Evidence Summary on the Use of RT-PCR Testing for COVID-19

Service Line	Evidence Summary
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Prepared by	Health Technology Assessment Council Health Technology Assessment Unit
Contact details	hta@doh.gov.ph | 8-875-7734 loc. 260 or 258

Acronyms Used in the Evidence Summary	
Acronym	**Definition**
Australia PHLN	Australia Public Health Laboratory Network
Australia TGA	Australian Therapeutic Goods Administration
CADTH	Canadian Agency for Drugs and Technologies in Health
Canada PHLN	Canada Public Health Learning Network
Canada PHO	Public Health Ontario
CDNA	Communicable Diseases Network Australia
CEBM	Center of Evidence-based Medicine (Oxford University)
China CDC	China Center for Disease Control and Prevention
Ct	Cycle threshold
DOH	Department of Health (Philippines)
ECDC	European Center for Disease Prevention and Control
FIND	Foundation for Innovative New Diagnostics
HIQA	Health Information and Quality Authority (Ireland)
HTW	Health Technology Wales
Japan MHLW	Ministry of Health, Labor, and Welfare of Japan
LRT	Lower Respiratory Tract
Malaysia MOH	Malaysian Ministry of Health
McMaster	McMaster University (Canada)
NIPH	Norwegian Institute of Public Health
NPS	Nasopharyngeal Swab
OPS	Oropharyngeal Swab
PHAC	Public Health Agency of Canada

PhilHealth	Philippine Health Insurance Corporation
PMDA	Japan Pharmaceutical and Medical Devices Agency
PSMID	Philippine Society for Microbiology and Infectious Disease
RITM	Research Institute for Tropical Medicine
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
Singapore MOH	Singapore Ministry of Health
South Korea MHW	South Korean Ministry of Health and Welfare
Thailand MOH	Thailand Ministry of Health
UK DOHSC	United Kingdom Department of Health and Social Care
UK MHRA	United Kingdom Medicines and Health Products Regulatory Agency
UK NHS	United Kingdom National Health Service
US CDC	United States of America Center for Disease Control and Prevention
US FDA	United States of America Food and Drug Administration
Vietnam MOH	Vietnam Ministry of Health
WHO	World Health Organization

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I. Background

The *World Health Organization (WHO)* declared the novel coronavirus disease (COVID-19), caused by severe acute respiratory syndrome 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 guidelines which set the real time reverse transcriptase polymerase chain reaction (RT-PCR) as the standard confirmatory test to diagnose COVID-19.

Nasopharyngeal specimens have been recommended by the Philippine Society for Microbiology and Infectious Disease (PSMID) for COVID-19 RT-PCR test. While nasopharyngeal specimen is the recommended specimen sample for RT-PCR, its method of collection poses several challenges such as its invasiveness and the need for skilled operators and more personal protective equipment.

Such challenges have led to exploration of alternative specimen samples for COVID-19 testing, specifically the use of saliva. Saliva specimens may be preferred by some testing centers over the other swab methods because it is much simpler, cheaper, quicker, non-invasive, and it does not require specialized personnel for sample collection. Recently, the US FDA authorized the emergency repurposed use of the RT-PCR test in saliva samples. Although there is a growing evidence on its actual use, its performance characteristics remain highly variable and conflicting results have been reported showing differences in brand kits, methods of collection, and laboratory procedures (Fernandes et. al., 2020; Ricco et. al.., 2020; Laszlo et. al., 2020).

This evidence summary looked into the systematic review of Buban, Villanueva, & Gregorio (2021) of the Living Clinical Practice Guidelines; the parallel testing results of the Philippine Red Cross, and the interim findings of the Research Institute for Tropical Medicine saliva study for the diagnostic performance of saliva specimens in RT-PCR testing. In addition, we also synthesized relevant information and evidence on the regulatory approval and validation requirements, performance characteristics, and existing recommendatory testing guidelines on the use of saliva specimens.

II. Policy Question

Should the DOH consider revising the current guidelines on RT-PCR tests for COVID-19 in view of the current evidence and presence of new variants?

Should the DOH consider the use of salivary clinical specimens for the detection of SARS-CoV-2 using RT-PCR?

III. Research Questions

A. Validation Requirements and Performance Specifications

- What are the current validation requirements and performance specifications for Emergency Use Authorization of RT-PCR kits regardless of sample type among stringent regulatory agencies?
- What are the new performance specifications for RT-PCR kits to detect new variants of concern issued by the stringent regulatory agencies?

B. Review of Guidelines and HTA Recommendations

- What are the current use cases of RT-PCR Tests based on country guidelines?
- What are the Cycle Threshold (Ct) values used by different agencies to delineate a positive from a negative RT-PCR result?
- What are the specimen samples recommended for RT-PCR and their respective use cases?
- What are the current recommendations of HTA agencies regarding the use of saliva as an alternate specimen for SARS-CoV-2 detection?

C. Diagnostic Performance

- What is the diagnostic performance of RT-PCR based on current evidence?
- What is the accuracy of tests for the detection of SARS-CoV-2 using saliva compared with nasopharyngeal, oropharyngeal, or lower respiratory tract clinical samples?
- What is/are the most appropriate method/s of collecting saliva for SARS-CoV-2 detection?
- What is the diagnostic performance (SN,SP) of RT-PCR in detecting new variants of concern?

D. Guidelines on New Variants

- Which genes would be best used to screen and confirm new variants (existing and expected variants in the future) of COVID-19?

E. Interpretation of Tests

- What are the guidelines in interpreting RT-PCR results seen in the latest body of evidence?

F. Estimated Cost of RT-PCR Testing

- What is the estimated cost of RT-PCR Testing using NPS/OPS?
- What is the estimated cost of RT-PCR Testing using saliva sample?

IV. Evidence Considered

A. Responsiveness to Disease Magnitude, Severity, and Equity

As of 18 March 2021, the COVID-19 pandemic has affected more than 219 countries and regions with at least 120, 915, 219 cases and 2,674,078 deaths worldwide (WHO, 2021). Locally, there are over 640,984 confirmed cases and 12,887 deaths(DOH, 2021). 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, 93.9% are mild cases, 1.1% are severe, 1.0% are critical, and 3.3% are asymptomatic. Currently, vaccinations on emergency use authorization have been initiated.

B. Safety and Effectiveness

1. Validation Requirements and Performance Specifications

a. What are the current validation requirements and performance specifications for issuance of emergency use authorization of RT-PCR kits regardless of sample type issued by the stringent regulatory agencies?

Of the 11 regulatory bodies reviewed, the reviewers found published guidelines on the validation requirements and performance specifications of RT-PCR kits from four countries, namely, the United States through the US FDA, United Kingdom through the UK MHRA, Japan through the PMDA, and the Philippines through the RITM. In addition, we also added the target product profile of the WHO for tests for diagnosis or confirmatory test for acute or subacute SARS-CoV-2 infection (e.g. during the first 2 weeks after symptom onset).

