Original paper

The Abbott LCx-MTB assay is more sensitive than culture for detection of Mycobacterium tuberculosis in respiratory specimens

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Abstract

Background: In countries with a high incidence of tuberculosis quick, reliable and cost effective testing for this disease is mandatory. With the introduction of new methods based on detection of amplified DNA fragment (Abbott LCx Mycobacterium tuberculosis assay) re-evaluation of the diagnostic panel (smear, culture and BACTEC versus LCx) is needed. We conducted a prospective study comparing clinical usefulness of these tests in detecting Mycobacterium tuberculosis in respiratory specimens from four clinical settings mandating transfer of respiratory specimens.
Material and methods: Respiratory specimens (320 sputum specimens and 54 bronchoalveolar lavage fluid specimens) collected from 374 patients under investigation for tuberculosis at the four clinical centers were divided into 2 portions. One portion was used for standard staining and cultures (auramine-fluorochrome and Ziehl-Neelsen staining, culture on Loevenstein-Jensen medium and in BACTEC Middlebrook 12B vials) and the second one was investigated for presence of Mycobacterium tuberculosis with LCx -MTB assay.
Results: In patients with a final diagnosis of tuberculosis we found LCx test more sensitive than culture and bacterioscopy (93.3% versus 81.3%; $p<0.01$ and 41.0%; $p<0.001$, respectively); while there was no difference in specificity and negative predictive value between LCx and culture.
Conclusions: LCx test is an effective diagnostic tool in the setting of the central laboratory to cooperate with different, distant, clinical centers for detection of Mycobacterium tuberculosis in respiratory specimens from subjects belonging to a population with a high incidence of tuberculosis.

Key words: tuberculosis; mycobacterium tuberculosis complex; direct amplification test; culture.

Introduction

For many years tuberculosis has been recognised as an important public health problem in Poland. Fortunately, there is a continuous decrease of tuberculosis case notifications except for the three year period (from 1991 till

1993) of stabilisation or even a slight increase. However, the annual incidence of tuberculosis is still estimated to be one of the highest in Western and Central Europe and reached 31.5 cases per 100 000 population in 1999 year. Moreover, there is a relatively high contribution (34.4%) of young adults (age between 20 and 44 years) with tuberculosis to all cases of this disease in Poland [1]. This represents a serious epidemiological problem because of the risk of recurrence and expectoration of bacilli resistant to at least one of antimycobacterial drug [2]. In addition, occurrence of tuberculosis in young adults is related to long-term sick-leave and thus economic loss.

Culture is usually required for the laboratory confirmation of tuberculosis. This gives opportunity to identify a pathogen and to determine drug susceptibility. The isolate may also be used for analysis of a molecular profile for epidemiological purposes. Unfortunately, the conventional culture methods are time consuming and need at least 8 to 12 weeks for isolation and identification of Mycobacteria [3, 4]. Even the radiometric BACTEC system that represents a major improvement in the cultivation of mycobacteria by early detection and high recovery rate needs usually 10 to 20 days to demonstrate tubercle bacilli [5, 6]. On the other hand, acid-fast bacilli microscopy lacks sensitivity and specificity being unable to distinguish tubercle bacilli from other mycobacteria [7, 8]. The microbiological confirmation of tuberculosis in Poland is still relatively low and was obtained only in 57.3 % of cases in 1998. This has not improved significantly over the last 30 years [9] in spite of introduction of BACTEC system into several regional microbiological laboratories in the early 1990s. In consequence, almost half of patients received empirical anti-TB treatment on the basis of a high index of suspicion (eg. suggestive chest X-ray picture and presence of nonspecific clinical symptoms) without the definitive diagnosis dependent on the isolation and identification of M. tuberculosis from a diagnostic specimen. This empirical treatment may be inadequate in view of the relatively high frequency of drug resistant bacilli strains in Poland. Determination of drug susceptibility in 1998 revealed that bacilli resistant to at least one anti-TB drug were found in 6.3% of sputum positive patients (expectorating tuberculosis germs) [9]. Alternatively, some cases of pulmonary tuberculosis may be misdiagnosed as pneumonia caused by nonspecific pathogens and initially treated with antibiotics including fluoroquinolones, augmentin or macrolides that possess antimycobacterial activity [10]. This treatment is not sufficient to cure the patient but may lead to the development of drug resistance and cause transitory negative results of sputum culture resulting in diagnostic problems.

