31st August 2020

Protocol for testing pooled specimens for SARS-CoV-2 on GeneXpert

Interim guidance to Pacific Island Countries and Territories

Background

Pooling of samples for testing is used for high throughput screening in large epidemic or pandemic where there is rapid expansion of testing that may exceed laboratory testing capacity. In such situation sample pooling may provide a pathway to maximise testing capacity while still preserving reagent supply or staffing resources and maintain a viable and functional testing algorithm that better meets Public health demand[1].

Sample pooling is the process of mixing multiple samples together and testing the 'pool' as if it were one sample. A negative result for a 'pool' means that all specimens in that pool are reported as negative, while a positive result means that the specimens that make up that particular pool must then be tested individually to determine which of the sample(s) in the pool returned the positive result(s)[1,3]. This method has most utility for nucleic acid tests (usually PCR) and potentially serology when mass screening is required.

GeneXpert SARS-CoV-2 test is an RT-PCR testing platform[3], therefore testing of pooled sample using GeneXpert will be the way forward in countries where the demand for testing is much higher than the available resources (GeneXpert testing cartridges). Pooled sample testing, however, is advisable in low disease prevalence settings where there is much higher chance of test results becoming negative and minimal chance for repeat testing[2]. When the infection rate is low and only a few people are infected, pool testing can significantly expand the testing capacity of the existing laboratory resources[5].

The number of samples pooled together will determine the sensitivity shift from baseline when compared to the standard individual sample testing regimen. The optimal pooling numbers and associated sensitivity shift should be determined for every separate event, as median detection readings (ie CT or OD) will influence the pooling-factor chosen for a given surge capacity or surveillance event. For example, if normal diagnostic analyte levels are low (e.g. high CT for PCR, or low SC/CO for serology), then pooling of 2-4 may be the maximum selected, while if the average value level of analyte is very high (such as parvovirus DNA) then the pooling-factor of 10 or more may be explored[2]. The pooling mathematics should be considered and determined for every event and may even be modified during an event as incidence numbers increase, to ease the burden of disentangling of pools and retesting.

The Victoria Infectious Disease Research Laboratory, Melbourne has assessed pools of 2, 4 and 8 samples condensed into one pool for viral testing on RTPCR. These had a corresponding 1,2 and 3 CT shift from original (as expected). Four samples were chosen to be pooled as 'one' as the optimal compromise of 75% reagent efficiency gain and limited sensitivity loss. Standard distribution of caseload and CT burden from

diagnosis samples were plotted and subsequent monitoring samples to see what percentage of samples may be close to the limit-of-detection before pooling. This was found to be approximately 5-10%[1]. Furthermore, the recent validation of GeneXpert SARS-coV testing using 4 and 6 pooled samples at the Doherty Institute showed E gene Ct values ranging between 20 and 28 when contained to 4 and 6 sample pools[9].

This protocol advocates the use of low viral load, high CT value testing protocol advocated by VIDRL since GeneXpert offers RT-PCR SARS-CoV-2 test and studies have shown low to moderate viral load of up to 10^6 in individuals infected with SARS-coV 2[6]. Pooling of four samples is also advocated to be the maximum while screening asymptomatic individuals in the Pacific Island countries where laboratory human resource protective gears are limited therefore double checking of pools by a second person may not be a viable option.

Situations where sample pooling for SARS-CoV-2 testing is recommended:

Sample pooling is recommended for countries or communities where there is low prevalence of COVID-19 infection, low test to positive ratio (TPR)[6] and a much lower chance of disentangling the pool for individual sample testing that will deem pool testing uneconomical.

Published papers have suggested pooling of up to 64 samples for open RT-PCR testing[4,7.] Becker et.al however recommended the number of samples per pool for SARS-coV-2 GeneXpert testing is unique for each testing site and should be determined by the positive test rates [8]. This protocol, therefore, errs on the side of caution and recommend sample pool to 4 specimens/samples until there is reliable evidence that larger pool size can be tested on GeneXpert without significant effect on positive results[9].

