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Glimpses of Concoction-Bane or Boon? Pooled Screening Test to diagnose COVID-19 infection by chip based Micro RTPCR of Asymptomatic Student Officers Attending Courses In Major Establishments in Armed Forces-An Institutional Experience

Brig Amit Sharma[1], Major Ragini Thapa[2], Hav Devender Singh[3]

[1]SM, Commandant Military Hospital Mhow
[2]Pathologist, Military Hospital Mhow
[3]lab Technical Staff, Military Hospital Mhow

ABSTRACT

Introduction:-COVID-19 Pandemic has quickly spread worldwide needs rapid testing on the prevalence of the virus in communities to enable rapid containment and enable parallel running of professional studying courses in various establishments under laid down guidelines. However, the equipment, human and laboratory resources required for conducting individual RT-PCR is taxing ,costly and time consuming. One technique to reduce the number of tests required is the pooling of samples for analysis bychip based RT-PCR test for COVID 19.

Objective :- To report and study our institutional experience in devising and implementing a pooling protocol and process for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using pooling of oropharyngeal swabs/samples collected and running micro reverse transcription polymerase chain reaction (RT-PCR) testing in Truenat (Duos) over an eleven months period in Covid 19 pandemic from (June 2020-April 2021) before conducting professional courses in military establishments under closed environment.

Materials and methods:-Pool testing was used for 1915 personnel attending different courses in army establishments with existing equipment(TRUENAT duos for micro RTPCR testing) to significantly increase thevolume of samples tested per day from asymptomatic course attending students arriving to military establishments for attending professional courses from different part of the country after 14 days of quarantine period. This is done by pooling multiple(05)swabs/samples in asingle test tube.

Results:-Total of 1915 army personnel were tested by pooled method of collection of throat swabs sample in Viral lysis media and using the TRUENAT system for screening and confirmation of COVID-19.Out of the 383pools collected from healthy course attending army officers, Junior commissioned officers, other ranks, health care workers and civil defence employees tonly10 pools tested out to be positive. Truenat (Molbio) is an indigenously developed portable version of CBNAAT, first time ever pooled testing was done by RTPCR in this machine in Military Hospital Mhow by permission of DGMS office and ICMR recommendations ,has successfully benefitted the various establishments to run courses much required professionally in armed forces setting.

Conclusion :- Light of the day can be seen by rigorously batting out Covid 19 infection by meticulous testing and attaining back normalcy under strict guidelines and following mandatory national protocols, facilitated by Pooled testing will greatly increase the volume of samples tested per day so that we can identify the asymptomatic carriers amongst healthy students attending armed forces professional courses . This approach should reducethe chance of infection and flatten the infection curve to baseline implementing 100% testing and cost effectiveness.

Key words:- Pooled chip based RTPCR testing ,COVID 19 Pandemic ,pooled variance, concordance

INTRODUCTION

The novel coronavirus, COVID-19, first detected in Wuhan, China, in late 2019, has encroached the world across posing threat that has spread throughout the world. Amidst crude case fatality rate COVID-19 poses the highest risk for serious disease and death [1]voluminous approach to testing for SARS-CoV-2 is one of themost important aspects of COVID-19 controlstrategy at present.[2]Meticulous Reporting on spot with acumen and right timing will result in

effective containment and progressive management against pandemic under prevailing measures and guidelines. Real-time reverse transcription-polymerasechain reaction (RT-PCR)-based molecular assays forvarious SARS-CoV-2 gene targets are the maintools of diagnosis for COVID-19 .[3,4] COVID-19 is diagnosed with RTPCR testing, which is common for virus monitoring and examines the presence of a unique genetic sequence(E GENE) of viruses in a samples taken from the patients. When carried out singly the test takes hours together and does not allow for monitoring of asymptomatic carriers in the population, which is vital to curb the epidemic, and run education and professional courses without a glitch in services, thus creating a bottleneck in identifying COVID-19 infected people.[5]According to the new pooling approach that we performed molecular testing on "combined sample," taken from course attending army personnels. This way we csignificantly accelerated the testing rate. Only in those cases, where the joint sample is found to be positive, we conducted individual test foreach of the specific samples, The widespread testing implemented for detecting SARS-CoV-2 infection screening for student attending army professional courses inresponse to the coronavirus disease 2019 pandemic ledto a significant decrease in turn around time and conservation of resources with implementation of courses on right duration without delay. To date, several institutions have implemented samplepooling, but publications documenting these experiences are rarity. Pooled screening for testing course officers by micro RTPCR(TRUENAt)attending courses in military establishments forms a regular feature of services provided by Military Hospital Mhowin current pandemic.

