POOLED TESTING

RECOMMENDATION

We suggest the use of pooled RT-PCR testing in targeted* low-risk and low-prevalence populations using a pool size of 5 in individuals suspected of COVID-19 infection. *(Moderate quality of evidence; Conditional recommendation)*

**For targeted populations refer to the list of Philippine Society of Pathologists and Department of Health*

Consensus Issues

The set recommendation included positivity rate and pool size. However, there were no studies that looked into the specificity and sensitivity of pooled testing for different pool sizes across different prevalence settings. Since the data presented did not clearly define the risk or prevalence settings, pooled testing was only suggested to be used at a specific target population despite the moderate quality of evidence.

EVIDENCE SUMMARY

Should pooled testing using RT-PCR for SARS-CoV-2 versus individual testing using RT-PCR be used for screening and surveillance for SARS-CoV-2 in individuals with suspected COVID-19 infection?

Evidence Reviewers: Rowena Natividad F. Genuino, MD, MSc; Beatrice J. Tiangco, MD, MSc; John Jefferson V. Besa, MD; Howell Henrian G. Bayona, MSc

Key Findings

21 cross-sectional studies (N = 220,253) were found that used pooled RT-PCR testing for SARS-CoV2; 6 were diagnostic accuracy studies that compared pooled testing with individual testing, while 15 were pragmatic clinical validation studies of pooled testing that did individual testing of positive pools. Studies had varying study population, use case, index test kit and pool size (5 to 16). There was moderately high pooled sensitivity, 81% (95% CI 72, 88; I^2=73.6%) (moderate certainty of evidence) and high pooled specificity, 99% (95% CI, 98 to 100; I^2=1.84%) (high certainty of evidence) (6 studies, N=5987), with a positivity rate of 2.7% to 15% in the study populations. Positive predictive value based on 21 studies ranged from 67% to 100%. Resource savings in number of test kits used ranged from 49 to 89%. Identified harms of pooled testing are delayed turnaround time for positive samples and laboratory errors. Overall risk of bias was low in 7 studies, 6 of which were diagnostic accuracy studies that contributed to pooled sensitivity and specificity, and low in 14 studies, mainly due to lack of independence of assessment between index and reference tests.

Introduction

Pooled testing is the process of combining equal parts of samples from a certain number of individuals and processing them in a single batch. Two approaches are used: (a) sample/media pooling which combines aliquots of transport media containing individual samples, and (b) swab

pooling which involves mixing multiple samples in a single transport media. When a pooled sample tests negative, all individual samples are presumed to be negative. On the other hand, pooled samples that test positive will require retesting of individual samples to identify the positive specimen. This is the simplest and most commonly used type of pooling, also called the Dorfman staged pooling.

Overall, pooling is an efficient method to increase throughput while saving resources. However, because the samples are diluted, less viral particles become available for detection, consequently increasing the likelihood of false negative results and the turnaround time for processing specimens. As such, its implementation has been limited to screening and surveillance in populations with low prevalence of COVID-19 [1–4]. The diagnostic accuracy of this testing method and its recommended use needs to be determined.

Review Methods

We searched for living CPGs, HTAs, or living reviews from the following sources on 10 February 2021: Australian National COVID-19 Clinical Evidence (https://covid19evidence.net.au/), UK Oxford COVID-19 Evidence Service (https://www.cebm.net/oxford-covid-19-evidence-service) and DOH Health Technology Assessment website (https://hta.doh.gov.ph/) .

To obtain additional cross-sectional studies or systematic reviews of cross-sectional studies that were not yet included in the existing reviews, we performed a comprehensive search of MEDLINE and Cochrane CENTRAL (31 Dec 2020) using search strategies that included the concepts of pooled testing and COVID-19 for studies that met the following inclusion criteria:

Population	Patients suspected with COVID-19, any age, any sex, any comorbidity, with or without symptoms, any onset/timing of symptoms
Intervention/ Index Test	Pooled testing using RT-PCR from different individuals, any brand of test kit, any specimen (nasopharyngeal, oropharyngeal, saliva, etc.), any pool size or method, whether samples pooled prior to or after RNA extraction
Comparator/ Reference Standard	Individual testing using RT-PCR of deconvoluted samples from the pools, any brand of test kit, any specimen (nasopharyngeal, oropharyngeal, saliva, etc.)
Outcomes	Any measure of diagnostic performance (sensitivity, specificity, PPV or NPV, or any data that can be used to compute these outcomes), resources saved, turnaround time, harms

We excluded retrospective case-control study designs that involved laboratory validation studies using previously known positive or negative samples, as well as modelling or simulation studies on pooling that did not include actual patient samples.

We derived individual and pooled diagnostic performance measures, namely, sensitivity, specificity, positive and negative predictive values, and likelihood ratios using pools as unit of analysis in 6/21 studies [5-9,13]. We computed using individuals as unit of analysis in 5 studies (all except Wang et al). The rest of 15 studies only had data for the calculation of the positive predictive value, positivity rate, and number of tests or resources saved.

Subgroup analysis was performed based on variables that were hypothesized to affect the sensitivity of pooled sampling, particularly those leading to higher levels of true positive and less levels of false negative results. These included the following:

a. test use case (i.e., screening, surveillance, or both)
b. symptom status (i.e., asymptomatic, symptomatic, unknown)
c. pool size (e.g., 5, 10, other)
d. specimen used (e.g., nasopharyngeal, nasal swab, etc.)
e. brand of PCR test kit
f. cycle threshold (Ct) values for RT-PCR positivity

We also did post-hoc subgroup analyses as to positivity rate, method of pooling (random vs non-random), amount of aliquot per sample, and timing of processing.

Results

Characteristics of included studies

We found a total of 21 cross-sectional studies that recruited persons suspected to have COVID-19 (N = 220,253). Two studies [10, 11] were previously included in a local rapid review in the PSMID website (12 studies, 21 July 2020) [12] and a scoping review (9 studies, 23 Nov 2020) [2], while five studies [6,8,13-15] were previously included in the Philippine DOH HTA rapid review [16-17]. We found 14 additional studies since the last search date of the DOH HTA review (3 October 2020). Only 6 studies had complete 2x2 tables and were eligible for meta-analysis. The characteristics of included studies are detailed in Appendix 1.

The studies were performed in 13 countries: USA (n=5), India (n=4), Spain (n=3) and one study each from Australia, Brazil, China, Germany, Iran, Israel, Italy, Latvia, Philippines and Thailand. The most common settings were hospitals (n=6), community (n=4), outpatient clinics (n=2), with one study each done in residents and workers at care homes, a textile factory with an ongoing outbreak, arriving travelers from high-risk areas (Wuhan) at an airport, a research institute and employees from a supermarket. The community prevalence in seven studies that reported it ranged from 0.5 to 10%. Only 6 studies reported the presence or absence of symptoms among participants. Participants were asymptomatic in five studies, among healthcare workers in US (Das 2020), volunteer employees in a supermarket in the Philippines [13], subjects at a research institute [18], unspecified healthcare facility [19], low-risk admitted patients in a hospital who did not have any history, PE, lab and imaging findings of COVID-19 [14]. Both symptomatic and asymptomatic participants were included in two studies [9,20] with the first study among patients in screening clinics and tertiary care hospitals, including suspect cases admitted in the COVID wards, while the second study included follow-up samples from known COVID-19 patients enrolled in clinical trials.