The development of amplification tests of Mycobacterium tuberculosis complex (MTBC) over the past few years is an attempt to dramatically shorten the time for detection of tubercle bacilli without losing the assay specificity. Amplification tests have been proved to be more sensitive than smear examination in detecting mycobacteria [11]. This technique gives an opportunity for fast detection of tubercle bacilli in respiratory specimens and introduction of a proper treatment thus decreasing the risk of disease transmission.

The Abbott LCx Mycobacterium tuberculosis assay (LCx-MTB) is one of the recently developed amplification tests to detect DNA from Mycobacterium tuberculosis complex in concentrated, decontaminated and digested respiratory specimens [12]. LCx-MTB assay uses the ligase chain reaction for the amplification of a segment of the single copy gene that encodes protein antigen b. This gene is specific for all members of the Mycobacterium tuberculosis complex (MTBC) which is the causative agent of tuberculosis in humans [13, 14]. Numerous studies evaluated sensitivity, specificity and clinical utility of rapid detection of MTBC in respiratory and nonrespiratory specimens with LCx-MTB test over the past few years [13-31]. They proved LCx-MTB assay a valuable diagnostic tool in smear positive and smear negative subjects with a suspicion of pulmonary tuberculosis – its sensitivity being similar to that obtained with culture [15, 18-22]. However, protocols of these studies did not fit well into real clinical-laboratory practice and had also some limitations that could have influenced the final evaluation of LCx-MTB assay: (A) – Respiratory specimens prepared for LCx-MTB assay were divided into two parts. One part was run on LCx-MTB test and the second one was stored and run again when discrepancies between culture and LCx results were noted [13, 15, 16, 19-23, 25, 31]. This approach may decrease the method sensitivity (decreased load of mycobacterial DNA is present in the sample) and increase the cost of the assay (two LCx runs for one sample and increased consumption of positive and negative control tests). (B) – LCx-MTB test was evaluated retrospectively with knowledge of the results of the previously performed smear-staining, culture and patient final diagnosis [14, 16, 19, 21-23, 25, 28, 29]. This may influence the results interpretation especially when the ratio of S/CO is on the border of LCx-MTB assay positivity. (C) – The majority of studies were performed in one microbiological centre wherein the conventional and LCx-MTB tests were performed [15-17, 19-21, 23, 26-28, 30, 31].

The Abbott LCx Probe System is rather expensive for the limited budget of the microbiological laboratory in developing countries where tuberculosis is still an important public health problem. Thus the LCx-MTB assay could be located in one central laboratory cooperating with regional microbiological laboratories and clinical centres. Therefore this assay needs evaluation under conditions that involve specimen storage and transportation from different centres.

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Taking it into consideration we decided to evaluate the LCx-MTB assay versus culture on L-J slants for detection of MTBC in respiratory specimens collected and transported from four clinical centres. The LCx-MTB test results were obtained from one run of the whole sample without its division into two parts and further retesting and without previous knowledge about patients' clinical status and results of any laboratory investigations.

Material and methods

Study design and clinical specimens

The LCx-MTB assay was performed at the Department of Experimental and Clinical Physiology, Institute of Physiology and Biochemistry Medical University of Lodz. Respiratory specimens, 320 sputum specimens and 54 bronchoalveolar lavage fluid (BAL fluid) specimens were collected from 374 patients under investigation for tuberculosis at the four clinical centers: Department of Bronchology Institute of Tuberculosis and Lung Diseases in Warsaw (131 km to the east of Lodz), Pulmonary Ward of District Chest Hospital in Otwock (161 km to the south-east of Lodz), Pulmonary Ward of District Chest Hospital in Kalisz (117 km to the south-west,) and from Department of Tuberculosis and Lung Diseases Medical University of Lodz (11 km to the north of the Department of Experimental and Clinical Physiology). The samples were divided into two portions. One portion was used for standard staining and cultures in Microbiology Laboratories localised at clinical centres. This involved auramine-fluorochrome and Ziehl-Neelsen staining, culture on Loevenstein -Jensen (L-J) medium and in BACTEC Middlebrook 12B vials (Becton Dickinson Diagnostic Instruments, Sparks, Md.).

