

# Laboratory procedures for the diagnosis of tuberculosis: a survey in ten Italian Regions

Maria Luisa Moro, Simona Nascetti, Filomena Morsillo,  
Matteo Morandi and the Italian "TB-SORV" Project Working Group\*

Area di Programma Rischio Infettivo, Agenzia Sanitaria e Sociale Regionale,  
Regione Emilia-Romagna, Bologna, Italy

**Summary.** The characteristics of laboratories performing tuberculosis (TB) diagnostic procedures were investigated in ten Italian Regions, through a mailed questionnaire. Three hundred and eighty laboratories answered (70.8% response rate), 250 of which performed directly at least one TB diagnostic procedure. Standard criteria concerning microscopy, culture, identification, and drug susceptibility testing were frequently not satisfied, particularly those related to the volume of activity (32% of laboratories performing microscopy examined < 10 samples and 36% of those performing culture performed < 20 cultures per week), processing time, biosafety requirements and participation to internal/external quality control programs. The survey's results highlight the need to promote the adoption of standardized procedures and to centralize the mycobacteriology testing in a reduced number of high quality laboratories, in order to improve diagnostic accuracy, resource management and quality of surveillance data.

*Key words:* *Mycobacterium tuberculosis*, laboratories, diagnostic tests, survey.

**Riassunto** (*Diagnosi di laboratorio della tubercolosi: una indagine in dieci regioni italiane*). Le caratteristiche dei laboratori che eseguono esami di micobatteriologia sono state indagate in dieci regioni italiane mediante un questionario. Hanno risposto alla indagine 380 laboratori (tasso di risposta del 70.8%), 250 dei quali eseguivano direttamente almeno un esame diagnostico per la tubercolosi (TB). I criteri standard per microscopia, coltura, identificazione e antibiogramma spesso non erano soddisfatti, in particolare i criteri relativi ai volumi di attività (il 32% dei laboratori che eseguivano microscopia esaminava < 10 campioni per settimana e il 36% che eseguiva coltura effettuava < 20 colture alla settimana), tempo di risposta, requisiti di biosicurezza e programmi di controllo di qualità interni ed esterni. Si evidenzia la necessità di implementare procedure standardizzate e di centralizzare l'esecuzione dei test di micobatteriologia in laboratori di elevata qualità per migliorare l'accuratezza diagnostica, la gestione delle risorse e la qualità della sorveglianza.

*Parole chiave:* *Mycobacterium tuberculosis*, laboratori, test diagnostici, indagine.

## INTRODUCTION

In the last years the incidence of tuberculosis (TB) has increased in some European countries [1, 2], while in Italy the trend of TB is stable and the incidence is relatively low. Since 1996, in fact, the annual crude incidence of TB in Italy is constantly lower than 10 cases for 100 000 inhabitants (the threshold for considering a country as at low prevalence) [3]. The steady decrease of the TB incidence in Italy since the beginning of the last century has come to an halt in the 80's due to the constant aging of the Italian population, the migrations from high prevalence countries, and the TB/HIV co-infection (all these conditions increase the risk of developing the disease) [4]. The spread of drug resistant tuberculosis (1 in 10 new cases is at

least resistant to one first line anti-tubercular drug) and the presence of multidrug resistant TB (MDR-TB) (resistance at least to isoniazid and rifampicin) and of extensively drug resistant TB (XDR-TB) (resistance to isoniazid, rifampicin, fluoroquinolons and at least 1 out of 3 second line drugs: capreomycin, kanamycin, amikacin) is emerging as the biggest threat to effective TB control [5-7].

Laboratory diagnosis of high quality is necessary to rapidly and accurately detect TB cases and antibiotic-resistance, to start effective therapy and, consequently, to stop the progression of disease and to prevent the spread of infection/disease to healthy people.

The quality of laboratory TB diagnosis depends on the type of laboratory procedures, the presence of standardized operating procedures, of internal and external quality control programs, the time nec-

\*The members of the Italian "TB-SORV" Project Working Group are listed before the References.

essary for referring specimens, the volume of activity and the respect of biosafety precautions [8-10].

In 2006 within the “Tuberculosis and Anti-tubercular Drug Resistance Surveillance” project funded by the Center for Diseases Prevention and Control (CCM) of the Italian Ministry of Health, the sub-project “Tuberculosis surveillance – TB SORV” (coordinated by the Emilia-Romagna Region) was implemented. Among other activities, a national survey aimed at describing the laboratory TB diagnostic procedures in private and public laboratories was conducted.

