Use of Pooled Testing for the Diagnosis, Screening, and Surveillance of COVID-19

Evidence Summary

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1. Background

The *World Health Organization (WHO)* declared the novel coronavirus disease (COVID-19), caused by severe acute coronavirus 2 (SARS-COV-2), a global pandemic affecting hundreds of countries and millions of people around the world. In response to this public health emergency, the *Philippine Department of Health (DOH)* issued testing guideline policies which currently sets the real time reverse transcriptase polymerase chain reaction (RT-PCR) as the standard confirmatory test to diagnose COVID-19.

As the number of COVID-19 cases increase, availability of diagnostic kits and reagents emerged as a major bottleneck in the laboratory testing of SARS-CoV-2 (Khodare, 2020). Current strategies involve testing only symptomatic individuals. However, evolving strategies for testing worldwide now also include testing asymptomatic individuals with pertinent contact history to curb the spread of infection in the community (Praharaj, 2020). Large scale population screening for COVID-19 infection is generally considered a necessary part of an exit strategy from the coronavirus lockdown (Wacharapluesadee, 2020).

In response to the growing demand for testing capacity, the recently issued *DOH Department Memorandum (DM) 2020-0439 Omnibus Testing Guidelines on Prevention, Detection, Isolation, Treatment and Reintegration Strategies for COVID-19* included pooled testing among the currently recommended testing options. The issuance, however, stated that pooled testing strategies are currently being evaluated and validated; hence, these guidelines shall be further amended as new developments emerge from new studies and pilot implementation. While the guidelines mentioned that pooled testing may be used for surveillance testing of asymptomatic workers, it emphasized that such methodology may only be used once results of on-going pilot testing are positive and favorable, based on the recommendations of experts.

A rapid review was conducted to search, appraise and synthesize currently existing synthesized relevant information and evidence on the regulatory approval and validation requirements, performance characteristics, existing recommendatory testing guidelines and assessment findings, as well as resource requirements on implementing pooled testing for the following use cases in COVID-19: (1) Diagnosis; (2) Screening; (3) Surveillance.

Policy Question

Which use case and for which population should the Philippine DOH consider the use of pooled testing for COVID-19?

Research Questions

1. Regulatory Approval

1.1. What are the approved uses of pooled testing by regulatory agencies in other countries?

1.2. What are the validation testing requirements of pooled testing of regulatory agencies in other countries?

1.3. What are the performance standards of pooled testing by regulatory agencies in other countries?

2. Performance Characteristics

2.1. What is the accuracy of pooled testing in the diagnosis, screening, and surveillance of COVID-19?

3. Global guidelines and position on use of pooled testing

3.1. Which countries have implemented testing strategies using pooled testing for diagnosis, screening, and surveillance of COVID-19?

3.2. What is the current position of HTA agencies regarding the use of pooled testing for diagnosis, screening, and surveillance of COVID-19?

4. Resource requirements

4.1. What are the resource requirements needed to implement pooled testing?

2. Responsiveness to disease magnitude, severity, and equity

2.1. Responsiveness to disease magnitude and severity

As of 28 October 2020, the COVID-19 pandemic has affected more than 219 countries and regions with at least 43, 766, 712 cases and 1, 163, 459 deaths worldwide (WHO, 2020). Locally, there are over 373, 144 confirmed cases and 7, 053 deaths. COVID-19 can have various clinical manifestations among those infected, ranging from mild pneumonia to having respiratory failure, septic shock, and multiple organ dysfunction or failure (Cascella, Rajnik, Cuomo et al., 2020). In the Philippines, among the active cases, 89.83% are mild cases, 0.49% are severe, 0.42% are critical, and 9.25% are asymptomatic. Currently, there are no known treatments for COVID-19.