Sample size in these studies ranged from 60 to 117,576 (median n = 3,509). The number of individual samples per pool ranged from 4 to 16. Majority (18/21, 85.7%) of the studies pooled nasopharyngeal, nasal/mid-turbinate, oropharyngeal swabs or various specimen combinations. Two studies pooled saliva [15,21], while one study pooled mostly throat washes [7]. Majority (17/21) pooled samples and media; only two pooled swabs [5,14], one study [22] pooled both samples and RNA, and one study [15] pooled RNA.

All the studies included in this review used pooled testing using RT-PCR versus individual testing of samples from deconvoluted pools as their reference standard. Validated RT-PCR tests were also used as the index test using the pooled human samples. In this review, "index test" refers to "RT-PCR used to diagnose SARS-CoV-2 virus in pooled human samples of saliva and/or oropharynx, nasopharynx". There were various brands of RT-PCR test kits (COBAS, Taq Path kit, unspecified, , CDC, Viasure, TaqMMan, AllPlex, LabGun, Xpert Xpress, Real Star, Gene Xpert, Sansure, Real Time Fluorescent, RealTime Multiplex Kit. Panther Fusion, Panther Aptima and Panther Aptima 450) while one just described it as laboratory-developed test (LDT) and a few did not specify. All except two studies [11,23] did preliminary laboratory validation of their planned pool size using known positive and n-1 negative samples.

Most studies (20, 21) did a two-stage Dorfman pooling wherein positive pools proceeded to individual testing of the samples contained in those pools. In addition, samples in negative pools were also tested individually in six studies [5-9, 13]. One study [13] also used three-stage (10-5-1) and four-stage (20-10-5-1) pooling wherein after initial testing of the biggest pool and testing positive, smaller subpools were tested sequentially and if positive, individual samples were finally tested. One study [18] did two replicates of each primary pool of 3 samples so that each mixed group (containing 6 samples) contained 2 different primary pools.

Overall quality of evidence

Overall risk of bias of included studies were rated high in 14/21 (67%) studies, and only seven studies that did individual testing of both positive and negative pools [5-9, 13] or stated blinded independent assessments [8,22] were at low risk of bias. Summary of the risk of bias ratings are detailed in Appendix 5.

Fifteen studies that did deconvolution (i.e., individual testing for the pools that tested positive for the index test), were likely not to have independent interpretation of the individual samples since they knew they were testing from a positive pool. Ideally, all pooled samples should have been tested individually by blinded lab technicians, regardless of the result of pooled sampling test (index test). Only six studies [5-9,13] also deconvoluted the negative pools, allowing them to measure the true and false negative rates in their studies. In the study by Wang et al, the authors only indicated the number of pools that tested positive, but did not specify the number of positive individual samples upon deconvolution, disallowing the measure of prevalence [9]. In the study by Lo, the authors only indicated the number of individuals that tested positive or negative for both index and reference tests, but not the number of positive pools with at least one positive individual sample in the report. However, subsequent communication from author provided the data for the derivation of the 2x2 table [13].

Outcomes

Diagnostic Accuracy

For the six studies that provided complete data for diagnostic accuracy using pools as unit of analysis, the pool size ranged from 5 to 16. Pooled sensitivity, using pools as the unit of analysis, was 81% (95% CI 72-88%) but the range was highly variable: from 28.6% [7], 75% [8], 74 to 85% [9], 93.4% [6] and 100% [5]. Specificity was high at 98 to 100%. Similarly, a high positive predictive value (PPV) was noted from 5 studies at 92.3% to 100%. Negative predictive value (NPV) was high for three studies with NPVs of 97.2% [6], 95.8% [8] and 100% [5] and moderate for two studies with NPVs of 80.8% [7] and 84 to 90% [9].

When we pooled using individuals as unit of analysis including only 5 studies (except Wang study), the sensitivity (83%, 95% CI 55 to 95; I2=92.8%) and specificity did not change much (100%, 95% CI 99 to 100%) but the heterogeneity for specificity increased to I2=87.6%.

Only PPV could be computed for the 15 other studies. Across all 21, it ranged from 66.7% to 100% with only 2 studies below 90%. Prevalence in all included studies was from 0.02% [24] to 15% [5].

The Lusebrink study, with a low sensitivity of 29% (2/7 pools), used a pool size of 10 for hospital staff screening in Germany, and collected specimens through mostly throat washes (88%) using saline solution gargle. They used a bigger aliquot (300 microliters per individual sample) than recommended 200 microliters by the Korean Society of Laboratory Medicine [25]. Out of 28 pools, there were five false negative pools, one of which turned out to have two positive individual samples. On the other hand, one of the two positive pools contained an invalid specimen. When they retested 17 pools with invalid specimens and false negative pools using lower pool size of 5, the sensitivity increased to 76.5% (13/17 pools). The authors concluded that the presence of PCR inhibitors in one invalid sample may have had a crucial effect on the pooled sample, and recommended against a Ct cutoff of 30 or above.

The local study by Lo et al [13] reported complete data for individuals as unit of analysis showed that sensitivity increased from 50% to 83% as they reduced the number of stages in the pooling strategy from 4-stage (Dorfman 20-10-5-1) to 2-stage (Dorfman 5-1). Specificity remained high at 100% in all 3 pooling strategies (Table 1)

Table 1. Summary of results of Lo study (individuals as unit of analysis)

Pooling method	Sn (%)	95% CI	Sp (%)	95% CI
Dorfman 5-1	83.33	51.59 to 97.91	100.00	99.14 to 100.00
Dorfman 10-5-1	58.33	27.67 to 84.83	100.00	99.14 to 100.00
Dorfman 20-10-5-1	50.00	21.09 to 78.91	100.00	99.14 to 100.00

Data source: Lo et al. An Evaluation of Pooling Strategies for qRT-PCR Testing for SARS-CoV-2 Infection by the PSP. Phil J Pathol.2020:5(2)

Subgroup analysis for sensitivity

Similar to the HTAC review, our subgroup analysis suggested that higher sensitivity was obtained with a use case of screening and diagnosis, use of saliva and nasopharyngeal specimens, pool size of 5, and Ct value of less than 40. (Appendix 6: Subgroup analyses). However, due to few studies, lack of at least two pooled studies in most subgroups and overlapping confidence intervals for sensitivity between subgroups, the effects of these explanatory variables may need further confirmation.

In terms of test brand, the 7500 Fast QuantStudio 6 Pro was found to have the highest sensitivity at 100% (95% CI 83.2 to 100%) based on a small-scale study (n=280, with 28 pools of 10 samples) that recruited both asymptomatic and pre-symptomatic patients in Brazil [5]. This test brand was not included in those reviewed in the HTAC review [17]. The local study by Lo et al that used the Sansure 2019-nCoV Diagnostic Extraction Kit on a MA6000 PCR machine (China) had a sensitivity ranging from 83% (if using a pool size of 5) to 50% (is using a pool size of 20). Evidence is insufficient at this time to conclude which specific test brand is superior.

Subgroup analysis according to disease prevalence could not be performed as only 1 study provided data on prevalence (4.8%, [8]). We note however, that the value of pooled testing comes from its cost-saving features, something much needed in low-income countries. While it makes sense that doing pooled testing in populations with high prevalence of the disease would yield higher true positives and lower false negative results, the cost of running RT-PCR in individual samples that yield positive tests in pooled sampling would increase rather than decrease the cost of diagnosis of the disease in high prevalence areas. To guide optimal pool size and obtain an acceptable sensitivity and cost-effectiveness, pooled testing should be conducted in targeted populations where the risk of disease and community prevalence are low.

Based on our subgroup analysis, we recommend that pooled testing be used for screening of SARS-CoV-2–using a pool size of 5. We suggest further study of the impact of other variables such as community prevalence, cut off values for positivity, presence and timing of symptoms, method of pooling (random vs non-random), amount of aliquot per sample, and timing of processing.