The objectives of this survey were: 1) to describe the general characteristics of laboratories performing TB diagnostic procedures and the characteristics of these procedures; 2) to identify critical issues.

## METHODS

The survey was performed in 2007 in 10 Italian Regions: Calabria, Campania, Emilia-Romagna, Friuli-Venezia Giulia, Piemonte, Puglia, Sardegna, Toscana, Veneto and Provincia Autonoma (PA) of Trento, by mean of a mailed questionnaire sent by the National coordination centre of the TB-SORV Project (Agenzia Sanitaria e Sociale Regione Emilia-Romagna) to the regional representatives and then forwarded to all public and private laboratories accredited by the National Health Service (Sistema Sanitario Nazionale – SSN).

In the Emilia-Romagna and Friuli-Venezia Giulia Regions and in the autonomous province of Trento, the questionnaire was not sent to private local laboratories, because they do not perform diagnostics for mycobacteria.

The questionnaire was organized in three sections. The first part explored the general and structural characteristics of the laboratories (*i.e.* denomination, location, private or public ownership, type of public health trust, availability of a computerized laboratory information system, -80 °C refrigerator for storing strains); the second part explored which procedures for TB laboratory diagnostic were performed directly or through other laboratories; the third part was aimed at describing the specific characteristics of TB diagnostic procedures performed (microscopy, culture, antibiotic-susceptibility testing, identification from culture, amplification methods); for each of these procedure, information were collected regarding the procedure itself, the timeliness of referring, the volume of activities, quality control programs and biosafety measures.

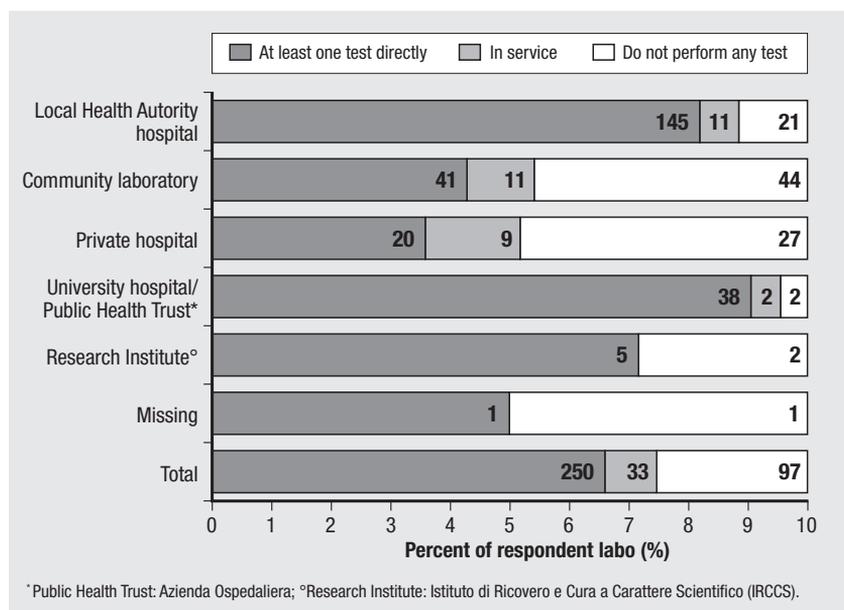
All the characteristics investigated were referred to the year 2006.

These five procedures were analysed according to the standard criteria reported in *Table 1*, based on existing European [10] and national recommendations [11].

