REVIEW

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False-Positive *Mycobacterium tuberculosis* Detection: Ways to Prevent Cross-Contamination

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The gold standard method for diagnosis of tuberculosis is the isolation of *Mycobacterium tuberculosis* through culture, but there is a probability of cross-contamination in simultaneous cultures of samples causing false-positives. This can result in delayed treatment of the underlying disease and drug side effects. In this paper, we reviewed studies on false-positive cultures of *M. tuberculosis*. Rate of occurrence, effective factors, and extent of false-positives were analyzed. Ways to identify and reduce the false-positives and management of them are critical for all laboratories. In most cases, false-positive is occurring in cases with only one positive culture but negative direct smear. The three most crucial factors in this regard are inappropriate technician function, contamination of reagents, and aerosol production. Thus, to reduce false-positives, good laboratory practice, as well as use of whole-genome sequencing or genotyping of all positive culture samples with a robust, extra pure method and rapid response, are essential for minimizing the rate of false-positives. Indeed, molecular approaches and epidemiological surveillance can provide a valuable tool besides culture to identify possible false positives.

Keywords: *Mycobacterium tuberculosis*; False-Positive Culture; Cross-Contamination; Genotyping

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Introduction

Despite global efforts to control tuberculosis, the number of patients in developing countries is high, and according to the World Health Organization, about 23% of people in the world have latent tuberculosis (TB) and about 10 millions of new cases of TB occurred in 2017 globally, which 16% of them died[1,2].

The control of disease will be possible and we can hope to reduce TB significantly, especially in developing countries, if timely diagnosis and proper drug treatment are made for TB. Planning to control TB requires recognition of infection, rapid diagnosis, and treatment of patients[3]. Despite increased

number of samples examined in diagnostic mycobacteriology laboratories, utilization of Lowenstein-Jensen solid culture medium and liquid culture medium are reduced due to molecular approaches include reverse transcription polymerase chain reaction, so duplicated simply, molecular approaches for diagnosis of TB[4]. However, contamination in conventional culture method is likely and may leading to false-positive results during the sampling of patients[5].

False-positive results in medical complications for patients which can cause significant costs for health care facilities[6]. There are no specific methods for the detection of contamination and different methods are used to reduce false-positives[7] which were not reliable methods until molecular methods were used to diagnose and confirm mycobacterial infection[8]. For a long time, the standard method for examining the contamination and transmission of tuberculosis was the IS6110 RFLP[9] which is done based on the number and genomic position of the IS6110 sequence, which the number of copies of the IS6110 sequence varies from one strain to another (0–25). Because it is not effective in strains with less than six copies of IS6110[10], the mycobacterial interspersed repetitive unit—variable number tandem repeat (MIRU-VNTR) and spoligotyping methods were replaced[11].

This review will consider possible ways of contamination and report of false-positive in laboratories with *Mycobacterium tuberculosis* studies and introduce the ways to prevent false-positive results.

The Rate of False-Positive Cultures

False-positive culture for *M. tuberculosis* is not low but a significant number are not recognized because laboratories usually do not have a continuous program to identify it and, contamination is suspected based on staff experience, unusual antibiotic susceptibility patterns and low colonies in a solid medium and sometimes genotyping[12]. However, the accuracy of diagnosis is increased with proper planning and accurate and complete genotyping, in addition to good laboratory practice, which is proposed as a suitable control method for mycobacterial laboratories[7]. In a meta-analysis study, the false-positive rate due to cross-contamination was 2% in 31 studies that examined a total of 29,839 cultures, and in those with one positive TB culture, the false-positive rate was 15% and those patients who had an initial diagnosis of TB and started treatment for them, had a false-positive rate of 9.2% (91/990)[13]. In the studied articles, the minimum contamination rate was 0.3%[14] and the maximum contamination rate was 18.2%[7]. In general, the contamination rate was low in the studies in which the samples number are high. For example, in the Ruddy et al.'s study[15] in London, the contamination rate was 0.54% in 2,042 samples, or in the Globan et al.'s study[16] in Victoria, Australia, the contamination rate was 0.7% in 2,298 samples whereas the contamination rate was high in the studies in which the samples number were low. For example, the contamination rate was 13.4% in the Thumamo et al.'s study[17] in Nigeria on 112 samples. It appears that the sources of contaminants are diverse, and contaminants can be introduced by a lack of equipped and specialized labs and highly skilled staff in the laboratories that the number of samples is low, can be considered as risk factors. Usually, the sample numbers are high in advanced and well-equipped centers where conditions are favorable for testing and pollution is low, and it can be concluded that coherent planning, the experience of staff and facilities are effective in preventing contamination.

