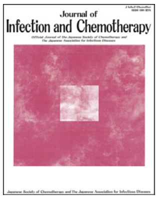
J Infect Chemother 25 (2019) 610–614

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic



Original Article

A case of *Mycobacterium tuberculosis* laboratory cross-contamination[☆]

Keita Takeda [a, b, e, *], Yoshiro Murase [a], Masahiro Kawashima [b], Maho Suzukawa [c], Junko Suzuki [b], Akira Yamane [b], Yuriko Igarashi [a], Kinuyo Chikamatsu [a], Yuta Morishige [a], Akio Aono [a], Hiroyuki Yamada [a], Akiko Takaki [a], Atsuhisa Tamura [b], Hideaki Nagai [b], Hirotoshi Matsui [b], Shigeto Tohma [d], Satoshi Mitarai [a, e]

[a] *Department of Mycobacterium Reference and Research, The Research Institute of Tuberculosis, Japan*
[b] *Center for Pulmonary Diseases, National Hospital Organization Tokyo National Hospital, Japan*
[c] *Clinical Research Center, National Hospital Organization Tokyo National Hospital, Japan*
[d] *Asthma, Allergy and Rheumatology Center, National Hospital Organization Tokyo National Hospital, Japan*
[e] *Department of Basic Mycobacteriology, Graduate School of Biomedical Science, Nagasaki University, Japan*

ARTICLE INFO

Article history:
Received 1 February 2019
Received in revised form
8 March 2019
Accepted 13 March 2019
Available online 11 April 2019

Keywords:
Variable number of tandem repeat
False-positive results
Mycobacterium tuberculosis

ABSTRACT

Setting: A laboratory cross-contamination event was suspected because *Mycobacterium tuberculosis* was unexpectedly detected at a high incidence in the cultures of several clinical specimens at the National Hospital Organization, Tokyo National Hospital, Japan.
Objective: To describe a case of *Mycobacterium tuberculosis* laboratory cross-contamination.
Design: We reviewed the medical records of 20 patients whose clinical specimens were suspected to have been contaminated by *Mycobacterium tuberculosis*. Variable number of tandem repeat analysis with 15 loci, the Japan Anti-Tuberculosis Association-12, and three additional hyper-variable loci, was performed to identify the cross-contamination event.
Results: The clinical, laboratory, and variable number of tandem repeat data revealed that the cross-contamination had possibly originated from one strongly positive specimen, resulting in false-positive results in 11 other specimens, including a case treated with anti-tuberculosis drugs.
Conclusion: Clinical and laboratory data must be re-evaluated when cross-contamination is suspected and variable number of tandem repeat analysis should be used to confirm cross-contamination. Furthermore, original isolates should be stored appropriately, without sub-culturing and genotyping should be performed at the earliest possible for better utilization of variable number of tandem repeat for the identification of cross-contamination.

© 2019 Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Isolation of *Mycobacterium tuberculosis* (MTB) is necessary for a definite diagnosis of active tuberculosis. However, laboratory cross-contamination is relatively common and the false-positive rate of MTB cultures has been reported to be as high as 2–4% [1–8], mainly during batch processing of large numbers of specimens containing one smear-positive sample [5–8]. Therefore, the possibility of a false-positive result should always be considered when the clinical manifestations do not match the disease. When cross-contamination is suspected, it is important to immediately confirm the event. However, the identification of cross-contamination is not a simple matter, especially in ordinary clinical laboratories.

Abbreviations: MTB, *Mycobacterium* tuberculosis; VNTR, variable number of tandem repeat; RFLP, restriction fragment length polymorphism; AFB, acid-fast bacillus; HV, hyper-variable; NALC-NaOH, *N*-acetyl-L-cysteine-sodium hydroxide.

[☆] All authors meet the ICMJE authorship criteria.

[*] Corresponding author. Center for Pulmonary Diseases, National Hospital Organization Tokyo National Hospital, 3-1-1 Takeoka, Kiyose, Tokyo, 204-8585, Japan.