**Table 1** | Characteristics of diagnostic tests of mycobacteriology considered as reference criteria for data analysis

Test	Methods	Referring time	Volume of activities	Biosafety
<b>Microscopy</b>	<ul style="list-style-type: none"> <li>- Ziehl-Neelsen staining, if &lt; 10 slides a day*</li> <li>- Fluorescent staining, if ≥ 10 slides a day*</li> <li>- Internal quality control of staining*</li> <li>- Standardized procedures*</li> </ul>	<ul style="list-style-type: none"> <li>- Within 24 h from sample receiving*</li> <li>- Positive results immediately communicated by phone*</li> </ul>	- 10 tests per week (40 for month)*	Laminar flow hood Biohazard Class I.* If effectuated also culture: Laminar flow hood Biohazard Class II and centrifuge with anti-aerosol protection
<b>Culture</b>	<ul style="list-style-type: none"> <li>- Association of solid medium with liquid medium*</li> <li>- Internal quality control of culture mediums*</li> <li>- External quality control*</li> </ul>	<ul style="list-style-type: none"> <li>- Solid mean: 30-40 days</li> <li>- Semi-automated radiometric system: 14-17 days</li> <li>- Liquid non radiometric medium: 10-15 days</li> </ul>	- 20 tests per week (80 for month)*	Laminar flow hood Biohazard Class II and centrifuge with anti-aerosol protection *
<b>Identification from culture</b>	<ul style="list-style-type: none"> <li>- Molecular tests or high performance liquid chromatography (HPLC) or traditional biochemical tests (solid mediums only)</li> <li>- Internal quality control*</li> <li>- External quality control*</li> </ul>	<ul style="list-style-type: none"> <li>- Identification from positive culture in 1-2 workdays</li> <li>- Culture, isolation and identification in 21 days from receiving of sputum (30 days for other pulmonary materials)</li> </ul>		Work area with containment level 3* Negative pressure room and filtered air by high efficiency filter*
<b>Antibiogram</b>	<ul style="list-style-type: none"> <li>- Method of proportions or method of absolute concentrations or method of resistance rate</li> <li>- Participation to internal quality control program of antibiogram*</li> <li>- Correct identification &gt; 90% samples in 2/3 rounds for isoniazid and rifampicin</li> </ul>	- Within 21-30 days from receiving of initial sample	- Test for second line drugs only in laboratories that perform at least 50 antibiotic-susceptibility test in new cases	See above

\*Characteristics considered in the analysis.



**Fig. 1** | Distribution of answering laboratories (number and percentage) and activities of mycobacterial diagnostics, by type of health care facility of affiliation.

According to the national recommendations [11] and the data reported by each laboratory, laboratories were classified as follows:

- level 1: laboratories which claimed to perform microscopy (independently from the number of slides examined per week), to have internal quality control procedures for the staining, standardized operating procedures and at least a Class I biological safety cabinet;
- level 2: laboratories with all the characteristics of level 1 which declared to perform cultures ( $\geq 80$  tests/month), having a Class II biological safety cabinet and centrifuges with aerosol containment devices, to be able to identify at least *Mycobacterium tuberculosis* complex (MTC), to perform antibiotic susceptibility tests for isoniazid, rifampicin, pyrazinamide and streptomycin or ethambutol, to participate to external quality control programs and to comply with structural biosafety requirements and human resource management;
- level 3: laboratories with all the characteristics of level 2, which declared to be able to identify all Mycobacteria (*Mycobacterium tuberculosis* and Mycobacteria other than tuberculosis – MOTT), to participate in international quality control programs and to have Class III biosafety containment system (level P3).

Laboratories, which did not satisfy requirements of level 1 or that were not classifiable, were considered of level 0.

Statistical analysis was performed using the SAS 9.1.3. software. To describe the variability of the number of mycobacteriology tests performed by type of laboratory, boxplot graphics were used. The spacings between the different parts of the box indicates the degree of dispersion (spread) and skewness in the data, and identify outliers.

## RESULTS

The questionnaire was sent to 537 laboratories of 10 Regions and has been returned by 380 of these (response rate 70.8%, inter-regional range 55-100%).

Among the 380 responding laboratories, 130 declared not to perform any mycobacterial laboratory diagnostic procedure. The remaining 250 laboratories (65.8%) performed at least one diagnostic procedure for mycobacteria (Figure 1), 4 of whom did not perform microscopy.

Fifty-eight percent of the 380 responding laboratories belonged to hospitals directly managed by Local Health Authorities (Aziende Sanitarie Locali), 17.2% by Hospital Trusts (Aziende Ospedaliere) or Research and Care Institutes (IRCCS), 8% by private hospitals and 16.4% was in the community. Seventy three percent of the laboratories had a public owner.

Microscopy was performed in 98% of the 250 laboratories, culture in 60%, identification from culture in 36%, amplification in 34%, antibiotic-susceptibility test in 28%, molecular tests in 20%, antibody test and cellular immunity in 12%, respectively.