OBJECTIVE

Pooling RT-PCR samples has the potential to cost-effectively generate estimates of COVID-19 prevalence in resource limited environment providing accurate results in minimum time has proven to be a boon for the serving personnel's for continuing courses and scheduled training without a breach in widespread COVID pandemic.

MATERIALS AND METHODS

The study was conducted in Military hospital Mhow from June 2020 to April 2021.Oropharyngeal samples(total of 5 in a single medium)were collectedfrom low-positivity (<5%) course attending army personnel's ,residing in same block with similar time of travel and quarantine from same courses,under full safety precautions.The pooled oropharyngeal samples were transferred to a viral lysis media immediately

after collection and transported to laboratory for testing The pools were run in MH Mhow molecular laboratory in Pools offive (Total 383 pools amounting to 1915 individuals)were run on the Truenat™ SARS COV-2 chip based Real Time RT PCR testing Molbio Diagnostics system nCoV Kit (TIB Molbiol, Verna, Goa, India).Truenat™ SARS CoV-2 (REF 601420005/601420020/601420050) is a chip-basedReal Time Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the semi quantitative detection of SARS CoV 2 RNA in human oropharyngeal swab specimen and aids in confirmation of COVID-19. Sample is collected in viral lysis buffer. TrueNat system is also now a multiplexed point of care test that includes a single assaycomprising of both the screening (E gene) and confirmatory (Orf1a) targets in a single test. The testdetects the RdRp gene of the virus and is recommended as a follow on test forconfirmation of positive results with other gene targets of the virus in a single step . All negatives are to be considered as true negatives. All samples that test positive by this assay must be consideredas true positives confirmed by ICMR. RT-PCR turnaround times between samplecollection to result reporting were monitored and compared, before and after sample pooling implementation and All patients with a Ct value of less than 35 were considered positive and greater than 35 negative. Less than 24 hrs were taken to run the tests where each pool took around 90 minutes.

RESULTS

Total of 1915individual samples that is 383 samples were pooled in batches of 5 individual each pooled were tested over a11-month period (reported from the Truenat micro RTPCR system) in Military Hospital Mhow, allowing our laboratory tosave 2000 tests with controls, equivalent to a conservation rate of 52.22%.A 24 -hour or less turnaround time was generally maintainedthroughout the pooling period.Amidst 1915 individuals 69.30% were (FLW)frontline workers/officers, followed by JCO(Junior commissioned officers),and health care workers in total of 2.76%(Fig 1). Pooled sample of asymptomatic course attending low risk personnel's was carried out after 14 days of preliminary quarantine ,after history of travel to Mhow cantonment by flight, road and railway facility for attending various professional courses. Pools were made out of students staying in the same room, in case of single occupant of a room sharing same block ,or staying in close proximity of less than 30 metres .Incase student was with a family the entire family was pooled. Pool samples of 5 army personnel each was taken according to ICMR and WHO ,pooling guidelines for testing. If pools were negative,no further testing was carried out for confirmation,in case of positive pools ,all the samples earmarked from individual pools of the clustered pool group were run again .Out of 383 pools carried out successfully in the hospital ,we had 10 pools positive(10/383) (2.6%).The combined CT scores for pools tested positive was mostly in the medium range of E gene=25, ORF value=26(Fig 2).When the tests were run separately, we obtained 1 sample positive out of 5 samples in 7 pools, and two samples out of 5 samples in one pool and lastly 3 samples positive out of samples of 5 combined. The individual CT scoring of these asymptomatic sample