The second part of the specimens was stored at -20°C for not longer than 14 days and then transported frozen by car to the Department of Experimental and Clinical Physiology. The detection of Mycobacterium tuberculosis complex (MTBC) with LCx-MTB test was performed once on the whole obtained specimen within two days after specimen delivery, without any knowledge of patient diagnosis, medication and results of previous assays for Mycobacterium tuberculosis. All details concerning the results of the microbiological examination of the first specimen portion and other respiratory specimens, patient clinical diagnosis, and results of anti-TB treatment (if it was introduced) were mailed to the Department of Experimental and Clinical Physiology from clinical centres five months later. To exclude any influence of LCx-MTB test on the clinical diagnosis, no information about the results was sent to clinical centres before ending the study. All patients involved in the study gave informed consent and the study protocol was approved by Ethics Committee in all participating centres.

Specimen processing

Respiratory specimens were transferred to 50 ml centrifuge tubes, mixed with 1 volume of 0.5% N-acetylcycteine (NAC) solution with 2% NaOH, incubated for 25 min at 37°C, diluted 2-fold with sterile phosphate buffered saline (PBS, pH 7.4) and centrifuged (15 min, 3000 xg). The pellet was resuspended in 1 ml PBS and used for auramine-fluorochrome and Ziehl-Neelsen staining, culture on L-J medium or BACTEC 12B vials. This was performed at microbiology laboratories at clinical centres. Respiratory specimens for LCx-MTB test were also digested and decontaminated by the NAC-NaOH procedure (but with some modifications) [32] under biosafety conditions in a separate room (Area 0) at the Department of Experimental and Clinical Physiology. The specimen (sputum or bronchoalveolar lavage fluid) was mixed with an equal volume of 0.5% NAC solution containing 2% NaOH and 1.45% sodium citrate and incubated for 30 min at 37°C with gentle shaking. Afterwards the sample was diluted with 10 volumes of PBS, centrifuged (15 min, 3000 xg), supernatant discarded and the pellet was resuspended in 0.5 ml of PBS with addition of 0.2% bovine serum albumin and run for LCx-MTB test.

Staining and culture protocols

Fluorochrome and/or Ziehl-Nelsen staining was performed by standard procedures [33]. After processing, BAL fluid specimens were inoculated into BACTEC 12B vials and onto L-J slants while sputum specimens only onto L-J slants. BACTEC 12B vials containing 0.1 ml of PANTA-plus supplement were inoculated according to the manufacturer's recommendations with 0.5 ml of the decontaminated specimen and were incubated for minimum 6 weeks and monitored by using the BACTEC 460 radiometric reader (Becton Dickinson) [34]. L-J slants received 0.2 to 0.6 ml of decontaminated sputum and were incubated at 37°C and examined twice a week for 12 weeks.

Mycobacteria grown on L-J slants were identified by conventional methods [35].

LCx assay protocol

The LCx -MTB assay was performed according to the manufacturer's instruction [12, 36] as previously described [15]. Results were expressed as ratios (S/CO) based on a cutoff value determined as 0.3 times the mean MEIA calibrator control value. Samples with an S/CO value ≥1 were considered positive. The LCx-MTB test was negative if the ratio S/CO was <1.

Analysis of results

The "combined gold standard" that involved all known results of microbiological assays (culture and bacterioscopy), and all relevant clinical data including

Table I. Clinical outcome of respiratory specimen donors that were under investigation for tuberculosis at four clinical centres

Final diagnosis	Respiratory specimen		
	Sputum	BALF	All
TB3	117	17	134
TB4	33	4	37
sarcoidosis	15	12	27
malignancy	62	10	72
Bacterial pneumonia	55	3	58
pulmonary disease caused by NTM	0	6	6
pleural disease	11	0	11
COPD	13	0	13
bronchial asthma	14	2	16