Two hundred and forty-six laboratories (100%) answered to all the items included in the "Microscopy" section of the questionnaire, 133 laboratories out of 150 (89%) to all the "Culture" items, 81 laboratories out of 85 (95%) to all the "Amplification" items, 75 laboratories out of 91 (82%) to all the "Identification from culture" items and 58 laboratories out of 70 (83%) to all the "Antibiogram" items.

On average, standard criteria for each method were met in less than 50% of laboratories, with wide inter-regional variations (Table 2).

None of the criteria explored reached 90%. The expected volume of activity was rarely satisfied (1/3 of laboratories that performed microscopy reported less than 40 tests for month; similarly, 1/3 of labora-

tories that carried out cultures, reported less than 80 tests for month).

Seventeen percent of laboratories that performed cultures declared not to have a Class II biological safety cabinet and centrifuges with aerosol containment devices (this proportion was as high as 35% in one region); laboratories which declared to perform the identification of *Mycobacterium tuberculosis*, had a separated room in 3 cases out of 4 and a negative pressure room in 1 out of 3.

On average, internal and external quality control programs were present in less than half of investigated laboratories; some criteria, reflecting the quality of the procedures performed, were not always satisfied: only 6% of the laboratories processing more than 10 specimens per day used the fluorescent microscopy; only 70% of laboratories performing cultures used a combination of solid and liquid cultures.

Another critical point was the ability to provide the microscopy results within 24 hours (14% of laboratories only).

When laboratories were classified according to the declared characteristics, 170 laboratories (69%) did not even satisfied level 1 criteria; 65 laboratories satisfied criteria for level 1 (26%), 10 for level 2 (4%), only 1 laboratory those for level 3 (0.4%).

Among the 170 laboratories that did not satisfy even the criteria for level 1 despite performing mi-

croscopy, criteria not always satisfied were: internal quality control program (15.9% only performed internal quality control of the staining procedure and 4.7% internal quality control of culture media); standardized operating procedures (18.8%); availability of biological safety cabinet (81.8%). Ten among these 170 laboratories, performed more than 80 cultures in a month.

Among the 65 laboratories which satisfied the level 1 criteria, but not those for level 2, criteria not always satisfied were: internal quality control programs (53.8% of laboratories only declared to do internal quality control programs for culture media; 26.2% for antibiograms); Class II biological safety cabinet (90.8%); at least 80 cultures in a month (50.8%); availability of centrifuges with aerosol containment devices (55.4%); capability to identify *Mycobacterium complex* (60%). Among these 65 laboratories, 33 performed more than 80 cultures in a month.

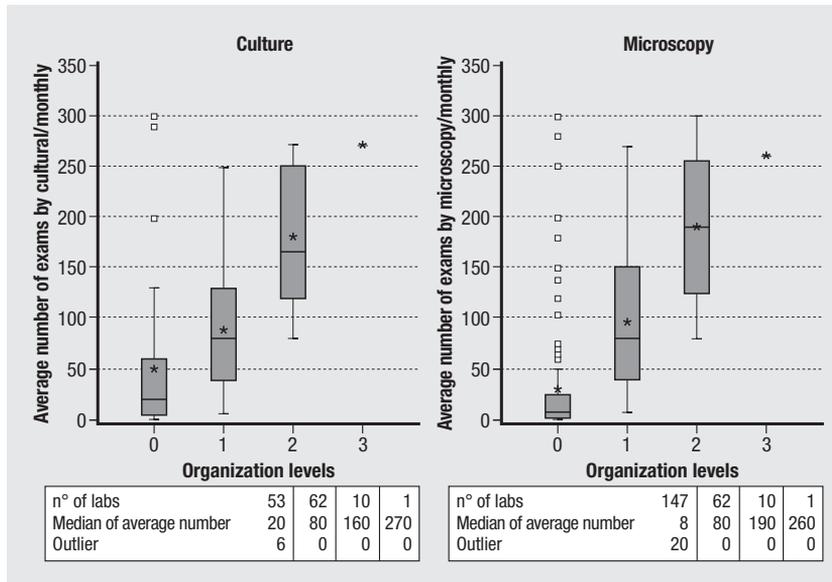
Among the 10 laboratories which satisfied the level 2 criteria, but not those for level 3, criteria not always satisfied were: participation to an external quality control program at international level (70% of laboratories) and availability of a negative pressure room (60% of laboratories).