Table 1. Validation Requirements and Performance Specifications of RT-PCR for COVID-19 Testing

Regulatory Domains	Regulatory requirements and specifications							
	WHO	US			UK	Japan	Philippines	
Specimen	Any Specimen	NPS/OPS	Saliva	Asymptomatic Screening	Any Specimen	NPS/OPS	NPS/OPS	Saliva
Validation requirements								
Sample size	Not reported	Minimum of ● 30 positive samples and ● 30 negative samples	Minimum of ● 30 positive samples and ● 30 negative samples	*For a previously unauthorized test*: At least 20 positive samples. A minimum of three geographically diverse sites is recommended. *For a previously authorized test*: At least 20 positive and 100 negative asymptomatic specimens that are consecutively collected.	Minimum of ● 150 positive and ● 250 negative clinical samples	Minimum of ● 10 positive samples in the range of approximately 10 to 200,000 copies. Including: ○ 2 or more of 10-20 copies ○ and 1 or more of 100-200 copies and ● 15 negative samples	Minimum of ● 30 positive samples and ● 30 negative samples	Minimum of ● 30 positive samples and ● 30 negative samples

Reference Test	Not reported	High sensitivity EUA RT-PCR assay which uses a chemical lysis step followed by solid-phase extraction of nucleic acid (e.g., silica bead extraction)	FDA recommends selecting a comparator assay that has established high sensitivity with an internationally recognized standard or FDA SARS-CoV-2 Reference Panel.	*For a previously unauthorized test*: Healthcare provider collected NP swabs ran in an assay with established high sensitivity with an internationally recognized standard or the FDA SARS-CoV-2 Reference Panel. *For a previously authorized test*: Another EUA Authorized Molecular Assay	Reference method against which the Negative/Positive Percent Agreement is calculated	Japan NIID Method - *"Pathogen Detection Manual 2019-nCoV Ver. 2.9.1"*	WHO-prequalified or emergency use-listed qRT-PCR;WHO-recommended rRT-PCR, ; qRT-PCR assay previously verified to be of equivalent or better performance as one that is WHO-prequalified or WHO-recommended or US FDA EUL qRT-PCR assay for detection of SARS-CoV-2.	Parallel NPS-OPS test
Performance standards								
Analytical Sensitivity	Equivalent to 103 genomic copies per mL in any respiratory tract specimen type.	100% detection of all SARS-CoV-2 sequences will be detectable with the selected primers and probes (inclusivity study)	None mentioned	None mentioned specific to this use case	≤1000 SARS-CoV-2 copies/mL (limit of detection)	N/A	Appraisal of the manufacturer reported limit of detection (LOD) using a LOD verification assay	

Analytical Specificity	N/A	The test does not react with related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in a clinical specimen	No standard mentioned but FDA suggests to refer to previous FDA decision summaries or contact them for recommended organisms	None mentioned specific to this use case	No clinically relevant cross-reactivity or interference Minimal interference caused by common interferents at clinically relevant concentrations (dependent on sample type and analyte)	N/A	No cross-reactivity with other respiratory viruses or bacteria as indicated in the manufacturer's instructions for use	
Minimum Acceptable Clinical Sensitivity/PPA	≥95%	PPA: ≥95%	PPA: ≥95%	PPA: ≥95% (lower bound of 95% CI: >76%)	Clinical sensitivity or PPA: >95% (with 95% two-sided confidence interval entirely above 90%)	N/A	**HTAC** (2020 recommendation): >99% (*based on FIND)* **RITM**: SN: >95% (*based on WHO TPP)* **FDA**: SN: ≥ 95% *(Memo 2021-009)*	**RITM** PPA: 90% *(DM 2021-0161)* **FDA** SN: ≥95% *(Memo 2021-009)*
Minimum Acceptable Clinical Specificity/ NPA	≥99%	NPA: ≥95%	None mentioned	NPA: ≥98% (lower bound of 95% CI: >95%)	Clinical specificity or NPA: >95% (with 95% two-sided confidence interval entirely	N/A	**HTAC** (2020 recommendation) >99% *(based on FIND)* **RITM**: SP: >98%	**RITM** NPA: 90% *(DM*

					above 90%)		**FDA:** SP: ≥ 99% *(Memo 2021-009)*	*2021-0161)* **FDA:** SP: ≥99% *(Memo 2021-009)*

b. What are the new performance specifications for RT-PCR kits to detect new variants of concern issued by the stringent regulatory agencies?

Of the 11 regulatory bodies reviewed, the reviewers did not find performance specifications for RT-PCR kits to detect new variants of concern. However, three regulatory bodies (i.e., US FDA, Health Canada, and ECDC) noted the potential impact of new variants on the performance of RT-PCR kits and provided brief recommendations on detection and identification of variants.

- The Australian Therapeutic Goods Administration noted that the performance of emerging SARS-CoV-2 PCR tests designed to detect specific variants of concerns is still being established, and these tests will not identify variants that they have not been designed for.
- The US FDA acknowledges that the performance of a diagnostic test can be impacted by viral mutation; hence, as of February 2021, it released recommendations on evaluating potential impact of emerging and future SARS-CoV-2 viral mutations for developers of molecular diagnostic tests:
    - To design their test to minimize the impact of viral mutations on test performance, including the use of highly conserved pan-SARS-cov targets that are not solely specific to SARS-CoV-2 alone in combination with several targets that are specific to the virus.
    - To routinely monitor for viral mutations that may impact test performance, which can include aligning of genetic sequences of new variants from public data sources to investigate mutations and its impact on test performance ; and,
    - To clearly convey any test limitations in the test's labeling especially when performance of a test has not been established yet for different variants.
- Health Canada published an updated guidance in February 2021 for those applying for emergency use authorization of nucleic-acid based tests. It noted that in the submission of requirements, the agency will refer to the guidance set by the US FDA. In order to address the growing number of SARS-CoV-2 variants of concern, the agency requires the manufacturers to: assess the impact of new variants on their test; and, to indicate their risk management plan pertaining to the variants.
- The ECDC published a guidance document on the methods to use for the detection and identification of COVID-19 variants. In the publication, they said that whole genome sequencing, or at least complete or partial S gene, should be used to confirm infection with a specific variant. Further, diagnostic screening PCR-based assays can be used for early detection and prevalence calculation of variants of concern.

More details can be found in Annex B.

2. Review of Guidelines and HTA Recommendations

a. What are the current use cases of RT-PCR tests based on country guidelines?

Of the 19 guidelines reviewed, the reviewers found 18 published guidelines *(Australia [PHLN and CDNA], Canada [PHLN and PHAC], China, European Union, FIND, Indonesia, Japan, Malaysia, Philippines, Singapore, South Korea, Thailand, UK, USA, Vietnam, and WHO)* which mention the role of RT-PCR in the testing strategies for COVID-19. Fifteen (15) of these still recommend the use of RT-PCR for COVID-19 diagnosis while the other three (China, Indonesia, and FIND) did not specify use cases in their guidelines. Of the 15 that recommended RT-PCR for diagnosis, four recommended it also for surveillance while three also recommended it for screening purposes. No published guidelines were found for the UK NHS since it links to the UK DOHSC web page as its reference.

b. What are the Cycle Threshold (Ct) values used by different agencies to delineate a positive from a negative RT-PCR result?

Among the 18 guidelines, only China has set Ct value thresholds for delineating positive and negative cases. All other countries reviewed did not mention Ct values in their guidelines. China, in discussing nucleic acid testing, determined that tests with: *no Ct value* or *Ct value that is 40* is a negative assay; a result with a *Ct value less than 37* is positive; and those with *results in between 37 and 40* are recommended to undergo repeated experiments with PCR wherein a Ct value less than 40 with apparent peaks in the amplification curve would be considered as positive (China CDC, 2020).

Meanwhile, the US CDC explicitly mentioned that Ct values from different RT-PCR tests cannot be directly compared. Ct values can be affected by factors other than the viral load in the patient sample. Moreover, the processes and parameters that will lead to a Ct value result such as the processing of genetic material, the specific genetic target being measured, and the amount of patient sample required for the test, will vary between RT-PCR tests. Due to these variations, Ct values from different RT-PCR tests cannot be compared (US CDC, 2021). Moreover, the WHO advises users of RT-PCR diagnostic tests to review and follow instructions for use carefully in cases where manual adjustments of positivity thresholds for Ct values are recommended by the manufacturer. The WHO also reminds users that the clinical presentation is important to correlate with diagnostic findings. As such, a new specimen should be taken and tested using the same or different nucleic acid testing technology in times of mismatch with clinical presentation.

c. What are the specimen samples recommended for RT-PCR and their respective use cases?