The Importance of False-Positive Results

False-positive results are important in several ways. Firstly, it delays the diagnosis of real disease and can sometimes be life-threatening by the procedure of treatment. By the previous studies, the drug was administered in 91+1 cases[6,15,18-32] with studies from the highest number of 34[6], 17[31], and 8[26] cases, to 8 cases of patient deaths[24,29-31] which two patients of them face toxic drug side effects[29,30]. Secondly, incorrect diagnosis of tuberculosis leads to hospitalization, requesting laboratory tests, radiography, nursing fees, tuberculosis rooms cleaning and non-medical expenses[6]. Third, severe psychological and social pressures are placed on the patient and the patient's family, losing their working days and making the patients poorer. Fourth, it shows the prevalence of tuberculosis falsely high in that area. Fifth, it is not possible to properly interpret the results of epidemiological studies that clustering among immigrants and indigenous people in countries such as Spain[7], Iran[33], Norway[34], and France[28] is not properly estimated. Sixth, the recent transfer rate is reported a lot, because the rate of recent transfer is determined from the number of samples in the cluster and the following formula[35].

$$\frac{\text{Number of clustered patients–Number of clusters}}{\text{Total number of patients}}$$

False-positive results in a larger number of clustered samples, resulting in a more recent incorrect estimation[25]. As in the study of Asgharzadeh et al.[19], intra-cluster samples decreased from 36 cases to 32 cases (23.1% to 20.8%) and recent TB transmission decreased from 13.5% (36-15/156) to 12.3% (32-13/154) with false-positive detection. Also in the study of Martinez et al.[7], intra-cluster samples were reduced from 69 to 32 (44.8% to 25.4%) with false-positives detection and the recent transmission rate decreased from 31.8% (69-20/154) to 16.7% (32-11/126). Therefore, epidemiological studies of tuberculosis should identify the actual number of cases of tuberculosis by identifying cases of contamination so as not to cause a false increase in recent TB transmission. Therefore,

molecular typing is required in isolated samples of *M. tuberculosis* to indicate the actual amount of recent transmission, and further investigation should be performed to reject the false-positive probability when the samples are placed in similar clusters in genotyping and there is no specific epidemiological link between patients[29].

Causes of False-Positive Cultures

Completely false-positive elimination is one of the goals of mycobacteriology laboratories, but various factors cause false-positive in culture (Table 1) which, the most prominent of them is the mistake of laboratory technician[31], contamination of reagents[23,25,26] and aerosol production[6,36], and it should be noted that aerosols containing live *M. tuberculosis*, obtained during the removal of live germs, can survive for a long time under very harsh environmental conditions, and play a major role in contaminating solutions and reagents and devices such as pipettes, caps, and utensil that play an important role in causing cross-contamination in small rooms[37].

Clinical devices such as bronchoscopy are among the factors contributing to the false-positive that can contribute to the transmission of infection to patients too[38]. The risk is doubled when the patient infected with a drug-resistant strain. In addition to the above mentioned, stimulating the sputum and use of nebulizer, the existence of plenty of equipment inside the hood that does not allow UV to shines on all the equipments, lack of professional staff and low experience of staff, and especially work pressure, can increase contamination. In Iran, even if technicians are trained and interested in work, which is usually not the case, people remain in TB centers just for having jobs. The work environment is not suitable for most employees because of the low benefits, inadequate fees, the difficulty of work and the probability of being infected. Therefore the above factors are effective in the contamination. Clerical errors, such as mislabeling, can also lead to false-positives[26,30,39].