E-mail addresses: takedak@tokyo-hosp.jp (K. Takeda), ymurase@jata.or.jp (Y. Murase), mkawashima-in@tokyo-hosp.jp (M. Kawashima), suzukawam@tokyo-hosp.jp (M. Suzukawa), junkosz-in@tokyo-hosp.jp (J. Suzuki), yamanea@tokyo-hosp.jp (A. Yamane), igarashi@jata.or.jp (Y. Igarashi), chikamatsu@jata.or.jp (K. Chikamatsu), ymorishige@jata.or.jp (Y. Morishige), a.aono1967@gmail.com (A. Aono), hyamada@jata.or.jp (H. Yamada), takaki@jata.or.jp (A. Takaki), tamura-in@tokyo-hosp.jp (A. Tamura), hnagai-tokyohosp@umin.ac.jp (H. Nagai), matsui.hirotoshi.sa@mail.hosp.go.jp (H. Matsui), touma-shigeto@tokyo-hosp.jp (S. Tohma), mitarai@jata.or.jp (S. Mitarai).

https://doi.org/10.1016/j.jiac.2019.03.012
1341-321X/© 2019 Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

Genotyping is useful for identifying cross-contamination [1–13]. One such method, variable number of tandem repeat (VNTR), is reported to be highly reproducible and discriminative, similar to IS*6110* restriction fragment length polymorphism (RFLP) [9–11]. VNTR analyzes the size of several mini-satellite loci in the MTB genome using the polymerase chain reaction (PCR) and nucleic acid electrophoresis system without the need for highly complicated techniques [12–14] or high amounts of DNA, as required for RFLP [15]. VNTR analysis is often used for the identification of MTB cross-contamination [4,7,15–17].

A laboratory cross-contamination event was suspected on February 3rd, 2016, at the National Hospital Organization (NHO), Tokyo National Hospital, which is a tertiary hospital for tuberculosis, because MTB was unexpectedly detected in the cultures of several specimens; humidifier water obtained from the house of a patient with hypersensitivity pneumonia, as well as sputa from patients with bronchial asthma, aspiration pneumonia, and bronchiectasis. During a single day, MTB was detected in 20 of 45 (44.4%) specimens of acid-fast bacillus (AFB) cultures, representing 83.3% (20/24) of isolated mycobacteria. The mycobacterial laboratory at the hospital processes approximately 11,000 to 12,000 AFB specimens by culture examination each year, of which approximately 9–11% are positive for mycobacteria and approximately 35–40% of the isolates in these positive cultures are MTB. This article reports a case of *Mycobacterium tuberculosis* laboratory cross-contamination.

2. Materials and methods

2.1. Clinical data

The medical records of 20 patients with positive MTB cultures from the time of the possible cross-contamination event were retrospectively reviewed. We analyzed the clinical course/manifestation and the laboratory data including specimen type, smear results, and interferon-gamma release assay. “No.” was used to designate patient number. The Institutional Review Board of NHO Tokyo National Hospital (approval date: May 30th, 2018; approval number: 180,015) approved this retrospective study and there was no requirement for written informed consent.

2.2. Bacteriological procedures

Forty-five clinical specimens, including sputum, gastric aspirate and fecal specimens, pleural effusions, and humidifier water, were handled in a single bio-safety cabinet on the day. A maximum of 20 specimens were simultaneously processed. For the sputum and gastric aspirate specimens, a pre-treatment procedure with a semi-alkaline protease (Sputazyme, Kyokuto Pharmaceuticals, Tokyo, Japan) [18] was performed. The fecal specimens were suspended and vortexed in sterile distilled water and the supernatant was then used for further examination. All specimens were further decontaminated using *N*-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH), followed by neutralization with phosphate buffer (PB, pH 6.8) and centrifugation (3000 *g*, 15 min at 4 °C). Next, the sediments were re-suspended at a 5:1 PB:sediment ratio and used to inoculate liquid medium (mycobacterium growth indicator tube; MGIT, Becton Dickinson, Sparks, MD, USA) or solid medium (2% Ogawa medium) for culturing at 37 °C for 6–8 weeks, respectively.

2.3. *Mycobacterium tuberculosis* isolates

In total, 24 MTB isolates were collected and examined. Nineteen MTB isolates were collected from sputum, pleural effusion, feces, and humidifier water specimens from patients treated during a single day. Unfortunately, one MTB isolate obtained from patient No. 19 became unculturable prior to VNTR analysis. Additional isolates were collected on the following days from three tuberculosis patients (No. 8, 10, and 11) to assess whether the VNTR profiles matched those obtained on the day of the possible cross-contamination event. MTB isolates could not be collected from tuberculosis patient No. 12 during the following days. The remaining two isolates were collected the next day to assess possible extended cross-contamination. The specimens were grown on 2% Ogawa medium and sub-cultured twice for 1.5 years until VNTR analysis. “#” was used to designate specimen number.