TB3 – active pulmonary tuberculosis; TB4 – not clinically active pulmonary tuberculosis; NTM- nontuberculous mycobacteria; COPD – chronic obstructive pulmonary disease

patient's history, signs, symptoms, chest X-ray, cytological and histological results of patient specimens, tuberculin skin test result, history of drugs administered, response to anti-TB treatment and final diagnosis was used to classify patients as those with active pulmonary tuberculosis (TB3) and those with other diseases. The "other diseases" group included also subjects with not clinically active pulmonary tuberculosis (TB4). Since one respiratory specimen was obtained from one patient the number of TB3 patients was assumed as the highest possible number of true MTBC positive results. Percentage sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) for LCx-MTB test, L-J culture and bacterioscopy were calculated according to formulas: Se=detected true positive/number of TB3 patients x 100; Sp=detected true negative/ number of patients with other diseases x 100; PPV=detected true positive/detected true plus false positive x 100; NPV=detected true negative/detected false plus true negative x 100. The differences between LCx-MTB test, L-J culture, BACTEC 460 system and bacterioscopy in respect of Se, Sp, PPV and NPV were

determined with chi-square test. A p-value less than 0.05 was considered to be significant.

Results

Respiratory specimen and patient clinical data

Of 374 analysed respiratory specimens (obtained from 374 patients) 104 samples (54 BAL fluid specimens and 50 sputum samples) were from the Department of Bronchology Institute of Tuberculosis and Lung Diseases in Warsaw, 98 sputa from the Pulmonary Ward of the District Chest Hospital in Otwock, 48 sputa from the Pulmonary Ward of the District Chest Hospital in Kalisz, and 124 sputum specimens were from the Department of Tuberculosis and Lung Diseases Medical University of Lodz.. No subject involved in the study was under anti-TB treatment nor received other antibiotics with antimycobacterial activity in monotherapy [10] for 12 months and 3 weeks from the day of specimen collection, respectively. Analysis of all relevant patient clinical data that came from clinical centres five months later revealed that 134 patients suffered from active pulmonary tuberculosis (TB3), 37 subjects had a diagnosis of not clinically active pulmonary tuberculosis (previously active disease, TB4), 27 had sarcoidosis, 72 malignancy, 58 bacterial pneumonia, 6 pulmonary disease caused by nontuberculous mycobacteria, 11 pleural disease, 13 COPD, and 16 patients had bronchial asthma (Table I). Subjects classified as TB4 had at least 3 further specimens (sputum, induced sputum or BAL fluid specimen) negative in culture on L-J slants and/or BACTEC 460 system, however, they were not assayed with LCx-MTB test.

Initial comparison of LCx-MTB test with culture without the analysis of patient clinical data

Of the 374 specimens, 109 (29%) were positive for MTBC by culture on L-J slants. Of the culture positive specimens, 55 (50%) had a positive smear result. LCx-MTB test gave a positive result in 127 specimens (34%). When the culture was used as a gold standard the overall sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of LCx-MTB assay was 91.7%, 89.8%, 78.7% and 96.4%, respectively. For samples

Table II. Initial comparison of LCx-MTB test results with culture for detection of Mycobacterium tuberculosis complex (MTBC) in respiratory specimens

Specimen (no)	Specimen positive by culture (109)		Specimen negative by culture (265)		Se%	Sp%	PPV%	NPV%
	LCx pos.	LCx neg.	LCx pos.	LCx neg.				
All (374)	100	9	27	238	91.7	89.8	78.7	96.4
Smear pos. (58)	54	1	0	3	98.2*	100	100†	75.0*
Smear neg. (316)	46	8	27	235	85.2	89.7	63	96.7

*pos. – positive; neg. – negative; Se – sensitivity; Sp – specificity; PPV – positive predictive value; NPV – negative predictive value. In parentheses, the number of analysed specimens. Significantly different versus smear-negative specimens; * – p=0.02; † – p=0.001*

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Table III. Assessment of LCx-MTB test, culture and bacterioscopy for detection of MTBC in respiratory specimens after analysis of patient clinical data including results of anti-TB treatment

Assay	TB3 (134)		Other diseases (240)		Se (%)	Sp (%)	PPV (%)	NPV (%)
	positive	negative	positive	negative				
LCX	125	9	2	238	93.3*†	99.2	98.4	96.4*†
Culture	109	25	0	240	81.3†	100	100‡	90.6†
Smear	55	79	3	237	41.0	98.7	94.8	75.0