The number of procedures, both for microscopy and culture (Figure 2), performed monthly by laboratories belonging to the same level was high-

**Table 2** | Proportion of laboratories that satisfy each reference criteria (global value and inter-regional range)

Test	Reference criteria	%	Inter-regional range (%)*
<b>Microscopy</b>		<b>(N = 246)</b>	
Methods	- Ziehl-Neelsen staining, if <10 slides/day	63	33-91
	- Fluorescent staining, if ≥10 slides/day	6	0-26
	- Internal quality control of staining	42	28-100
	- Standardized procedures	44	26-76
Referring time	- Within 24 h from sample receiving	14	0-23
	- Positive results immediately communicated by phone	78	57-100
Volume of activities	- 10 tests for week (40 for month)	32	13-71
Biosafety	- Laminar flow hood Biohazard Class 1	35	10-57
<b>Culture</b>		<b>(N = 133)</b>	
Methods	- Association of solid medium with liquid medium	70	57-100
	- Internal quality control of culture mediums	41	30-75
	- External quality control	52	18-100
Volume of activities	- 20 tests for week (80 for month)	36	
Biosafety	- Laminar flow hood Biohazard Class 2, centrifuge with anti-aerosol protection	83	65-100
<b>Identification</b>		<b>(N = 75)</b>	
Methods	- Internal quality control	51	38-100
	- External quality control	59	20-100
Biosafety	- Work area separated from other room	75	42-100
	- Negative pressure room and filtered air by high efficiency filters	35	0-78
<b>Antibiogram</b>		<b>(N = 58)</b>	
Methods	- Participation to internal quality control program of antibiograms	55	33-100

\*Excluded the P.A. of Trento where only one laboratory is present.



**Fig. 2** | Box-plot of the distributions of the number of tests for month by level of the laboratory.

ly variable, and this number significantly varied among laboratories of different levels. For example, the median number of cultures performed monthly in the 53 laboratories classified as belonging to level 0 was 20, but 25% of those performed more than 50 cultures/months (75° percentile) and there were 6 outliers processing up to 300 cultures per month.

Among the 133 laboratories that performed cultures, 41 (31%) declared more frequently than the others to process an high number of cultures (90% executed more than 80 cultural tests in a month), to perform internal and external quality control, to comply with biosafety measures, and to provide periodical training course to the staff.

## DISCUSSION

This survey, carried out in 10 Italian Regions, highlights that the organization and functioning of laboratories performing TB laboratory diagnostic procedures is significantly defective and that specific interventions are urgently needed.

The number of laboratories performing mycobacteriological procedures is clearly excessive compared to the relatively low number of notified TB cases; this is particularly true for second level procedures, such as culture, Mycobacteria identification and drug susceptibility testing (Table 2). Most of the activities are concentrated in few laboratories, while it is evident the dispersion of activities among a number of laboratories which do not process a sufficient number of tests per month, as recommended by international guidelines [12] to guarantee both an acceptable level of quality and an optimal use of the resources. The same problem has been reported in high prevalence areas [13], but it is more critical in Italy given that TB is a relatively rare disease. Due to the low incidence, the number of false positives results may be high, as described in other low-prevalence countries [14].

The most important quality indicators (performing quality control activities, carrying out training activities) were frequently lacking in several laboratories. Moreover, the infrequent use of fluorescent staining in laboratories performing >10 microscopy tests/week represents a critical issue compared to other surveys [15]. Other authors have underlined that, to reach and maintain good quality level in laboratories performing TB diagnostics, appropriate financial resources are needed [16].

In a high percentage of responding laboratories, minimal biosafety criteria were not satisfied. The Decreto Legislativo 81/2008 (*Testo Unico sulla Sicurezza e Salute sul Lavoro*) [17], as well as the previous DL.vo 626/94 [18], prescribes (*Titolo X*) and recommends (*Annex XLVIII*), containment measures when working with biological agents of group III, that should be applied in any laboratory where mycobacterial cultures are performed, regardless of the number of cultures or the type of procedures.

In addition to the excessive number of laboratories performing level 2 and 3 procedures, without satisfying the necessary standards, the survey showed that basic criteria for good laboratory practice, such as internal quality control programs, were frequently lacking. This was true both for basic laboratories and for more advanced ones. Several laboratories, classified as being level 0 could have been classified as level 1, if standard operating procedures and internal quality control programs were present. Similarly, some advanced laboratories, despite satisfying several standards, were not classifiable as reference laboratories because of the lacking of internal quality control programs.

