an aseptic technique is required, placing the collected material in a sterile container.
5. The sample container should be labelled with the patient’s initials before shipment to the laboratory. Identification preferably should be made on both the container and cover.
6. Serial studies are advised to ensure improved performance. In direct sputum smear microscopy, one sample will yield on average 85% of positive readings, while two samples will yield 95% (i.e., the second sample raises the possibility of positivity by 10%), and three samples will afford 100%. This is why the collection (and shipment) of three samples per patient is advised. Sending of more than three samples would add no further benefit.
7. Since the most viable sputum sample is that collected in the morning, it is advisable, in the case of hospitalised patients, to obtain the three
samples early in morning on 3 consecutive days. However, this procedure is problematic when sampling is performed at the peripheral level, since the patient would be required to present to the health care centre on 4 consecutive days: the first day being the consultation and the next 3 days to deliver the samples. Thus, under control programme conditions, the first sample should be obtained at the time of consultation, while providing the patient with a second container for collection of the second sample early the next morning. When the patient returns on the next day to deliver this second sample, the final third sample is collected. In this way, the patient is only required to report to the health care centre twice, and three samples are obtained, with one sample being an early morning sample. A similar procedure can be carried out in the case of urine sampling.

8. The collected samples are to be sent immediately to the laboratory. If this is not possible, the samples should be kept in a refrigerator. However, many peripheral health centres in poor countries lack these facilities. In such instances, it may be advisable to perform staining in situ and to send the slide, instead of the sample, for examination. If only a smear microscopy evaluation is requested, there is no problem storing the sample for 7 to 10 days before shipment. The problem only occurs when cultures are required. In which case, sample shipment should not be delayed for more than 4 to 5 days; storage in a refrigerator will be required. If this measure is not adopted, the sensitivity of the culture decreases considerably, since the bacteria tend to die and will be unable to grow in culture. Still, identification by direct smear microscopy remains possible.

9. The patient should be instructed on the correct way to collect sputum; if difficulties are encountered, induced sputum sampling can be attempted by clapping and/or using a physiological saline aerosol.

10. Efforts should be made to obtain the best sample possible. If the patient only expectorates saliva, the latter should never be discarded for two reasons. First, collection of the saliva reflects patient effort and also ensures that the programme adheres to the requirements concerning the collection of such samples. If this saliva is not collected, it becomes difficult to determine whether poor sample quality is involved or whether this important step in the case detection procedure is failing. Second, while the diagnostic yield of saliva is very low, it is not negligible, and many studies have shown that it can contribute a percentage of positivity readings that, although low, should not be ignored. Sputum stained with
blood should also not be rejected, since they yield some diagnostic information.

11. If the patient fails to expectorate, sputum can be induced with a physiological saline aerosol, with the procedure performed in open areas or well-ventilated rooms. Due to the high prevalence of TB in countries with low or middle incomes, when there is a clinico-radiological picture compatible with the disease but negative serial smear findings, the diagnosis can be assumed and treatment started—except when no serial smear microscopy evaluation has been made. Therefore, despite the acknowledged usefulness of bronchoalveolar lavage and other samples obtained via bronchoscopy, these techniques are not indicated in low- and middle-income countries, except in very specific cases, in particular, those in whom there are sufficient criteria for considering other alternative diagnoses. In any case, if these samples are obtained, they must be shipped and processed immediately since the lidocaine used in bronchoscopy inhibits the growth of *M. tuberculosis*.

12. In children who do not expectorate, gastric lavage can be performed on 3 consecutive days. The microbiological study of these samples has been shown to be useful, since in the course of the night children swallow their respiratory secretions. The major inconvenience here is that in order to obtain an adequate sample, gastric lavage must be performed when the child is awake, since subsequent intestinal peristalsis rapidly eliminates the secretions swallowed during the night. As a result, patient admission for sampling is necessary. Unless the clinical picture is serious, it is not necessary to hospitalise children simply to perform gastric lavage.

13. All biopsy samples should also be sent to the microbiology laboratory, without fixation and with only a few drops of distilled water added to prevent dehydration. Formalin is to be avoided, although it is appropriate for samples sent to the pathology laboratory.

14. In HIV-infected patients, in whom disseminated TB is much more frequent, collection of all possible samples (including sputum, urine, cerebrospinal fluid, biopsy specimens of different organs) to confirm the diagnosis should be considered. In patients with severe immune deficiency and fever of unknown origin, three blood cultures for *M. tuberculosis* may be adequate.
Evolution of the microbiological techniques to diagnose tuberculosis

The evolution of microbiological diagnostic techniques since the time Robert Koch first used smear microscopy in 1882 has undergone four well-differentiated phases, during which progress has been very uneven. The first and very prolonged phase extended up until the mid-1970s and was characterised by few advances. Laboratories used conventional technology, with limitations involving the low sensitivity of smear microscopy and the excessively prolonged duration of culture, identification, and the performance of susceptibility testing. The lack of important developments during this period was attributable not only to technical limitations, which were only overcome recently, but also to the fact that since TB posed less of a problem in the developed world, there was no great pressure to develop new, faster, and more sensitive techniques. This phase, which has since ended in the developed countries, remains the norm in low- and middle-income countries. As a result, only a small proportion of the world population is currently benefiting from the advances characterising the rest of the evolutionary phase.