*374 respiratory specimens (320 sputum specimens, 54 BAL fluid specimens) were obtained from 374 patients. Of these patients, 134 subjects had diagnosed active pulmonary tuberculosis (TB3) according to ATS classification of tuberculosis infection. Se – sensitivity; Sp – specificity; PPV-positive predictive value; NPV – negative predictive value. Significantly different; * – vs culture – p = 0.01; † – vs bacterioscopy – p = 0.001; ‡ – vs bacterioscopy – p = 0.01*

Table IV. Assessment of LCx-MTB test, culture and bacterioscopy for detection of MTBC in sputum specimens after analysis of patient clinical data

Assay	TB3 (117)		Other diseases (203)		Se (%)	Sp (%)	PPV (%)	NPV (%)
	positive	negative	positive	negative				
LCX	110	7	0	203	94.0 *†	100	100	96.7 *†
Culture L-J	96	21	0	203	82.1	100	100 ‡	90.6 †
Smear	55	62	3	200	47.0	98.5	94.8	76.3

*320 sputum specimens were obtained from 320 patients. Of these patients, 117 subjects had diagnosed active pulmonary tuberculosis (TB 3). Significantly different; * – vs culture – p = 0.01; † – vs bacterioscopy – p = 0.001; ‡ – vs bacterioscopy – p = 0.05*

that were positive in bacterioscopy the LCx-MTB test reached higher sensitivity (98.2 % vs 85.2 %, p=0.02) and PPV (100% vs 63.0% p = 0.001) than for samples that were smear-negative (Table II). However, NPV was lower for smear- positive specimens (75.0% vs 96.7%, p=0.02).

Comparison of LCx-MTB test with culture and bacterioscopy for detection of MTBC in respiratory specimens after the analysis of patient clinical data.

The analysis of combined gold standard (results of culture performed also with additional respiratory specimens, and all relevant patient clinical data) revealed 134 patients with active pulmonary tuberculosis (TB3) (Table I). LCx-MTB test detected MTBC in 125 respiratory specimens from these patients while culture and bacterioscopy in 109 and 55 specimens, respectively. LCx-MTB test gave 2 false positive results in 2 BAL fluid specimens obtained from patients with lung cancer. Bacterioscopy gave also 3 false positive results with sputa from 2 patients with bacterial pneumonia and one with lung cancer. The sensitivity of LCx-MTB assay for detection of MTBC in respiratory specimens (sputum and BAL fluid) reached 93.3 % and was higher than that obtained with L-J culture (p=0.01) and bacterioscopy (p=0.001), respectively (Table III). There were no significant differences between specificity and PPV obtained with LCx-MTB test and culture on L-J slants. The NPV for bacterioscopy reached only 75% and was lower than that for LCx-MTB test and culture (p=0.001).

Of 320 sputum donors, 117 subjects had active pulmonary tuberculosis (TB3). LCx-MTB assay

revealed MTBC in 110 sputum specimens from these patients while culture on L-J slants and bacterioscopy detected mycobacteria in 96 and 55 patients, respectively. No false positive results were obtained with LCx-MTB assay and culture in the case of sputum investigation. The sensitivity, specificity, PPV and NPV calculated for LCx-MTB assay, culture, and bacterioscopy of sputum specimens are shown in Table IV. LCx-MTB test revealed significantly higher sensitivity than culture (94.0% vs 82.1%, p=0.01) and bacterioscopy (94.0 % vs 47.0 %, p=0.001). The same was observed for NPV, while specificity and PPV did not differ between LCx-MTB assay and culture. Of 54 donors of BAL fluid specimens,17 had active pulmonary tuberculosis. LCx-MTB test detected MTBC in 15 patients classified as TB3 and gave 2 false positive results with specimens obtained from 2 patients with lung cancer. Both, culture on L-J slants and BACTEC 460 system revealed MTBC in 13 BAL fluid specimens from TB3 patients. No false positive result was obtained with these methods. Thus the calculated sensitivity of LCx-MTB test, culture and BACTEC system for detection of MTBC in BAL fluid specimens reached 88.2 %, 76.5% and 76.5% and did not differ from one another significantly. Also other calculated parameters such as specificity, PPV and NPV were similar for these methods (Table V). It should be pointed out that 6 BAL fluid specimens that were negative in LCx-MTB test and in L-J culture gave a positive result with BACTEC 460 system and nontuberculous mycobacteria were identified (M. avium complex –