Samples/specimens for Pooled sample/specimen testing:

Specimens to be used for pooled sample testing are Nasopharyngeal, mid turbinate, nasal and oropharyngeal swabs in VTM, and nasal aspirate and wash.

Testing of pooled specimen for SARS-CoV-2 on GeneXpert at this point is recommended only for screening of asymptomatic individuals who are likely to be negative or if infected would have low viral load, the number of samples to be pooled therefore should not exceed 4.

Samples from patients who meet the case definitions and/or have clinical features of COVID-19 should be tested singly, not pooled with specimens from asymptomatic individuals for screening purposes.

Role of clinicians and Public health officials, pathologist and laboratory medical officers:

Clear communication between clinicians and laboratory officials on the choice of specimens to be pooled and those to be tested singly is highly recommended to ensure rational use of the limited supply of GeneXpert test cartridges.

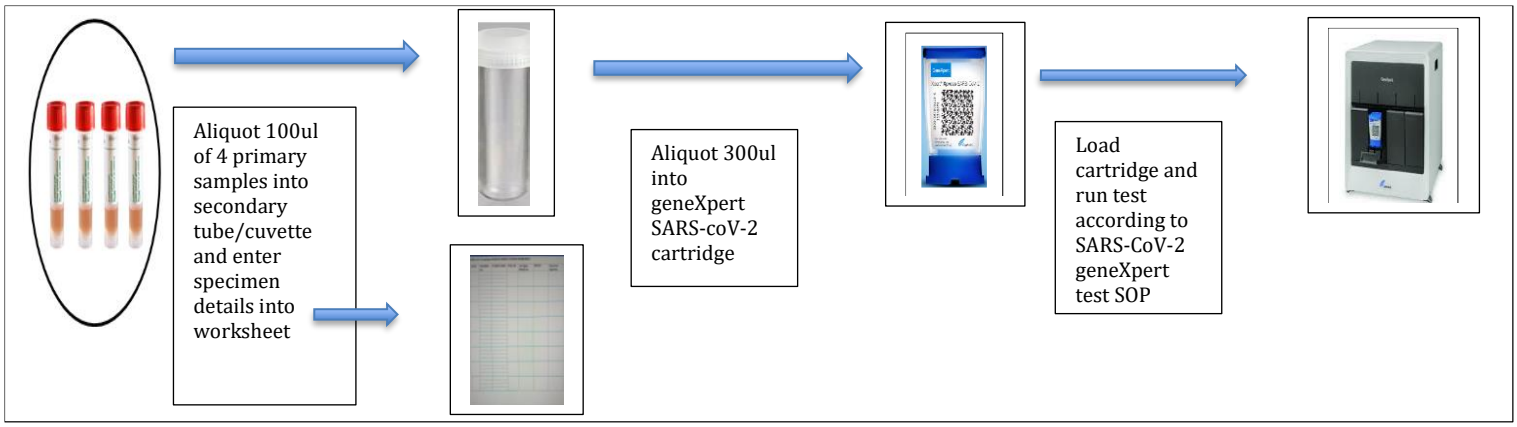
The laboratory medical officer/pathologists and managers should make sure there is ongoing communication between clinicians and the laboratory and proper guidance is offered to testing scientists and technicians.

The laboratory medical officer/pathologist should review results of pooled specimen testing at regular intervals, seek advice and/or communicate decisions to stop or change pooled specimen testing strategies when disease incidence and prevalence increase.

Review of protocol

This protocol will be reviewed and may change as more information on pooled specimen testing for SARS-CoV-2 on GeneXpert becomes available in future.

