



**COVID-19 (nCorona) Virus Outbreak Control and Prevention State Cell  
Health & Family Welfare Department  
Government of Kerala**

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**No.31/F2/2020/Health- 1 – 12<sup>th</sup> May 2020**

**ADVISORY ON RT-PCR POOLED TESTING FOR COVID-19**

**REF: ICMR Advisory on Pooled testing dated 13<sup>th</sup> April 2020**

**Background:**

As the Government of India has given permission for people to return to their home state post lock down relaxation, the number of people are coming back from vulnerable areas and hot spots abroad and from within the country. This has necessitated to have a proper COVID19 screening and confirmed detection mechanism. The COVID 19 pandemic has resulted in international supply chain break down. Therefore, in order to use the available resources optimally, it is decided to conduct RT-PCR pooled sample testing strategy.

Indian Council of Medical Research (ICMR) has recommended the use of pooled testing based on the feasibility study conducted at DHR/ICMR Virus Research & Diagnostic Laboratory (VRDL) at King George's Medical University (KGMU), Lucknow (annexure-1).

This advisory stipulates the concept of pooled sample technique. The field teams may select the category of patients and the number from each category as per the Sentinel Surveillance Advisory issued from time to time.

**Objective:**

The objective of the pooled sample testing strategy is to enable screening of wider and larger number of persons at risk for COVID-19.

The **District Surveillance Officer (DSO)** for COVID-19 shall be in charge of the process of implementing the collection and management of samples for pooled testing. Directions and follow up shall be done by the DSO to ensure the proper implementation of the pooled sample testing strategy at the district level.

**Eligible persons or samples** for pooled sample testing are as follows:

1. Samples of persons selected under sentinel surveillance as per the Advisory regarding Sentinel Surveillance.
2. Samples taken from persons who have come from outside Kerala by Air, surface or Sea route from 7<sup>th</sup> May 2020 onward. The details shall be provided in the Sentinel surveillance advisory.

**Biological material to be collected:**

Nasopharyngeal or Oropharyngeal secretions.

Each individual's sample is to be collected in the modified Viral Transport Media (mVTM) 0.5 ml provided. The samples are to be collected individually in the VTM. The samples shall **NOT** to be pooled at the point of collection.

**Modified Viral Transport Media (mVTM):**

The samples eligible for pooled sample testing should be collected **ONLY** using the **modified VTM of 0.5 ml**. These modified VTMs shall be provided to all the districts for the purpose of pooled sample testing.

**Methodology on pooled testing by Laboratories:**

1. The tests are to be conducted as pooled test; maximum of five samples in each pool.
2. 50  $\mu$ l from each of the five samples is to be taken and pooled.
3. From this 250  $\mu$ l of pooled sample, RNA should be extracted using the required volume specified in the RNA Extraction Kit protocol
4. Do real time PCR.
5. Report all individual samples in a negative pool as negative.
6. If the pool is positive, do RNA extraction from individual VTM and do real time PCR individually.
7. Report each sample individually.

**Sample package and transportation:**

The sample packing and transportation procedures are the same as per the existing guidelines on sample testing, collection and transport for laboratory diagnosis of COVID-19.

**Labelling of samples:**

All samples collected under the pooled testing strategy should be labelled as **"pooled testing"**.

**Designated Laboratories:**

The designated laboratories for Sentinel Surveillance samples shall also be the designated laboratories for the pooled sample testing.

The lab in charge shall conduct the RT-PCR pooled tests on the samples labelled as **"pooled test"** as per the methodology for pooled sample testing. The reporting of results shall also be done accordingly on the digital platform provided.



**Principal Secretary**

INDIAN COUNCIL OF MEDICAL RESEARCH  
DEPARTMENT OF HEALTH RESEARCH

Date: 13/04/2020

**Advisory on feasibility of using pooled samples for molecular testing of COVID-19**

**Background:** Number of COVID-19 cases in India is rising exponentially. In view of this, it is critical to increase the numbers of tests conducted by laboratories. Positivity rate in cases is still low. Hence, it may help to use the pooled samples for screening. A pooled testing algorithm involves the PCR screening of a specimen pool comprising multiple individual patient specimens, followed by individual testing (pool de-convolution) only if a pool screens positive. As all individual samples in a negative pool are regarded as negative, it results in substantial cost savings when a large proportion of pools tests negative.

**Objectives:** To increase capacity of the laboratories to screen increased numbers of samples using molecular testing for COVID-19 for the purpose of surveillance.

**Methods & Results:** A feasibility study was conducted at DHR/ICMR Virus Research & Diagnostic Laboratory (VRDL) at King George's Medical University (KGMU), Lucknow. It has been demonstrated that performing real-time PCR for COVID-19 by pooling 5 samples of TS/NS (200 ul/sample) is feasible when the prevalence rates of infection are low. All individual samples in a negative pool to be regarded as negative. Deconvoluted testing is recommended if any of the pool is positive. Pooling of more than 5 samples is not recommended to avoid the effect of dilution leading to false negatives.

**Recommendations** for sample pooling for real-time RT-PCR screening for COVID-19 are as follows (based on the KGMU study):

1. Use only in areas with low prevalence of COVID-19 (initially using proxy of low positivity of <2% from the existing data. Still a watch should be kept on increasing positivity in such areas
2. In areas with positivity of 2-5%, sample pooling for PCR screening may be considered only in community survey or surveillance among asymptomatic individuals, strictly excluding pooling samples of individuals with known contact with confirmed cases, Health Care Workers (in direct contact with care of COVID-19 patients). Sample from such individuals should be directly tested without pooling
3. Pooling of sample is not recommended in areas or population with positivity rates of >5% for COVID-19

**Preferable number of samples to be pooled is five**, though more than two samples can be pooled, but considering higher possibility of missing positive samples with low viral load, it strongly discouraged to pool more than 5 samples, except in research mode.

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