Table V. Assessment of LCx-MTB test, culture and BACTEC 460 system for detection of MTBC in bronchoalveolar lavage fluid specimens after analysis of patient clinical data

Assay	TB3 (17)		Other diseases (37)		Se (%)	Sp (%)	PPV (%)	NPV (%)
	positive	negative	positive	negative				
LCX	15	2	2	35	88.2	94.6	88.2	94.6
CultureL-J	13	4	0	37	76.5	100.0	100.0	90.2
BACTEC-460	13	4	0	370	76.5	100.0	100.0	90.2

54 BAL fluid specimens were obtained from 54 patients. Of these patients, 17 subjects had diagnosed active pulmonary tuberculosis (TB3). No significant differences between LCx-MTB test, L-J culture and BACTEC 460 system were found

Table VI. The sensitivity and specificity of LCx-MTB test in previously performed studies that involved more than 200 respiratory specimens

Se	Sp	Specimens			No. of patients	Reference no.
		no	Smear positive %	R		
90.8*	100*	520	22.1	1/4	326	21
70.2	99.9	2083	3.2	<1/2	<1411	19
77.0	99.0	493	2.6	1/3	205	25
90.2	98.4	2001	3.4	<1/2	1130	24
90.2	99.2	482	19.3	≤1.2	457	20
89.3	98.5	226	8.4	1/4	208	23
75.7	98.8	273	13.2	≤1/4	205	27
63.8†	99.5 †	499	7.4	1/4	230	26
93.7	99.2	1971	8.2	<1/2	979	39

*R – part of the tested respiratory sample; * – did not differ significantly from L-J culture, for other results the statistical analysis was not made;*
† – calculated by us on the basis of the data given by the authors

5 isolates, M. fortuitum – 1 isolate).Thus in 6 patients the pulmonary disease caused by nontuberculous mycobacteria has been diagnosed (Table I). These results were excluded from Table VI since LCx-MTB assay detects only MTBC (12).

Discussion

We performed the evaluation of LCx-MTB assay (Abbott Laboratories) for detection of MTBC in respiratory specimens from subjects investigated for tuberculosis under the following conditions: (A) – LCx-MTB test laboratory cooperated with four different clinical centres with local microbiological laboratories for smear staining and culture performance; (B) – the result of LCx-MTB test was obtained from one run of the whole received sample without knowledge of any patient clinical data; (C) – one respiratory specimen was obtained from one patient not treated with anti-TB drugs; (D) – all patient data consisting of "combined gold standard" for diagnosis of active pulmonary tuberculosis and other diseases were received 5 months after LCx-MTB test performance. Thus, the LCx evaluation was performed in conditions that excluded the possibility to retest part of specimen

from patient if any doubts or technical problems occurred and results were immediately classified as positive or negative just after receiving the printout from MEIA LCx analyser. This is important especially for specimens that gave S/CO ratio between 0.90 and 1.00. Knowledge of the patient clinical data including previous positive LCx-MTB test if the patient gave more than one respiratory specimen may provoke the laboratory staff to decrease the recommended by the manufacturer border of S/CO positivity and classify these specimens as positive thus increasing the assay sensitivity [14]. Despite these study protocol limitations the sensitivity of MTBC detection in respiratory specimens and NPV were higher for LCx-MTB assay than for L-J culture, while Sp and PPV did not differ significantly between these two methods. The same was observed for Sp, Se, PPV and NPV calculated only for sputum specimens. For 54 BAL fluid specimens LCx-MTB test gave results that did not differ significantly from those obtained with L-J culture and BACTEC 460 system. This suggests that specimen transportation from clinical centres to LCx laboratory, lack of clinical data on specimen donor when the assay was performed and exclusion of the possibility to retest the same sample had no