2.4. DNA preparation

One loopful of cells (approximately 10 μL) harvested from cultured colonies grown on 2% Ogawa medium was suspended in 400 μL of Tris/EDTA buffer (pH 8.0) and the bacteria were then heat-killed in boiling water for 10 min. Following centrifugation (18,000 g, 5 min at 4 °C), the supernatant was used for VNTR analysis.

2.5. VNTR analysis

VNTR analysis were performed for 15 loci; JATA-12 [19] and three additional hyper-variable (HV) loci (3232, 3820, and 4120) [20]. PCR analysis was performed for all loci and the sizes of the amplified DNA fragments were estimated according to previously described conditions [19]. Two isolates were deemed to be the same when their VNTR profiles matched completely or differed in only one locus.

3. Results

Twenty cases of possible cross-contamination were identified based on the clinical, laboratory, and VNTR data (Table 1). The VNTR profiles demonstrated very similar results for 11 specimens (#8–18) on the day of the possible cross-contamination. The VNTR profiles of the other two specimens from the second day were distinctly different.

VNTR analysis revealed differences in two loci among similar VNTR profiles (#8–18). For the MIRU 26 locus, two bands were present at a size of seven and eight copies in three of the 11 isolates (#8–10). For VNTR 3232, a difference in copy number (12 or 14) was observed for one HV locus.

Based on the clinical and laboratory data, seven cases (No. 9, 13–18) with similar MTB VNTR profiles were inconsistent with tuberculosis. In four other cases (No. 8, 10–12) the VNTR profiles of MTB isolated from the specimens were consistent with tuberculosis.

Specimen #8 was considered as the index as this specimen was strongly AFB-positive (3+) and was the first suspected cross-contamination specimen handled.

The VNTR profile of patient No. 8, completely matched that of a subsequent specimen obtained from the same patient. In contrast, the VNTR profiles of specimens obtained on different days from both patient No. 10 and No. 11 did not match (>5/15 loci; Table 1).

Two cases with discordant clinical and VNTR data were observed. Patient No. 9 was first diagnosed as having pulmonary empyema and was treated with ampicillin/sulbactam, which improved the clinical findings. However, the patient was subsequently re-diagnosed as having concomitant pleuritis caused by an uncultured bacterium and MTB, based on a single MTB isolate identified at that time. The VNTR profile of this MTB isolate was the same as that of the cross-contamination specimen; thus, patient No. 9 was thought to have been misdiagnosed as tuberculosis.

Table 1
Clinical, laboratory, and VNTR data of suspected cross-contamination cases.