The Identification Methods of False-Positive

Attention to false-positive and accurate interpretation of the results is needed and genotyping is necessary when the sample is smear-negative and the clinical symptoms are inconsistent with the culture result or the patient has nonspecific symptoms commonly seen in immunocompromised patients[40]. Infected specimens are identified and sources of contamination are tracked by genotyping. Genotyping is also a very useful method for detecting reinfection from relapse[41]. Genotyping is one of the most suitable methods for false-positive detection. There are several genotyping methods in

Table 1. Common risk factors for false-positive detection of tuberculosis

No.	Risk factor	Rate of possibility	Way to prevent
1	Contamination of reagents	***	Single-use material, dispensed reagents, staff training
2	Improper work of technicians	***	Staff training
3	Creation of aerosols	**	Material quality, centrifuge cap, Staff training
4	Mislabeling	***	Double checked labeling Automated label systems
5	Contaminated bronchoscope	****	Proper decontamination, programmed cleaning, Staff training
6	Poor laboratory techniques	***	Improved standard and use of advanced techniques
7	Contaminated equipment	***	Daily cleaning and check list for cleaning possible equipments
8	Splashing	****	Staff training for safe preparation
9	Reprocessing of contaminated specimens	***	Single-use materials Standard methods Staff training
10	BACTEC needle carryover	**	Single-use specific needle
11	Unsuitable safety cabinet	**	Check filters Use of standard cabinet Check for aerosols inside cabinets Annual inspection
12	Use of 70% alcohol for decontamination of laboratory equipment	*	Use of proper anti-tuberculosis reagents
13	Small room for processing of the sample	**	Use of proper spaces for processing

*: rare; **: sometimes; ***: mostly; ****: very often.

which the appropriate identifying method is IS6110-RFLP which needs high amounts of DNA and it is difficult to compare the results of different laboratories with this method (Figure 1). Another method is spoligotyping, which is a rapid method that requires little amounts of DNA and can be performed on clinical specimens and cultured strains[42] and well identifies Beijing strains lacking 1–34 bp spacer but the identifying strength of it is low[43] and so it is not reliable for cross-contamination testing. Another method is MIRU-VNTR typing that from 41 MIRU loci, 12, 15, or 24 loci are used for epidemiological studies and is a well-automated and good method for studying the genetic diversity of strains[44]. The power is significantly increased and can be considered as the standard method when the MIRU-VNTR method is used in conjunction with spoligotyping[39]. Other genotyping methods are not a priority due to some bugs. However, these methods cannot distinguish between the recent transmission in the host country from the new importation from the origin country[45]. So it would be ideal if the whole genome sequencing (WGS) method is used[46]. The WGS method enables the identification of strains, false-positives, and accurate epidemiological data preparation[47] and can also identify drug-resistant strains rapidly[46]. Therefore, it can be very useful in reducing TB cases significantly. WGS has the potential to revolutionize the diagnosis of *M. tuberculosis* infection. However, the utility of WGS is currently limited due to the major drawbacks of sequencing, such as the costs associated with the test, the technical skill required, complex bioinformatic procedures and the unavailability of sequencing facilities. There are currently no plans for routine implementation of WGS in resource-limited, high-TB burden countries.

Ways to Reduce Contamination

Various factors appear to be involved in contamination in several studies that are influenced by laboratory planning, adequate experience, accuracy and interest of staff, facilities and methods used in the laboratory. Therefore, changes in *in-vitro* methods and techniques and long-term epidemiological surveillance in the laboratory are essential in cases where contamination is high. To reduce contamination, it is recommended that (1) the solutions used for decontamination and digestion should be prepared specially for each sample as aliquot, and each aliquot should be used for only one sample and avoid common containers. (2) Attention to good laboratory practice in the mycobacteriology laboratory and periodically provide necessary training. (3) Provide a suitable questionnaire for each sample entered into the laboratory, including the time of preparation, sample entry, and testing, patient demographics, history of the disease, clinical symptoms of the patient and the physician requesting the test, to contact the doctor easily in cases of suspected infection. (4) Do not opening the lids of the tubes for five minutes after mixing or centrifuging the tubes so that the aerosol particles containing bacteria do not spread out of the tube and do not transfer to containers and other tubes. (5) At least one negative-control sample should be processed with samples every working day. (6) Only the microscopic examination of acid-fast bacilli should be carried out in satellite laboratories and they must be sent to the reference laboratories for culture. (7) Planned external quality control is necessary to reduce errors in different countries, especially in countries where the prevalence of TB is not low. It should also be noted that there are many

