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Editorial Adress:
d/a Laboratorium Patologi Klinik RSUD Dr. Soetomo, Gedung Diagnostik Center Lt. IV
Jl. Mayjend. Prof. Dr Moestopo 6–8 Surabaya, Indonesia
Telp/Fax. (031) 5042113, 085-733220600 E-mail: majalah.ijcp@yahoo.com, jurnal.ijcp@gmail.com
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RESEARCH

DIAGNOSTIC VALUE OF FASTSURE TB DNA RAPID TEST FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS

(Nilai Diagnostik dari Uji Cepat Fastsure TB DNA untuk Diagnosis Tuberkulosis Paru)

Diyan Wahyu Kurniasari, Jusak Nugraha, Aryati

ABSTRAK

Diagnosis tuberkulosis (TB) di Indonesia menurut Kemenkes RI 2014 masih mengandalkan pemeriksaan mikroskopis Basil Tahan Asam (BTA) dari hapusan dahak namun memiliki kepekaan dan kekhasan diagnostik yang rendah. Kepekaan diagnostik kultur Mycobacterium tuberculosis (M.tuberculosis) pada media Lowenstein Jensen (LJ) lebih tinggi daripada mikroskopis BTA namun hasilnya memerlukan waktu 6-8 minggu. Uji cepat Fastsure TB DNA menggunakan metode Cross Priming Amplification (CPA) merupakan uji deteksi kualitatif DNA M.tuberculosis yang diamplifikasi pada satu suhu tetap dan menggunakan nucleic acid lateral flow strip dalam perangkat plastik tertutup. Penelitian ini dilakukan untuk menilai nilai diagnostik uji cepat Fastsure TB DNA untuk diagnosis tuberkulosis paru. Metode penelitian secara observasional potong lintang selama Juni sampai November 2015. Total 58 spesimen dahak dari 33 orang terduga tuberkulosis paru dan 25 orang non-tuberkulosis dari RS Paru Karang Tembok Surabaya. Setiap spesimen dilakukan pemeriksaan mikroskopis BTA, kultur M.tuberculosis pada media LJ sebagai standar baku emas dan ekstraksi DNA dan amplifikasi pada uji cepat Fastsure TB DNA. DNA terekstraksi dimasukkan ke dalam tabung kemudian ke cartridge yang tersedia dalam perangkat. Hasil diamati dalam waktu 30 menit berupa munculnya garis uji dan pembanding pada pita. Berdasarkan penelitian ini, diperoleh kepekaan dan kekhasan diagnostik uji cepat Fastsure TB DNA masing-masing sebesar 84,8% dan 92% dengan koefisien kappa adalah 0,757 menunjukkan kesesuaian yang cukup baik. Uji cepat Fastsure TB DNA merupakan pemeriksaan yang cepat, praktis, relatif tidak mahal untuk diagnosis M.tuberculosis dari spesimen klinis khususnya pada BTA negatif.

Kata kunci: *Hapusan mikoskopik, uji cepat Fastsure TB DNA, kultur dari M.tuberculosis*