Patient Number (No.)	Specimen Number (#)	Process date	Specimen type	Smear result	MTB-positive culture on different days	IGRAs	Final diagnosis	JATA-12												HV		
								0424	MIRU 10	1955	2074	2163b	2372	MIRU 26	QUB15 (3155)	MIRU 31	3336	QUB26 (4052)	4156	3232	3820	4120
1	1_1	03/02/2016	sputum	2+	+	T-SPOT (−)	Pulmonary tuberculosis	3	2	3	2	3	2	5	6	3	12	8	3	14	5	4
2	2	03/02/2016	sputum	−	+	T-SPOT (+)	Pulmonary tuberculosis	2	4	3	3	7	3	7	4	5	7	8	5	15	12	5
3	3	03/02/2016	sputum	2+	+	T-SPOT (−)	Pulmonary tuberculosis	1	4	2	3	7	4	7	4	5	7	8	5	17	14	12
4	4	03/02/2016	sputum	−	+	T-SPOT (+)	Pulmonary tuberculosis	1	4	2	3	7	4	7	4	5	7	8	5	16	14	11
5	5s	03/02/2016	sputum	3+	+	ND	Pulmonary tuberculosis	3	2	3	1	4	2	5	4	4	10	3	3	5	5	2
5	5f	03/02/2016	fecal specimens	2+	+	ND	Pulmonary tuberculosis	3	2	3	1	4	2	5	4	4	10	3	3	5	5	2
6	6	03/02/2016	sputum	2+	+	ND	Pulmonary tuberculosis	3	4	3	4	8	3	7	4	5	7	8	3	14	14	9
7	7	03/02/2016	sputum	−	+	T-SPOT (+)	Pulmonary tuberculosis	3	2	3	2	2	5	5	5	2	6	2	2	5	6	4
8	8	03/02/2016	sputum	3+	+	ND	Pulmonary tuberculosis	4	3	4	3	2	3	7/8[a]	4	5	7	8	4	12	12	10
9	9	03/02/2016	pleural effusion	−	−	T-SPOT (−)/QFT-3G (−)	Empyema + Tuberculous pleuritis misdiagnosed	4	3	4	3	2	3	7/8[a]	4	5	7	8	4	12	12	10
10	10	03/02/2016	pleural effusion	−	+	T-SPOT (+)	Tuberculous pleuritis	4	3	4	3	2	3	7/8[a]	4	5	7	8	4	12	12	10
11	11	03/02/2016	sputum	−	+	T-SPOT (+)	Pulmonary tuberculosis	4	3	4	3	2	3	7[b]	4	5	7	8	4	12	12	10
12	12	03/02/2016	sputum	−	+	T-SPOT (+)	Pulmonary tuberculosis	4	3	4	3	2	3	7[b]	4	5	7	8	4	12	12	10
13	13	03/02/2016	sputum	−	−	ND	Bronchial Asthma	4	3	4	3	2	3	7[b]	4	5	7	8	4	12	12	10
14	14	03/02/2016	sputum	−	−	T-SPOT (+)	Chronic pulmonary aspergillosis + Old pulmonary tuberculosis	4	3	4	3	2	3	7[b]	4	5	7	8	4	12	12	10
15	15	03/02/2016	sputum	−	−	T-SPOT (−)/QFT-3G (−)	Bacterial pneumonia	4	3	4	3	2	3	7[b]	4	5	7	8	4	12	12	10
16	16	03/02/2016	humidifier water	−	−	T-SPOT (+)	Hypersensitive pneumonia	4	3	4	3	2	3	7[b]	4	5	7	8	4	12	12	10
17	17	03/02/2016	sputum	−	−	T-SPOT (−)	Bronchiectasis	4	3	4	3	2	3	7[b]	4	5	7	8	4	14[b]	12	10
18	18	03/02/2016	sputum	−	−	T-SPOT (−)	Bronchiectasis	4	3	4	3	2	3	7[b]	4	5	7	8	4	14[b]	12	10
19	−	03/02/2016	sputum	−	−	T-SPOT (−)	Aspiration pneumonia	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	20	04/02/2016	sputum	−	+	T-SPOT (+)	Pulmonary tuberculosis	2	2	3	1	4	2	5	4	3	17	5	3	5	5	2
1	1_2	04/02/2016	sputum	2+	+	T-SPOT (−)	Pulmonary tuberculosis	2	2	3	2	3	2	5	6	3	12	8	3	14	5	4
8	8_2	04/02/2016	sputum	3+	+	ND	Pulmonary tuberculosis	4	3	4	3	2	3	7/8[a]	4	5	7	8	4	12	12	10
10	10_2	05/02/2016	pleural biopsy specimen	−	+	T-SPOT (+)	Tuberculous pleuritis	4	3	3	3	3	3	7	4	4	1	9	4	12	14	12
11	11_2	02/02/2016	sputum	−	+	T-SPOT (+)	Pulmonary tuberculosis	4	1	4	2	6	4	7	4	5	7	7	5	17	13	12

MTB: *Mycobacterium tuberculosis*
JATA: the Japan Anti-Tuberculosis Association.
HV: hyper-variable.
IGRAs: Interferon-Gamma Release Assays.
T-SPOT: T-SPOT TB.
QFT-3G: QuantiFERON TB Gold In Tube.
ND: not conducted.

[a] Two bands were present corresponding to a size of seven and eight copies.

[b] the rectangles in the MIRU26 and 3232 locus columns indicate differences between specimens with similar VNTR profiles.

Based on the clinical course and on the evidence of cross-contamination in other samples, it was concluded that patient No. 19 was also false-positive for MTB, although VNTR analysis was not performed.

4. Discussion

This report describes a suspected laboratory cross-contamination event deduced based on an unexpectedly high number of MTB-positive cultures in a single day. Clinical, laboratory, and VNTR data indicated that the cross-contaminations occurred during the processing of specimen #8, which may have contaminated the following 11 specimens (#9–18 and the subsequent specimen obtained from patient No. 19).