3. Safety and effectiveness

3.1. Regulatory Standards

Eleven regulatory agencies were searched for guidelines on the regulation and authorization of the use of pooled testing. Only the *US Food and Drug Administration (US FDA)* has established

guidelines on pooled testing and on how to validate the test when requesting for *an Emergency Use Authorization (EUA).* In the EUA diagnostic template, patients suspected of COVID-19 infection and individuals without symptoms or other reasons to suspect the said infection are the targeted population for pooled testing. The US FDA guidelines stipulate that the use of pooling in certain SARS-CoV-2 tests (i.e., screening and diagnostic tests) can be an option and authorized granting that there exist proper mitigation and validation studies, thereby, ensuring its proper implementation. Furthermore, on the use of SARS-CoV-2 diagnostic test for surveillance purposes (e.g., determining the prevalence of acute infections in a population), the US FDA explicitly stated that it does not generally regulate the use of a test for this purpose or use case. Nonetheless, it was mentioned by the US FDA that if surveillance testing is performed by a non-Clinical Laboratory Improvement Amendments (CLIA) certified laboratory, a confirmatory test on the detected positive individuals should be performed by a CLIA-certified laboratory.

As for the validation testing requirements, if commercial test kit manufacturers would like to include sample pooling and swab pooling to their authorized uses, they must submit an EUA request together with the data from their clinical validation studies with the following guidelines:

- The reference standard used is the same EUA-authorized assay RT-PCR kit for which the added indication is being requested for.
- If the RT-PCR has not been previously granted an EUA, the reference standard should be a comparator assay that has an established high sensitivity with an internationally recognized standard or the FDA SARS-CoV-2 reference panel.
- The index test must have at least 20 positive individual samples for an EUA RT-PCR kit and at least 30 positive individual samples for a new RT-PCR kit. Each positive n-sample pool shall have 1 positive sample and n-1 negative samples. An equal number of negative pools must also be tested.
- The pool size should be chosen in the context of the positivity rate and percent of weak positives in the intended test population, and the sensitivity of the test. However, the US FDA recommends to test developers that they begin validating their test kits using a pool size of 5.

The US FDA also requires two additional studies for swab pooling to evaluate the inhibition observed when (1) the sample has high concentrations of swab specimen (e.g. mucin) and (2) when the sample has a high concentration of viral load.
The table below summarizes the performance standards set by the US FDA for the validation studies required when requesting for an EUA for pooled testing:

Performance standard domain	Sample/ Media Pooling	Swab Pooling
Performance for pooled testing vs. Individual testing	• ≥85% PPA • Individual weak positive samples with high Ct values (viral loads close to LoD of the assay) can be accurately detected when pooled with n-1 negative samples	
Interference of swab specimens	N/A	• 95% agreement with expected results and, • <5% invalid rate
Interference of viral load	N/A	• 100% positivity rate or • ≤5% invalid rate

The US FDA currently lists a total of ten molecular diagnostic tests that can be used for pooled testing. Of these, only one brand is currently authorized by the Philippine FDA as an RT-PCR test kit for COVID-19 but for individual testing and not specifically for pooled testing as regulatory standards for the latter do not exist yet in the Philippines.

3.2. Guideline Recommendations

Of the fourteen guidelines reviewed, only four (US CDC, ECDC, Public Health Ontario and Philippine Department of Health) have existing recommendations on pooled testing for COVID-19. The US CDC recommendation has the widest scope of use which is for diagnosis, screening and surveillance. Both the ECDC and the Philippine DOH recommend its use only for screening or surveillance. We also note that two guidelines explicitly stated that they do not recommend pooled testing for diagnosis. Public Health Ontario (PHO) recommends its use for diagnosis and surveillance. To further retrieve information on the use of pooled testing among different countries specifically on pilot implementation efforts or plans which are not usually reported in guidelines, the reviewers conducted a targeted search for news articles from different ministries of health websites as well as independent news agencies. Available news articles from the United Kingdom, Singapore, Malaysia, Korea and Vietnam have mentioned the use of pooled testing as screening tests in different settings and populations. Indonesia, Thailand and China have mentioned the use of pooled testing for surveillance under different circumstances.