SARS-CoV-2 POOLED SPECIMEN TESTING WORKFLOW



SARS-CoV-2 POOLED SPECIMEN TESTING STANDARD OPERATING PROCEDURE

1. Establish a **SARS-CoV-2** specimen pooling worksheet (sample provided Annex 1)
2. Arrange your samples preferably in numerical order to avoid confusion that can arise in random sample pooling
3. Enter the 4 primary sample IDs (sample numbers and or patient IDs) in the worksheet
4. Enter the number of secondary (pooled sample tube) in the corresponding column of the worksheet
5. Arrange the 4 primary samples and label corresponding sterile secondary tube (pooled sample tube)
6. Using a calibrated pipette, aliquot 100ul from the 1st primary sample into the secondary tube (pooled samples). Ensure that pipette tips are filter/barrier tips, clean and lifted directly from pipette-tip racks. Discard tip after use.

Note: Ensure that pipette tip does not touch any other object including your gloves, to avoid contamination.

7. Repeat **step 6** above for 2nd, 3rd and 4th Primary sample ensuring that a new tip is used for each primary sample. Your secondary (pooled specimen) tube will now have 400ul of pooled sample
8. Close secondary tube or cuvette tightly and mix secondary sample by rapidly inverting the tube 5 times, if you are using uncapped sterile tube then mix sample by aspirating and squirting 3-4 times within the tube using GeneXpert testing pipette before aspirating 300ul for testing.

Note: Do not vortex sample to avoid aerosolization.

9. Follow **SARS-CoV-2 GeneXpert testing SOP** for the rest of the testing procedures.
10. Enter test results and test module number into your worksheet and testing register or LIS
11. For all pools with **negative results**, report results for all 4 patients in that pool as **negative**
12. For pools with **positive or presumptive positive results, do not enter result but re-run test** on each individual specimen following the SARS-CoV-2 GeneXpert testing SOP
13. Report results according to your laboratory procedure.
14. Refer specimen with presumptive positive results to reference laboratory for further testing as according to SARS-CoV-2 testing SOP.

References:

1. Druce Julian, Protocol for high throughput screening using Pooled Samples, unpublished protocol, May 2020
2. Druce Julian, Advise on Pooled specimen testing, unpublished email advise, May 2020
3. Muhammad Saeed, Mobeen Ahmad,et al, GeneXpert technology, a breakthrough for the diagnosis of tuberculous pericarditis and pleuritis, Saudi Medical Journal 2017
4. Idan Yelin, Noga Aharony, Einat Shaer Tamar, et.al, Evaluation of COVID-19 RT-qPCR test in multi-sample pools, *Clinical Infectious Diseases;* May 2020
5. Smola Sigrun, Virologists show that sample pooling can massively increase coronavirus testing capacity, The Lancet Infectious diseases, April 2020
6. Fengting Yu, Liting Yan, Nan Wang, et. al, Quantitative Detection and Viral Load Analysis of SARS-CoV-2 in Infected Patients *Clinical Infectious Diseases*, March 2020
7. Dr Liji Thomas, Smart sample pooling for COVID-19 could solve test kits shortage problem, www.new-medical.net/news/20/200/429, Apr 2020
8. Michael G Becker, Tracy Taylor, Sandra Kiazyk, Dana R Cabiles, Recommendation for sample pooling on the Cepheid GeneXpert system using the Cepheid Xpert Xpress SARS-coV-2 assay, Europe pubmed Central, May 2020.
9. Maryza Graham, Eloise Williams, Nicole Isles, Eka Buadromo, Tebuka Toatu, Julian Druce, Sample pooling on Cepheid Xpert®Xpress SARS-coV-2 assay, Manuscript to be published.

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Annex 1

SARS-CoV-2 GeneXpert POOLED SAMPLE TESTING WORKSHEET

DATE	SPECIMEN NO.	PATIENT NAME	POOL NO	Gen Xpert Module no	RESULT	Operators signature

This document has been developed by the Pacific Community (SPC) and the Pacific Islands Society of Pathology (PISP) to suit the Pacific context.