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negative effect on the overall results obtained with LCx-MTB assay. Moreover, the results with BAL fluid specimens are in agreement with our previous study showing LCx-MTB test equal to BACTEC 460 system in detection of MTBC in BAL fluid specimens from subjects with smear-negative pulmonary tuberculosis [15]. In this study LCx-MTB assay equipment and BACTEC 460 system were located at the same laboratory, no blinding of clinical data was performed and half of each specimen was stored for possible retesting. We found the overall sensitivity and specificity of LCx-MTB test 93.3% and 99.2%, respectively. Table VI shows the results of 9 previous studies on LCx-MTB test that involved more than 200 respiratory specimens. Only study performed by the test manufacturer reported similar, while the remaining 8 reports revealed lower LCx-MTB assay sensitivity than in our study. The assay specificity did not differ from those shown in Table VI [14, 18, 19, 21-25, 36]. Involvement of more than one respiratory specimen from one patient, possibility to retest the same specimen, and lack of blinding of the clinical data at the moment of test performance are the main differences between the protocol of our study and those 9 studies. As mentioned above these differences may rather increase the assay sensitivity and cannot explain higher LCx-MTB test sensitivity in our trial. To solve this problem we checked what part of specimen was run for LCx-MTB test. In our study the respiratory specimen was divided into two equal portions and one portion was analysed by LCx-MTB assay and the second was used for culture and smear staining. Thus the ratio of the sample volume assayed by LCx-MTB test to the whole specimen volume was 1/2. This ratio was reached only in one study (but not for all specimens) presented in Table VI. Other 4 studies had the ratio <1/2, in 3 the ratio was 1/4 and in 1 study the ratio was equal or lower than 1/4. The lowest sensitivity 63.8% was obtained by authors who tested only 1/4 of the whole specimen by LCx-MTB assay [24]. The analytical sensitivity of LCx-MTB assay reported by the manufacturer is 56.5 colony forming units (CFU) of MTBC per specimen [36]. Quantitative culture results showed that 13 of 25 (52%) culture-positive specimens containing no more than 20 CFU were falsely negative when tested with LCx-MTB test [24]. Other authors reported occurrence of false negative results even in specimens containing up to 500 CFU [23]. The low ratio of the sample tested by LCx-MTB assay to the whole specimen may result in a low number of DNA copies subjected to amplification reactions and be responsible for false negative results and decreased assay sensitivity. This may have importance for both smear-positive and paucibacillary, smear-negative tuberculosis. Even in the case of smear-positive specimens rich with MTBC the clumping of bacilli may cause its nonuniform distribution during specimen sampling and result in false negative readings [19].

Our material involved 15.5% smear-positive respiratory specimens. Higher ratio of smear-positive specimens in a cohort study may result in higher LCx-MTB test sensitivity [14]. Since the ability of LCx-MTB assay to detect MTBC DNA depends on the number of bacilli in the sample [14, 36] there is a bigger chance to obtain a positive result with smear-positive and culture-positive specimen than with smear-negative and culture-positive one. This is supported by greater consistence between culture and LCx-MTB assay results in smear-positive that in smear-negative respiratory specimens (Table II). On the other hand, 3 studies (Table VI) with the ratio of smear-positive specimens 22.1%, 19.3% and 13.2% reported LCx-MTB assay sensitivity 90.8%, 90.2% and 75.7%, respectively [18, 19, 25]. This did not exceed our sensitivity (93.3%), however the ratio of the sample tested by LCx-MTB assay to the whole specimen was lower than that in our protocol. In addition, studies with a low rate of smear positive specimens but with a higher part of respiratory specimen run for LCx-MTB assay gave higher sensitivities [22, 23, 36]. This clearly indicates that higher LCx-MTB assay sensitivity can be obtained when a higher part of the respiratory specimen is designed for a single assay. Division of the specimen into 1 or 2 additional samples for retesting and/or testing for inhibitors by the spike-back procedure may decrease the overall LCx-MTB sensitivity and therefore could be omitted when easy to obtain material is tested. If any problems with the test performance occur (e.g. technical, abnormal result of positive or negative control leading to difficulties in result interpretation) the better solution is to ask for additional sputum specimen from the patient than retest the previously prepared reserve sample of the same material with a decreased number of bacilli due to the sampling procedure. Such an approach may also decrease the average cost of the assay since lower amounts of compounds including positive and negative controls are consumed. This may have importance in countries with high incidence of tuberculosis. As a rule these countries have a relatively low budget for dealing with this disease.