A similar survey, conducted in 18 Regions in 1999-2001 by the Associazione Microbiologi Clinici Italiani (AMCLI), highlighted similar results [19]. As a consequence of that survey, specific indications were issued by the Ministry of Health. The present study, even if data are not immediately comparable because of dif-

ferent population included, did not show a significant improvement over time. Therefore, political commitment and specific interventions are necessary to improve the mycobacteriology laboratory network in Italy and to optimize the use of resources in this field.

A limit of this survey is the fact that only some regions were involved and that the response rate within the participating regions was not always optimal, even if overall it was quite high in comparison with other studies [19, 20]. The fact that all existing laboratories were not included could lead to an underestimation of the number of laboratories that perform mycobacteriological tests: as a consequence, the observed dispersion of tests in a myriad of different laboratories may be even bigger.

Despite the above-mentioned limits, the survey outlines that, to provide accurate and efficient TB diagnostics [21] at national level, it is necessary to promote the reduction of mycobacteriology laboratories performing second and third level tests and to adopt standardized operating procedures and internal and external quality control programs in all laboratories.

## CONCLUSIONS

The study highlights a substantial non compliance of laboratories to national and international recommendations for high quality mycobacterial diagnostics. This obviously affects both the quality of TB diagnostics and the accuracy of TB surveillance systems based on laboratory data. Increased govern-

mental and local efforts are needed to improve the status of TB diagnostic procedures.

## Acknowledgements

We really thank Claudio Piersimoni (Azienda Ospedale Umberto I, Ancona) for his precious suggestions for the analysis and presentation of data.

The survey was funded by CCM-Health Ministry (Referent: Stefania D'Amato).

## Conflict of interest statement

There are no potential conflict of interest of any financial or personal relationship with other people or organizations that could inappropriately bias conduct and findings of this study.

## Members of the Italian "TB-SORV"

### Project Working Group

Lorenzo Surace, Laura Pontoriero, Nisticò Salvatore (*Regione Calabria*); Renato Pizzuti, Bruno Sarnelli (*Regione Campania*); Guglielmo Pitzalis, Paolo Monte (*Regione Friuli-Venezia Giulia*); Anna Pavan, Regina Esposito (*Regione Lombardia*); Gualtiero Grilli, Enrica Carducci (*Regione Marche*); Sergio Rago, Roberto Patriarchi (*Regione Molise*); Massimiliano Bugiani, Dianella Bardelli, Lorenza Ferrara (*Regione Piemonte*); Rosa Prato, Anna Rita Fusco, Miragliotta Giuseppe (*Regione Puglia*); Donatella Campus, Giovanna Rossi (*Regione Sardegna*); Emanuela Balocchini (*Regione Toscana*); Augusto Dalpiaz, Dino Sella (*Provincia Autonoma Trento*); Anna Tosti (*Regione Umbria*); Angelo Zanello (*Regione Valle D'Aosta*); Antonio Ferro (*Regione Veneto*).

Received on 24 February 2010.

Accepted on 10 May 2010.