Of the 18 published guidelines reviewed, only 15 included recommendations on the specimen types for COVID-19 RT-PCR testing while two guidelines (Singapore MOH and Vietnam MOH) have not specified sample specimens for RT-PCR testing. Excluding China, Indonesia, and the Foundation for Innovative New Diagnostics (FIND) which did not specify use cases in their guidelines, only 12 of these 15 guidelines had specified sample types per use cases and population.

Across the reviewed guidelines, nasopharyngeal swab (NPS) turned out to be the most common type of specimen recommended across all use cases and testing population. Next to NPS is oropharyngeal swab (OPS) which is another form of upper respiratory specimen. Majority of guidelines [five of eight] which recommended OPS, also recommended it to be collected together with NPS. Some of the guidelines also recommended the collection of lower respiratory tract and saliva specimens but these are usually for diagnosis in symptomatic populations only.

The table below shows the use case and testing population of different guidelines per specimen types.

Table 2: Review of Guidelines and HTA Recommendations for COVID-19 RT-PCR Testing

Recommended Specimen	RT-PCR for Diagnosis	RT-PCR for Screening	RT-PCR for Surveillance
Nasopharyngeal swab (NPS)	*For both symptomatic and asymptomatic:* Australia PHLN; PHAC; Japan MHLW; DOH Philippines, UK DOHSC, WHO *For symptomatic only:* Canada PHLN; South Korea MHW *No specified target population:* ECDC; Malaysian MOH; Thailand MOH; US CDC	*For asymptomatic:* PHAC DOH Philippines WHO *No specified target population:* ECDC	PHAC Thailand MOH
Oropharyngeal swab (OPS)	*For both symptomatic and asymptomatic:* Australia PHLN; DOH Philippines*; UK DOHSC*; WHO* *For symptomatic only:* South Korea MHW* *No specified target population:* ECDC; US CDC; Malaysia MOH*	WHO	DOH Philippines
Lower Respiratory Tract Specimen (LRT)	*For symptomatic only:* Canadian PHLN[1,3] ; Thailand MOH[1,2]; DOH Philippines[1]; South Korea; MHW[3]; US CDC[3] ; WHO[3] *No specified target population:* Japan MHLW[3]; ECDC; Australia; PHLN; Malaysia MOH[3]	ECDC	ECDC
NPS Combined with Other Respiratory Tract Specimens	Canada PHLN		
Saliva Specimen	*For symptomatic only:* Japan MHLW *No specified target population:* ECDC; US CDC	ECDC	ECDC
Other Non-Respiratory Specimen	WHO (Fecal Specimen)		US CDC (Wastewater)

**Indicated OPS to be collected with NPS; 1 For hospitalized patients or those with severe respiratory illness/ lower respiratory tract infection; 2 Thailand MOH recommended sputum specimens for non-intubated LRI patients, tracheal suction secretion for intubated LRI patients, and lung biopsy for non-intubated fatal LRI patients; 3 Specimen via spontaneous production*

d. What are the current recommendations of HTA agencies regarding the use of saliva as an alternate specimen for SARS-CoV-2 detection?

Of the 28 HTA agencies reviewed, four (HIQA of Ireland, HTW of Wales, McMaster University [McMaster] of Canada, and NIPH of Norway) have published a full rapid review report assessing the use of saliva as alternative specimen for SARS-CoV-2 RT-PCR testing, while one, the CADTH of Canada published a health technology update, and another cited an individual study (CEBM of Oxford University in the United Kingdom).

None of the reviewed HTA reports have explicitly recommended use of saliva-based testing for COVID-19 diagnosis. The reported ranges of point estimates from the HTA reports are summarized in the supplemental document.
No agency was able to investigate the appropriate saliva collection method and the appropriate target population.

- HTW, HIQA, and NIPH concluded that the current evidence reveals a lack of consistency in terms of method of sample collection (i.e. how collected, timing of test); poor reporting of test parameters; and are generally low in quality based on appraisals.
- CADTH and McMaster emphasized that the ability of saliva samples to be self-collected would be especially beneficial for those in remote locations who have limited access to COVID-19 testing centers and that adapting this technology may allow for greater capacity to test large groups of people. On the other hand, CEBM noted that a study in Korea (Jeong et al., 2020) found that SARS-CoV-2 is present in saliva, urine, and stool from COVID-19 patients until days 11-15 after symptom resolution. This study, however, was performed with a small sample size of five participants.
- Overall, the existing reports from HTA agencies are scanty. All six agencies mentioned that while saliva samples may result in safer and more comfortable testing to the healthcare workers and the patients, evidence on the detection of SARS-CoV-2 in saliva should be interpreted with caution as any emerging evidence can easily tip the result to either side of the benefit scale.

3. Diagnostic Performance

In 2020, the HTAC recommended RT-PCR test kits to have a clinical sensitivity and specificity of at least 99%. In terms of analytical performance, the HTAC recommended i) an analytical sensitivity wherein a confirmatory and screening gene has been tested and ii) an analytical specificity where no significant cross-reactivities have been identified. With this updated review, we noted the changes in recommended specifications on the clinical sensitivity and specificity which is at least 95% and at least 99%, respectively,

following the required specifications for COVID-19 RT-PCR test kits set by the Philippine FDA (FDA Memo No. 2021-009). A full discussion on performance standards for regulatory requirements can be found in Section 3.1.

a. What is the diagnostic performance of RT-PCR based on current evidence?

Based on the systematic review and meta-analysis of Tsang et al (2021) that included 23 studies (n=7,393 participants; 16,762 samples), the reviewers observed comparably high specificity (range 97–99%) and negative predictive values (range 95–99%) among different clinical specimens (i.e. nasal swabs, saliva, throat swab, pooled nasal and throat swab) when compared to the gold standard, nasopharyngeal swab. Additionally, the following results were obtained when comparing these samples to NPS:

- Sensitivity (SN) values:
  - Highest SN were achieved by pooled nasal and throat swabs: 97% (95% CI 0.93–1.00; I^2: 18.3%; 3 studies).
  - Lower SN were achieved by saliva (85%, 95% CI 0.75–0.93, I^2: 92.7%; 13 studies) and nasal swabs (86%, 95% CI 0.77–0.93; I^2: 69%, 7 studies)
  - Much lower SN by throat swabs (68%, 95% CI 0.35–0.94; I^2: 73.6%, 2 studies).
- Positive predictive values (PPV) values:
  - High PPVs were reported by pooled nasal and throat (97%, 95% CI 90–100, I^2=57.4%, 3 studies) and nasal swabs (96%, 95% CI 87–100, I^2=84.0%, 7 studies)
  - Slightly lower PPVs were obtained by saliva (93%, 95% CI 88–97, I^2=79.3%, 13 studies)
  - Lowest PPVs were reported for throat swabs at 75% (95% CI 45–96, I^2=64.5%, 2 studies).

b. What is the accuracy of tests for the detection of SARS-CoV-2 using saliva compared with nasopharyngeal, oropharyngeal, or lower respiratory tract clinical samples?