Diagnosis

Two guidelines (US CDC and PHO) currently recommend the use of pooled testing for diagnosis of COVID-19 infection.

- As a diagnostic tool, the US CDC currently recommends pooled testing among patients with symptoms or recent exposure or to determine the resolution of infection.
- PHO uses pooled testing for a portion of specimens submitted to its laboratory from assessment centers. PHO did not further elaborate on the target population nor on the characteristics of population being tested for diagnosis
- US CDC and PHO both recommend a two-stage specimen pooling strategy in which samples are pooled together, and if a pooled test result is negative, then all specimens within the pool can be presumed negative with the single test; whereas if a pooled test result is positive or indeterminate, all the specimens within the pool need to be retested individually.

The ECDC and the Philippine DOH explicitly stated in their guidelines that they do not recommend the use of pooled testing for diagnosis. The Philippine DOH does not recommend it for certain populations which include individuals with symptoms (regardless of severity), recovered patients and close contacts of positive individuals, while ECDC does not recommend it due to the possibility of error.

Screening

Three guidelines (US CDC, ECDC, and Philippine DOH) currently recommend the use of pooled testing in screening infected individuals without, or have not yet developed symptoms who may be contagious so that measures can be taken to prevent further transmission.

- The US CDC does not specify the target population of this use case.
- The ECDC specifically recommends it for mild and asymptomatic patients.

- The Philippine DOH recommends the use of pooled testing in screening the following populations: inbound travellers, overseas Filipino workers (deployment and returning), and locally stranded individuals.

In addition to testing guidelines, several news articles from countries like the United Kingdom, Singapore, Malaysia, Korea and Vietnam have mentioned the use of pooled testing as screening tests in different settings and populations.

- The United Kingdom is introducing the use of COVID-19 screening using pooled testing in universities to help prevent outbreaks and allow campuses to stay open (Mahase, 2020).
- Singapore on the other hand, allows the use of pooled testing in migrant worker dormitories and nursing facilities, with their ministry of health recommending its use in testing sub- populations with very low prevalence rates of COVID-19, or for mass screening purposes. (Singapore MOH, 2020a) (Singapore MOH, 2020b) (Sin.Y., 2020) (Sun.D., 2020)
- Malaysia recommends the use of pooled testing in mass testing groups with high risk of infection such as members of the Kuching church where the biggest clusters occurred due to mass gatherings. (Choong.J., 2020)
- Korea also recommends using pooled testing in local clusters at a higher risk of acquiring COVID-19 infection using pool sizes of 10 (Sung-sun, 2020)
- Vietnam uses pooled testing on returnees from Da Nang population, which is considered to be the outbreak epicentre in Vietnam by using pool size of three to five individuals per laboratory test. (Kiet, 2020) (WHO, 2020)

Surveillance

Across the three use cases, pooled testing is most commonly used for surveillance purposes, based on the guidelines reviewed. All four guidelines (US CDC, ECDC, PHO, and Philippine DOH) recommend the use of pooled testing for surveillance.

- The US CDC recommends the use of pooled testing to monitor a community of population level occurrence, such as an infectious disease outbreak or to characterize the occurrence once detected, as well as to look at incidence and prevalence of occurrence.
- The ECDC recommends the use of pooled testing in determining prevalence of disease in the community or to enhance testing of mild and asymptomatic patients;
- PHO recommends it in testing asymptomatic patients especially during outbreak investigations.
- The Philippine DOH recommends the use of pooled testing in the surveillance of the following populations: healthcare workers and all workers in health facilities, essential workers including market vendors, transport workers, frontline government workers and other economy workers.

In addition to testing guidelines, several news articles from countries like Indonesia, Thailand and China have mentioned the use of pooled testing for surveillance under different circumstances.