ABSTRACT

The diagnosis of tuberculosis (TB) in Indonesia based on the Ministry of Health 2014 still relies on microscopic examination of acid fast bacilli (AFB) of sputum smear but this has a low diagnostic sensitivity and specificity. The diagnostic sensitivity of *Mycobacterium tuberculosis* (*M.tuberculosis*) culture on Lowenstein Jensen (LJ) media is higher than microscopic smear but needs 6-8 weeks to provide a positive result. Fastsure TB DNA Rapid Test uses Cross Priming Amplification (CPA) method, a qualitative detection of amplified *M.tuberculosis* DNA at a constant temperature and using nucleic acid lateral flow strip in a sealed plastic device. In this study, the diagnostic value of Fastsure TB DNA Rapid Test for the diagnosis of pulmonary tuberculosis was evaluated. This study design was observational cross-sectional and was done in June until November 2015. A total of 58 sputum specimens from 33 subjects with suspected pulmonary tuberculosis and 25 subjects of non tuberculosis from Karang Tembok Pulmonology Hospital, Surabaya was obtained. Each specimen underwent microscopic smear examination, *M.tuberculosis* culture on LJ media as the gold standard and DNA extraction and amplification for Fastsure TB DNA Rapid Test. Extracted DNA was inserted into the tube and then into the cartridge provided with the Fastsure TB DNA Rapid Test kit. The presence of test and control lines of the strip was observed within 30 minutes. Based on this study, the diagnostic sensitivity and specificity of Fastsure TB DNA Rapid Test were 84.8% and 92%, respectively and kappa coefficient was 0.752 thus showing a good agreement. Fastsure TB DNA Rapid Test is rapid, relatively inexpensive and simple for diagnosis of *M.tuberculosis* from clinical specimens particularly for negative acid fast bacilli.

Key words: Microscopic smear, Fastsure TB DNA rapid test, culture of *M.tuberculosis*

Department of Clinical Pathology, Faculty of Medicine, Dr. Soetomo Hospital, Surabaya, Indonesia. E-mail: diyan_pk@yahoo.co.id

INTRODUCTION

The burden of tuberculosis (TB) disease is the greatest threat to the country's economy and Indonesia now ranks fourth in the world of the group of countries with a high burden of pulmonary TB (high-burden countries).[1] It is a challenge for all parties to control this infection. One important effort to suppress the transmission of TB in the community is to make an early definitive diagnosis.

The diagnosis of tuberculosis in Indonesia based on the Ministry of Health 2014 still relies on microscopic examination of acid fast bacilli (AFB) of sputum smear by Ziehl Neelsen (ZN) staining but has a low diagnostic sensitivity and specificity. This method is relatively easy, inexpensive, can be widely applied and is highly specific for *Mycobacterium tuberculosis* (*M.tuberculosis*) in TB endemic countries.[2] Sputum smear is less sensitive because AFB show positive results when there are at least 5000–10000 organisms/mL of sputum.[3] Some researchers reported that in patients with pulmonary TB, the sensitivity of sputum smear examination was more than 80% compared to culture. Other researchers have reported that the sensitivity of sputum smear examination was lower, varying between 20–80%.[2,4,5] Sensitivity microscopic examination is also low in cases with a lesser number of bacteria (paucibacillary), as in the case of children and co-infection with Human Immunodeficiency Virus (HIV) -TB.[2,4]

Culture has an important role in the diagnosis of TB because its sensitivity and specificity are better than acid-fast bacilli staining. Lowenstein-Jensen (LJ) culture is the gold standard for identification *M.tuberculosis* with 99% sensitivity and 100% specificity, but requires a long time to obtain results, which is about 6–8 weeks. This will cause a significant delay in diagnosis and starting the therapy.[3,6]

World Health Organization recommends rapid molecular detection in suspected TB adult patients and in children but microscopically negative.[7]

Fastsure TB DNA Rapid Test is a Nucleic Acid Amplification Test (NAAT) method using Cross Priming Amplification (CPA) which detects *M.tuberculosis* nucleic acid in human clinical specimens. Cross priming amplification technology is a recent invention of isothermal DNA amplification with a high sensitivity, specificity and efficiency, using multi cross linked primers, to amplify the DNA or RNA target sequence at a temperature.[8]

Fastsure TB DNA Rapid Test uses a simple tool, the constant process is faster and relatively inexpensive than PCR so it is suitable for use as Point of Care Testing (POCT), in small hospitals and clinics in areas with limited resources.[8] This study evaluated the diagnostic value of Fastsure TB DNA Rapid Test for the diagnosis of pulmonary TB compared to the gold standard of *M.tuberculosis* culture in sputum specimens using LJ media. This method is expected to help the diagnosis of TB with a fast, easy, and safe test so that it can be done routinely in developing countries, including Indonesia.