The second stage spanned the second half of the 1970s and was characterised by the introduction of a new culture technology that has still not been surpassed today: the so-called radiometric growth detection systems. The main limitation of this new technology was the need to work with radioactive isotopes, which was a major obstacle for many laboratories that did not possess the license to store and work with such materials.

The third phase took place during the 1980s and was characterised by two important events that greatly influenced the evolution of diagnostic techniques. One event was the advent of HIV, while the other was the accelerated development of new technologies. The appearance of HIV in the early 1980s led to an increase in TB cases and in disseminated infections caused by environmental mycobacteria in severely immunosuppressed patients. The evolution of diagnostic techniques during this period was characterised by the development of rapid non-radiometric culture techniques, the standardisation of effective systems for isolating mycobacteria from blood (blood cultures), and the development of rapid identification techniques (e.g., genetic probes, chromatography) as alternatives to traditional biochemical methodology. This period in turn gave way to the fourth phase, comprising the last 10 years, which involves the development of new genetic amplification techniques for the rapid diagnosis of TB.

Still, these important advances in microbiological diagnosis are used almost exclusively in the richest countries, since their cost and complexity render them unfeasible in those poorer countries with the greatest TB burden.
Conventional microbiological techniques for diagnosing tuberculosis

Conventional microbiological techniques for the diagnosis of TB are the only methods recommended for routine application in low- and middle-income countries. Only in exceptional situations, which will be analysed later, are other techniques justified.

The conventional microbiological diagnosis of TB is based on four successive stages: 1) sample staining for direct visualisation under the microscope (smear microscopy); 2) solid medium culture; 3) identification of the microorganism using biochemical techniques; and 4) drug susceptibility testing.

Smear microscopy

*M. tuberculosis* is a gram-positive or frequently colourless bacterium, as a result of which it is often not visualised in samples subjected to routine processing.

The detection of acid-fast bacilli in stained preparations examined under the microscope constitutes the first evidence of the presence of mycobacteria in a clinical sample. The acid-fast characteristics of the microorganism are attributable to the high lipid content of the bacterial wall (see Chapter 4). This technique is the easiest and fastest option available, and offers preliminary confirmation of the diagnosis, which, under the conditions of a TB control programme, allows confirmation of the case and implementation of treatment. It also addresses a public health concern by identifying infectious cases and offering the possibility of removing these infectious sources in the community through adequate treatment.

The recommended approach comprises the classic Ziehl-Neelsen technique, which reveals *M. tuberculosis* as small, red-coloured curved rods (bacilli) over a bluish background (Figure 11). This technique is simple, very economical, and reproducible in any setting. Visualisation is carried out under x1000 magnification in immersion oil and should last for at least 10 to 15 minutes. When reporting the results of the microscopic examination, the microbiologist should provide the clinician with an estimation of the number of acid-fast bacilli detected. A decrease in the number of bacteria is indicative of the efficacy of treatment. The number of bacilli observed is preferably scored by means of the following cross-system:

- (...) Absence of acid-fast bacilli/100 microscopic fields
- (+) 1-9 acid-fast bacilli/100 fields. Report numerically
- (++) 10-99 acid-fast bacilli/100 fields
- (+++) 1-10 acid-fast bacilli per field (observation of only 50 fields required)
- (++++) > 10 acid-fast bacilli per field (observation of only 20 fields required)
Smear microscopy is the technique of choice for the diagnosis of TB in all settings, in view that it is still better than the other more sophisticated techniques in five areas: 1) simplicity and reproducibility in any setting; 2) speed; 3) low cost; 4) high specificity; and 5) the ability to delimit contagiousness. For this reason, any method that is to replace smear microscopy must at least offer these five characteristics, in addition to improving test sensitivity. The major limitation of smear microscopy is its relatively low sensitivity; indeed, the great majority of cases are detected at fairly advanced stages of the disease. The non-visualisation of acid-fast bacilli in a clinical sample does not rule out the diagnosis of TB, since the lowest detectable concentration of bacilli is 10,000/ml of sample. Thus, the technique only serves to detect very advanced and contagious cases of TB. For instance, if a sputum sample only contains 5000 acid-fast bacilli/ml—still a high figure—and 0.01 ml is extended on the slide, the latter will contain only 50 bacteria, i.e., a single bacterium per 200 microscopic fields. If the technician examines 100 fields, the probability of seeing a bacterium is only 50%.

In effect, the sensitivity of smear microscopy is relatively limited. This implies that a negative result does not exclude the disease, since many false-negative results may occur. This possibility of yielding false-negative results