## References

1. European Centre for Disease Prevention and Control/WHO Regional Office for Europe. *Tuberculosis surveillance in Europe 2007*. Stockholm: European Centre for Disease Prevention and Control; 2009.
2. European Centre for Disease Prevention and Control. *Annual Epidemiological Report on Communicable Diseases in Europe 2009*. Stockholm: European Centre for Disease Prevention and Control. p. 43-47.
3. D'Amato S, Rizzato E, Virtuali L, Cenci C, Pompa MG. *Epidemiologia della tubercolosi in Italia (anni 1995-2007)*. Ufficio V Malattie Infettive e Profilassi Internazionale Dipartimento della Prevenzione e della Comunicazione – DG Prevenzione Sanitaria Ministero del Lavoro, della Salute e delle Politiche Sociali. Available from: [www.ministerosalute.it](http://www.ministerosalute.it).
4. Moro ML. Epidemiologia dell'infezione e della malattia tubercolare. In: Cassone A. *Tubercolosi. Epidemiologia, diagnosi e terapia*. Collana "I manuali" dell'Accademia Nazionale di Medicina. Genova: Forum Service Editore; 1998. p. 7-49.
5. Migliori GB, Loddenkemper R, Blasi F, Raviglione MC. 125 years after Robert Koch's discovery of the tubercle bacillus: the new XDR-TB threat. Is "science" enough to tackle the epidemic? *Eur Respir J* 2007;29(3):423-7. Available from: <http://erj.ersjournals.com/cgi/content/full/29/3/423>.
6. Migliori GB, De Iaco G, Besozzi G, Centis R, Cirillo DM. First tuberculosis cases in Italy resistant to all tested drugs. *Euro Surveill* 2007;12(20):pii=3194. Available from: [www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3194](http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3194).
7. Fattorini L, Migliori GB, Cassone A. Extensively drug-resistant (XDR) tuberculosis: an old and new threat. *Ann Ist Super Sanità* 2007;43(4):317-9.
8. Centers for Disease Control/National Institutes of Health. *Proposed guidelines for goals for working safely with Mycobacterium tuberculosis in clinical, public health and research laboratories*. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention; 1997.
9. Barenfanger J. Making your lab safe against multi-drug resistant *Mycobacterium tuberculosis*. *Clin Microbiol News* 1993; 15:76-80.
10. Drobniwski FA, Hoffner S, Rusch-Gerdes S, Skenders G, Thomsen V. Recommended standards for modern tuberculosis laboratory services in Europe. *Eur Respir J* 2006;28(5):903-9.
11. Italia. Ministero della Salute. Circolare del 26 febbraio 2004. *Manuale tecnico per la diagnosi microbiologica della tubercolosi*. Available from: [www.ministerosalute.it/imgs/C\\_17\\_publicazioni\\_614\\_allegato.pdf](http://www.ministerosalute.it/imgs/C_17_publicazioni_614_allegato.pdf).
12. American Thoracic Society. Level of laboratory services for mycobacterial diseases. *Am Rev Respir Dis* 1983;128:213.
13. DeRiemer K, Martins Moreira F, Werneck Barreto AM, Uelères Braga J. Survey of mycobacteriology laboratory practices in an urban area with hyperendemic pulmonary tuberculosis. *Int J Tuberc Lung Dis* 4(8):776-83.
14. De Boer AS, Blommerde B, de Haas PEW *et al*. False-positive *Mycobacterium tuberculosis* cultures in 44 laboratories in the Netherlands (1993-2000): incidence, risk factors, and consequences. *J Clin Microbiol* 2002;40:4004-9.

15. Tokars JI, Rudnick JR, Kroc K, et al. US Hospital mycobacteriology laboratories: status and comparison with State Public Health Department Laboratories. *J Clin Microbiol* 1996;34:680-5.
16. Denniston MM, Bird BR, Kelley KA. Contrast of survey results between state and a cohort of nonstate mycobacteriology laboratories: changes in laboratory practices. *J Clin Microbiol* 1997;35:422-6.
17. Italia. Decreto Legislativo 9 aprile 2008, n. 81 2008. Attuazione dell'articolo 1 della legge 3 agosto 2007, n. 123, in materia di tutela della salute e della sicurezza nei luoghi di lavoro. *Gazzetta Ufficiale – Serie Generale* n. 101, 30 aprile 2008.
18. Italia. Decreto Legislativo 19 settembre 1994, n° 626. Attuazione delle direttive 89/391/CEE, 89/654/CEE, 89/655/CEE, 89/656/CEE, 90/269 /CEE, 90/270/CEE, 90/394/CEE, 90/679/CEE, 93/88/CEE, 97/42/CEE e 1999/38/CE riguardanti il miglioramento della sicurezza e della salute dei lavoratori durante il lavoro. *Gazzetta Ufficiale* n. 265, 12 novembre 1994 (Suppl. ord. n. 141).
19. Piersimoni C, Mandler F, Marchetti D, Molinari GL, Riva R, Tortoli E, Tronci M, Scarparo C. Mycobacterial testing in hospital laboratories: results from a questionnaire survey in Italy. *Clin Microbiol Infect* 2004;10:1014-7.
20. Drobniowski FA, Watt B, Smith EG, et al. A national audit of the laboratory diagnosis of tuberculosis and other mycobacterial diseases within the United Kingdom. *J Clin Pathol* 1999;52:334-7.
21. Drobniowski FA, Nikolayevskyy V, Hoffner S, Pogoryelova O, Manissero D, Ozin AJ. The added value of a European Union tuberculosis reference laboratory network – analysis of the national reference laboratory activities. *Euro Surveill* 2008;13(12):pii=8076. Available from: [www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8076](http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8076) .