tested positive turned out to be almost in moderate range to low range near to the positive pool values.(Fig 3). Positive samples included in creating pools ranged from a Ct value of E gene-24 cycles to a maximum of 30 Cycles and ORIF values ranging from 25 to 28 .On an average, Ct values obtained with the 5-sample pooled testing exceeded individual sample testing by1.5±1 cycles(E Gene values) &2±1.5(ORIF value)Fig 4.5-samplePooling concordance rates with individual sample testing werehigh and false-negative rates were low and specificity high(61%). This was especially true for samples with Ct values less than 30 cycles (in mild to moderate categorical load of infections).The pooled variance calculated for the 10 pooled sample CT values and independent sample tested from positive pools CT value was 3.267(S^2 p=(SS1+SS2)/(df1+df1)=58.8/18=3.267),also the Kendell Coefficient of concordance for the individual samples and 10 combined pools of 5 samples each is falling in substantial range with 96% concordance(range 0.95-0.96){Formula used;- W(Kendell's Coefficient of concordance weighed on CT values =12s(sum)/m^2(n^3 -1) .[6]The concordance rates between 5-sample pools and individual sampletesting for samples with Ct values less than or equalto 30 cycles were 96% for ourlab. Fig 4.

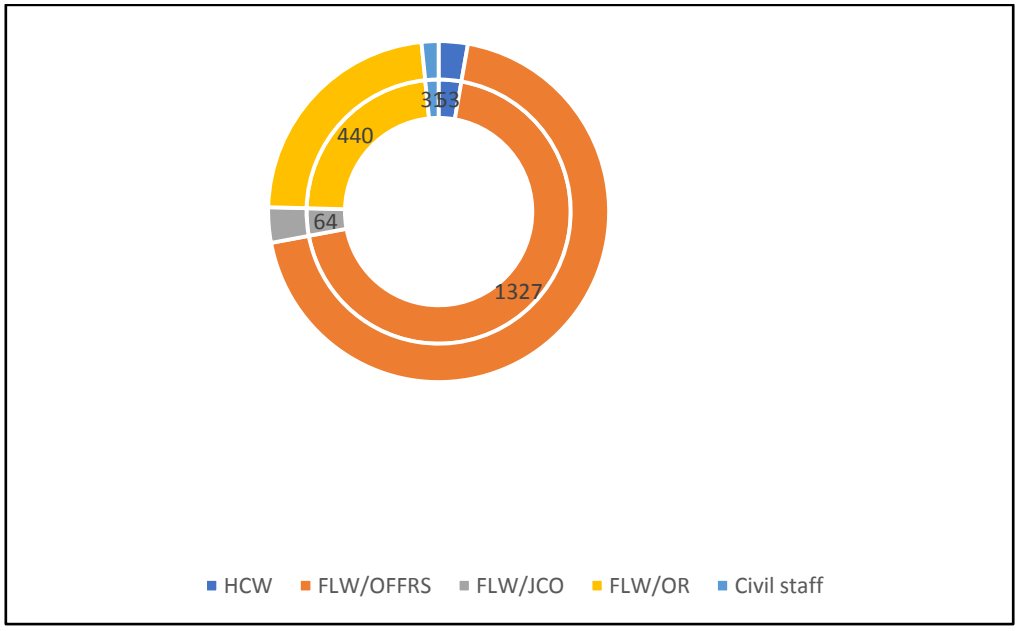

Fig1:-Shows Distribution Of Health Care Workers(HCW),(Front Line Workers)FLW And Civil Employees In Military Hospital MHOW