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**Figure 11.** Direct smear microscopy based on the Ziehl-Neelsen technique. *M. tuberculosis* appears as small, dark-red rods over a bluish background.
(sensitivity) can be influenced by three important factors. The first pertains to the stage of the disease. In this sense, sensitivity is high (80-90%) in a patient with TB who has a cavitary pattern on chest radiograph, but decreases in those who present with only TB infiltrates (50-80%), decreasing particularly in patients with nodular forms or masses (under 50%). The second factor pertains to sample quality and performance of the technique. As mentioned before, it is essential to obtain the best samples possible, and many studies have shown that the highest sensitivities are achieved with purulent sputum, followed by mucopurulent and mucous samples, and saliva. Sample quality is often not taken into account, but nevertheless it is an important consideration when trying to compensate for the low sensitivity of smear microscopy. The last factor involves the time spent by the technician or microbiologist in examining the sample under the microscope. It is known that 100 fields of the slide correspond to 1% of the smear, 200 fields to 2%, and 300 fields to 3%. In this context, the examination of 300 fields, which is the number needed to ensure a negative result with a high degree of certainty, would take 15 to 20 minutes. Persons studying the samples, however, often spend less time in examination. If the smear microscopy is clearly positive (++ or ++++) (Figure 11), very little observation time is needed to confirm the result. The problem of time mostly occurs with apparently negative results, which is the case with most slides. Therefore, not spending enough time to examine the sample can lead to a false-negative result. In turn, false-negative results imply that contagious cases remain unidentified and are left untreated in the community. The need to spend adequate time in examining samples means that a technician working full time on smear microscopy can only examine a maximum of 25 to 30 slides in a day.

The acid-fast staining characteristics with smear microscopy are common to all species belonging to the genus *Mycobacterium*, as well as to some fungal species. As a result, the rest of the environmental mycobacteria appear the same under the microscope. Moreover, while lacking the typical bacillary shape, some fungi, *Nocardia* species, or even food particles, dirt, or scratches on the slide can mislead the inexperienced observer. This may slightly reduce the specificity of the technique. Still, in countries with high and medium burden of TB disease, over 99% of all cases of positive smear microscopy are effectively attributable to *M. tuberculosis*. For this reason, under the norms of a TB control programme, in countries with low- or middle-income levels a positive smear microscopy result in itself is sufficient to accept a case as having TB and to start treatment.

Sometimes the culture becomes negative before smear microscopy because the treatment provided to the patient makes the bacilli non-viable.
The mycobacteria though continue to be eliminated by the host and continue to exhibit acid-fast staining characteristics. This situation gives rise to false-positive results owing to the existence of “non-viable bacilli”. Despite these results, such patients have very little potential to infect, and their course is favourable. As will be commented later on, culture (not smear microscopy) is the only acceptable method for following up on a TB patient and for ensuring that cure has been effective. However, under TB control programme conditions, the logistical problems posed by culture examination make it necessary to resort to smear microscopy for patient follow-up, despite the fact that microscopy may not indicate the true course of the disease (e.g., smear-positive with negative culture results).

Mycobacterial culture

Mycobacterial culture is the only means of ensuring a definite diagnosis of TB (with the corresponding identification), and the only acceptable method available for assessing patient follow-up and confirming cure. For this reason, in countries with sufficient economic resources, all clinical samples suspected of containing mycobacteria should be grown in adequate culture media. However, there are limitations that reduce the use of culture in low- and middle-income countries.

The results of culture are largely dependent on the previous steps of sample decontamination and digestion. Most clinical samples contain abundant commensal flora that grow faster than *M. tuberculosis*. These contaminating microorganisms must be eliminated from the sample since they would prevent the development of any mycobacteria present. It is also important to liquefy the organic remains (e.g., tissues, mucus, serum, and other proteinic materials) surrounding the microorganisms, so that the decontaminating agents can destroy the undesired bacteria. This would allow the mycobacteria to survive and access the nutrients contained in the medium. Mycobacteria are more resistant to strong bases and acids than are other microorganisms, as a result of which such digestion-decontamination techniques can be successfully used. However, decontamination should be performed with care because excessive or insufficient decontamination may adversely affect the viability of the mycobacteria in the sample, giving rise to false-negative results.

Culture offers several advantages that define it as the gold standard for the diagnosis and follow-up of TB cases. These advantages can be summarised as follows:
1. Cultures are much more sensitive than smear microscopy, and are able to detect as few as 10 bacteria per millilitre of sample.
2. Isolation in pure culture is necessary to correctly identify the isolated strains, since other mycobacteria appear identical to *M. tuberculosis* by smear microscopy.

3. Culture provides definitive confirmation of negative conversion and healing of patients with treatment. In poor countries where problems associated with treatment (e.g., suspected resistance) make culture-based follow-up necessary, the number of colonies obtained must be quantified, since this parameter is vital for monitoring of treatment and for assessing possible treatment failure. The same considerations apply to TB cases in rich countries.

However, the logistical problems posed by culture limit its use, particularly in poorer countries. The main inconveniences of culture can be summarised as follows:

1. The main limitation of conventional culture is related to the slow divisional capacity of *M. tuberculosis*. This causes the time elapsed from sample receipt to reporting of the result to be no less than 4 to 6 weeks in conventional solid media, and much longer in poor countries. This is too long a wait for establishing a firm diagnosis.

2. The cost of culture is far greater than that of smear microscopy, and specific media are needed, with subsequent storage in an oven. Moreover, more specific training of personnel is required to perform cultures.

In view of the above considerations, it is not possible to use culture at the most peripheral levels of health care, unlike with smear microscopy. Thus, when TB is clinically suspected and smear microscopy proves positive in this setting, treatment should be started and the patient registered as a TB case.