METHODS

This study was an observational study design with descriptive cross-sectional and was conducted in June to November 2015 from patient's sputum with suspected pulmonary tuberculosis who were treated at the Karang Tembok Pulmonology Hospital Surabaya and fulfilling the inclusion criteria and signed an informed consent. Detection of TB DNA in sputum with Fastsure TB DNA Rapid Test was done in the Clinical Pathology Laboratory of the Dr. Soetomo, Hospital Surabaya and culture at the Public Health Laboratory, Surabaya. Inclusion criteria were patients aged more than 21 years with suspected pulmonary tuberculosis and minimal sputum sample volume was 5 mL. Suspected pulmonary tuberculosis defined by criteria that met one or more of the following symptoms: disturbances in the respiratory tract (cough>2 weeks, coughing blood, shortness of breath, chest pain), systemic symptoms (fever, malaise, night sweats, anorexia, weight loss).[9] Sputum was taken from each respondent 3 times, at least one early morning specimen. Each sample underwent three examinations simultaneously, Ziehl-Neelsen (minimal 1 mL), culture of *M.tuberculosis* in LJ media (minimal 3 mL) and TB DNA examination with Fastsure TB DNA Rapid Test (minimal 1 mL).

The preliminary study on Fastsure TB DNA Rapid Test has been conducted in accordance with the instructions manual of MP Diagnostic Fastsure TB DNA Rapid Test, but there were some differences in procedure. Minimal specimen volume was 1 mL and mucopurulent specimen should be homogenized by vortex after washing first. Vortex treatment after washing first was done for all specimens to be more homogeneous and uniform.

Fastsure TB DNA Rapid Test included sample preparation, nucleic acid isothermal amplification and hybridization in a constant temperature. Detection of amplification CPA products was performed on nucleic

Figure 1. Detection of TB DNA

acid lateral flow strips that were in a sealed plastic device to prevent cross-contamination of amplicons and reduce false-positive results. All these steps were completed within 3 hours. Unlike the polymerase chain reaction (PCR) which required a minimum of 30-35 thermal cycles to complete the reaction.[8]

Sputum was mixed with NaOH 4% (ratio 1: 1–2.5 or more if mucopurulent) and incubated for 10 min (at room temperature) while vortexed 4–5 times to make it more homogeneous. One mL of the mixture was transferred into a 1.5 mL centrifuge tube and centrifuged at 10,000 rpm for 10 min and the supernatant was removed to obtain a precipitate (pellet). The pellet was washed (with 1 mL of NaCl 0.9%) and vortexed two times then centrifuged at 10,000 rpm for 10 min and the supernatant was discarded. This process was performed in a biosafety cabinet. DNA extract solution (40 mL) consisting of a pair of specific primers and probes, was added to the pellet, homogenized and incubated in a water bath (95–100°C) for 10 min, then removed and allowed to stand for 10 min at room temperature. The tube was centrifuged at 10,000 rpm for 5 min and the supernatant was stored as an amplification template. Amplification templates can be examined or stored at a temperature of 20°C (stable for 1 week). Resuspension buffer was transferred into a test tube (tube amplification) and incubated at room temperature for 2–3 min to dissolve completely. Each sample/negative control/positive control was added to the test tube and homogenized with a micropipette. The negative control used ddH20, control reagents as the positive control and sample used amplification template. Twenty mL of mineral oil was transferred into the test tube, without shaking. All reaction tubes were centrifuged at 4,000 rpm for 3–5 min and then incubated in a water bath (63°C) for 1 hour. The test tube was placed in the cartridge then into the detection kit and locked. The results were read visually after 15–30 minutes on a lateral flow strip in the instrument (Figure 1). The appearance of test and control lines indicated a positive result, appearance of control line only indicated a negative result and if the control line did not appear it was considered as invalid. The intensity of the color line was compared to the intensity scale of card in kit.[10]

RESULTS AND DISCUSSION

During the study period, 58 suspected tuberculosis patients were divided into 33 TB patients and 25 non-TB patients. TB samples which volumes were not enough were excluded from this study so 64 samples were obtained. In accordance with the diagnosis of tuberculosis based on the Ministry of Health 2014, someone is diagnosed with pulmonary TB if positive smear results were found from one specimen with clinical symptoms of TB.[11] TB sample analysis was performed on a TB culture as a gold standard and resulted 33 positive samples from 33 TB patients. Distribution of TB patients by gender obtained 20 males and 13 females, while the non-TB patients obtained 16 males and 9 females (Table 1).