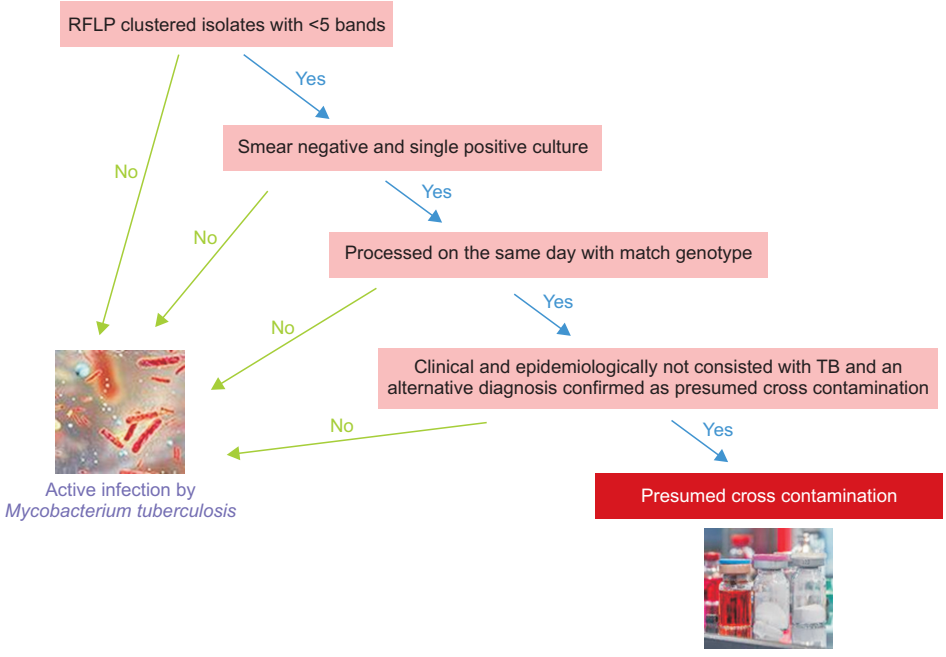

Figure 1. Molecular typing (RFLP) based method for identification of false-positive *Mycobacterium tuberculosis* in molecular laboratories. This algorithm defines a way to distinguish correct identification of positive cases from false-positive cases. TB: tuberculosis.

false-negatives in TB centers that cause delays in treatment, endangering patient's live, increasing mortality, and increasing other people's infections[48]. (8) Using whole-genome sequencing techniques on positive or at least samples that are smear-negative, and only have a positive culture, otherwise, the genotyping test should be used at least with eight loci MIRU-VNTR 10, 26, 31, 40, QUB11b, Mtub21, QUB-3232, and QUB-26 for rapid diagnosis of infections. Nowadays, polymerase chain reaction is widely used to identify *M. tuberculosis*, especially in extra-pulmonary tuberculosis[49,50]. The polymerase chain reaction (PCR) method is highly sensitive but false-positive results are probably due to the contamination of it[51]. So it is necessary to aliquot the raw material, frequent washing of the rooms, especially the electrophoresis room, using of ventilators and UV light, autoclaving materials such as sampler, microtube, and deionized water, separate performing of DNA extraction (in the pre-PCR room), PCR (PCR part) and Electrophoresis (in the post-PCR room) and no transfering of the equipment and materials, especially from the post-PCR room to the PCR and pre-PCR room, and using of negative controls in each experiment in nucleic acid proliferation-dependent methods to avoiding contamination in the PCR, which can reduce false-positives.

Conclusion

It can be concluded that relies on laboratory results is increases due to the increasing number of immunocompromised patients in the world who exhibit nonspecific symptoms of tuberculosis but false-positive cases are still high. Therefore, to prevent inappropriate treatment with toxic drugs and to prevent impose a cost on patients, identifying false-positives is essential which requires close collaboration between technicians and laboratories. Whole-genome sequencing or genotyping with high identifying power and rapid response to positive culture samples is essential to minimize false-positives definitive identification and prolonged epidemiological surveillance after necessary laboratory changes.

Authors' Contributions

Conceptualization: Asgharzadeh M, Kafil HS. Methodology: Asgharzadeh M, Kafil HS. Formal analysis: Ozma MA, Rashedi J, Poor BM, Agharzadeh V. Data curation: Asgharzadeh M, Ozma MA, Rashedi J, Poor BM, Agharzadeh V, Vegari A, Shokouhi B, Ganbarov K, Ghalehlou NN, Kafil HS. Validation: Asgharzadeh M, Kafil HS. Investigation: Asgharzadeh M, Kafil HS. Writing - original draft preparation: Asgharzadeh M, Kafil HS. Writing - review and editing: all authors. Approval of final manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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