The indication for culture is therefore dependent on the extent of the endemic disease in the area, and on the available health care infrastructure and resources. It can generally be concluded that in industrialised countries, which for many years have been successful in diagnosing cases using smear microscopy among mildly ill patients, and which possess many health care centres and laboratories that do not have economic constraints, culture should be performed whenever a clinical sample is received from a patient suspected of having TB. However, in poor countries, where the main challenge continues to be access to smear microscopy evaluation for all symptomatic respiratory cases, culture is only indicated in special situations. Priority in these poorer countries must be given to smear microscopy and treatment. Culture would be reserved for cases of suspected resistance (although the
need for culture in this situation depends on the availability of second-line drugs). Culture would almost never be included in the diagnostic algorithm of patients initially presenting with negative smear microscopy results.

An intermediate position is represented by middle-income countries, where patients with positive smear microscopy results are often dealt with adequately, and where the economic constraints are not as severe as in poorer countries. Here, culture examination should be performed whenever the initial patient course with therapy proves negative (e.g., suspected failure, defaulter, relapse). However, in patients with persistent respiratory symptoms following an initial negative smear microscopy result and with no clinical response to broad-spectrum antibiotic treatment, a new smear microscopy evaluation is required, with culture of one of the sputum samples, in addition to a chest radiograph (Figure 10).

Traditional culture has always been made in solid medium, using coagulated egg (e.g., Löwenstein-Jensen, Coletsos) or agar (Middlebrook 7H10 and 7H11) as a base. These should be the only media indicated for routine use in countries with low- or middle-income levels, with preference going to Löwenstein-Jensen medium (Figure 12). Some mycobacteria, many of which are associated with patients with AIDS, such as \( M. \) haemophilum, \( M. \) malmoense, \( M. \) genavense, and \( M. \) avium subs. paratuberculosis, require culture medium supplemented with special growth factors, such as hemin, blood, mycobactin, or ferric ammonium citrate. Incubation of the seeded media in an atmosphere enriched with 5% to 10% carbon dioxide favours the growth of \( M. \) tuberculosis.

These solid media offer the advantages of increased culture simplicity, counting bacterial colonies (which is important in the follow-up of patients exhibiting a poor bacteriological course), detecting growths of more than one mycobacterium in the clinical sample, and cost-effectiveness. However, solid media have the inconvenience of slow bacterial growth and manual reading of results (which can lead to errors). These drawbacks have led to the search for faster and more sensitive techniques, and have yielded new culture media such as radiometric methods (Bactec\textsuperscript{®} system), biphasic culture media (MB-Septi-Check\textsuperscript{®}), and techniques for isolating mycobacteria from blood. These methods will be dealt with in detail in the chapter on non-conventional methods and new microbiological techniques for the diagnosis of TB.
Figure 12. Löwenstein-Jensen solid culture medium showing the growth of colonies (rough, breadcrumb appearance) of *M. tuberculosis*.
Identification of mycobacteria

The mycobacteria comprising the *M. tuberculosis* complex can easily be differentiated using a set of biochemical tests, since these microorganisms are niacin positive, reduce nitrates to nitrites, and possess pyrazinamidase (which allows the distinction between *M. tuberculosis* and *M. bovis*) as well as a heat-sensitive (thermolabile) catalase. On the other hand, any identification strategy aiming to go beyond the simple separation of *M. tuberculosis* from the other mycobacteria entails the use of complex identification techniques capable of addressing a minimum of 20 differentiating features. The main limitations of biochemical techniques are their complexity, slowness, and lack of reproducibility, although they are considerably less expensive than other options. These limitations have led to the development of fast, alternative identification techniques, such as chromatography and genetic probes (see Chapter 8). However, only biochemical tests are indicated for routine application in countries with low or middle-income levels.

Species identification, while one of the steps in the microbiological diagnosis of TB, is of less importance in comparison with culture and, particularly, smear microscopy. As has been mentioned, in countries with high and medium rates of TB disease, about 99% of the cases of positive smear microscopy are attributable to *M. tuberculosis*. In this context, and from the logistical perspective, in these countries a diagnosis of TB can be accepted in all cases of positive smear microscopy, without the need for species identification.

In low- and middle-income countries, the cases of disease caused by environmental mycobacteria are so infrequent that they justify the existence of only one laboratory per country with the capacity to perform identification based on biochemical tests. Often, in countries where culture is indicated, it is only necessary to identify *M. tuberculosis*—this being simpler than having to identify the rest of the environmental mycobacteria. The identification of other types of mycobacteria would only be justified in patients exhibiting a poor clinical response to initial treatment and standard re-treatment. Nevertheless, the percentage of disease caused by these other mycobacteria is increasing in wealthier countries, particularly where AIDS is more prevalent. In such countries, techniques for speciation of mycobacteria can be justified in all TB patients with a positive culture because these countries often possess simpler automated techniques for identification and have a higher number of these cases.
In vitro M. tuberculosis susceptibility testing

*M. tuberculosis* susceptibility testing can be carried out directly with a concentrates specimen when abundant acid-fast bacilli are observed under the microscope (direct method) or on a bacterial culture in the exponential growth phase (indirect method). The standardised techniques for *in vitro* sensitivity testing are the Canetti proportions and multiple dilutions technique, the Meissner absolute concentration method, and the Mitchison resistance level procedure—with all cultures conducted in Löwenstein-Jensen medium. The Centers for Disease Control and Prevention (CDC) in the United States recommend the proportions technique, but using a semi-synthetic medium (Middlebrook 7H10). At present, sensitivity studies can be performed based on radiometric technology (Bactec®), using a simplified and adapted version of the proportions method developed by Canetti. Of all these options, the only one recommended for routine use in low- and middle-income countries is the Canetti proportions technique using Löwenstein-Jensen medium.

