

**INDIAN COUNCIL OF MEDICAL RESEARCH
DEPARTMENT OF HEALTH RESEARCH**

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 deconvolution) 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 is strongly discouraged to pool more than 5 samples, except in research mode.