Evidence from Local Diagnostic Studies

- In the Research Institute for Tropical Medicine (RITM) validation study completed on April 16, 2021, the reported sensitivity (i.e., 79.9%, 95% CI: 75.3 to 84.1) did not meet the 90% target performance of RITM. Meanwhile, the reported specificity (i.e., 98.9%, 95% CI: 98.2 to 99.4) was close to the target performance. The results were based on 339 positive samples or 20.4% positivity rate. The validation study enrolled a total of 1,661 participants from January to March 2021 in selected areas from National Capital Region and Region IV-A. The test kit brands used in the study were *MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit*

*I*l and *MiRXES Fortitude Kit 2.1* which are both not yet authorized locally for saliva-based RT-PCR. Currently, there are 2 brands authorized by the Philippine FDA for saliva-based RT-PCR: *Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay*; and, *AMT Resolute 2.0 (100 reactions) (CKFG0002)*.

Evidence from International Diagnostic Studies

- Based on a systematic review conducted by Buban, Villanueva, and Gregorio (2021), the overall pooled sensitivity of saliva samples is 84% (95% CI: 80-88; I^2 statistic: 90.13; 51 studies) while the overall pooled specificity is 96% (95% CI: 94-98; I^2 statistic: 98.14; 51 studies). Sensitivity point estimates of included studies ranged from 46% to 100% while specificity estimates ranged from 0% to 100%.
- Given the heterogeneity from both estimates in the review of Buban, Villanueva, and Gregorio (2021), subgroup analysis was conducted according to diagnostic status, presence of symptoms, setting, saliva collection method, saliva collection location, and RT-PCR kit brand.
    - Sensitivity of saliva test was found to be 85% (95% CI: 76-91, I^2=87.77%) on symptomatic individuals and 89% (95% CI: 83-93, I^2=0.53%) for asymptomatic individuals. On the other hand, specificity of saliva test was found to be 97% (95% CI: 90-99, I^2=94.53%) on symptomatic individuals and 93% (95% CI: 73-99, I^2=19.61%) for asymptomatic individuals.
    - In terms of setting, sensitivity of saliva samples in hospital settings had a sensitivity of 86% (95% CI: 81-90, I^2= 84.91%) while that of community or outpatient settings had a sensitivity of 78% (95% CI: 66-86, I^2= 90.09%). Specificities for hospital and community settings were 94% (95% CI: 88-97, I^2=96%) and 98% (95% CI: 97-99, I^2=95.32%) respectively.
    - Sensitivity of saliva samples collected from oral saliva and posterior oropharyngeal saliva had almost similar values at 82% (95% CI: 77-86, I^2= 88.51%) and 89% (95% CI: 83-93, I^2= 81.65%) respectively. On the other hand, specificity of posterior oropharyngeal swab was lower at 79% (95% CI; 50-93, I^2=95.37%) compared to oral saliva which was at 97% (95% CI: 96-98, I^2= 95.73%)
    - In terms of saliva collection method, saliva drool/spit had a sensitivity of 83% (95% CI: 78-97, I^2=91.64%) while saliva swab had a sensitivity of 71% (95% CI: 46-87, I^2=95.81%). Both methods showed almost similar specificity estimates at around 97-98%.
    - In confirmed COVID-19 patients, sensitivity was found to be 81% (95% CI: 74-87, I^2=82.20%) while suspected COVID-19 patients had sensitivity of 74% (95% CI: 62-82, I^2=85.87%). Specificity for confirmed COVD-19 patients (65%; 95% CI: 42-83, I^2=88.97%) were significantly lower compared to suspected COVID-19 patients (96%; 95% CI: 92-98,

I^2=83.54%).

- Sensitivity and specificity of different brands varied greatly ranging from 49%-95% and 43%-100% respectively. *Xpert Xpress SARS-CoV-2* by Cepheid had the highest sensitivity (95%; 95% CI: 91-99; 3 studies) while *Charite E-gene assay* had the lowest sensitivity (49%; 95% CI: 39-58). On the other hand, a specificity of 100% was observed in *Cobas SARS-CoV-2* by Roche (95% CI: 99-100; 1 study) and *Coronavirus Typing (8-well Assay)* by AusDiagnostics (95% CI: 98-100, 1 study).
  - Among the brands analysed that were approved in other countries for saliva RT PCR use as of 22 March 2021, there were only 2 brands which were included in the analysis. *SalivaDirect (Yale University)* had a higher sensitivity at 92%(95%CI: 0.84-0.91) but the specificity is lower 87% (95%CI: 0.82-0.91)] compared to *TaqPAth COVID-19 Combo Kit (Thermo Fisher Scientific)*. In terms of specificity, *TaqPAth COVID-19 Combo Kit (Thermo Fisher Scientific)* had which had a higher specificity [80% (95%CI: 0.95-1.00)] but its sensitivity is lower than *SalivaDirect (Yale University)* [80% (95%CI: 0.73-0.86)].
  - Among brands analysed that were approved in other countries for saliva RT PCR use but approved in the Philippines only for conventional samples, there were only 3 brands which were included in the analysis. *Xpert Xpress SARS-CoV-2 test (Cepheid)* had the highest sensitivity [0.95 (0.91, 0.99)] with a high specificity as well [0.99 (0.95, 1.00)]. While *Cobas SARS-CoV-2 Assay (Roche) had the highest specificity [1.00 (0.99, 1.00)], its sensitivity [0.92 (0.88, 0.95)] is lower than Xpert Xpress SARS-CoV-2 test (Cepheid). Meanwhile, the remaining brand Genesig RT-PCR SARS-coV-2 Kit (Primerdesign)* had the lowest sensitivity [0.77 (0.61, 0.94)] and specificity [0.98 (0.96, 1.00)] among the three.
- **Subgroup analysis of studies compliant with RITM's sample size requirement** The meta-analysis of 30 studies (n=13,774) with at least 30 true positives yielded a sensitivity of 84% (95%CI: 0.78-0.88) and specificity of 96% (95%CI: 0.93-0.98) for saliva-based RT-PCR. Heterogeneity was noted to be 93.42% and 98.80%, respectively. The high heterogeneity merited a downgrade in quality of evidence due to inconsistency.
- **Subgroup analysis on the timing of collection based on onset of symptom/s**
  - **0-7 days after onset of symptoms [5 studies (n=295)]:**
    Substantial heterogeneity and wide confidence interval was noted.
    - Sensitivity: 82% (95% CI 0.68-0.91), I^2 = 52%
    - Specificity of 93% (95% CI 0.50-0.99), I^2 = 89.57%
  - **0-14 days after onset of symptoms [7 studies (n=398)]:**

Substantial heterogeneity was noted.
    - Sensitivity: 84% (95% CI 0.75-0.90), I^2 = 74.89%
    - Specificity: 94% (95% CI 0.77-0.99), I^2 = 87.77%
- **15-30 days from onset of symptoms [2 studies (n=68)]:**
  Meta analysis could not be done due to insufficient data. The following are the point estimates reported in the included studies:
    - Sensitivity: 0.65 (95%CI: 0.25, 0.81) [Gavars, 2020], 0.54 (95% CI: 0.46, 0.80) [Iwasaki, 2020]
    - Specificity: 0.83 (95% CI 0.59, 0.96) [Gavars, 2020], 1.00 (95% CI 0.29, 1.00) [Iwasaki, 2020]
- **More than 30 days from onset of symptoms [1 study (n=20)]:**
  Meta analysis could not be done due to insufficient data. The following are the point estimates reported in the included study:
    - Sensitivity: 0.71 (95% CI 0.29, 0.96) [Gavars, 2020]
    - Specificity: 0.85 (95% CI 0.55, 0.98) [Gavars, 2020]

■ **Subgroup analysis on timing of collection based on onset of exposure**
- **Asymptomatic patients based on time since exposure:** No information on exposure was provided by the included studies; hence, further subgroup analysis could not be done.