Susceptibility tests in Löwenstein-Jensen medium require 4 to 5 weeks, versus 2 to 4 weeks in semi-synthetic media (Middlebrook 7H10 or 7H11), and versus 5 to 8 days with the radiometric technique. These susceptibility tests require their respective periods of time because they are performed on the positive culture, and not on the direct sample (direct method). As a result, in addition to the above-mentioned durations, we must add the time required for the culture to yield a positive result: 3 to 6 weeks in the case of solid media, and 12 to 21 days for the Bactec system. Thus, a sensitivity test using the Bactec method can be completed in less than 1 month, while in practice it takes more than 3 months when solid media are used.

The laboratory must inform the clinician of the amount of growth occurring in the media with antituberculous drugs, compared with in an untreated control medium. When susceptibility testing is performed correctly, the control will contain countable colonies. Thus, the counting of colonies in the medium containing drug and in the control will allow calculation of the proportion of resistant bacteria in the total population, expressed as a percentage. In general, when 1% or more of the bacterial population proves resistant to a critical concentration of a given drug, the drug should no longer be used as treatment, since the resistant population will become dominant in only a short period of time.

Here again, the indication for performing the technique will depend on the available resources and the extent of the disease in each area. Thus, in rich countries where there are few TB cases and a ready availability of second-line drugs, susceptibility testing is indicated at least in all patients
exhibiting a poor microbiological course, as well as in cases of failure, relapse, and treatment default. Controversy exists over whether all initial patients should be subjected to susceptibility testing before starting treatment. The CDC has recommended this practice since 1994, owing to a marked increase in treatment resistance in the United States. However, many other industrialised countries consider this measure to be unnecessary, instead advocating periodic representative studies (ideally every 5 years) to monitor resistance rates. In the case of low- and middle-income countries, susceptibility testing would only be indicated in cases of treatment failure, relapse, and default, as well as for the periodic surveillance of resistance in the country. However, in some very poor nations where the problem of TB is very serious, it may be questioned whether it is advisable to spend markedly limited resources on susceptibility testing at the expense of other priorities, such as ensuring initial therapy and access to smear microscopy evaluation for all symptomatic respiratory cases.

In effect, susceptibility testing poses an important practical limitation for use in poor countries, since the information they afford is obtained late, is not always reliable, and is based on a group of patients (re-treated patients) that constitute a less important epidemiological priority (priority is given instead to initial cases with smear-positive results). The recommended practice is to perform drug susceptibility testing in Löwenstein-Jensen medium. It is necessary to take into account that this test may require 4 to 5 months before yielding results: 2 months for culture, 1 month for identification, 1 month for the actual drug susceptibility testing results, and as much as 1 extra month lost as a result of sample shipment delays and the preparation of reports. Furthermore, the in vitro (drug susceptibility result) and in vivo correlation (diseased host) is close to 100% only for isoniazid and rifampicin. In most disease TB cases, clinical management decisions must be taken many months before drug susceptibility testing becomes available.

**Recommended reading**

Carcinoma
Section summary
Carcinoma in situ is a highly sensitive technique for diagnosing pulmonary TB in immunocompetent individuals, even though it is unspecific, since TB generates no pathognomonic radiological signs, regardless of how suggestive the images may seem. Thus, while there are radiographic images highly suggestive of TB, these findings are only an inferential aid in the diagnosis and suggest that microbiological evaluations should be carried out to confirm the diagnosis. In the same way, the prognosis and response to treatment cannot be decisively assessed by the radiographic course, since lesion regression can take place in a period of 3 to 9 months.

The role of chest X-rays in the diagnostic algorithm of TB is again dependent on the available resources and on the prevalence of the disease in the population seen. In rich countries, radiographic evaluation, together with smear microscopy, is recommended in all cases of suspected TB. In poorer countries, radiographic evaluation would take a second place. Its use in poorer countries should be restricted to patients with two negative serial smear microscopy results in whom antibiotic treatment is unsuccessful. Here again, it should be remembered that the focus is on patients with smear-positive results who are easily diagnosed at the peripheral level by means of microscopy. In very exceptional cases, computed tomography and other imaging techniques are indicated for the diagnosis of TB. Their application on a routine basis should be discouraged.
Both pulmonary and extrapulmonary TB present no pathognomonic radiological signs. Thus, although there are radiological manifestations highly suggestive of TB (e.g., upper lobe cavitations), and the accompanying clinical picture may be compatible with the disease in the context of a favourable epidemiological setting, the diagnosis of TB should never be accepted merely on the grounds of a radiological study. In effect, chest X-rays suggestive of TB can only indicate the need for an opportune microbiological evaluation. On many occasions, TB is diagnosed based only on the chest X-ray findings in patients who actually do not have the disease (Figure 13). In the same way, the disease is often not diagnosed in patients who do have TB (Figure 14).

In addition, the radiological course should not be used to decisively evaluate the prognosis and response to treatment, since lesion regression can take place in a period of 3 to 9 months. There may even be a paradoxical increase in the number of lesions in the first month of therapy, without the suggestion of treatment failure. In view of the above, all patients with uncomplicated TB should undergo only two radiological studies (even in places where the technique is readily available): at the start and the end of treatment. Radiographs should not be obtained on each control visit, unless complications develop.