Resource savings and turnaround time

Resource savings reported from 21 studies ranged from 48% (pool size of 5 to 16; 15% positivity rate) [5] to 90% (pool size of 10; 0.07% positivity rate) [11]. In the local study by Lo et al, the percent savings increased with more stages of pooling; from 69% (2-stage Dorfman 5-1) to 79% (3-stage Dorfman 10-5-1) and 83% (4-stage Dorfman 20-10-5-1) at a constant positivity rate of 2.7%, but with decreasing sensitivity from 83.3% to 50%, and increasing delay in turnaround time from 2 to 4 batch runs for positive pools. For negative samples, the average turnaround time was from 1.09 to 1.44 batch runs, which meant that most of the negative samples tested using Dorfman 5-1 would still be released on the same batch run. (Table 2)

Table 2. Turnaround times (Lo et al)

Method	Turnaround time (TAT) (no. of batch runs)	
	Positive pools	Negative pools
Dorfman 5-1	2	1.09
Dorfman 10-5-1	3	1.21
Dorfman 20-10-5-1	4	1.44

Laboratory errors

One study [24] reported two significant human errors that occurred during the first period of high throughput testing using the pooling strategy; one involving inaccurate manipulation of a sample, and the other being incorrect orientation of a 96-well sample block. They attributed these to the extremely high test throughput and relative novelty of pooling protocol, and was mitigated by holding back reporting of negative pooled samples until individual testing and analysis of positive pools was complete.

Recommendations from Other Groups

The use of pooled testing for either screening, diagnosis or surveillance has been recommended by several local and international health agencies.

- The **CDC** (23 Oct 2020) published an interim guidance that authorizes certified laboratories to use specimen pooling to expand SARS-CoV-2 nucleic acid diagnostic or screening testing capacity [2].

- The **US FDA** (25 Aug 2020) encourages developers to consider validating their tests first for screening asymptomatic individuals and for testing pooled samples in settings with low prevalence, wherein more negative results are expected [26].

- The **European CDC** (28 May 2020) recommended [27] and provided methodology [28] for a binary splitting pooling method or a single stage non-adaptive group-testing approach for up to 1.3% positivity without the need to subsequently test individual samples.

- The **College of American Pathologists (CAP)** (n.d.) stated that although pooled testing may conserve RT-PCR test kits in low-prevalence setting, the challenges for proper implementation include lack of infrastructure such as automated equipment, reduced sensitivity due to dilution of specimens, and increased turnaround time for positive tests due to multistage process [29].

- The **Philippine Society of Pathologists Inc. (PSP)** (29 May 2020) recommended in its position paper the implementation of pooled RT-PCR COVID testing to expand testing capacity, reduce turnaround time, and conserve reagents and human resources [30]. It recommended screening asymptomatic persons in targeted populations, including low-prevalence communities (10% or less) (Table 3), and a pool size of 5 until an accurate prevalence of cases with SARS-CoV-2 is identified in the population [13].

- The **Philippine Department of Health (DOH) Health Technology Assessment Council (HTAC)** (11 Dec 2020) noted that pooled testing works well in low-prevalence (<5%) and small pool sizes. They recommended a pool size of 5 and the use of RT-PCR test kits that are validated by the Research Institute of Tropical Medicine and approved by the Food and Drug Administration or other authorized institutions for pooled testing use. A minimum sensitivity of 90% was recommended, and that Ct cutoff must be extended 3 Ct beyond the recommended value of the manufacturer [31].

- The **Philippine DOH** (23 Nov 2020) has issued interim guidelines through Department Memorandum 2020-0539 [29] specifying eligible populations for pooled testing based on the pooling protocols set by the Philippine Society of Pathologists, Inc., RITM, and DOH [28]. (Table 4)

Table 3. Targeted populations for pooled testing based on Philippine Society of Pathologists (Appendix C in Lo et al 2020)

A. Low prevalence communities (10% or less) for epidemiologic surveillance and aggressive contact tracing; B. Targeted community testing in areas that are under lockdown to identify additional infected individuals and to guide in decisions for lifting the lockdown; C. Surveillance of health care workers and all workers in the health care facility D. Workplace testing to include factory workers, market vendors, call center agents, transportation workers, and others; E. Border testing at airports and seaports for inbound foreign travelers and returning residents; F. Overseas deployment of OFWs; G. Returning OFWs; H. Frontline government workers (police, military, quarantine, immigration officers to name a few); I. Locally Stranded Individuals (LSI)

J. Any other vulnerable populations to be determined in the future

Table 4. Eligible populations for pooled testing based on DOH Memorandum 2020-5039[11]

1. Screening of population groups a. Inbound international travelers, including returning Filipinos, Overseas Filipino Workers, and foreigners; and b. Interzonal domestic travelers, including returning residents. **2. Surveillance of population groups** a. Health care workers in health facilities; b. Frontline government workers (police, military, quarantine, immigration officers, to name a few); c. Factory workers, market vendors, call center agents, transportation workers, and others in workplace settings; d. Other populations to be determined in the future. **3. Surveillance of communities that fulfill any of the two (2) criteria** a. COVID-free Municipalities: No cases reported yet since the start of the pandemic and/or for 2 weeks by date of report b. Attack Rate: Municipalities/cities with attack rate less than 100 per 100,000 population based on the data for the last two weeks. c. Should results of pooled testing manifest an Attack Rate of more than 100 per 1,000 Population or a Prevalence Rate of more than 10%, pooled testing shall be discontinued.

Research Gaps

Pooled testing methods in included studies differed in pool size, number of pooling stages, brand of test kit, Ct cut-off for positivity, with positivity rates ranging from <1% to as high as 15%. Since each laboratory has its own workflow and validation methods, the optimal pooling strategy would depend on their initial validation findings and the current community prevalence.

There are 2 ongoing studies: a validation study on RT-PCR, pooled RT-PCR, LAMP and pooled LAMP in diagnosis of COVID-19 (NCT04581083; recruiting; estimated study completion: 30 Nov 2020) [12] and a multi-center validation of SARS CoV-2 RT-PCR testing using combinatorial tapestry pooling (CTRI/2020/06/026005; registered 21 Jun 2020; not yet recruiting).

References

[1] Center for Devices and Radiological Health. (2020, August 24). Pooled Sample Testing and Screening Testing for COVID-19. U.S. Food and Drug Administration. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/pooled-sample-testing-.

[2] Centers for Disease Control and Prevention. (2020, October 23). Interim Guidance for Use of Pooling Procedures in SARS-CoV-2 Diagnostic, Screening, and Surveillance Testing. https://www.cdc.gov/coronavirus/2019-ncov/lab/pooling-procedures.html.

[3] Department of Health. (n.d.). Pooled testing for screening and monitoring COVID-19. Health Technology Assessment Department of Health. https://hta.doh.gov.ph/2020/12/11/pooled-testing-for-screening-and-monitoring-covid-19/.

[4] Iacobucci, G. (2020). Covid-19: Study findings strongly support use of pooled testing, say researchers. BMJ, m4267. https://doi.org/10.1136/bmj.m4267

[5] Christoff AP, Cruz GNF, Sereia AFR, Boberg DR, de Bastiani DC, Yamanaka LE, et al.

Swab Pooling for Large-Scale RT-qPCR Screening of SARS-CoV-2. SSRN Electron J. 2020;

[6] de Salazar A, Aguilera A, Trastoy R, Fuentes A, Alados JC, Causse M, et al. Sample pooling for SARS-CoV-2 RT-PCR screening. Clin Microbiol Infect. 2020;26.