■ **Subgroup analysis on timing of collection based on disease progression (i.e. mild, moderate, severe, critical COVID-19)**
This subgroup analysis was not included in the rapid review of Living CPG.

■ **Subgroup analysis based on extraction type (e.g., direct)** Based on the meta-analysis of five studies (n=807) that used direct RT-PCR (i.e. no extraction) of saliva samples, the pooled sensitivity was reported at 96% (95% CI 0.68-1.00, I^2=96.61%) while the pooled specificity was found to be 92% (95% CI 0.36-1.00, I^2=97.22%). However, there was an observed substantial heterogeneity among these studies. These figures are not significantly different from the overall meta-analysis of all 51 studies. However, the high heterogeneity may merit a downgrade of quality of evidence for inconsistency, and the wide confidence interval for specificity may merit a further downgrade for imprecision.

- Risk of bias assessment using the criteria for appraising validity by Dans et al. showed that the majority of the studies (80.8%) had a moderate risk of bias with no studies showing a high risk of bias. Meanwhile, the remaining 19.2% of the studies had low risk of bias. Majority of issues found in the studies were in terms of the lack of reporting on the independence of interpretation of the tests.
- Buban, et al., recommended the use of saliva drool/spit and oral saliva specimens for RT-PCR diagnosis of COVID-19 of symptomatic and asymptomatic patients with suspected COVID-19 in hospital and community/outpatient settings. (Moderate quality of evidence, Strong recommendation)
- However, Buban et al. did not recommend the use of saliva swab and posterior oropharyngeal saliva specimens for RT-PCR diagnosis of COVID-19 in symptomatic and asymptomatic patients with suspected COVID-19 in hospital and community/outpatient settings. (Low quality of evidence, Conditional recommendation)

c. What is/are the most appropriate method/s of collecting saliva for SARS-CoV-2 detection?

Based on the subgroup analysis in the SR of Buban, Villanueva, & Gregorio (2021) of the Living Clinical Practice Guidelines, saliva drool/spit had a sensitivity of 83% (95% CI: 78-97) based on 44 studies; while saliva swab had a sensitivity of 71% (95% CI: 46-87) based on 6 studies. Both methods showed almost similar specificity estimates.

d. What is the diagnostic performance of RT-PCR in detecting new variants of concern?

Two reviewers performed a literature search of relevant studies in PubMed from inception to April 19, 2021 using MeSH terms for RT-PCR and COVID-19 as well as the supplementary concept for the SARS-CoV-2 variants. No filters on study type, language and publication date were applied. We initially aimed to search for studies looking at the diagnostic performance of RT-PCR tests to detect new COVID-19 variants. However, no studies that fit this criterion were found. Hence, we shifted the question to map out available relevant evidence on COVID-19 variants and diagnostic testing. Studies that looked at the implications of COVID-19 variants of concern for screening and diagnosis testing using RT-PCR in various healthcare settings were included without restrictions on the study type or design.

Based on an independent screening by five reviewers, the search yielded 24 records. Of these, 7 were included in the full-text assessment after initial screening. One study was then excluded as the methods for identifying variants were focused on sequencing methods rather than on RT-PCR methods leaving six studies for inclusion in the qualitative synthesis.

Of the six included studies (Bustin et al, Ibba et al, Jain et al, Penarrubia et al, Surelac et al, Vanaerschot et al) none has provided diagnostic performance data. However, the studies analyzed genome data and have provided implications for the testing of COVID-19 variants. The key findings are as follows:

- Four studies (Bustin et al, Ibba et al, Surelac et al, Vanaerschot et al) conducted their studies in specific countries, namely, the UK, Italy, Romania, and the USA, respectively. The remaining two studies (Jain et al, Penarrubia et al) utilized genome databases from Global Initiative on Sharing All Influenza Data (GISAID) and GenBank.
- Specific genes were identified as having potential for testing in three studies (Ibba et al, Surelac et al, Vanaerschot et al). Specifically, Ibba et al noted that analysis of the shape of S gene amplification curve may be used as a potential proxy for S gene variants (ie. B.1.1.7 variant). Similarly, Surelac et al, noted that screening for S gene mutations using RT-PCR and whole genome sequencing led to the identification of the B.1.1.7 and B.1.258 strains. On the other hand, Vanaerschot et al concluded that the G29140U mutation led to a 67-fold reduced sensitivity in Ct values of the N-gene assay.
- Bustin, et al studied a specific PCR assay (i.e. Cov2-ID) and noted that the assay targets three viral genes since multiple gene targets provide a more accurate finding. Similar findings were noted by Penarrubia et al which found that multiple assay targets in RT-PCR tests can mitigate the risk of loss of sensitivity or specificity.
- Three studies (Jain et al, Surelac et al, Vanaerschot et al) identified the need for molecular monitoring to be included in public health strategies. The purpose of such would be to assess diagnostic efficacies, screening for COVID-19 variants, and the monitoring for mismatches to avoid false negative results. Jain et al utilized pairwise sequence alignment using an EMBOSS needle to realign the Wuhan-Hu-1 reference genome followed by mapping using a basic local alignment search tool (BLAST). Surelac et al also studied the relationships of sequences by aligning them using a ClustalW algorithm and conducting phylogenetic analysis. On the other hand, Vanaerschot et al. ran samples in three different RT-PCR tests namely an N-gene, E-gene, and Simplex assay. The samples with Ct value difference [ΔCT(N−E)] of 2.5 standard deviations above the mean from the three RT-PCR assays were then sequenced to determine which mutations were associated with the lowered sensitivity.

4. Guidelines on the detection of New Variants

Among the 19 testing guidelines searched, only the following organizations have published guidelines on the detection of new variants and mentioned the use of RT-PCR in the process of variant of concern detection for surveillance:

Table 3. Guidelines on the Detection of SARS-COV-2 Variants

Organization	Published Guidelines on Detection of New Variants (Y/N)	Mentioned the Use of RT-PCR in the Guidelines on the Detection of New Variants (Y/N) and the Use Case	Recommended test in the guidelines to detect New Variants	Number of WGS performed per week
Centre for Disease Control and Prevention of the United States (US CDC)	Y	Y Surveillance	Genomic Sequencing	6,000
Public Health England of the United Kingdom (UK PHE)	Y	Y *Surveillance* *Screening*	Genomic Sequencing	10,000
Department of Health of the Philippines (PH DOH)	Y	Y *Surveillance*	Genomic Sequencing	*No data available*
Center for Disease Network of Australia (CDNA)	Y	Y *Surveillance*	Genomic Sequencing	All COVID-19 positive samples
World Health Organization (WHO)	Y	Y *Surveillance*	Genomic Sequencing	None specified
Centre for Disease Control and Prevention of the European Union (ECDC)	Y	Y *Surveillance*	Genomic Sequencing	*recommends testing at least 500 random/representative samples per country per week.*
Japan Ministry of Health, Labor and Welfare (MHLW)	Y	Y Screening Surveillance	RT-PCR and Genomic Sequencing	5% of all domestic cases have been sampled
Public Health Canada	Y	Y Surveillance	Genomic Sequencing	800

- Across all these guidelines, the recommended method for detection of new variants is genome sequencing. Japan, on the other hand, uses RT-PCR for screening of strains that have the N501Y mutation then conducts genome sequencing to confirm the variant of the mutant strain. The PCR test was developed by the Japan National Institute for Infectious Diseases after successful isolation of the mutant strain. However, the NIID has not yet published additional information on the developed PCR test or guidelines on the use of the test.
- To date, none of the eight organizations with published guidelines on the detection of new COVID - 19 variants have specified gene targets to be used for the confirmation or

detection of the new variants. The following countries and organizations, however, have specified testing guidelines to enhance the screening and surveillance of different variants of concern:

