TAKSHASHILA INSTITUTION

Takshashila Policy Advisory

A Policy Guide to Pooled testing

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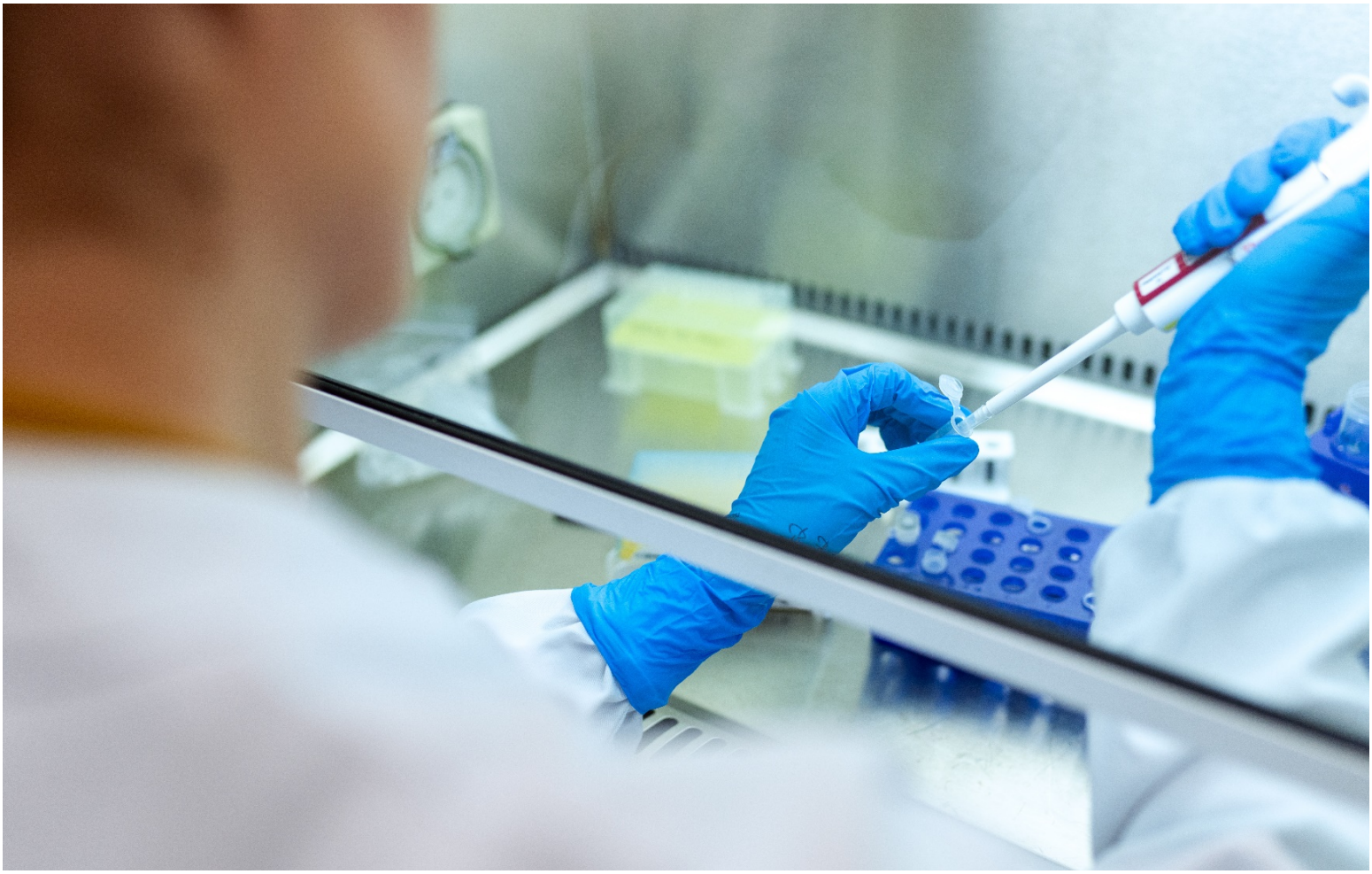

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Executive Summary:

Pooled testing is a valuable tool in the routine surveillance of diseases. However, it is not a substitute for conventional diagnostic testing. The Indian Council of Medical Research (ICMR) advisory on pooled testing suggests using pooled testing approach in areas of low prevalence of the disease. This advisory builds on the ICMR advisory and recommends the following:

1. For routine surveillance, employ pooled testing at the smallest administrative unit – ward in urban area and village in rural areas.
2. To confidently determine 2% positivity, a threshold of 8-10% of target population should be tested using conventional testing approach.
3. Businesses may use pooled testing for periodic screening of their employees.
4. Pooling strategies should be scientifically driven and adapted to the context.