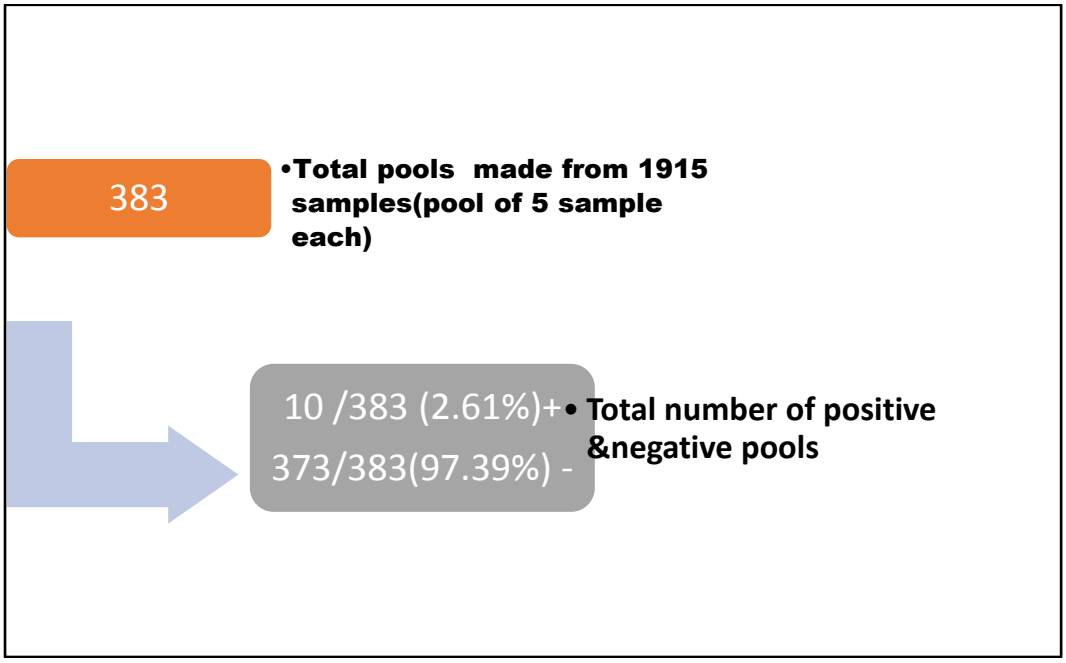

Fig 2:-Total pools made from individual course attending army personnel

Infected pool	Pool CT scoring	Individual sample CT Score Nd-not detected	No. of tests positive in pool	Kendels Concordance Coeficient	Pooled variance value
Pool 1	E- 27, ORIF-28	E1- 25 ORIF-26E2- ND ORIF-ND E3- ND ORIF- NDE4- ND ORIF- ND E5- ND ORIF- ND	01/05	Falls in the range 0.95-0.99	3.267 for 10 Positive pools
Pool 2	E- 28, ORIF-29	E1- 27 ORIF-28E2- ND ORIF- ND E3- ND ORIF- NDE4- ND ORIF- ND E5- ND ORIF- ND	01/05	Strength Of Agreement Between Original Pool And Individual Pool Is Substantial	
Pool 3	E- 26, ORIF-27	E1- ND ORIF- NDE2- ND ORIF- ND E3- ND ORIF- NDE4- ND ORIF- ND E5- 25 ORIF- 26	01/05		
Pool 4	E- 30, ORIF-28	E1- ND ORIF- NDE2- ND ORIF- ND E3- ND ORIF- NDE4- 29 ORIF-28 E5- ND ORIF- ND	01/05		
Pool 5	E- 27,ORIF-26	E1- 26 ORIF- 26E2- ND ORIF- ND E3- ND ORIF- NDE4- ND ORIF- ND E5- ND ORIF- ND	01/05		
Pool 6	E- 27,ORIF-28	E1- ND ORIF- NDE2- ND ORIF- ND E3- ND ORIF- NDE4- ND ORIF- ND E5- 25 ORIF-26	01/05		
Pool 7	E- 27,ORIF-28	E1- ND ORIF- NDE2- ND ORIF- ND E3- ND ORIF- NDE4- ND ORIF- ND E5- 26 ORIF-27	01/05		
Pool 8	E- 24,ORIF-25	E1- 22 ORIF-24E2- ND ORIF- ND E3- ND ORIF- NDE4- ND ORIF- ND E5- 24 ORIF-25	02/05		
Pool 9	E- 26,ORIF-25	E1- 25 ORIF-26E2- ND ORIF- ND E3- ND ORIF- NDE4- ND ORIF- ND E5- 24 ORIF-24	02/05		
Pool 10	E- 24,ORIF-26	E1- 22 ORIF-24E2- ND ORIF- ND E3- 22 ORIF-24E4- ND ORIF- ND E5- 24 ORIF-25	03/05		

