lipL32 and secY Based-Quantitative Real-Time Polymerase Chain Reaction as Rapid Diagnosis to Detect Clinically Suspected Leptospirosis Patient in Indonesia

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INTRODUCTION

Similar symptoms between leptospirosis and other infectious disease causing delayed in the treatment of the patient increasing fatality rate

Fatality

The existing serodiagnosis test had several drawbacks that would become a challenges to the Leptospirosis diagnostic.

Serodiagnostic MAT Test

- requires highly skilled lab workers,
- a long time to analyze data, and
- assurance culture maintenance of reference serovars to detect antigens of *Leptospira*.

Lateral Flow Assay (LFA)-IgM Rapid Test

had various sensitivity levels and were less sensitive for early diagnosis of leptospirosis

Developing a more reliable diagnostic test to detect *Leptospira* with qRT-PCR by a specific gene.

OBJECTIVE

The objective of this study is to detect leptospirosis through qRT-PCR using *lipL32* and *secY* genes.

METHOD

Eligible patient according to WHO guidelines of leptospirosis symptoms

Acquired 30 serum sample

MAT test
Performed at referral hospital for MAT test in Indonesia, Dr. Kariadi General Hospital Medical Center, Semarang, Central Java

DNA Extraction
Using DNAeasy Blood and Tissue (QIAGEN) from 200 µL serum

qRT-PCR Test
Performed by 7500 Fast Applied Biosystem using the DNA-binding dye technique, SYBR Green

Specificity Test of Primers
against pathogen Malaria, Hepatitis B virus, Dengue Virus, *Escherichia coli*, *Enterobacteria* and *Mycobacterium tuberculosis*

Data Analysis

RESULT

Table 1. Comparison of *lipL32* qRT-PCR with MAT result

qRT-PCR	MAT Result		Total
	Positives	Negatives	
Positives	14	16	30
Negatives	0	0	0
Total	14	16	30
Parameter	**Estimate**		
Sensitivity	100.00 % (14/14)		
Specificity	0.00 % (0/16)		
Positive Predictive Value	46.67 % (14/30)		
Negative Predictive Value	undetermined (0/0)		

Table 2. Comparison of *secY* qRT-PCR with MAT result

qRT-PCR	MAT Result		Total
	Positives	Negatives	
Positives	9	5	11
Negatives	5	11	16
Total	14	16	30
Parameter	**Estimate**		
Sensitivity	64.29 % (9/14)		
Specificity	68.75 % (11/16)		
Positive Predictive Value	81.82 % (9/11)		
Negative Predictive Value	68.75 % (11/16)		

Table 3. qRTPCR result of *Lipl32* and *SecY* genes against other pathogens

Pathogens	qRTPCR Result	
	Lipl32	*SecY*
Hepatitis B virus	-	-
Dengue Virus	-	-
Escherichia coli	-	-
Enterobacteria	-	-
Mycobacterium tuberculosis	-	-
Malaria	-	-

It should be noted that MAT showed better results in confirming serum that developed more than five days of infection while qRT-PCR is better to diagnose in early infection of leptospirosis. Hence, the discrepancies between these two-diagnosis method are unavoidable.

CONCLUSION

Our study indicate that *lipL32* genes showed promising results than *secY* to support diagnosis of leptospirosis where the gold standard method is difficult to access.