**Figure 13.** Chest X-ray of a 72-year-old patient diagnosed with active pulmonary TB on the basis of the present image, when in fact the subject did not have active disease (inactive residual TB).
Figure 14. Chest X-ray showing a large mass in the upper right lung lobe. The initial clinical suspicion was bronchogenic carcinoma, although the pathology study and transbronchial biopsy culture confirmed the diagnosis of TB.

A suspected diagnosis of pulmonary TB is mainly based on the existence of a suggestive chest X-ray. Thus, the technique is highly sensitive but it is also unspecific. For instance, a normal chest X-ray can be found in the context of pulmonary disease, only in patients with some forms of primary TB, in few cases of very early detection of the disease, and in severely immune suppressed HIV-infected individuals. In the rest of cases, including almost all instances of adult TB, the presence of radiological abnormalities is a constant finding.

When primary TB manifests radiologically, it usually does so in the form of an alveolar infiltrate with or without adenopathies (hilar or within the mediastinum) (Figure 15), or as lymph node involvement without parenchymal lesions (Figure 16). Other possible presentations include normal chest X-rays, pulmonary consolidation, adenopathies (generally unilateral), cavitation (rare and isolated), pleural effusion (more frequent in young patients and adolescents), and even atelectasia of certain lobes secondary to compression by mediastinal adenopathies (more common in children and adolescents).

In post-primary TB, lung parenchymal involvement is a common finding, most often involving the apical segments and posterior portions of the
superior lobes, or the apical segment of the inferior lung lobes (Figures 17 and 18). Cavitation is frequent in such cases (Figures 17 and 18), as is bronchogenic spread to other parts of the lungs (Figure 19) and pleural effusion (Figure 20). Tuberculomas (nodules of variable size) with variations in morphology may preferentially be located in the upper lobes (Figure 21). However, while the above are the most commonly affected sites, any portion of the lung could be involved, with unusual or atypical radiographic findings (Figures 22 and 23). Among these atypical findings are parenchymal consolidations that are indistinguishable from those found in pneumonia of other infectious aetiologies (Figure 24). This fact often leads to delays in diagnosis, particularly if the clinical presentation is sudden rather than gradual. It is sometimes necessary to obtain chest X-rays with the patient in hyperlordosis, to better visualise small lesions present in both lung vertexes (Figure 25).
Figure 16. Chest X-ray of a 7-month-old boy with lesions typical of primary TB. A large, mediastinal, adenopathic mass is observed, causing collapse of the left upper lobe. Diagnosis of TB was confirmed by histopathological study and culture of the biopsy specimen obtained via bronchoscopy.

Figure 17. Chest X-ray of a 36-year-old patient showing lesions typical of post-primary TB. Infiltrates with necrosis and cavitation are observed in both superior lung lobes, with retraction of structures towards that zone. The diagnosis of TB was confirmed by smear microscopy and culture.
Figure 18. Chest X-ray of a 39-year-old patient showing lesions typical of post-primary TB. Infiltrates with necrosis and cavitation are observed in both superior lung lobes. There was evidence of bronchogenic seeding in the left lower lobe. The diagnosis of TB was confirmed by smear microscopy and culture.

Figure 19. Chest X-ray of a 52-year-old patient showing lesions typical of post-primary TB. Infiltrates with necrosis and cavitation are observed in both superior lung lobes, with retraction of structures towards that zone. There was evidence of bronchogenic seeding in the rest of the lobes in both lungs. The diagnosis of TB was confirmed by smear microscopy and culture.
Figure 20. Chest X-ray of an 18-year-old patient showing a large left pleural effusion. The diagnosis of pleural TB was based on histopathological study of the percutaneous biopsy specimen.

Figure 21. Chest X-ray of a 49-year-old patient showing an irregular nodule with poorly defined margins in the right upper lobe. Bronchogenic carcinoma was initially suspected, although sputum culture confirmed the diagnosis of TB.
Figure 22. Chest X-ray of a 24-year-old woman showing a cavitary lesion in the right lower lobe. The diagnosis of TB was confirmed by smear microscopy and culture.

Figure 23. Chest X-ray of a 73-year-old patient showing an irregular nodule with poorly defined margins in the right lower lobe. Bronchogenic carcinoma was initially suspected, although the histopathology and culture of the biopsy specimen obtained by thoracoscopy confirmed the diagnosis of TB.
Figure 24. Chest X-ray of a 24-year-old patient showing a segmental condensation in the right upper lobe. Community-acquired pneumonia was initially suspected, although smear microscopy and culture confirmed the diagnosis of TB.

Figure 25. Chest X-ray of a 42-year-old patient. The projection was made with the patient in hyperlordosis (lordotic radiography), which allowed the identification of a nodule in the right upper lobe that otherwise could not be visualised in either poster-anterior or lateral projection since the image of the nodule coincided with that of the collarbone. Sputum culture confirmed the diagnosis of TB.
Miliary TB can be a manifestation of both primary and post-primary TB. The typical radiographic findings consist of multiple fine nodules measuring less than 3 mm in diameter and generally very profusely distributed in the lower lung lobes (Figures 26 and 27). Visualization in these cases is greatly facilitated by identification of the retrocardiac space in the lateral chest X-ray projection (Figure 28). These nodules are sometimes better seen with systems that are able to magnify certain parts of the chest X-ray (Figure 29). It is important to know that these fine nodules may persist, even after the disease has been cured.