Fig 3:- Pool Ct (cycle threshold) of 10 positive pools out of 383 tested, with individual CT scores (E gene, ORIF values)of 5 samples in each poolwithKendels concordance coefficient and pooled variance score;-

DISCUSSION

Pooling of samples can help accelerate the surveillance for COVID-19 identification in a community or group of asymptomatic students /personnels attending professional courses in closed establishments after travel history and 14

days of quarantine, living together under well enacted quarantine guidelines by government of India MOHFW dated February 2021. If found positive, tracing back to the individual(s) can be done by individual sampling exercise of the pooled sample found positive. In some countries this technique is already being used to make COVID-19 testing more cost-effectivelikeIn Israel, researchers at Technion – Israel Institute of Technology and Rambam Health Care Campus successfully identified a positive carrier from a pooled analysis of 64 samples.[7] In the US, the Nebraska Public Health Lab pooled 60 samples obtained from across the state.[8]The first pooled RT-PCR method suggested for classifying the infection rate in a population using group testing is viable when population prevalence is low. When prevalence is high, then it is likely that the optimal size of the pool will have to be very small [9]. The optimal and accurate pool size depends on the sensitivity of the test, and this is a function of viral load. Salivary viral load has been found to be highest in the first week after the onset of symptoms..One limitation of pooling multiple RT-PCR samples is that the sensitivity of testing is reduced as in our experience the sensitivity ranged between 46-50 % and specificity was 60-61% for pooled samples.Pooled sample testing has been considered as asimple and practical approach for improving the testingoutput while minimizing the resources being utilizedfor real-time RT-PCR. Several studies from differentcountries have reported different pooling strategies10-16and are included in Fig5.Pooling of oropharyngeal swabsamples in VLM was done before RNA extraction. Recent studies and pre-prints have reportedsuccessful pooling of extracted RNA for up to 32different samples[7,8,17,18]. PerformingRNA extraction methodology and then pooling only saves resources. The RNA extraction step remains one of the most ratelimitingsteps for SARS-CoV-2 RT-PCR15 in terms ofusage of reagents as well as time management.

Country	Pooling strategy	Size of pool	References
Israel	Pooling of extracted RNA	(32/pool)	11
Chile	Chile Pooling of nasopharyngeal samples in universal transport medium	5 samples/pool	12
Germany	Pooling of extracted RNA before RT‑ PCR amplification	Range of pool sizes of extracted RNA(4‑ 30/pool)	10
Germany	Pooling of swabs directly in a ‘pool container’ after being placed in an ‘archive’ container	5 samples/pool	14
Israel	Israel Combinatorial pooling strategy where each sampleis a part of multiple pools. Liquid dispensing robot used to create pools.	348 patient samples were tested in 48 pools	15
USA	Pools of 5 samples 50 µl each with one positive in each pool were evaluated	5 samples/pool	13
Spain	Pooling of nasopharyngeal specimens from universal transport medium	Pool sizes of 5 samples/pool and 10 samples/pool evaluated	16

Figure5;- Strategy for pooled real‑ time RT‑ PCR testing reported from different countries

CONCLUSION

It is recommended under existing WHO and ICMR guidelines, use of the pooled sample(5 sample) method for the detection of SARS-CoV-2 in low risk groups by RT-PCR in community surveillance aiding in facilitation of professional courses regularization in pandemic, particularly in resource limited settings where testing kits, facilities, and technical man power are scarce. Our best estimates are that for the currently estimated prevalence of COVID-19 in closed establishments conducting courses, a single cluster/pool of5 tests could be combined from asymptomatic course undergoing students for SARSCoV-2 detection by chip based Micro RT-PCR is a beneficial andacceptable strategy for low viral loads saving our resources and timely fore front management on war footing. Hence Pooling of samples and testing is a cost-effective technique for providing much-needed effective screening and detection of COVID-19 infection in asymptomatic low risk groups for facilitating institutional training.

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