- The UK PHE has undertaken investigations to study the S gene target failure (SGTF) for VOC 202012/01 and the E848K mutation in the B.1.1.7 VOC 202012/01 variant detectable in RT- PCR used to monitor the UK variant of concern (Public Health England, 2021). Further, the UK is currently implementing surge testing which is extensive testing including door-to-door and testing of asymptomatic subjects. All samples that test positive will be subjected to genome sequencing. For international arrivals, all travelers are required to take a test on or before day 2 of quarantine for variant surveillance and on or after day 8 of quarantine. According to the guidelines, the assay to be used for variant surveillance must be semi-quantitative and include a minimum of 2 distinguishable SARS-CoV-2 gene targets not including the S gene or performance reference controls.
- The US CDC noted that the new variants have potential consequences in the spread of the disease due to its ability to evade detection by specific viral diagnostic tests, including RT-PCR. Thus, the Center of Disease Control developed the National SARS-CoV-2 Strain Surveillance (NS3) as part of their public health response, and with it, they released a guidance document for all laboratories, detailing the acceptable specimen types for sequencing and potential virus characterization.
- The National Institute of Infectious Disease (NIID) of Japan has reported the successful isolation and distribution of the VOC-202012 / 01 and 501Y.V3 mutant strains to be used for experimental research in various institutions. In addition to genomic sequencing, Japan MHLW and local government health units in Japan follow the mutant screening system wherein a patient suspected of COVID-19 is tested using RT-PCR. At least 40% of the patients who test positive will be tested with the mutant strain PCR test developed by the Japan NIID to identify the presence of the N501Y mutation. Samples positive for the mutation will then undergo genome analysis to confirm the SARS-CoV-2 variant. However, neither the MHLW nor the NIID published guidelines or information on the use of the developed mutant strain PCR method.
- The Public Health Canada also notes that the UK Variant VOC 202012/01 variant can test negative for S-gene target but positive for other targets using the three-target assay (N, ORF1ab, S) from *Thermo Fisher (TaqPath)*. And therefore recommended that multi-target reverse transcription polymerase chain reaction (RT-PCR) assays that include a S-gene target which are affected by the deletions present in the variant can be used as a signal for follow up confirmatory genome sequencing.
- The WHO reminds users of in vitro diagnostic medical devices (IVDs) to regularly review test results to detect unexpected increases or decreases in positivity rate,

target detection rate, invalid or unreturnable result rate, etc. They also warn that certain mutations may increase the risk of delayed diagnosis as well as misdiagnosis. The WHO also urges manufacturers of IVDs to proactively study published literature for mutations possibly impacting the safety, quality, or performance of the products.

- Locally, the Philippine Genome Center (PGC) employs whole genome sequencing which sequences 30,000 bases of the viral genome; therefore, no particular genome is targeted. Documented positive samples with a cycle threshold (Ct) value of not greater than 30 in any gene channel of interest shall be submitted to the PGC for sequencing as stated in the DOH Department Memorandum 2020-0420.

5. Interpretation of Tests

What are the guidelines in interpreting RT-PCR results given in the latest body of evidence?

Among the 19 guidelines searched, 16 organizations have published guidelines on the interpretation of PCR tests. Among them, four (Thailand MOH, UK NHS, Japan MHLW, Philippines DOH) mentioned that positive RT-PCR test results mean the presence of SARS-CoV-2 in a specimen coming from a symptomatic individual or person under investigation. Meanwhile, three guidelines (Indonesia MOH, Malaysia MOH, South Korea MHW) consider a positive RT-PCR result to mean the presence of SARS-CoV-2 material in the test regardless of the occurrence of symptoms in an individual. Others did not specify the symptom status of patients needed in their guidelines. Five guidelines (Malaysia MOH, South Korea MHW, China CDC, Singapore MOH, FIND) did not provide an operational definition for a negative RT-PCR in their guidelines. Notably, only the US CDC, in addition to interpreting a negative result as an indication of having no current infection, differentiated the need for quarantine based on the vaccination status of the individual. Unvaccinated individuals with known SARS-CoV-2 exposure but presenting no symptoms still need to be quarantined, while fully-vaccinated individuals presenting no COVID-like symptoms need not quarantine or be tested when exposed to a suspected or confirmed case as their risk of infection is low. Details of the guidelines in interpreting RT-PCR results of each country are summarized in the Annex.

6. Technical Specifications

What are the desired technical specifications for PCR tests for COVID-19 based on the guidelines from other settings?

Among the 19 guidelines searched, 15 organizations have published guidelines on the desired technical specifications for PCR tests for COVID-19. There were a variety of requirements for each of the parameters reviewed. The summary of requirements and the number of guidelines specifying them are discussed below:

Storage Requirement:

- **No specified requirement:** Four guidelines (Australia TGA, US CDC, Vietnam MOH) did not mention a storage requirement. However, Vietnam MOH mentioned a "refrigerator" in the list of equipment needed for RT-PCR testing.
- **2 to 8°C Storage temperature requirement:** Six guidelines (Public Health Ontario [Canada PHO], ECDC, Indonesia MOH, RITM, Thailand MOH, US CDC) mentioned the use of 2-8°C as a storage requirement. ECDC and Thailand MOH goes further and qualifies this temperature for specimens to be used within 48 hours and 72 hours, respectively.
- **-20°C Storage temperature requirement:** Three guidelines (UK MHRA, RITM, China CDC) mentioned the use of a colder temperature for storage. UK MHRA indicates this temperature for the storage of test kits and reagents for at least 12 months. RITM, on the other hand, mentions a range of -20°C to -30°C for the storage of aliquot samples and RNA extracts. Meanwhile, China CDC mentioned the use of this temperature for storage of specimens
- **4°C Storage temperature requirement:** Four guidelines (China CDC, Japan MHLW, South Korea MHW, WHO). China CDC mentions this temperature for specimens to be used within 24 hours. On the other hand, this is indicated for specimens to be used within 48 hours for Japan MHLW. WHO mentions a range of 4°C to 10°C as a storage temperature at 3000 m altitude with 70% humidity. China CDC also mentions this storage temperature for serum samples.
- **-70°C Storage temperature requirement:** Four guidelines (China CDC, ECDC, RITM, Thailand MOH) mentioned this temperature for long-term storage of more than 24 hours, more than 48 hours, long term storage and banking, and more than 72 hours, respectively.
- **-80°C Storage temperature requirement:** Only Malaysia MOH mentioned a storage temperature of samples for retesting.
- **Specimen and reagent to be stored separately:** Only Malaysia MOH mentioned that the specimens and the reagent should be stored separately.

Transport Requirement:

- **No specified requirement:** Three guidelines (Australia TGA, FIND, Indonesia MOH) did not mention a transport requirement.
- **2°C to 8°C Transport Temperature, or Refrigerator Temperature:** Five guidelines (Malaysia MOH, RITM, WHO, Japan MHLW, RITM) mentioned this temperature for transport. RITM specifies this temperature for biopsy specimens while WHO mentions that test kits should be stable for at least 12 days at 2°C to 8°C.
- **Follow pre-existing transport rules:** Three guidelines (ECDC, UK MHRA, Vietnam MOH) follow pre-existing transport rules. The EU cites the P650 packaging instructions for Category B infectious substances assigned to UN 3373 in the 2019-2020 WHO Guidance on regulations for the transport of infectious substances. The packing instruction P650 for UN3373 samples specified by the UK states that the specimens should be packed in triplicate with the position of dry ice or ice to be outside the second or the outermost bag (WHO, 2019).