Figure 26. Chest X-ray of a 21-year-old female patient showing a disseminated miliary pattern. The diagnosis of miliary TB was confirmed by the histopathological study and culture of the transbronchial biopsy specimen.

In HIV-infected patients, the different radiographic findings that may be seen depend on the degree of immune suppression involved. If immune suppression is not severe and the patient was already infected with the TB in the past, endogenous reactivation of these bacilli is common, with the production of the typical lesions of post-primary TB (Figure 30). If immune suppression is severe, any exposure to a recent source of transmission, and even endogenous reactivation, will result in typical findings associated with primary TB, with frequent lymphatic involvement (Figure 31) and haematogenous dissemination. Among these findings, normal chest X-rays are common (Figure 32) and extrapulmonary involvement is frequent.

The pulmonary radiographic manifestations of environmental mycobacteria are very similar to those of TB when immunocompetent hosts are involved (Figures 33 to 35). The only apparent difference appears to be the
Figure 27. Chest X-ray of an 18-year-old female patient showing a disseminated miliary pattern. The diagnosis of miliary TB was confirmed by the histopathological study and culture of the transbronchial biopsy specimen.

Figure 28. Lateral projection chest X-ray of an 18-year-old female patient showing a disseminated miliary pattern. This miliary pattern can be better visualised in the retrosternal and retrocardiac space. The diagnosis of miliary TB was confirmed by the histopathological study and culture of the transbronchial biopsy specimen.
Figure 29. Amplification of the zone of the right upper lung lobe corresponding to a chest X-ray of an 18-year-old female patient showing a disseminated miliary pattern. The diagnosis of miliary TB was confirmed by the histopathological study and culture of the transbronchial biopsy specimen.

Figure 30. Chest X-ray of a 28-year-old female patient with AIDS showing a cavitary infiltrate in the right upper lung lobe. The CD4+ T-lymphocyte count was 468 cells/mm³. The diagnosis of pulmonary TB was confirmed by smear microscopy and sputum culture.
Figure 31. Chest X-ray of a 25-year-old patient with AIDS showing a large adenopathic mass in the region of the mediastinum and right hilum. No intraparenchymal lesions were observed. The CD4+ T-lymphocyte count was 84 cells/mm³. The diagnosis of pulmonary TB was confirmed by smear microscopy and culture of the specimen obtained by fine needle aspiration biopsy through the tracheal carina (bronchoscopy).

Figure 32. Chest X-ray of a 45-year-old AIDS patient showing no significant lesions. The chest X-ray can be considered normal. The CD4+ T-lymphocyte count was 43 cells/mm³. The diagnosis of pulmonary TB was confirmed by smear microscopy and sputum culture.
absence of lesions suggestive of primary TB and the fact that some of these infections caused by environmental mycobacteria may manifest as solitary lung nodules (Figure 36). A relatively common presentation of lung disease caused by *M. avium* comprises small micronodules in the middle lung lobe, the visualisation of which may be greatly facilitated by computed tomography.

**Figure 33.** Chest X-ray of a 75-year-old female patient showing infiltration of the right lower lobe and middle lobe. Fibrous tracts are seen in the right upper lobe and lingula, with calcifications present in the zone. The initial positive smear microscopy study led to the diagnosis of TB, although the patient’s symptoms failed to improve with the antituberculous treatment provided. Culture confirmed the involvement of *M. avium* complex.

**Figure 34.** Chest X-ray of a 48-year-old female patient showing a cavitary infiltration of the right upper lobe. The initial positive smear microscopy study led to the diagnosis of TB, although the patient’s condition failed to improve with the antituberculous treatment provided. Culture confirmed the involvement of *M. kansasii*. 
Figure 35. Chest X-ray of a 58-year-old female patient showing bronchial dilatations of the right lower lobe, suggestive of bronchiectasis. The initial positive smear microscopy study led to the diagnosis of TB, although the patient’s condition failed to improve with the antituberculous treatment provided. Culture confirmed the involvement of *M. abscessus* complex.

Figure 36. Chest X-ray of a 52-year-old female patient showing a nodule in the left upper lobe. The patient was evaluated for haemoptysis, and bronchogenic carcinoma was initially suspected. However, bronchial aspirate culture confirmed the involvement of *M. abscessus* complex.
For a more correct assessment of the mediastinum, computed tomography is very useful, although apart from very exceptional cases, both computed tomography and other imaging techniques are not indicated for the diagnosis of TB. Their routine use is therefore not advised, although it must be stressed that TB can yield a series of characteristic images with imaging (Figures 37 to 39). Imaging can also be useful in detecting areas suitable for diagnostic puncture procedures in suspicious cases (Figure 40).

When deciding whether to include chest radiography in the diagnostic algorithm of TB, it is necessary to consider not only its good sensitivity and low specificity, but also to consider that it is more costly and less accessible than smear microscopy. For this reason, the inclusion of radiology in the diagnostic algorithm will vary according to the available resources and epidemiological characteristics of the region. Thus, in rich countries that have few TB cases, no restrictions in terms of resources, and radiological services available to the entire population, the ideal approach in the event of suspected TB would be to combine the good sensitivity of radiology with the high specificity of smear microscopy. The recommended approach in these countries whenever a suspected TB case is encountered is to always perform radiography and serial smear microscopy, with strict emphasis on the need to confirm the diagnosis by culture examination.
Figure 38. Computed tomographic scan of a 28-year-old patient showing bronchogenic dissemination in the form of centrolobular nodules. TB was diagnosed with smear microscopy and culture.