Introduction:

On 14th April, 2020 ICMR released an advisory on pooling samples for diagnosis of COVID-19[1]. The objective of this advisory is to increase capacity of testing laboratories to screen samples for COVID-19.

The advisory recommends pooling of up to 5 samples in areas of low prevalence of COVID-19. Low prevalence area is defined as area with <5% positivity, with pooled testing highly recommended in areas with <2% positive cases.

Pooled testing has been previously used for surveillance of other diseases such as HIV and malaria[2,3].

This document builds on the ICMR advisory to outline key conditions under which pooled testing can be undertaken. We recommend pooled testing to be done at the level of the smallest administrative unit – a ward in a city/urban area or a village in rural areas. Further before starting pooled testing, at least 8% of the target population must have been screened using conventional Real Time-Polymerase Chain Reaction (RT-PCR). Finally, the benefit of pooled testing is only accrued when majority of samples tested are negative. Hence it is important to use pooled testing for samples not suspected to be positive.

What is pooled testing?

There are various methods to diagnose COVID-19. Real Time-Polymerase Chain Reaction (RT-PCR) is the gold standard method, recommended by both WHO and ICMR. RT-PCR detects the virus by amplifying and identifying the genetic material of the virus in the sample. In conventional RT-PCR, one sample obtained from one patient is individually tested. This means that for the screening test, 5 samples obtained from 5 different individuals require 5 RT-PCR test reactions.

Note: Sometimes 2 or more samples may also be taken from the same individual and tested in one pool. In the context of this paper, pooling refers to mixing of samples obtained from different individuals as described below.

Pooled testing is a technique of combining samples obtained from a few individuals into one test sample. This one test sample is then used for RT-PCR analysis to determine if any of the individuals suffer from COVID-19. ICMR recommends pooling 5 samples into one test sample. This means that for screening test, 5 samples requires one RT-PCR test reaction.

Note: The number of samples pooled is limited by the ability of the test to detect viral genetic material. As more samples are pooled, the sample material used per individual sample reduces. This leads to a reduction in the viral genetic material present in the test sample. If too many samples are pooled, it is possible that the amount of genetic material used per sample becomes too low for the test to detect it. This can result in false negatives – that is, samples which actually contain the virus, would also appear to be negative in the result. The recommendation to pool 5 samples is based on experiments performed at King George's Medical University, Lucknow, according to the ICMR advisory. It may be advisable to do similar experiments using locally used kits to determine the optimum number of samples for pooling. For the purposes of this document, a pooled sample of 5 individuals is considered, as recommended by ICMR.

If the test sample gives a negative result, all individual samples are treated as negative for COVID-19. The advantage of pooled testing is that it reduces the number of tests required to screen a population for COVID-19. However, this advantage of pooled testing works only when majority of samples are negative.

If the pooled test sample gives a positive result, the individual samples from that pool have to be individually tested. In this case, 5 samples require 6 RT-PCR test reactions. For positive samples, a second confirmatory test would also need to be performed.

If the target population has a high positive rate, it is likely that many pooled samples would show positive result and willvhave to be re-tested. This scenario

squanders the advantage of using pooled testing strategy and hence should be avoided.

COVID-19 status	Conventional RT-PCR for 5 individuals	Pooled testing (5 samples pooled)
All negative samples	5	1
One or more positive samples	5	6

Table 1: Number of RT-PCR reactions required under various conditions of pooled and conventional testing.

At what unit of administration can pooled testing be employed?

The decision of employing pooled testing is best made at the smallest administrative units – in urban areas, at the ward level and in rural areas at the village community level.

Hot spot areas within a city or district can skew the results of pooled testing and hence it would be recommended to deploy pooled testing approach to as small an administrative unit as possible.

Pooled testing can also be employed at the level of businesses/factories or business parks for routine surveillance of employees.

How to determine if existing positivity is 2%?

The ICMR guideline suggests pooled testing can be used for areas where positive cases are <2% of population of that area.

We would recommend that at least **8-10% of population** be initially tested to determine COVID-19 positivity for the population. India's test positivity rate is roughly 5%, which means for every 100 tests done 5 are positive.

At 2% positivity rate a population of 5000 people will have 100 cases. Given a Total Positivity Rate (TPR) of 5%, at least 500 individuals would have to be tested to reasonably deduce the 2% positivity rate to the population. The determination of positivity rate should not be based on fewer samples.

Samples from areas considered as hotspots and those with >5% positive rates should not be pooled.

Additional note: While ICMR recommends a 2% threshold for low prevalence of COVID-19, this may not be universally applicable. For example, a village which has reported no COVID-19 cases and has not allowed visitors post the beginning

of lockdown will likely be of low disease prevalence levels. In such areas, pooled testing may be allowed without the 8-10% population being initially tested.