[7] Lüsebrink J, Schildgen V, Schildgen O. Pooling is an insufficient strategy to avoid health care staff to patient transmission of SARS-CoV-2. Infect Control Hosp Epidemiol. 2020;1–3.

[8] Singh AK, Nema RK, Joshi A, Shankar P, Nema S, Raghuwanshi A, et al. Evaluation of pooled sample analysis strategy in expediting case detection in areas with emerging outbreaks of COVID-19: A pilot study. PLoS One [Internet]. 2020;15(9 September 2020):1–8. Available from: http://dx.doi.org/10.1371/journal.pone.0239492

[9] Wang H, Hogan CA, Miller JA, Sahoo MK, Huang C, Mfuh KO, et al. Performance of Nucleic Acid Amplification Tests for Detection of Severe Acute Respiratory Syndrome Coronavirus 2 in Prospectively Pooled Specimens. Emerg Infect Dis [Internet]. 2020;27(1). Available from: http://www.ncbi.nlm.nih.gov/pubmed/33183494

[10] Abdalhamid B, Bilder CR, McCutchen EL, Hinrichs SH, Koepsell SA, Iwen PC. Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources. Am J Clin Pathol. 2020;153(6):715–8.

[11] Hogan CA, Sahoo MK, Pinsky BA. Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2: Letter. JAMA. 323(1967–9).

[12] Tumanan-Mendoza B, Flores-Genuino RNS, Bermudez-delos Santos A. Should pooled sample testing using RT-PCR be used in screening patients suspected to have COVID-19? 2020.

[13] Lo R, Barrientos A, Espiritu B, Santiago FK, Iii AT, Veloso J, et al. An Evaluation of Pooling Strategies for qRT-PCR Testing for SARS-CoV-2 Infection : A pragmatic multi-site parallel operational study by The Philippine Society of Pathologists , Inc . Philipp J Pathol. 2020;5(2):1–22.

[14] Mastrianni D, Falivena R, Brooks T, McDermott B, Tan J, Vandell R, et al. Pooled Testing for SARS-CoV-2 in Hospitalized Patients. J Hosp Med. 2020;15(9):538–9.

[15] Pasomsub E, Watcharananan SP, Watthanachockchai T, Rakmanee K, Tassaneetrithep B, Kiertiburanakul S, et al. Saliva sample pooling for the detection of SARS-CoV-2. J Med Virol [Internet]. 2020;0–3. Available from: http://dx.doi.org/10.1002/jmv.26460

[16] Health Technology Assessment Department of Health. Pooled Testing for COVID-19 [Internet]. [cited 2020 Jan 1]. Available from: https://hta.doh.gov.ph/2020/12/11/pooled-testing-for-screening-and-monitoring-covid-19/

[17] Philippine DOH. Use of Pooled Testing for the Diagnosis, Screening and Surveillance of COVID-19: Rapid Review V 4.0.

[18] Alizad-Rahvar AR, Vafadar S, Totonchi M, Sadeghi M. False-negative mitigation in group testing for COVID-19 screening. medRxiv. 2020;1–18.

[19] Cesselli D, Bulfoni M, Marzinotto S, Marcon B, Cmet S, Cussigh AR, et al. Implementation and validation of a pooling strategy for a sustainable screening campaign for the presence of SARS-CoV-2. medRxiv [Internet]. 2020;2020.08.28.20174946. Available from: https://doi.org/10.1101/2020.08.28.20174946

[20] Mohanty S, Ravindra A, Gupta K, Hallur V, Behera B, Mahaptra A, et al. Intricacies in characterizing positivity in pooled sample testing for SARS-CoV-2. J Med Virol. 2020;0–2.

[21] Gavars D Perminovs D Stasulans J Stana J Metla Z Pavare J Tauckels E Gulbis E Dumpis U GM. Saliva as testing sample for SARS-CoV-2 detection by RT-PCR in low prevalence community setting (preprint). Medrxiv [Internet]. 2020;2020.10.20.20216127. Available from: http://medrxiv.org/content/early/2020/10/21/2020.10.20.20216127.abstract

[22] Chhikara K, Kanta P, Ghosh A, Prakash RC, Goyal K, Singh MP. Validation of SARS CoV-2 detection by real-time PCR in matched pooled and deconvoluted clinical samples before and after nucleic acid extraction: a study in tertiary care hospital of North India. Diagn Microbiol Infect Dis. 2020;99(January):115206.

[23] Li H, Sun K, Persing DH, Tang Y-W, Shen D. Real-time Screening of Specimen Pools for Coronavirus Disease 2019 (COVID-19) Infection at Sanya Airport, Hainan Island, China. Clin Infect Dis. 2020;2019(Figure 1):2019–21.

[24] Chong BSW, Tran T, Druce J, Ballard SA, Simpson JA, Catton M. Sample pooling is a viable strategy for SARS-CoV-2 detection in low-prevalence settings. Pathology. 2020;52(7):796–800.

[25] Kim SY, Lee J, Sung H, Lee H, Han MG, Yoo CK, et al. Pooling upper respiratory specimens for rapid mass screening of COVID-19 by real-time RT-PCR. Emerg Infect Dis. 2020;26(10):2469–72.

[26] FDA U. FDA Provides Update on Pooled Sample Testing and Screening Testing for COVID-19.

[27] ECDC - European Centre for Disease Prevention and Control. COVID-19 Testing Strategies and Objectives [Internet]. ECDC Technical Report. 2020. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/TestingStrategy_Objective-Sept-2020.pdf

[28] Centre for Disease Prevention E. Methodology for estimating point prevalence of SARS-CoV-2 infection by pooled RT-PCR testing [Internet]. 2020. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Methodology-estimating-point-prevalence -SARS-CoV-2-infection-pooled-RT-PCR-testing.pdf

[29] College of American Pathologists. Pooled Testing: Guidance from the CAP's Microbiology Committee [Internet]. 2021. Available from: https://www.cap.org/covid-19/pooled-testing-guidance-from-cap-microbiology-committee

[30] Padua Jr. R. Diagnostic testing strategies to manage COVID-19 pandemic: proposed by the Philippine Society of Pathologists, Inc. Philipp J Pathol [Internet]. 2020;5(1):5–8. Available from: https://doi.org/10.21141/.PJP.2020.08

[31] DOH. Right test for the right reason: Updates on new testing technologies for COVID-19 [Internet]. Available from: https://doh.gov.ph/press-release/RIGHT-TEST-FOR-THE-RIGHT-REASON-UPDATES-ON-NEW-TESTING-TECHNOLOGIES-FOR-COVID-1

Appendix 1: Characteristics of Included Studies

	Study ID	Use Case	N	Symptom Status	Specimen Used	Brand of RT-PCR test kit	Ct value for positivity	Pool size	Prevalence / Rate of Positivity	Pooling method[b]
1	Abdalhamid (USA)	Screening	60	At risk for COVID-19 (determined by the public health dept)	NP	CDC (2019-nCoV) Real-Time RT-PCR Diagnostic Panel kit (CDC, Atlanta, GA)	<40	5	3.33	2-stage/ pooled sample/media
2	Alcoba-Florez (Spain)	Not stated	4475	persons tested at University Hospital Nuestra Señora de Candelaria in August 2020	NP	Real Accurate Quadruplex corona-plus PCR Kit (PathoFinder, The Netherlands) TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific, Waltham, MA, USA)	< 40	5	N/A	2-stage/ pooled sample/media
3	Alizad-Rahvar (Iran)	Screening	263	Not stated	Throat swabs	(2019-nCoV) Real-Time Multiplex kit (Liferiver)	<45	6	7.6	2-stage w/ groupMix method/pooled sample/media
4	Barak(1) (Israel)	Not stated	117576	symptomatic but also asymptomatic carriers	NP	TaqPath qPCR Master Mix on the QuantStudio 5 Real-Time PCR Instrument	Not stated	8	1.7	2-stage/ pooled sample/media
	Barak(2)		16240[					5	5.76	
5	Cesselli (Italy)	Screening	3592	Asymptomatic subjects	NP	DiaSorin Molecular SimplexaTM COVID-19 direct assay system	< 37	8	0.64	2-stage/ pooled sample/media

