



GOVERNMENT OF KERALA

Abstract

Health & Family Welfare Department - Standard Operating Procedure for Molecular Diagnosis of Leptospirosis - orders issued - Reg

HEALTH & FAMILY WELFARE (F) DEPARTMENT

G.O.(Rt)No.2249/2022/H&FWD Dated, Thiruvananthapuram, 17-09-2022

Read Letter No. PH4/52179/2022/DHS dated 29.08.2022 from the Director of Health Services, Thiruvananthapuram.

ORDER

In order to ensure early testing for effective confirmatory diagnosis of the Leptospirosis disease, Government are pleased to issue "*SoP for Molecular Diagnosis of Leptospirosis*", as annexed to this order.

(By order of the Governor)
B SURENDRAN PILLAI
ADDITIONAL SECRETARY

To:

The State Mission Director - National Health Mission, Thiruvananthapuram.

The Director of Health Services, Thiruvananthapuram.

The Director of Medical Education, Thiruvananthapuram.

The Director, Public Health Labs

The Managing Director, Kerala Medical Services Corporation Ltd

All District Medical Officers (Health)

Principal Accountant General (A&E/Audit) Kerala.

Health & Family Welfare (B) Department

Information & Public Relations (Web & New Media) Department

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Forwarded /By order

Signed by Savitha M V

Date: 17-09-2022 12:06:08

Section Officer

Copy to:

Private Secretary to the Hon'ble Minister (Health)
PA to Principal Secretary (Health)

S.O.P for Molecular Diagnosis (PCR) of Leptospirosis

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Molecular Diagnosis of Leptospirosis

1. Background:

Human is the accidental host in leptospirosis and gets infection through contact with infected urine or from infected animals or water and soils contaminated with infected animal urine. The routes of entry are cuts or abrasions of the skin, or through mucosal membranes. Leptospire will not get transmitted from human to human. Serological Tests/Antibody tests are commonly used currently for the diagnosis of Leptospirosis in Regional Laboratories. Detection of specific antibody of the IgM class to leptospira genus specific antigen by ELISA is a valuable screening procedure for the diagnosis of acute infection. However they have their own limitations. Molecular diagnostic tests, (RT-PCR) can conclusively diagnose Leptospirosis as early as the first week in patient's blood and urine specimens.

The gold standard for the diagnosis of leptospirosis is isolation of *Leptospira* bacteria. However, isolation of bacteria is time and resource-intensive; it requires level III or above biocontainment facilities and highly skilled technical personnel to handle samples and live bacteria for eventual identification and typing. Handling all live leptospira involves risk of laboratory infection and very strict bio safety rules must be observed. In order to avoid these disadvantages, methods based on the polymerase chain reaction (PCR) are becoming very useful and considerable progress has been made recently to improve their sensitivity, specificity, and technical ease and to lower costs.

Detection of Leptospiral DNA from clinical material by various methods has been developed. Dot-Blotting, In-situ Hybridization were used before the age of PCR and the sensitivity of radio labelled probes was lower approx. 10^3 leptospire /ml. Polymerase chain reaction (PCR) based assays are increasingly used for the detection of leptospire in tissues and body fluids of humans and animals because of their perceived sensitivity and capacity to give an early diagnosis. Real-time PCR is faster than regular PCR and less sensitive to contamination.

2. Molecular Tests (real time PCR) for the Diagnosis of Leptospirosis:

Early diagnosis of Leptospirosis is a diagnostic challenge as serological tests are often time consuming and unable to detect the infecting serovar. PCR tests are confirmatory tests for Leptospirosis and will aid in the early disease confirmation and public health Surveillance. In the wake of rising number of cases of suspected Leptospirosis, Government of Kerala has set up Molecular Diagnosis Facility for **Leptospirosis in designated Laboratories (Annexure 1)**.

Indications for Sending Samples for RT-PCR for Leptospirosis:

RT-PCR is a **confirmatory test** and should not be used as a screening tool for Leptospirosis infection.

Only in the following cases, the samples should be send for Leptospirosis Molecular Tests.

- Samples from Suspected Cases of Leptospirosis forwarded through **District Surveillance Officer** of the District for Epidemiological **Confirmation**
- Specimens from Suspected Deaths of Leptospirosis Cases
- *Hospitalised Critically ill/Severely ill cases in the first week of illness (as identified by treating clinician and forwarded by District Surveillance Officer) for early diagnosis.*
- Hospitalised/Severely Ill Cases presenting as ARDS/Multi-Organ Failure but without any attributable cause but suggestive clinical features of Leptospirosis *(as identified by treating clinician and forwarded by District Surveillance Officer)*

Recommended samples for RT-PCR Test:

- Whole blood collected in the first week of illness (in the first 4 days is ideal, but can be sent up to 10 Days . (The Whole Blood Sample to be collected and sent in EDTA bottle)
- Urine (collected at least 10 days after symptom onset is ideal)

Collection, Transportation and Storage of Samples :

Blood: 5 ml of Whole Blood to be collected in EDTA bottle. (If facility available, centrifuge the EDTA sample at low speed- 1000-1500 rpm for 5 minutes and send the plasma with buffy coat to the referral lab). Three-layer Packing to be done and sealed and transported to the laboratory by maintaining cold chain. Avoid freezing and multiple thawing of the whole blood specimen.