Meanwhile, Vietnam follows their guidance entitled, 2018 *Regulation on the Collection, Preservation, Packaging, Transportation, Storage, Use, Research, Exchange, Disposal Of Medical Samples* by the Vietnam MOH. This issuance indicated that samples should be transferred to the suitable testing facility in the shortest possible time or at least 2 hours after collection to processing. If not complied within 2 hours, upper respiratory samples for molecular testing (not necessarily RT-PCR) shall be stored in a dedicated shipping environment while in transport at a storage temperature of 4 - 8°C if stored for ≤ 48 hours or -20 ° C or -70 ° C if stored for > 48 hours.

- **Requiring a Viral Transport Medium:** Four guidelines (Australian PHLN, South Korea MHW, Thailand MOH, US FDA) require the use of a viral transport medium on transport.
- **Triple packaging during transport:** This is required in three guidelines (Japan MHLW, Thailand MOH, US CDC)

PCR machine compatibility Requirement:

- **Off the shelf Equipment:** Only WHO mentioned that the PCR tests should be compatible with off-the-shelf equipment such as PC and at least 2 most commonly available thermocytes with thermocycler-specific CT cut-off values for assay determined
- **No specified requirement:** All other guidelines did not specify a PCR machine compatibility requirement.

Human resource Requirement:

- **No specified requirement:** Six guidelines (Canada PHO, Australia TGA, China CDC, ECDC, Indonesia MOH, Thailand MOH) did not specify this requirement
- **Trained medical practitioners:** All other guidelines specified the need for trained medical practitioners such as practitioners in the laboratory

Cost Requirement:

- **PCR Costing Mentioned:** Three guidelines (Canada PHO, FIND, Philippine DOH) mentioned costs for RT-PCR testing. For Canada PHO it amounts to CAD 47.50 or PHP 1,847.97 (exchange rate: 1 CAD = PHP 38.90, as of 16 March 2021). FIND amounts the test to be USD 30 or PHP 1,455.92 (exchange rate: 1 USD= PHP 48.53, as of 16 March 2021).The Philippines prices the test as PhP3,800 for public and PhP 4,500-5,000 for private institutions.
- **No specified requirement:** All other guidelines did not specify their cost requirements.

Processing Time:

- **Turnaround Time:** Six guidelines (FIND, UK MHRA, Australian PHLN, Philippine DOH, Thailand MOH, WHO) mentioned their ideal turnaround time to be 15 minutes or less with FIND mentioning a maximum time to be 1 hour (without indication of stages covered) and an acceptable time for UK MHRA to be less than five hours from sample to result. Thailand MOH specified that 12 hours is the maximum time from sample to result. Meanwhile, the remaining guidelines (WHO, Australia PHLN, Philippine DOH) did

not indicate in their guideline which stages of testing are covered in the ideal turnaround time. For the WHO, the acceptable time is less than 4 hours. As for Australian PHLN, the turnaround time should be less than 24 hours after a laboratory received a specimen. Meanwhile the Philippine DOH set the turnaround time for rRT-PCR at 24-72 hours and 24 hours for GeneXpert. On the other hand, Japan mentioned an implementation time of 2-4 hours but did not qualify if this pertained to their turnaround time.

- **No specified requirement:** All other guidelines did not mention processing time in their requirement.

Other Requirements:

- **Preventive Maintenance Requirement:** UK MHRA noted that preventive maintenance should not be needed until after one year or 10,000 samples
- **Fluorescence Cycle Requirement:** China CDC noted that a fluorescence threshold at 10-fold the standard deviation of the fluorescence signal of the first three to 15 cycles should be used
- **Biosafety Requirement:** A level two biosafety is specified for three guidelines (ECDC, UK MHRA, Vietnam MOH)
- **ISO Requirement:** Compliance with ISO 13485:2016 standards were mandated by two guidelines (WHO, UK MHRA)

C. Household Financial Impact

Currently, the Philippine Insurance Corporation (PhilHealth) Circulars covers the cost of RT-PCR testing per Philhealth Circular 2020-0017.

Aside from this, the DOH has also set price ceilings for laboratory-based RT-PCR testing per Department Circular No. 2020-0391 released in November 2020. For private institutions, a price range of PhP 4,500 to 5,000 was set for plate-based RT-PCR tests. Meanwhile, a PhP 3,800 price ceiling was set for plate-based RT-PCR tests in public laboratories. This was in line with the Executive Order No. 118 which was issued to regulate COVID-19 testing prices (DOH, 2020). The measures from PhilHealth and DOH were made to assist the Filipino household with the expenses in getting tested for COVID-19.

D. Cost-effectiveness

The evidence was not reviewed. A full-blown cost-effectiveness analysis is currently not done for rapid reviews under a pandemic situation due to its emergency nature. A full blown cost-effectiveness analysis that takes on a societal perspective (i.e., including the economic and social impacts) will be performed once sufficient evidence is available and when full market authorization has been granted.

E. Affordability and Viability

1. Costing analysis based on RITM data

a. What is the estimated cost of RT-PCR Testing using NPS/OPS?

Based on RITM costing data (as of April 2021), the estimated cost per test for RT-PCR using NPS/OPS varies depending on the procedure, brand, and cost of consumables (which are affected by the brand and quantity procured) which may range from PHP 2,968.54 to PHP 3,932.28.

Below are their calculated costs per test, while the details of their inputs in the calculation are provided in Appendix C.

Table 4. Estimated Cost per Test for RT-PCR using NPS/OPS sample (RITM, 2021)

	Manual Extraction using BGI test kit	Manual Extraction using MirXes Fortitude 2.1	Automated Extraction using Sansure	Automated Extraction using BGI	Automated Extraction using MirXes Fortitude 2.1
Estimated Cost per Test (PHP)	3,293.54	4,257.38	2,350.94	2,968.54	3,932.38

b. What is the estimated cost of RT-PCR Testing using saliva sample?

Based on RITM costing data (as of April 2021), the estimated cost per test for RT-PCR using saliva varies depending on the procedure, brand, and cost of consumables (which are affected by the brand and quantity procured) which may range from PHP 2,150,94 to PHP 3,732.38. Table 5 presents their calculated costs per test.

Below are their calculated costs per test, while the details of their inputs in the calculation are provided in Appendix C.

Table 5. Estimated Cost per Test for RT-PCR using Saliva sample (RITM, 2021)

	Manual Extraction using BGI test kit	Manual Extraction using MirXes Fortitude 2.1	Automated Extraction using Sansure	Automated Extraction using BGI	Automated Extraction using MirXes Fortitude 2.1

Estimated Cost per Test (PHP)	3,093.54	4,057.38	2,150.94	2,768.54	3,732.38

2. Costing analysis based on PhilHealth Coverage

- PhilHealth released the PhilHealth Circular No. 2020-0010, which aims to establish the policy for the implementation of the benefit packages for SARS CoV-2 testing in the Research Institute for Tropical Medicine (RITM) and all RITM-accredited testing centers. In addition, PhilHealth Circular No. 2020-0017 was also released, which aims to update the benefit package for SARS-CoV-2 testing using RT-PCR specifically to decrease the financial coverage (i.e, cost of the benefit package) after testing and laboratory supplies have stabilized.
- The services included in this benefit package are the following: screening/clinical assessment, diagnostic workup, as indicated; specimen collection; specimen transport; conduct of RT-PCR testing (including the test kit); and analysis and reporting of results. The case-based payment of the benefits for SARS-CoV-2 testing that shall be available for any Filipino patient are indicated in Table 1 of PhilHealth Circular 2020-0010. A PhilHealth-accredited testing laboratory may claim any of the following packages:
    - Package Code C19T1: If all testing services are procured and provided by the testing laboratory, the package amount is Php 3,409.
    - Package Code C19T2: If the test kits are donated to the testing laboratory, the package amount is Php 2,077.
    - Package Code C19T3: If the test kits are donated to the testing laboratory, and the facility budget includes the cost of running the laboratory and the cost of the RT-PCR machine for testing, the package amount is Php 901.