	Study ID	Use Case	N	Symptom Status	Specimen Used	Brand of RT-PCR test kit	Ct value for positivity	Pool size	Prevalence / Rate of Positivity	Pooling method[b]
6	Chhikara (India)	Screening	500	Suspected COVID-19 patients	Throat swabs and NP	Taq Man probe–based commercial kit (Taq Path kit) in Applied Biosystem 7500 real-time machine (ABI, USA).	< 37	5	4.2	2-stage/ pooled samples & RNA
7	Chong(1) (Australia)	Not stated	10,312	Samples from lower acuity settings	Variety of swabs, including dry swabs	SARS-CoV-2 rRT-PCR Pooled sample: RdRp gene Individual sample: E or N gene	45	8	0.63	2-stage/ pooled samples/media
	Chong(2)	Not stated		Samples from lower acuity settings	Variety of swabs, including dry swabs	SARS-CoV-2 rRT-PCR Pooled sample: RdRp gene Individual sample: E or N gene	45	4		
	Chong(3)	Not stated	19,388	Samples from lower acuity settings	Variety of swabs, including dry swabs	SARS-CoV-2 rRT-PCR Pooled sample: RdRp gene Individual sample: E or N gene	45	4	0.02	
8	Christoff (Brazil)	Screening	613	Asymptomatic and presymptomatic patients	NP 'swab from nostril into 5 ml of saline"	7500 Fast,QuantStudio 6 Pro Real Time PCR (Applied Biosystems, USA), or in a CFX 384 (BioRad, USA); genes E and RdRp	< 40	5 to 16; ave 13.67	15.33	2-stage/pooled swabs
9	Das (USA)	Screening	7000	Asymptomatic HCWs	MidTurbinate[	CDC (2019-nCoV) Real-Time RT-PCR Diagnostic Panel kit (CDC, Atlanta, GA) & Panther Fusion SARS-COV-s LDT	< 36	10	0.11	2-stage/ pooled

	Study ID	Use Case	N	Symptom Status	Specimen Used	Brand of RT-PCR test kit	Ct value for positivity	Pool size	Prevalence / Rate of Positivity	Pooling method[b]
10	De Salazar (Spain, 9 sites)	Screening and diagnosis	3509	Patients or health professionals (at least 24 were for clearance after testing positive for SARS-CoV2)	NP Random pooling according to availability at each site; Processed within 24 h; Aliquot amount not stated	Viasure SARS-CoV-2 Real Time PCR (CerTest) TaqMan2019-nCoV Assay Kit v1 (Thermo Fisher Scientific) Allplex 2019-nCoV Assay (Seegene) Light Mix E gene (Roche)	<35	9,10	6.87	2-stage/pooled samples/media
11	Garg 2020a (1) (India)	Screening	4620	Samples referred for testing to COVID laboratory, Dr Ram Manohar Lohia Institute of Medical Sciences, Lucknow.	NP and OP	LabGun COVID-19 RT-PCR Kit (Lab Genomics)	Not stated	10	1.56	2-staged/ pooled samples/media
	Garg 2020a(2)		14950					5	1.38	
12	Gavars (Latvia)	Screening	3660	workers in a textile factory with an outbreak; 68% asymptomatic	Saliva	laboratory-developed and validated test method which detects S and N genes of the SARS-CoV-2 virus (LoD=1 cp/rxn)	Not stated	5	1.17	2-stage/ pooled
13	Hogan (USA)	Screening	3660	Samples for routine respiratory virus testing Stanford Health Care Clinical Virology Laboratory	NP and OP	Not stated (Germany kit)	Not stated	10	0.07	2-stage
14	Li 2020 (China)	Screening	944	High risk exposure	NP	Xpert Xpress SARS-CoV-2 assay (Sunnyvale, CA); cartridge-based	Not stated	9 or 10	0.21	2-stage/ pooled samples/media

	Study ID	Use Case	N	Symptom Status	Specimen Used	Brand of RT-PCR test kit	Ct value for positivity	Pool size	Prevalence / Rate of Positivity	Pooling method[b]
15	Lo 2020 (Philippines)	Screening	440	volunteer employees from a supermarket chain excluded those with fever, cough, colds, or shortness of breath at the time of interview, those with previous RT-PCR testing, pregnant women, less than 18 years of age	NP and OP 50 µL	Sansure Novel Coronavirus (2019-NCoV) Nucleic Acid Acid Diagnostic kit; RT-PCR performed on MA6000 PCR machine (China)	For pooled testing, any target gene amplification (ORF1 and N genes) regardless of Ct value, degree of amplification of curve properties (sigmoid or non-sigmoid) will be considered positive Individual testing will undergo the same interpretation as manufacturer's specifications	5 10-5-1 20-10-5-1	2.73	2-, 3-, 4-stage pooling/ pooled samples/media
16	Lusebrink (Germany)	Screening	280	Healthcare staff	Throat washes (n=327), swabs (n=32), bronchoalveolar lavages[ABC5] (n=1) 30 µL	RealStar SARS-CoV-2 RT-PCR Kit (Altona Diagnostics, Hamburg, Germany)	<30	10	2.86	2-stage/ pooled samples

	Study ID	Use Case	N	Symptom Status	Specimen Used	Brand of RT-PCR test kit	Ct value for positivity	Pool size	Prevalence / Rate of Positivity	Pooling method[b]
17	Mastrianni (USA)	Screening patients for admission into either COVID or non-COVID units	530	patients at low risk for SARS-CoV-2 admitted to a community hospital; without complaints8suggestive of COVID-19, may have had negative inflammatory markers, no significant lymphopenia and negative imaging	NP	Gene Xpert, cartridge-based	Not stated	2 to 3	0.75	2-stage/ pooled swabs
18	Mohanty (India)	Screening	7228	symptomatic and asymptomatic individuals with either travel history or active contacts of a laboratory-confirmed case.	nasal, NP, and throat swabs	Taqman primer probe designed by NIV, Pune, India and Invitrogen SuperScriptTM III Platinum One-Step Quantitative RT-PCR Kit/AgPath-ID TM One-Step RT-PCR Kit on a AriaMx Real-time PCR Instrument (Agilent, California, USA).	≤ 35	4	2.07	2-stage/ pooled samples/media
19	Pasomsub(1) (Thailand)	Screening	200	Patients under investigation for COVID-19 during the outbreak in Bangkok, Thailand	Saliva	SARS-CoV-2 Nucleic Acid Diagnostic Kit (Sansure, Changsha, China)	≤ 45.1	5	8.5	2-stage/ pooled RNA
	Pasomsub(2)							10		
20	Singh (India)	Screening	545	Suspected COVID-19 patients	NP and OP 200 µL	Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2 (BGI, Hong Kong)	≤ 35	5	4.58	2-stage/ pooled sample/media

	Study ID	Use Case	N	Symptom Status	Specimen Used	Brand of RT-PCR test kit	Ct value for positivity	Pool size	Prevalence / Rate of Positivity	Pooling method[b]
21	Wang(1) (USA)	Screening and diagnosis	880	Samples from tertiary-care academic hospitals and affiliated outpatient facilities in the San Francisco Bay Area of California.	NP and OP 500 µL/8 = 62.5 µL	Laboratory-developed test (LDT) targeting the envelope gene with the Rotor-Gene Q Instrument (QIA-GEN) *As per manufacturer's recommendation & ROC curve analysis of pooled relative light unit (RLU) values, with individual test as reference standard, to determine optimal RLU discriminated threshold	40-45 (for indeterminate) <35 (positive)	8	6.6	2-stage/ pooled samples/media
	Wang(2)					Panther Fusion SARS-CoV-2 assay (Hologic)				
	Wang(3)					Panther Aptima SARS-CoV-2 assay (Hologic)				
	Wang(4)					Panther Aptima-450 SARS-CoV-2 assay (Hologic)				

Appendix 2: GRADE Evidence Profile

Question: Should pooled testing using RT-PCR for SARS-CoV-2 be used to screen for COVID-19 in suspected patients with COVID-10?