MTB genotyping should be examined as soon as possible in order to prevent unnecessary medical interventions [8,21]; although a prompt examination was not performed in the present case. In fact, patient No. 9 was misdiagnosed as having tuberculosis and therefore unnecessarily treated with anti-tuberculosis drugs. The present case emphasizes an important message: clinical data must be re-evaluated and VNTR analysis should be performed to identify cross-contamination.

Based on the VNTR results, specimen #8 also affected the following specimens of true tuberculosis patients (No. 10–12). As these specimens were smear-negative, it was difficult to assess whether MTB was present in the specimens or whether contaminated MTB had been cultured.

The VNTR profiles of MTB isolates considered as cross-contamination demonstrated differences in two loci. For the MIRU 26 locus, two bands were present in only three isolates, suggesting the presence of clonal variants [21] and that one of the variants had contaminated the other specimens. A difference in copy number was observed for the VNTR 3232 locus; it is possible that mutations occurred during the sub-culturing step, as HV loci have been reported to be more evolutionary than standard loci [20].

Original isolates should be stored appropriately to assess cross-contamination events. In the present case, VNTR analysis could be performed because nearly all of the isolates had been stored and sub-cultured for 1.5 years. However, repeated sub-culturing may cause changes in the VNTR profile. Thus, it is recommended that the original isolates from the specimens are stored without sub-culturing, preferably at −80 °C.

In the present case, MTB contamination had most likely occurred in the reagents (i.e., the semi-alkaline protease, the NALC-NaOH solution, or PB) during batch processing via a contaminated lid or pipette [5,22,23]. Cross-contamination occurred after the processing of specimen #8 on a certain day and ended within a day and these reagents are changed daily. The maximum number of specimens simultaneously handled at the NHO Tokyo National Hospital was reduced to 15 following this event because one of the factors contributing to cross-contamination is a large batch number [5–8].

In conclusion, clinical and laboratory data must be re-evaluated when cross-contamination is suspected and VNTR analysis should be applied to identify cross-contamination. We further recommend that in cases of suspected cross-contamination, the MTB isolates should be stored appropriately; original isolates should be stored without sub-culturing and genotyping should be immediately performed for better utilization of the VNTR results.

Conflicts of interest

None to declare.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

The authors wish to thank the Medical Microbiology Laboratory Team at NHO Tokyo National Hospital for specimen processing.

References

[1] de Boer AS, Blommerde B, de Haas PE, Sebek MM, Lambregts-van Weezenbeek KS, Dessens M, et al. False-positive mycobacterium tuberculosis cultures in 44 laboratories in The Netherlands (1993 to 2000): incidence, risk factors, and consequences. J Clin Microbiol 2002;40:4004–9.

[2] Jasmer RM, Roemer M, Hamilton J, Bunter J, Braden CR, Shinnick TM, et al. A prospective, multicenter study of laboratory cross-contamination of *Mycobacterium tuberculosis* cultures. Emerg Infect Dis 2002;8:1260–3.

[3] Centers for Disease Control and Prevention (CDC). Multiple misdiagnoses of tuberculosis resulting from laboratory error–Wisconsin, 1996. MMWR Morb Mortal Wkly Rep 1997;46:797–801.

[4] Lai CC, Tan CK, Lin SH, Liao CH, Chou CH, Huang YT, et al. Molecular evidence of false-positive cultures for *Mycobacterium tuberculosis* in a Taiwanese hospital with a high incidence of TB. Chest 2010;137:1065–70. https://doi.org/10.1378/chest.09-1878.

[5] Burman WJ, Reves RR. Review of false-positive cultures for *Mycobacterium tuberculosis* and recommendations for avoiding unnecessary treatment. Clin Infect Dis 2000;31:1390–5.

[6] Ruddy M, McHugh TD, Dale JW, Banerjee D, Maguire H, Wilson P, et al. Estimation of the rate of unrecognized cross-contamination with *Mycobacterium tuberculosis* in London microbiology laboratories. J Clin Microbiol 2002;40:4100–4.

[7] Burman WJ, Stone BL, Reves RR, Wilson ML, Yang Z, El-Hajj H, et al. The incidence of false-positive cultures for *Mycobacterium tuberculosis*. Am J Respir Crit Care Med 1997;155:321–6.

[8] Wurtz R, Demarais P, Trainor W, McAuley J, Kocka F, Mosher L, et al. Specimen contamination in mycobacteriology laboratory detected by pseudo-outbreak of multidrug-resistant tuberculosis: analysis by routine epidemiology and confirmation by molecular technique. J Clin Microbiol 1996;34:1017–9.