- Indonesia plans to conduct pooled testing with pool sizes of five each in eight provinces that have been hardest hit by the coronavirus. A thousand samples will be taken from these provinces using a multi-step random sampling. (Sutrisno.B, 2020)

- Thailand also employs pooled testing using saliva samples to accelerate testing 100,000 persons in targeted groups including health and medical professionals, prison inmates, drivers for public buses and migrant workers. (WHO, 2020)
- China used pooled testing on the entirety of Wuhan population as mass, indiscriminate testing, using pool sizes of five to ten individuals in one laboratory test (BBC, 2020)

We note that of the guidelines reviewed, the WHO and Australia do not have any guidelines regarding pooled testing, nor are there any articles which cites the use of this strategy.

3.3. HTA Review Recommendations

We searched for existing reviews from 10 HTA agencies. Of these, there was no existing review with recommendations specific to pooled testing. Moreover, none of the HTA agencies included in our search have published any information regarding any ongoing studies on the use of pooled testing in COVID-19.

3.4. Diagnostic Performance

A total of 20 studies were included in the qualitative synthesis of the rapid review. Nineteen studies were primary diagnostic accuracy studies and one was a clinical trial protocol. The 18 primary studies were included for quantitative synthesis. Eight studies looked at pooled testing for screening, two studies explored pooled testing for surveillance, five were for diagnosis and three did not mention a particular use case. Majority of the studies did not describe the study population used and were laboratory-based simulations rather than field validation. However, one data set from one study mentioned use of pooled testing among asymptomatic healthcare personnel, employees of essential industries, and residents and employees of nursing homes while one study mentioned use of pooled testing among asymptomatic residents of a nursing home.

The sensitivity of pooled testing greatly varies when the unit of analysis is by pool (sensitivity point estimates ranging from 25 –100%) wherein the number of pools tested is small, compared to when the analysis is by individual (sensitivity point estimates ranging from 92 –100%). The overall pooled sensitivity of pooled testing analyzed by pool was found to be 87%, (95% CI: 81-91, I2=71%) while pooled sensitivity analyzed by individual was 97% (95% CI: 95-99, I2=0%). The overall pooled sensitivity analyzed by individual suggests that pooled testing has a good rate of correctly identifying COVID-19 positive individuals and consequently, a low rate of false negatives. Meanwhile, the pooled sensitivity analyzed by pools should be interpreted with caution due to the observed high level of heterogeneity.

On the other hand, the specificity of pooled testing was consistently high ranging from 97% to 100%. The overall pooled specificity of pooled testing analysed by pool [98.9% (95% CI: 89-96, I2=12%)] and analysed by individual [99.99%, CI: 98.9-100, I2=0%)] implies that the test has a very low rate of both false positive pools and both false positive individuals.

Given the substantial heterogeneity present in the pooling of sensitivity, we performed subgroup analysis to assess the impact of several factors (e.g., pool size, brand of index test, CT value threshold, presence of symptoms, onset of symptoms, use case, specimen, and prevalence of disease) that may have served as sources of heterogeneity across the studies. The results from the subgroup analysis suggest that:

- Based on low to moderate quality of evidence from 18 studies, pooled testing showed higher sensitivity estimates when pool sizes used are smaller. This finding was very intuitive because the more specimens in the same pool, the more diluted the shared reagent becomes losing the power of the test.
- Based on low to moderate quality of evidence from 18 studies the sensitivity varies greatly from one brand to another possibly because of the different processes and protocols each one takes, as well as the different criteria set by their respective manufacturers.
- Based on low and moderate quality evidence from 2 studies, an increase in the CT value of positive samples in a pool decreases the sensitivity estimate. However, this needs further investigation since the number of studies analyzed for this variable is small.
- The sensitivity of pooled testing for screening (6 studies, low to moderate validity) was the highest but was closely followed by those that indicated diagnosis (4 studies, low to moderate validity) as its use case. Though, both use cases were found to have substantial variation within the included studies.
- Sensitivity estimates for symptomatic individuals, although based only on one moderate quality study, were higher compared to the asymptomatic (2 studies, low and moderate validity) and unspecified population (13 studies, low to moderate validity). Caution must also be taken in the interpretation of this analysis since very few studies had available information regarding the clinical characteristics of the sample.
- Pooled testing that used saliva had the highest sensitivity but was comparable to those that collected test material using nasopharyngeal specimen. However, it should be noted that only one moderate quality study used saliva as its specimen. Those that used mixed samples of nasal and oral specimen also had an acceptable sensitivity.
- Few studies indicated the prevalence of the disease where the sample was obtained so our numerical results for studies with low prevalence still warrant further investigation.