Sensitivity	0.81 (95% CI: 0.72 to 0.88)
Specificity	0.99 (95% CI: 0.98 to 1.00)

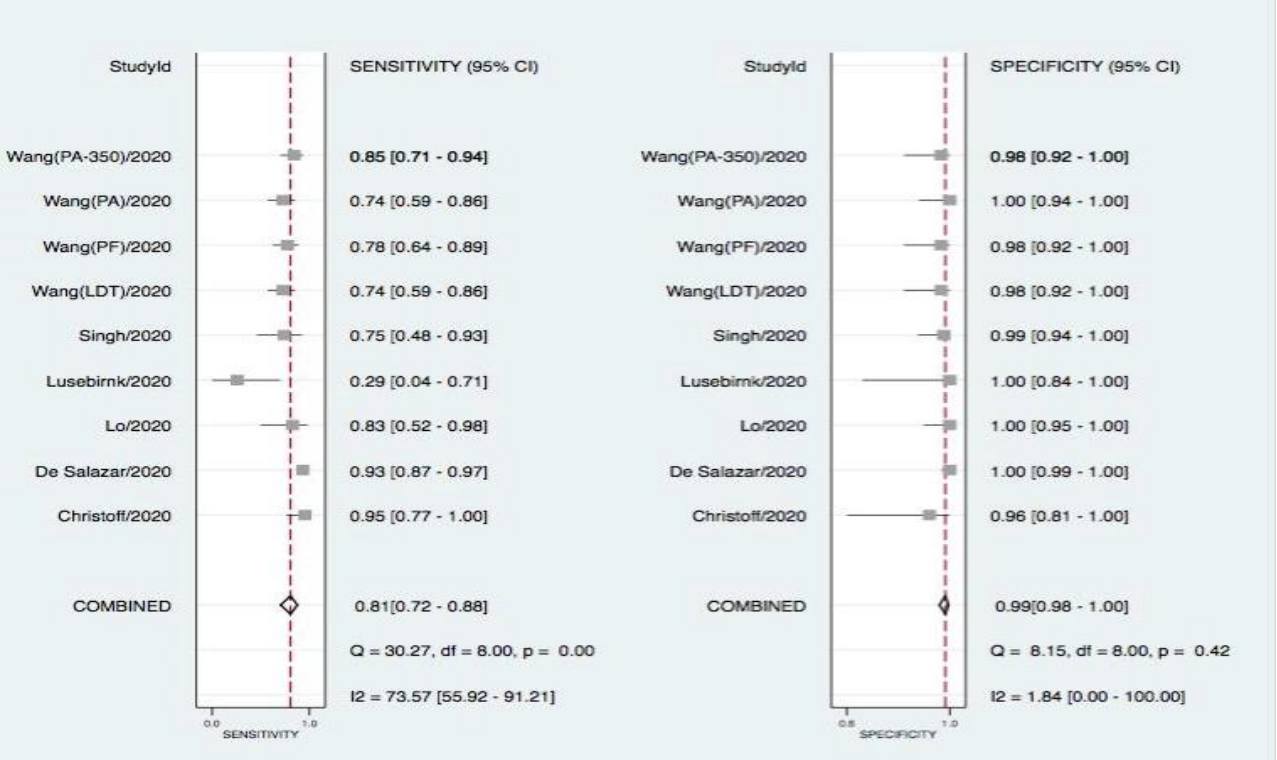
Prevalences	1%	5%	10%

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 1%	pre-test probability of 5%	pre-test probability of 10%	
True positives (patients with COVID-19)	6 studies 5987 patients	cross-sectional (cohort type accuracy study)	not serious	not serious	serious [a]	not serious [b]	none	8 (7 to 9)	41 (36 to 44)	81 (72 to 88)	⨁⨁⨁◯ MODERATE
False negatives (patients incorrectly classified as not having COVID-19)								2 (1 to 3)	9 (6 to 14)	19 (12 to 28)	
True negatives (patients without COVID-19)	6 studies 5987 patients	cross-sectional (cohort type accuracy study)	not serious	not serious	not serious [c]	not serious	none	980 (970 to 990)	941 (931 to 950)	891 (882 to 900)	⨁⨁⨁⨁ HIGH
False positives (patients incorrectly classified as having COVID-19)								10 (0 to 20)	9 (0 to 19)	9 (0 to 18)	

Explanations

a. I2=77.7%
b. wide CIs that crosses line of significance
c. different study population, pool size, brand of test kit

Appendix 3: Forest plot for sensitivity and specificity for pools as unit of analysis (N=6 studies)



Appendix 4: Characteristics of Ongoing Studies

No	Clinical Trial ID / Title	Study design, sample size	Country	Population	Intervention Group	Comparison Group	Clinical Outcomes
1	**Validation of SARS CoV-2 RT-PCR testing using combinatorial tapestry pooling of samples: a multi-centre study (CTRI/2020/06/026005)**	Retrospective observational; N=321 Not yet recruiting Registered on: 21/06/2020	India	Age 1 day to 99 years, both genders, NP swabs submitted for COVID-19 testing	Combinatorial tapestry pooling	Individual testing	Primary outcome: Sn and Sp of combinatorial tapestry pooled testing vs individual testing Secondary outcome: Sn and Sp of simple pooled testing vs individual testing

No	Clinical Trial ID / Title	Study design, sample size	Country	Population	Intervention Group	Comparison Group	Clinical Outcomes
2	**Validation of Laboratory Techniques, Strategies, and Types of Samples for Epidemiological Control in the Covid-19 Pandemic** (NCT 04581083) • Recruiting • Estimated study completion date: November 30, 2020	Cross-sectional; N=30 Completed: October 30, 2020	Bolivia	Age 21-64 Symptomatic: Subjects with signs and symptoms of respiratory infection less than or equal to 3 days, preferably with clinical and molecular diagnosis compatible with Covid-19. Asymptomatics: Subjects who have had direct contact with people infected and who have not shown any symptoms related to Covid-19. Negative: Individuals with negative RT-PCR testing for SARS-CoV-2 (reference test) who have not manifested any symptoms seven days prior to sampling.	Pooled RT-PCR	individual RT-PCR	Validation

Appendix 5: Appraisal of Included Studies

Clinical Question: Among patients with suspected COVID-19, how accurate is pooling of samples as compared to individual testing using RT-PCR?