Table 1. Characteristics of patients with suspected pulmonary tuberculosis and non-TB based on gender

Characteristics	Suspected lung TB	Non-TB	Total
Males	20 (60.6%)	16 (64%)	36 (64%)
Females	13 (39.4%)	9 (36%)	22 (36%)
Total	33 (100%)	25 (100%)	58 (100%)

Based on gender, this study showed that most patients were males. This was accordance with Hussain *et al.*[12] and Buntuan[13] who revealed that the proportion of males was more than females.[12,13] It can be caused due to smoking in males that facilitate the spread of lung TB.[9,12] The habit of smoking will damage lung defense mechanisms/mucociliary clearance.[12]

The age range of patients with suspected tuberculosis and non tuberculosis were mostly 40–49 years. Mean age of the patients with suspected tuberculosis was 44.33 years (SD 12.9 years) with a range of 23–68 years. These results were consistent with the WHO report and Aderemi *et al.* study.[14,15] Mean age of non-TB patients was 51 years (SD 15.98 years) with a range of 23–73 years.

The results of AFB (Ziehl Neelsen staining) in 33 specimens of pulmonary tuberculosis patients with positive cultures of *M.tuberculosis* was 28 (84.8%) specimens with positive smear and 5 (15.2%) specimens with negative smear. The result of *M.tuberculosis* culture in 25 non-TB patients showed 23 (92%) patients with negative smear and 2 (8%) patients with positive smear suffering from lung abscess (Table 2).

Culture of *M.tuberculosis* is still the gold standard for TB diagnostics. Negative smear results can be caused by a number of bacteria which were too small (less than 5000 bacteria/mL sputum), the quality of sputum and low skilled laboratory assistants.[16,17]

The result of Fastsure TB DNA Rapid Test in 33 specimens from 33 patients with positive *M.tuberculosis* culture showed 28 (84.8%) as positive and 5 (15.2%) as negative. The result of Fastsure TB DNA Rapid Test in 25 non-TB patients with negative *M.tuberculosis* culture showed 2 (8%) positive in patients with lung abscess and 23 (92%) negative in other lung diseases (COPD, bronchial asthma, lung carcinoma, pneumonia, bronchitis) (Table 3).

Tabel 2. The results of microscopic examination of smear compared to the gold standard of *M.tuberculosis* in patients with pulmonary tuberculosis and non-TB

AFB examination	*M.tuberculosis* culture of TB patients		*M.tuberculosis* culture of non-TB patients	Total
	Positive	Negative	Negative	
Positive	28 (84.8%)	0 (0%)	2 (8%)	30 (51.7%)
Negative	5 (15.2%)	0 (0%)	23 (92%)	28 (48.3%)
Total	33 (100%)	0 (0%)	25 (100%)	58 (100%)

Tabel 3. The result of Fastsure TB DNA rapid test compared to culture of *M.tuberculosis* of whole specimens

Fastsure TB DNA rapid test	*M.tuberculosis* culture of TB patients	*M.tuberculosis* culture of non-TB patients	Total
	Positive	Negative	
Positive	28 (84.8%)	2 (8%)	30 (51.7%)
Negative	5 (15.2%)	23 (92%)	28 (48.3%)
Total	33 (100%)	25 (100%)	58 (100%)

This study obtained positive *M.tuberculosis* smear and Fastsure TB DNA Rapid Test in patients with lung abscess but negative cultures. Lung abscess can also be caused by *M.tuberculosis*.[17] Possibly, these patients suffered from pulmonary tuberculosis with lung abscess and already received TB treatment so the bacteria died and could not grow in LJ media. Staining of TB smear and Fastsure TB DNA Rapid Test can not distinguish live or dead bacteria.