Figure 39. Computed tomographic scan of a 32-year-old patient showing infiltration with cavitation. The arrow indicates the presence of so-called “tree-in-bud” lesions. TB was diagnosed with bacilloscopy and sputum culture.
Figure 40. Computed tomographic scan of a 56-year-old patient showing a cavitary lesion. TB was diagnosed by histopathological study and culture of the sample obtained by fine needle aspiration biopsy through the chest wall. Computed tomography was used to guide the needle biopsy.

However, in low- and middle-income countries, the primary focus should be on the smear microscopy-positive patient, who can be easily diagnosed at the peripheral health care level using microscopy. Moreover, these countries often have no access to radiographic equipment, and the cost of such studies allows these techniques only to be used in patients presenting with two negative serial smear microscopy results and in whom broad-spectrum antibiotic treatment has been ineffective (Figure 10).
Recommended reading


Tuberculin testing

Section summary

Tuberculin testing is of very limited value in the diagnosis of TB. However, in children, particularly those under the age of 5 years, in whom the prevalence of M. tuberculosis infection is very low, a positive tuberculin test indicates either very recent infection or actual disease. This is why the tuberculin test offers a high positive predictive value (PPV) for diagnosing tuberculous disease in this age group. The other group in which the test also yields a high PPV for diagnosing tuberculous disease is persons with severe immunosuppression. It should be noted, however, that a negative result never definitively rules out active disease.

The information relating to tuberculin testing in the diagnosis of tuberculous infection has been addressed in detail in the previous chapter. However, there are several points regarding the possible usefulness of the test in diag-
nosing TB. The positive predictive value (PPV) of the tuberculin test for diagnosing active tuberculous disease (i.e., the probability that a Mantoux-positive individual has active TB disease) is greatest in children, in whom the prevalence of TB infection is low, and in those with a positive tuberculin test result and a suggestive clinical picture of disease. The test can also be very useful in infected, high-risk patients, such as patients with HIV infection, silicosis, diabetes, or immunosuppressive disease; who use intravenous drugs; who suffer from malnutrition; or who are undergoing chronic immunosuppressive therapy.

In addition, it should be noted that a negative result does not necessarily rule out the diagnosis of TB disease, since the patient in question may have an impaired host response to the tuberculin (Table 4), as in the case of disseminated TB and pleural TB. It should also be taken into account that with the exception of HIV-infected individuals, elderly patients are the subjects who most often have negative tuberculin test results.

**Recommended reading**


**Histopathological diagnosis**

**Section summary**

In certain situations of negative smear microscopy, and when haematogenous dissemination, extrapulmonary TB, or neoplastic disease is suspected, biopsy samples may be required. Here, the diagnosis is based on the identification of caseating granulomas, although other disease processes can also produce caseating granulomas—particularly when other environmental mycobacteria or certain fungal species are involved. Consequently, the biopsy sample must always be sent to the microbiology laboratory for culture. Histopathology offers a high-probability diagnosis that justifies the start of treatment if the clinical and radiological pictures are suggestive, while waiting for culture confirmation.
In certain situations, TB is diagnosed on the basis of granulomas in samples collected via different organ biopsy techniques (e.g., bronchial, transbronchial pulmonary, thoracotomy pulmonary, hepatic, lymph node, bone marrow). These situations generally involve cases that are difficult to interpret, that have repeatedly negative microbiological findings (haematogenous dissemination, extrapulmonary TB), or that involve patients with suspected neoplastic disease. Thus, the diagnosis may be unexpected, as in the case where a solitary lung nodule was actually found to be TB (Figures 21 and 23).

The diagnosis is based on the identification of caseating granulomas with Langhans’ cells (Figures 41 and 42), which are highly suggestive of TB. However, other disease processes can also produce a very similar histopathological picture, in particular other environmental mycobacteria and some fungal species. Moreover, such microorganisms can produce clinical and radiological pictures very similar to those associated with TB. In AIDS patients, however, it is very rare to find typical granulomatous lesions because of the changes in the immune system. As a general rule, the pathologist uses staining techniques to identify acid-fast bacilli (e.g., environmental mycobacteria, certain fungi). However, since this approach is not 100% reliable, a biopsy sample must be sent to the microbiological laboratory for culture whenever TB is suspected.

Figure 41. Microscopic view of a granuloma showing caseous necrosis typically associated with TB.
The detection of tuberculous granulomas in biopsy specimens in patients with clinical and radiological evidence suggestive of TB is sufficient indication to start treatment, while waiting for the culture results to confirm the diagnosis.

When a biopsy sample is obtained to identify the cause of death, macroscopic lesions highly suggestive of TB may also be identified. Such lesions may consist of cavitations (Figure 43), extensive bronchogenic disseminations (Figure 44), or even highly significant miliary TB lesions (Figure 45).

**Recommended reading**

Figure 43. Macroscopic view of a lung affected by TB. Caseous necrosis is extensive, and a large cavitation can also be seen.