Factors to consider when allowing pooled testing:

1. How likely is the target population to be COVID-19 positive – for example, metros or villages where cases have been already reported or which have seen population migrations since March 2020.
2. What is the likelihood of spread of disease if there were asymptomatic cases in the population – high population density areas are likely to host more cases than low density areas where social distancing is more feasible.
3. Travellers – Travellers by any mode of public transport are exposing themselves to new environments and people. During transit, a traveller from a low-risk area may come in contact with a traveller from a high-risk area and hence, public transport may itself prove to be a hub for disease spread. Hence, travellers should not be subjected to pooled testing.

Areas with previous history of COVID-19 cases and/or significant migration of people in the past 3 months should strictly follow the ICMR guidelines.

When to use pooled testing?

Community screening: Pooled testing is best suited to understand the prevalence of disease, if any, within a target population. For example, this approach can be used for screening a population that has been locked down and is now going to open up its operations. Pooled testing is best not used as a diagnostic measure for identifying positive individuals – while pooled testing will identify positive samples, there will be additional cost to verifying the infected individual(s).

Pooled testing for businesses/factories:
Businesses/factories can utilise pooled testing for routine surveillance of their employees. This purpose of pooled testing is different from testing big swathes of population, since businesses will test samples periodically and can take decisive steps on finding a positive sample.

It is however, important that such businesses continue to employ social distancing measures, temperature checking and hygiene measures to prevent the spread of COVID-19. There is an assumption here that people who come to work will likely be self-selected to be healthy. However, since COVID-19 causes asymptomatic infections, the business should be prepared to ramp up testing procedures, if a pooled sample does come positive.

For pooling samples, it would be optimal to pool samples from individuals who share a common working space. Since the disease is likely to spread to co-workers across shared spaces, there is a likelihood of such pools to give the same result.

What would be the ideal strategy to pool samples?

ICMR recommends pooling up to 5 samples in one test sample.

For community screening purposes, if enough tests are available for testing the entire target population, it would make sense to pool samples from family members into one test sample. If enough tests for the target population are not available, take one representative sample from a family member and combine with samples from within their adjoining social network.

Reasoning: Since social contact is highest within family members, it is likely that if one member is COVID-19 positive, at least a few other members would also be infected with the virus.

For any other patients who samples are to be tested by doctor's referral, the doctor should mark if the sample can be used for pooled testing based on their assessment of the patient. Samples suspected to be positive, even from a low prevalence area should not be recommended for pooled testing.

How many tests would I need per 1000 population to do the testing?

For a population of 5000 people, approximately 1000 pooled tests will be needed instead of 5000 tests for the single sample approach for screening.

At 2% positive rate – we would expect 100 positive samples. In worst case (if 100 samples get scattered across 100 pools, 500 tests would need to be repeated). Then the 100 positive samples would need to be subjected to confirmatory test as well. So total tests required will be 1600, instead of approximately 5100 tests.

Conclusion

Pooled testing is an excellent approach to increasing testing coverage in areas of low prevalence of disease. However, excessive pooling of samples can lead to false negatives, leading to a false perception of the disease spread. A threshold amount of testing (8-10% of population) needs to be first done, before embarking on pooled testing. Pooled testing might be useful to businesses in the routine disease surveillance of their employees.

References:

[1]Indian Council of Medical Research, Department of Health Research. Advisory on feasibility of using pooled samples for molecular testing of COVID-19. April 2020. https://www.mohfw.gov.in/pdf/letterregguidanceonpoolingsamplesfortesting001.pdf

[2]Krajden Mel, Cook Darrel, Mak Annie, Chu Ken, Chahil Navdeep, Steinberg Malcolm, Rekart Michael and Gilbert Mark. "Pooled nucleic acid testing increases the diagnostic yield of acute HIV infections in a high-risk population compared to 3rd and 4th generation HIV enzyme immunoassays." *Journal of Clinical Virology*, 61, Issue 1 (2014): 132-137 https://doi.org/10.1016/j.jcv.2014.06.024

[3]Hsiang Michelle S., Hwang Jimee, Kuene Simon, Drakeley Chris, Kandula Deepika, Novotny Joseph, Parizo Justin, Jensen Trevor, Tong Marcus, Kemere Jordan, *et al.* "Surveillance for malaria elimination in Swaziland: A national cross-sectional study using pooled PCR and serology." *PLoS One*, 7 (2012):e29550. doi: 10.1371/journal.pone.0029550

[4]Pradeep Thakur. "Covid-19: Positivity rate per 100 tests rises to 5% in last 2 weeks." Times of India. Published 25 May 2020. http://timesofindia.indiatimes.com/articleshow/75958512.cms?utm_source=contentofinterest&utm_medium=text&utm_campaign=cppst