Fastsure TB DNA Rapid Test is a molecular examination using sputum for TB diagnosis and only requires a small number of bacteria less than 10/mL sputum. Consistency of mucopurulent sputum may cause false negative results if sample preparation was not done properly. There was a possibility of bacteria trapped inside of solid particles and thick mucus so the bacteria would be discarded. Extra buffer and vortex is needed to homogenize and aspirate supernatant carefully. False-negative results on Fastsure TB DNA Rapid Test with CPA method was also caused by CPA inhibitor like hemoglobin in sputum containing blood.[19] Bloody sputum can inhibit amplification and hybridization.

Statistical analysis of Fastsure TB DNA Rapid Test with LJ culture as the gold standard was presented in Table 4.

This study showed that the sensitivity and specificity of Fastsure TB DNA Rapid Test compared to culture of *M.tuberculosis* were 84.8% and 92%, respectively with a kappa coefficient of 0.757, indicating a good agreement.[20,21] If the patients with lung abscess were TB patients, the specificity became 100%. This is consistent with a previous study by Hussain *et al.*[13] who examined Fastsure TB DNA Rapid Test with 82.45% sensitivity and 100% specificity.[13] Fastsure TB DNA Rapid Test is specific enough for the detection of *M.tuberculosis* in sputum.

Fastsure TB DNA Rapid Test with CPA method is a device that detects the amplified products using nucleic acid lateral flow strip in a sealed plastic device. Hybridization and amplification product is detected in cross contamination-proof lateral flow DNA strip device, thereby reducing false positive results. The results are then read visually.

Some factors that should be considered in the examination of Fastsure TB DNA Rapid Test were minimal sputum volume was 1 mL and vortex after the first washing for increasing the positivity. Mucopurulent sputum required dilution with a larger buffer volume (greater than 1: 2.5) and shaking more often to obtain homogeneous samples. Sputum volume less than 1 mL may cause false negative results. Not properly pipetting can cause that small amounts of bacteria were discarded so that the results will be negative. Samples containing blood can inhibit amplification and hybridization if not done properly. Fastsure TB DNA Rapid Test can not distinguish the live and dead bacteria so clinical evidence of TB is needed to establish TB diagnosis.

CONCLUSION AND SUGGESTION

Fastsure TB DNA Rapid Test is a method that combines NAAT and chromatographic lateral-flow immunoassay. It should be noted that minimal sample volume is 1 mL and vortex is needed after the first washing. This examination is fast, simple, relatively inexpensive for the diagnosis of *M.tuberculosis* from clinical specimens, especially in negative smears. Sensitivity and specificity of diagnostic Fastsure TB DNA Rapid Test were 84.85% and 92%, respectively. Further study with certain diluents prior to examination would be required for pretreatment of

Table 4. The result of Fastsure TB DNA Rapid Test with culture of *M.tuberculosis* on LJ media as the gold standard

Diagnostic test		95% confidence interval
Sensitivity	84.85%	69.08–93.35
Spesificity	92%	75.03–97.78
Positive predictive value	93.33%	78.68–98.15
Negative predictive value	82.14%	64.41–92.12
Eficiency of diagnostic	87.93%	77.12–94.03
Likelihood ratio (+)	10.61	3.931–28.61
Likelihood ratio (-)	0.1647	0.1105–0.2455

mucopurulent sputum and need to investigate other causes of false positive and negative in a research. Correct motivation is needed for suspected TB patient in expectorating the sputum. Specimen from saliva can cause false negative results because they do not contain acid fast bacilli or false positive if contaminated by bacteria causing cross-reactions, such as those listed in the kit.

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