An unpublished local study by Lo et al (2020) had a lower sensitivity in both units of analysis than the overall pooled sensitivity estimate of our quantitative synthesis. The reported sensitivity estimates of the study for analysis by pools were 83% (95% CI: 67-94), 72% (95% CI: 55-86), and 67% (95% CI: 49-81) while the sensitivity estimate for analysis by individuals were reported to be at 83% (95% CI: 52-98), 58% (95% CI: 28-85), and 50% (95% CI: 21-79) for pool sizes of 5, 10, and 20, respectively. Furthermore, the study of Lo et al showed consistent trend with the observations in the subgroup analysis which indicates that lower pool sizes were seen to have higher sensitivity estimates. In contrast, the specificity of the local study with individuals as unit of analysis (100%, 95% CI: 99-100) was comparable to the overall specificity estimate of the included studies in meta-analysis.

As for the quality of these studies on diagnostic performance, our critical appraisal shows that eight studies had high risk of bias and eleven had moderate risk of bias. Some factors that have affected the validity of these studies include non-independence of the definition, performance, and interpretation of the index and reference test.

Based on low to moderate quality evidence, the use of pooled testing for COVID-19 shows high specificity but varied sensitivity. Further, the prevalence of the population to which pooled testing can be applied remains unclear.

4. Household financial impact

Evidence not reviewed.

5. Cost-effectiveness

Evidence not reviewed.

6. Affordability and viability

Resource Requirements

Originally, we had planned to look at the resources needed to implement pooled testing. However, among the nineteen studies that were reviewed, nine studies included only information on the resources saved when using their proposed pooling strategy at a certain prevalence and positivity rate. Three additional studies were also obtained to give us more information on the reduction in resource requirements when pooled testing strategies are implemented. The studies expressed resources saved in terms of money amounts of costs or in terms of specific resource requirements such as the number of tests saved, reduction in testing hours, and changes in human resources required. The included studies have reported positivity rates ranging from 0.12-3% or prevalence of 4-6.86% while pool sizes used range from 3-20 samples.

Five studies relayed cost savings in terms of money as an advantage of pooled testing over individual testing. It should be noted that not all declared whether their calculations included costs saved in compensation of personnel, cost saved in utilities, costs saved in test kits, and other costs saved due to the decrease in tests required when pooled testing is employed so their estimates can still vary.

For the studies specifying reduction in number of tests saved when implementing pooled testing in comparison to the standard individual PCR testing, they show that given low positivity rates, using a pooled testing strategy may reduce the number of tests to be conducted individually from 62 – 87%, consequently increasing the capacity of testing for COVID –19. In terms of testing hours, studies show that pooled testing, when done using a strategic pool size among a population with low prevalence, will save the laboratory hours of processing time even when positive pools have to be deconvoluted and tested individually. . As with the changes in personnel required, the one study concluded that when pooled testing is employed, less laboratory staff would be required but there will be a small increase in the need for clerical staff.

In the study by Lo et al (2020) conducted in the Philippine setting, savings were reported in terms of the number of test kits saved when employing multi-stage Dorfman pooled testing. They found that for a population with a positivity rate of 3%, employing pooling strategies can reduce the number of tests required by 69-83%. The study also showed that test savings is a function of the positivity rate or prevalence rate by calculating the average savings per batches of 100 samples using simulations. The calculations showed that as the positivity/prevalence rate increases, the number of test savings decreases. In terms of testing hours, the study found that pooled testing strategies would increase the turnaround time since more than one batch run would be required to release the results of a positive or negative sample.

