Memo No: HPH/9M-21/2020/92 Dated: 18.04.2020

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

In order to increase the capacity of the laboratories to screen more numbers of samples using molecular testing for COVID-19 for the purpose of surveillance and as per ICMR Advisory published on 13.4.2020 on the subject, it is decided to use pooled samples for screening of suspected COVID 19 cases in West Bengal.

Recommendations for sample pooling for real-time RT-PCR screening for COVID-19 are as follows:

**Inclusion criteria**
- During community survey AND surveillance among asymptomatic individuals in areas where the prevalence rate of infection are low.

**Exclusion criteria**
- Exclude pooling samples of individuals with known contact with confirmed cases, and where the rate of infection is expected to be high.

**Interpretation:** 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 (FIVE) samples is not recommended to avoid the effect of dilution leading to false negatives.

The related guideline of ICMR dated 14/04/2020 is enclosed for the ready reference and guidance.

Director of Medical Education
West Bengal

[Signature]

Director of Health Services
West Bengal
Dear Colleague,

I congratulate you all for your outstanding efforts in containing the COVID-19 outbreak in India.

Taking cognizance of the increased load of sample testing at various laboratories, ICMR has developed guidance on pooling samples for testing. The same is attached for ready reference.

You may consider implementing this strategy after careful analysis of the recommendations given in the enclosed document.

With regards,

Yours sincerely,

(Balram Bhargava)

Prof. (Dr.) Balram Bhargava, Padma Shri
MD, DM, FRCP (Glasg), FRCP (Edin.),
FACC, FAHA, FAMS, FNASc, FSAc, FNA, DSc
Secretary to the Government of India
Department of Health Research
Ministry of Health & Family Welfare &
Director-General, ICMR

Indian Council of Medical Research
Department of Health Research
Ministry of Health & Family Welfare
Government of India
V. Ramalingaswami Bhawan, Ansari Nagar
New Delhi - 110 029

D.O.No.ECD/Misc./COVID-19/2020
14th April, 2020

Secretaries/Principal Secretaries Health (All States)

Principal Secretary
H & F.W. Dept.

Tele.: 26586204, 26589620, Fax (Off.): 91-11-26588662, E-mail: secy-dg@icmr.gov.in
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.

Contributors:

- Dr. Shantanu Prakash, Dr. Amita Jain, DHR/ICMR VRDL, KGMU, Lucknow
- Dr. Kiran Rade, WHO-India