Directness

	Clinical Question	Research Question
Population	Patients suspected of having COVID-19	Patients suspected of having COVID-19
Exposure	Pooled sampling compared to individual sampling using RT-PCR	Study of unknown samples: For all 19 studies, pools with positive results were compared with individual testing using RT-PCR detection of SARS-CoV-2 For 5 studies, pools with negative results were also compared with individual testing using RT-PCR detection of SARS-CoV-2
Outcome	Presence or Absence of SARS-CoV-2 RNA	Presence or Absence of SARS-CoV-2

Validity

	Study ID	APPRAISAL #1: Was the reference standard an acceptable one?	APPRAISAL#2: Was "definition" of the index test and the ref standard independent?	APPRAISAL#3: Was "performance" of the index test and the ref standard independent?	APPRAISAL#4: Was the ref standard interpreted independently of the index test?	Overall risk of bias 1-2 high risk; 3-4 low risk
1	Abdalhamid	YES	YES	NO	No information	HIGH
2	Alcoba-Florez	YES	YES	NO	No information	HIGH
3	Alizad-Rahvar	YES	YES	NO	No information	HIGH
4	Barak	YES	YES	NO	No information	HIGH
5	Cesselli	YES	YES	NO	No information	HIGH
6	Chhikara	YES	YES	NO	YES	LOW
7	Chong	YES	YES	NO	No information	HIGH
8	Christoff	YES	YES	YES	No information	LOW
9	Das	YES	YES	NO	No information	HIGH
10	De Salazar	YES	YES	YES	No information	LOW
11	Garg 2020a	YES	YES	NO	No information	HIGH

	Study ID	APPRAISAL #1: Was the reference standard an acceptable one?	APPRAISAL#2: Was "definition" of the index test and the ref standard independent?	APPRAISAL#3: Was "performance" of the index test and the ref standard independent?	APPRAISAL#4: Was the ref standard interpreted independently of the index test?	Overall risk of bias 1-2 high risk; 3-4 low risk
12	Gavars	YES	YES	NO	No information	HIGH
13	Hogan	YES	YES	NO	No information	HIGH
14	Li	YES	YES	NO	No information	HIGH
15	Lo	YES	YES	YES	No information	LOW
16	Lusebrink	YES	YES	YES	No information	LOW
17	Mastrianni	YES	YES	NO	No information	HIGH
18	Mohanty	YES	YES	NO	No information	HIGH
19	Pasomsub	YES	YES	NO	no information	HIGH
20	Singh	YES	YES	YES	YES	LOW
21	Wang	YES	YES	YES	No information	LOW

Appendix 6: Subgroup Analyses

Covariate	Studies		Number of Pools	Sensitivity	
Pool Size	**N**	**Study ID**	**n**	**Sn (%)**	**95% CI**
5	2	Lo, Singh	197	0.73	0.51-0.95
6-10	3	De Salazar, Lusebrink , Wang(1), Wang(2), Wang(3), Wang(4)	820	0.52	0.08-0.97
5-16	1	Christoff	45	1.00	0.83-1.00
Positivity rate (%)	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**
0-5	3	Lo, Lusebrink, Singh	225	0.52	0.08-0.95
>5-10	2	De Salazar, Wang	792	0.78	0.60-0.97

>10-15	1	Christoff	45	1.00	0.83-1.00
Ct value for positivity	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**
Any amplification	1	Lo	44	0.83	0.56 – 0.98
<40	2	Christoff, Wang	484	0.84	0.68-1.00
<35	1	Singh	109	0.75	0.48-0.93
<30	1	Lusebrink	28	0.29	0.04-0.71
Not stated	1	De Salazar	352	0.93	0.87-0.97
RT-PCR Brand	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**
Sansure	1	Lo	44	0.83	0.56 – 0.98
7500 Fast QuantStudio 6 Pro	1	Christoff	45	1.00	0.83-1.00
Viasure+TaqMan+Allplex	1	De Salazar	352	0.93	0.87-0.97
RealStar SARS-CoV-2	1	Lusebrink	28	0.29	0.04-0.71
Real-Time Fluoroscent RT-PCR	1	Singh	109	0.75	0.48-0.93
Laboratory-developed test	1	Wang(1)	110	0.75	0.48-0.93
Panther Fusion	1	Wang(2)	110	0.74	0.59-0.86
Panther Aptima 450	1	Wang(3)	110	0.78	0.64-0.89
Panther Aptima	1	Wang(4)	110	0.74	0.59-0.86
Symptom presence	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**
Asymptomatic	1	Lo	44	0.83	0.56 – 0.98
Mixed	2	Christoff, Wang	485	0.84	0.68-1.00

Unspecified	3	de Salazar, Lusebrink, Singh	489	0.52	0.08-0.97
Use Case	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**
Surveillance	0	-	-	-	-
Screening	4	Christoff, Lo, Lusebrink, Singh	270	0.82	0.40-0.97
Screening and diagnosis	2	De Salazar, Wang(1), Wang(2), Wang(3), Wang(4)	1144	0.78	0.60-0.97
Specimen Used	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**
Nasopharyngeal (NP)	2	Christoff, de Salazar	397	0.92	0.84-1.00
NP + Oropharyngeal (OP)	3	Lo, Singh, Wang	636	0.73	0.51-0.95
Throat washes	1	Lusebrink	28	0.29	**0.04-0.71**
Timing of processing	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**
Within 24 hours	2	De Salazar, Singh	461	0.74	0.51-0.97
Within 48 hours	1	Christoff	45	1.00	0.83-1.00
Not stated	3	Lo, Lusebrink, Wang	556	0.50	0.08-0.95
Method of pooling	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**
Random	1	De Salazar	352	0.93	0.87-0.97
Non-random (e.g. consecutive)	1	Singh	109	0.75	0.48-0.93
Both random and non-random	**1**	Wang(1), Wang(2), Wang(3), Wang(4)	440	0.76	0.60-0.92
Not stated	**2**	Christoff, Lusebrink	73	0.54	0.08-1.00
Amount of aliquot per individual sample in pool (µL)	**n**	**Study ID**	**N**	**Sn (%)**	**95% CI**

50	1	Lo	44	0.83	0.56 – 0.98
200	**1**	Singh	109	0.75	0.48-0.93
300	**1**	Lusebrink	28	0.29	0.04-0.71
500	**1**	Wang	440	0.76	0.60-0.92
“swab from nostril into 5 ml of saline”	**1**	Christoff	45	1.00	0.83-1.00
Not stated	**1**	De Salazar	352	0.93	0.87-0.97

Appendix 7: Summary of diagnostic accuracy results (by pools as unit of analysis)

	STUDY ID	TP	FN	FP	TN	Sn %	95% CI	Sp %	95% CI	PPV %	95% CI	NPV %	95% CI	Accuracy%	95% CI	Positivity rate %	95% CI	% Savings in Tests Used
1	Abdalhamid	2	NA	0	NA	NA	NA	NA	NA	100%	–	NA	NA	NA	NA	3.33	0.41 to 11.53	63.33
2	Alcoba-Florez	162	NA	0	NA	NA	NA	NA	NA	100	–	NA	NA	NA	NA	NA	NA	61.90
3	Alizad-Rahvar	3	NA	0	NA	NA	NA	NA	NA	100	29.2 to 100	NA	NA	NA	NA	7.6	0.09 to 2.72	62.74
4	Barak (1) (P8)	1313	NA	54	NA	NA	NA	NA	NA	96.05	94.88 to 97.02	NA	NA	NA	NA	1.7	1.62 to 1.77	78.20
	Barak (1) (P5)	679	NA	38	NA	NA	NA	NA	NA	94.70	92.6 to 96.22	NA	NA	NA	NA	5.76	5.41 to 6.13%	80.00
5	Cesselli	20	NA	0	NA	NA	NA	NA	NA	100	83.16 to 100	NA	NA	NA	NA	0.64	.41 to 0.96	83.05
6	Chhikara	11	NA	0	NA	NA	NA	NA	NA	100%	–	NA	NA	NA	NA	4.2	2.62 to 6.35	69.00
7	Chong(1)	8	NA	0	NA	NA	NA	NA	NA	100%	–	NA	NA	NA	NA	0.08	0.03 to 0.15	74.90
	Chong(2)	49	NA	0	NA	NA	NA	NA	NA	100%	–	NA	NA	NA	NA			
	Chong (3)	3	NA	0	NA	NA	NA	NA	NA	100	–	NA	NA	NA	NA	0.29	0.22 to 0.38	74.94
8	**Christoff**	**20**	**0**	**0**	**25**	**100**	**83.16 to 100**	**100**	**86.28 to 100**	**100**	**–**	**100**	**–**	**100**	**92.13 to 100**	**15.33**	**12.57 to 18.43**	**48.06**
9	Das	8	NA	0	NA	NA	NA	NA	NA	100%	–	NA	NA	NA	NA	0.11	0.05 to 0.23	88.86