Because the culture result depends on the number of viable bacilli in the inoculated sample, a higher proportion of culture positive specimens to the whole studied material may also influence the overall LCx-MTB test sensitivity. For instance, in a recent study that involved 350 respiratory specimens and 3.6 % of them were culture-positive the LCx-MTB test sensitivty in comparison with culture was 87.5% [37]. We had 8-times higher ratio of culture-positive specimens and the overall sensitivity reached 91.7% when culture was a gold standard (Table II).

Moore and Curry reported 74% sensitivity of LCx-MTB test comparing to culture on the basis of 493 respiratory specimens of which 6.9% were culture positive [23]. They also showed that culture-positive specimens containing less than 500 CFU/ml may be negative in LCx-MTB assay [23]. When the analysed material is derived from a population with a low incidence of tuberculosis one may expect the relatively high contribution of culture-positive specimens with low bacilli load to the whole number of culture-positive specimens. This apart from the low ratio of sample investigated by amplification test to the whole specimen may be responsible for lower LCx-MTB assay sensitivity described in these papers. Taking the above into consideration the requirements for effective performance of LCx-MTB assay (high sensitivity, low cost of single tuberculosis case detection) should involve localisation of LCx laboratory in an area with a high incidence of tuberculosis and/or analysis of specimens from patients with a high suspicion of tuberculosis and the part of the specimen designed for a single run should be as large as possible.

We noted 2 false positive results obtained with LCx-MTB test in BAL fluid specimens from two patients with lung cancer. It is possible that contamination of BAL fluid specimens with residual genetic material of MTBC in bronchoscopes may be responsible for false positive results. Moreover, these bronchoscopes had been used in the previous week for 3 TB3 patients that in our study were positive in LCx-MTB test, culture and BACTEC system. Previous studies also reported PCR false positive results in specimens obtained with contaminated bronchoscope [38]. To test this possibility we randomly washed with PBS two sterile bronchoscopes used in the study and then the buffer was concentrated and run for LCx-MTB test. This procedure was performed four times, however, no positive result was observed. Another cause of false positive results could be a laboratory DNA contamination of the tested respiratory specimen. During the whole study we never observed any positive LCx-MTB test result with salmon sperm DNA (negative control) and the procedures of specimen digestion and decontamination (area 0), removing of potential amplification inhibitors and specimen addition to amplification vials (area 1), amplification and automatic detection (area 2) were performed in three separate rooms. Therefore, this explanation of false positive results seems unlikely.

Jouveshomme et al. reported constantly negative LCx-MTB test results when specimens were derived from culture-negative TB3 patients and concluded that direct amplification tests may have limited significance in these subjects [24]. In our study 25 (18.7%) of 134 patients with TB3 were culture-negative while LCx-MTB test gave positive results. On the other hand, only 9 patients with culture-positive pulmonary tuberculosis (6.7%) were negative in LCx-MTB assay (false negative results). This indicates that LCx-MTB assay may be useful in culture-negative TB3 patients. The ratio of the sample tested by LCx-MTB assay to the whole specimen was two times higher in our study than that described by Jouveshomme et al. and this may explain the above mentioned differences. Nine LCx-MTB test false negative results, especially one with sputum from a patient with smear- and culture-positive pulmonary tuberculosis could be caused by the presence of inhibitors of enzymatic amplification reactions. However, due to study protocol limitations we were not able to prove this with a spike-back procedure [36]. The nonuniform distribution of microorganisms during specimen sampling seems to be not responsible for these false negative results because they did not occur with respiratory specimens (probably with low bacilli load) obtained from culture-negative TB3 patients.

We conclude that the LCx- laboratory may effectively cooperate with different, distant, clinical centres for detection of MTBC in respiratory specimens from subjects belonging to population with a high incidence of tuberculosis. The most critical point for obtaining a high sensitivity of LCx-MTB test is to run for single assay the part of obtained specimen as much as it is possible. If this condition is fulfilled the knowledge of patient clinical data at the moment of test performance is not necessary for obtaining the higher overall test sensitivity than with L-J culture.

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