[9] Kanduma E, McHugh TD, Gillespie SH. Molecular methods for *Mycobacterium tuberculosis* strain typing: a users guide. J Appl Microbiol 2003;94:781–91.

[10] Barlow RE, Gascoyne-Binzi DM, Gillespie SH, Dickens A, Qamer S, Hawkey PM. Comparison of variable number tandem repeat and IS6110-restriction fragment length polymorphism analyses for discrimination of high- and low-copy-number IS6110 *Mycobacterium tuberculosis* isolates. J Clin Microbiol 2001;39:2453–7. https://doi.org/10.1128/JCM.39.7.2453-2457.2001.

[11] Cowan LS, Mosher L, Diem L, Massey JP, Crawford JT. Variable-number tandem repeat typing of *Mycobacterium tuberculosis* isolates with low copy numbers of IS6110 by using mycobacterial interspersed repetitive units. J Clin Microbiol 2002;40:1592–602.

[12] Supply P, Mazars E, Lesjean S, Vincent V, Gicquel B, Locht C. Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. Mol Microbiol 2000;36:762–71.

[13] Frothingham R, Meeker-O'Connell WA. Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. Microbiology 1998;144:1189–96.

[14] Smittipat N, Palittapongarnpim P. Identification of possible loci of variable number of tandem repeats in *Mycobacterium tuberculosis*. Tuber Lung Dis 2000;80:69–74.

[15] Gascoyne-Binzi DM, Barlow RE, Frothingham R, Robinson G, Collyns TA, Gelletlie R, et al. Rapid identification of laboratory contamination with *Mycobacterium tuberculosis* using variable number tandem repeat analysis. J Clin Microbiol 2001;39:69–74.

[16] Yan JJ, Jou R, Ko WC, Wu JJ, Yang ML, Chen HM. The use of variable-number tandem-repeat mycobacterial interspersed repetitive unit typing to identify laboratory cross-contamination with *Mycobacterium tuberculosis*. Diagn Microbiol Infect Dis 2005;52:21–8.

[17] Johnson MG, Lindsey PH, Harvey CF, Bradley KK. Recognizing laboratory cross-contamination: two false-positive cultures of *Mycobacterium tuberculosis*–Oklahoma, 2011. Chest 2013;144:319–22. http://doi.org/10.1378/chest.12-2294.

[18] Burdz TV, Wolfe J, Kabani A. Evaluation of sputum decontamination methods for *Mycobacterium tuberculosis* using viable colony counts and flow cytometry. Diagn Microbiol Infect Dis 2003;47:503–9.

[19] Murase Y, Mitarai S, Sugawara I, Kato S, Maeda S. Promising loci of variable numbers of tandem repeats for typing Beijing family *Mycobacterium tuberculosis*. J Med Microbiol 2008;57:873–80. http://doi.org/10.1099/jmm.0.47564-0.

[20] Allix-Béguec C, Wahl C, Hanekom M, Nikolayevskyy V, Drobniewski F, Maesa S, et al. Proposal of a consensus set of hypervariable

mycobacterialinterspersed repetitive-unit-variable-number tandem-repeat loci for subtyping of *Mycobacterium tuberculosis* Beijing isolates. J Clin Microbiol 2014;52:164–72. http://doi.org/10.1128/JCM.02519-13.

[21] Braden CR, Templeton GL, Stead WW, Bates JH, Cave MD, Valway SE. Retrospective detection of laboratory cross-contamination of *Mycobacterium tuberculosis* cultures with use of DNA fingerprint analysis. Clin Infect Dis 1997;24:35–40.

[22] Iwamoto T, Yoshida S, Suzuki K, Tomita M, Fujiyama R, Tanaka N, et al. Hypervariable loci that enhance the discriminatory ability of newly proposed 15-loci and 24-loci variable-number tandem repeat typing method on *Mycobacterium tuberculosis* strains predominated by the Beijing family. FEMS Microbiol Lett 2007;270:67–74. https://doi.org/10.1111/j.1574-6968.2007.00658.x.

[23] Van Duin JM, Pijnenburg JE, van Rijswoud CM, de Haas PE, Hendriks WD, van Soolingen D. Investigation of cross contamination in a *Mycobacterium tuberculosis* laboratory using IS*6110* DNA fingerprinting. Int J Tuberc Lung Dis 1998;2:425–9.