Urine: 15-20 ml of freshly voided Mid Stream Urine sample can be sent to the referral laboratory by maintaining cold chain (as applicable).

CSF: 1.5-2 ml of CSF can also be sent in cases of suspected cases of Meningitis (if clinically indicated)

Points to be noted (while sample collection):

Urine should be collected in a sterile wide-mouth container

(The following instructions to be communicated to the Patient)

- Carefully clean the peri-urethral area with soap and plenty of water.
- Discard first voided sample and subsequent midstream urine is collected in sterile wide mouth container and handed over to the health care personnel.

The sample to be transported from the collection centre to the Molecular Lab immediately to avoid multiplication of contaminants preferably (However if delay is expected, specimen should be kept cool preferably at 4 to 8^o C).

Each sample should be properly labelled mentioning the name, date of collection, and accompanied with a duly filled proforma with relevant clinical details.

Sample Rejection Criteria (For the Molecular Laboratory):

- Samples without proper labelling and packaging
- Samples without referral letter /intimation from the District Surveillance Unit
- Samples without proper maintenance of Cold-Chain
- Samples received with spillage/leakage
- Blood Samples not received in EDTA

In case of rejection of samples, the Nodal Officers of the Molecular Laboratory to duly intimate the same to the concerned District Surveillance Officer.

Testing and Reporting (For the Molecular Laboratory):

- The Molecular Laboratories performing the PCR test to follow the technical S.O.P for the test procedure provided from State Public Health Laboratory, Thiruvananthapuram (for using the in-house kits) and the Kit Literature of PCR kits (Commercial Kit).
- The PCR laboratories to complete the test and communicate the test results within 24-32 hours of receipt of the samples in the laboratory to the District Surveillance Officer and the Sample Collection Centre (Institution) from where the samples were sent to the referral laboratory.
- A Clinical Microbiologist / Molecular Biologist to be assigned as the Nodal Officer -in-charge of the PCR tests who shall also be the authorised signatory for the PCR test reports.

Procurement of the Test Kits (For the Molecular Laboratory) :

- The tests kits for the PCR tests (Extraction and PCR kits) to be purchased either using the Communicable Diseases Plan fund or the NHM-PIP (Communicable Diseases fund/PPCL)

through KMSCL upon request received from the Medical Officer in charge of the concerned laboratory to the respective District Surveillance Officers. The provision of the purchase of the same through the XV-FC (Health) Grant and LSGD funds can also be sought.

Quality Control & Monitoring:

District Surveillance Officers are encouraged to send blinded QC (Quality Control) samples to the referral testing laboratories at periodic intervals to ensure continued quality of the test results after consultation with the State Nodal Officer (Leptospirosis/Zoonoses) and State Surveillance Officer.

Internal QC- Blinded Samples (One PCR Positive Specimen and One PCR Negative Specimen) to be sent to State Public Health Laboratory, Thiruvananthapuram every three months by all the testing Labs.

External QC- Three Leptospirosis PCR positive specimens and three Leptospirosis PCR negative specimens to be sent to ICAR-NIVEDI, Bangalore every six months by all the testing labs after intimating the State Nodal Officer (Leptospirosis/Zoonoses) and State Surveillance Officer. The samples to be send blinded to ICAR-NIVEDI with the details shared with State Nodal Officer/ State Surveillance Officer.

For Technical Issues regarding the same, the following Officer may be contacted:

(Dr Nikhilesh Menon R Clinical Microbiologist & Field Epidemiologist, Ernakulam - 9446407443)

Annexure**List of the existing Laboratories with Facility for Leptospirosis PCR**

<u>Sl No</u>	<u>Name of the Testing Labooratory</u>	<u>Districts from which samples to be send</u>
1	State Public Health Lab, Thiruvananthapuram	Thiruvananthapuram, Kollam
2	Regional Public Health Lab, Pathanamthitta	Idukki, Kottayam, Pathanamthitta
3	Regional Public Health Lab, Ernakulam	Ernakulam
4	Regional Public Health Lab, Kozhikode	Palakkad, Malappuram, Kozhikode
5	Regional Public Health Lab, Kannur	Kannur, Kasargode
5	ICMR- National Institute of Virology, Field Unit, Alappuzha	Alappuzha
6	VRDL, Government Medical College, Thrissur	Thrissur
7	VRDL, Government Medical College, Thiruvananthapuram	Thiruvananthapuram
8	VRDL, Government Medical College, Kozhikode	Kozhikode
9	District Public Health Lab, Sulthan Bathery, Wayanad	Wayanad

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