7. Recommendation

Pooled RT-PCR testing can be utilized for screening and surveillance in low prevalence population. The responsible unit of DOH must undertake the necessary prevalence study to determine appropriate populations for pooled RT-PCR testing. For diagnosis of COVID-19, individual RT-PCR testing must be done regardless of the prevalence.

Provided that RT-PCR test kits to be used for pooled COVID-19 testing must undergo and pass the validation requirements for pooled testing by RITM, local authorities (e.g., FDA), or other authorized institutions. Likewise, the RT-PCR test kits to be used for pooled COVID-19 testing must meet the **minimum technical specifications** and **requirements of HTAC**.

Parameter	Requirement
Regulatory Requirement	RT-PCR test kits **must be authorized by the Philippine FDA** for pooled COVID-19 testing and must be validated for use in pooled testing by local or international agencies.
Validation	All kits to be used for pooled testing should also undergo additional validation for pooled testing by RITM or other authorized institutions similar to what the US FDA requires
Cost	Must include all necessary accessories per test, including extraction reagents, consumable, & viral transport media. The detailed breakdown of the cost must be provided by the supplier The ceiling cost is Php 1,800 per assay, excluding the cost of the PCR machine, and the consumption of personal protective equipment.
PCR Machine Compatibility	Must be compatible with the existing machine/s of the testing facility, noting other prerequisites needed in order to operate such as appropriate containment and biosafety procedures.
Storage, expiration and stability	The expiration date must be no less than six (6) months from date of manufacture. The storage and working temperature must be -20 degrees Centigrade. Must pass the acceptance testing by RITM at the cost of the winning supplier.
Human resource	Must not require more than the basic competency of personnel equipped with skills on RT-PCR techniques and in-vitro diagnostic procedures and instrumentation, with **additional training** conducted by the RITM and

	the Philippine Society of Pathologists (PSP) for pooled testing
Analytical Sensitivity (Gene Targets)	Must have been tested for confirmatory gene (i.e., RdRP, ORF1ab, & N) and screening gene (i.e., E gene)
Analytical Specificity (Cross-Reactivity)	Must have no significant cross-reactivities identified among the RT-PCR test kits. For cross-reactivity testing, must use at least both of the following organisms: Influenza A and Influenza B
Clinical Sensitivity	Must have at least **90% clinical sensitivity**
Clinical Specificity	Must have at least 99% clinical specificity
Processing Time	Must be six (6) hours or less (excluding repeat test and specimen transport)
Reference Standard	Refer to RITM validation protocol for pooled RT-PCR testing
Sample Size Requirement	Refer to RITM validation protocol for pooled RT-PCR testing
Cycle threshold (Ct) values	Refer to RITM validation protocol for pooled RT-PCR testing
Pool size	Should be at most five (5)
Pooling method	Any appropriate pooling method is acceptable
List of accredited laboratories for pooled testing	The pooled COVID-19 testing must only be performed by laboratories identified and authorized by RITM.

In terms of research, the HTAC recommends exploring the **correlation of cycle threshold (Ct) values** with **viral load concentration**, and the **correlation of Ct values** with **infectivity**.

Given the available evidence from rapid review and the study of Lo et al., pooled COVID testing is recommended to be used for screening and surveillance in populations or settings with low prevalence. Use of pooled COVID-19 testing for specific populations is yet to be determined pending the prevalence data from the Epidemiology Bureau. As the national reference laboratory, there should be clear validation standards from the RITM. Lastly, it is recommended that the FDA Philippines issue guidance as to the appropriate device to be used for pooled testing. The FDA should have authorization for RT-PCR test kits that may be used for pooled COVID-19 testing.

9. References

Unless specified, the references cited in this document were derived from the rapid review report on pooled COVID-19 testing.