	STUDY ID	TP	FN	FP	TN	Sn %	95% CI	Sp %	95% CI	PPV %	95% CI	NPV %	95% CI	Accuracy%	95% CI	Positivity rate %	95% CI	% Savings in Tests Used
10	**De Salazar**	**99**	**7**	**0**	**246**	**93.40**	**86.87 to 97.30**	**100**	**98.51 to 100**	**100%**	**–**	**97.23%**	**94.50% to 98.63%**	**98.01%**	**95.95% to 99.20%**	**6.87**	**6.05 to 7.76**	**61.76**
11	Garg 2020a	61	NA	0	NA	NA	NA	NA	NA	100	–	NA	NA	NA	NA	1.56	1.22 to 1.96%	76.80
		194	NA	0	NA	NA	NA	NA	NA	100	–	NA	NA	NA	NA	1.38	1.2o to 1.58%	73.51
12	Gavars	43	NA	0	NA	NA	NA	NA	NA	100	–	NA	NA	NA	NA	1.20	0.87 to 1.61	78.25
13	Hogan	2	NA	1	NA	**100**		NA		66.7	20.6 to 93.9	NA	NA	NA	NA	0.07	0.01 to 0.25	88.90
14	Li	NA	0	NA	NA	NA	NA	NA	NA	100	–	NA	NA	NA	NA	0.21	0.03 to 0.76	87.92
15	**Lo(1) (5-1)**	**10**	**2**	**0**	**76**	**83.33**	**51.59 to 97.91**	**100**	**99.14 to 100**	**100**	**–**	**99.53**	**98.37 to 99.87**	**–**	**–**	**2.73**	**1.42 to 4.72**	**69.00**
16	**Lusebrink**	**2**	**5**	**0**	**21**	**28.57**	**3.67 to 70.9**	**100**	**83.89 to 100**	**100**	**–**	**80.77**	**72.44 to 87.03**	**82.14**	**63.11 to 93.94**	**2.86**	**1.24 to 5.55**	**65.00**
17	Mastrianni	4	NA	0	NA	NA	NA	NA	NA	100		NA	NA	NA	NA	0.75	0.21 to 1.92	64.34
18	Mohanty	150	NA	49	NA	NA	NA	NA	NA	75%	68.8 to 81.2	NA	NA	NA	NA	19	16 to 23	63.99
19	Pasomsub(1)	13	NA	0	NA	NA	NA	NA	NA	100		NA	NA	NA	NA	8.5	5.0 to 13.3	47.50
	Pasomsub(2)	13	NA	0	NA	NA	NA	NA	NA	100		NA	NA	NA	NA			40.00

Pooled Testing

	STUDY ID	TP	FN	FP	TN	Sn %	95% CI	Sp %	95% CI	PPV %	95% CI	NPV %	95% CI	Accuracy%	95% CI	Positivity rate %	95% CI	% Savings in Tests Used
20	**Singh**	**12**	**4**	**1**	**92**	**75.00%**	**47.62% to 92.73%**	**98.92%**	**94.15% to 99.97%**	**92.31%**	**62.60% to 98.85%**	**95.83%**	**90.78% to 98.17%**	**95.41%**	**89.62% to 98.49%**	**4.58**	**2.99 to 6.70**	68.07
21	**Wang(1)LDT]**	**34**	**12**	**1**	**63**	**73.91%**	**58.87% to 85.73%**	**98.44%**	**91.60% to 99.96%**	**97.14%**	**82.84% to 99.58%**	**84.00%**	**76.33% to 89.53%**	**88.18%**	**80.64% to 93.55%**	**6.59**	**5.04 to 8.44**	55.68
	Wang(1)PF	**36**	**10**	**1**	**63**	**78.26%**	**63.64% to 89.05%**	**98.44%**	**91.60% to 99.96%**	**97.30%**	**83.66% to 99.61%**	**86.30%**	**78.44% to 91.60%**	**90.00%**	**82.81% to 94.90%**			53.86
	Wang(1)PA	**34**	**12**	**0**	**64**	**73.91%**	**58.87% to 85.73%**	**100.00 %**	**94.40% to 100.00 %**	**100**	**–**	**84.21%**	**76.63% to 89.66%**	**89.09%**	**81.72% to 94.23%**			56.59
	Wang(1)PA-350	**39**	**7**	**1**	**63**	**84.78%**	**71.13% to 93.66%**	**98.44%**	**91.60% to 99.96%**	**97.50%**	**84.75% to 99.64%**	**90.00%**	**81.97% to 94.69%**	**92.73%**	**86.17% to 96.81%**			51.14

Note: In bold font are 6 studies with complete data for 2x2 table for unit of analysis as pools; In italics are data of Lo for 10-5-1 and 20-10-5-1 pooling methods for unit of analysis as individuals, while rest of studies provided data for unit of analysis as pools

Appendix 8. Summary of diagnostic accuracy (by individuals)

STUDY ID	TP	FN	FP	TN	Sn %	95% CI	Sp %	95% CI	PPV %	95% CI	NPV %	95% CI	Accuracy	95% CI
Christoff	94	0	0	519	100.00%	96.15% to 100.00%	100.00%	99.29% to 100.00%	100%		100%		100.00%	99.40% to 100.00%
De Salazar	234	7	0	3268	97.10%	94.11% to 98.82%	100.00%	99.89% to 100.00%	100%		99.79%	99.56% to 99.90%	99.80%	99.59% to 99.92%
Lo(1)	10	2	0	428	83.33%	51.59% to 97.91%	100.00%	99.14% to 100.00%	100%	–	99.53%	98.37% to 99.87%	99.55%	98.37% to 99.94%
Lo(2)	7	5	0	428	58.33%	27.67% to 84.83%	100.00%	99.14% to 100.00%	100%	–	98.85%	97.77% to 99.41%	98.86%	97.37% to 99.63%

STUDY ID	TP	FN	FP	TN	Sn %	95% CI	Sp %	95% CI	PPV %	95% CI	NPV %	95% CI	Accuracy	95% CI
Lo(3)	6	6	0	428	50.00%	21.09% to 78.91%	100.00%	99.14% to 100.00%	100%	–	98.62%	97.59% to 99.21%	98.64%	97.06% to 99.50%
Lusebrink	2	6	0	272	25.00%	3.19% to 65.09%	100.00%	98.65% to 100.00%	100%		97.84%	96.81% to 98.54%	97.86%	95.39% to 99.21%
Singh	21	4	5	460	84.00%	63.92% to 95.46%	98.92%	97.51% to 99.65%	80.77%	63.34% to 91.08%	99.14%	97.91% to 99.65%	98.16%	96.54% to 99.16%