- Based on PhilHealth claims data from March 2020 to January 2021, the total amount spent for the package covering all testing supplies and services is Php 308,586,796.00 claimed by 70,058 individuals. On the other hand, for donated test kits, the total amount spent for the package including the other services is Php 162,750,944.00 claimed by 46,277 individuals as of January 18, 2021. For the last package where the test kits were donated and the facility budget already subsidizes the laboratory and testing costs, PhilHealth has spent Php 8,793,088.00 as claimed by 4,525 individuals. It should be noted that the inconsistencies in the unit package prices can be attributed to the revision of the cost coverage after setting the price ceiling for RT-PCR testing.
- The most comprehensive testing package (i.e, Package Code C19T1) accounts for 58.0% of the total number of claims. The remaining testing packages account for 38.3% (Package Code C19T2, test kits donated) and 3.7% (Package Code C19T3, test kits donated and subsidized facility) of the total number of claims, respectively. If one assumes this proportion of package claims based on latest available data, the projected

costs for daily testing of 50,000 covered individuals is Php 140,254,044.35/day using the revised package coverage. Further, a daily testing coverage of 200,000 individuals would incur a total cost of Php 561,016,177.40/day.

V. Recommendations *(as of 30 April 2021)*

A. HTAC recommendation for RT-PCR testing using NPS and OPS specimens:

RT-PCR testing using nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) remains to be the most sensitive test for COVID-19. **The HTAC recommends the use of RT-PCR at or shortly after the onset of illness for symptomatic patients; or at least five (5) to seven (7) days after exposure to a suspected index case.** In Table 4, the recommended use cases, target population, and timing of testing are presented.

Table 4. Recommendations for RT-PCR testing using NPS and OPS specimens

Recommended Use Cases	Target Population	Timing of Testing
Diagnosis *DM 2020-0512: Diagnostic testing / Testing for diagnosis looks for presence of COVID-19 at the individual level and is performed when there is a particular reason to suspect that an individual may be infected (i.e. manifestation of symptoms or known history of exposure). Diagnostic testing intends to diagnose an infection in patients suspected of COVID-19 by their healthcare provider, such as in symptomatic individuals, individuals who have had recent exposure, and individuals who are in a high-risk group such as healthcare providers with known exposure. In these guidelines, this shall be applied to close contacts and suspect cases identified after symptoms-based screening.*	• Symptomatic • Asymptomatic with exposure	Per *DOH Department Memorandum (DM) 2020-0512* or the "*Omnibus Interim Guidelines on Prevention, Detection, Isolation, Treatment, and Reintegration Strategies for COVID-19*", it is best to conduct RT-PCR at or shortly after the onset of illness for symptomatic patients; or at least five (5) to seven (7) days after the exposure to a suspected index case.
Screening	• Asymptomatic individuals with or without known	The timing of testing using RT-PCR for screening is based on program needs.

DM 2020-0512: Screening testing / Testing for screening intends to identify infected individuals prior to development of symptoms or those infected individuals without signs or symptoms who may be contagious, so that measures can be taken to prevent them from infecting others. This includes broad screening of asymptomatic individuals without known exposure and then deciding on the next courses of action based on individual test results. In these guidelines, this shall be applied to travelers from high prevalence areas.	exposure, especially those travelling from high prevalence areas.	
Surveillance *DM 2020-0512: Surveillance testing / Testing for surveillance is primarily used to obtain information at a population level, rather than an individual level. Surveillance testing may be random sampling of a certain percentage of a specific population, to (1) monitor for increasing or decreasing prevalence, and (2) determine the effects of community interventions such as social distancing at the population level. In these guidelines, these shall be applied to frontliners and essential workers.*	• Random sample of the targeted population	The timing of the test using RT-PCR is based on the surveillance program.

B. HTAC recommendation on the use of saliva as an alternative specimen for RT-PCR testing:

The HTAC **recommends the use of saliva as an alternative specimen for RT-PCR testing for diagnosis, screening and surveillance as indicated in Table 4. The collection method, interpretation of results and recommended in-house verification procedures are described in Table 5.**

Table 5. Recommendation for the use of saliva as an alternative specimen for RT-PCR testing

Parameter	Specifications	Recommendations
Collection Method	Passive drool method using a wide-mouth sterile container with screw-cap or pop-op cover	Per DM 2021-0161, the following are the recommended method collection: i. Advise patients to avoid eating, drinking, brushing teeth, using mouthwash, and smoking for at least 30 minutes prior to sample collection. ii. Provide patients with a properly labeled, graduated, sterile, wide-mouth container, along with instructions on how to provide saliva sample 1. Advise patient to pool his/her saliva in the mouth. 2. Ask the patient to spit at least 2-3 mL of saliva to the container.
Interpretation of Results	Positive saliva test result - positive for SARS-CoV2 Negative saliva test result - negative for SARS-CoV2	Interpretation shall follow guidelines in *DM 2020-0512* or the "*Omnibus Interim Guidelines on Prevention, Detection, Isolation, Treatment, and Reintegration Strategies for COVID-19"*, where a confirmed case using a positive RT-PCR test must be isolated and triaged according to clinical status. A negative RT-PCR test may indicate absence of SARS-CoV-2 but does not rule out COVID-19. Per the US FDA Letter, *"Genetic Variants of SARS-CoV-2 May Lead to False Negative Results with Molecular Tests for Detection of SARS-CoV-2 - Letter to Clinical Laboratory Staff and Health Care Provider"* recommendations on the interpretation of results are as follows: Per US-FDA clinical laboratory staff and health care providers who use molecular tests for the detection of SARS-CoV-2 must be cognizant of the following:

		● Genetic variants of SARS-CoV-2 arise regularly, and false negative test results can occur. ● Increased prevalence of genetic variants less likely affects tests that use multiple genetic targets to determine a final result. In addition, clinical laboratory staff and health care providers who use molecular tests for the detection of SARS-CoV-2 must consider the following: ● Negative results in combination with clinical observations, patient history, and epidemiological information; and, ● Repeat testing with a different test (with different genetic targets) if COVID-19 is still suspected after receiving a negative test result.
In-house Verification procedures	Must follow in-house verification methods set by the RITM	Following DM 2021-0161, or "*Interim Guidelines on the use and administration of Saliva-based RT-PCR testing*", the HTAC recommends, in house verification by COVID-19 Laboratories adhering to the guidelines set by RITM. 1. The COVID-19 laboratories shall perform in-house verification of the new RT-PCR methods using the FDA registered RNA extraction kit and RT-PCR detection kit validated for saliva specimens. 2. The COVID-19 laboratories shall submit the saliva-based RT-PCR verification report to RITM. 3. RITM shall issue a certification to the COVID-19 laboratory for saliva-based RT-PCR testing. 4. RITM shall endorse to HFSRB the copy of the certification of COVID-19 laboratories capable of performing saliva-based RT-PCR testing. 5. HFSRB shall regularly provide a census of COVID-19 laboratories certified to perform saliva-based RT-PCR testing.

C. RT-PCR kits using NPS/OPS and Saliva as specimens must satisfy the following recommended minimum regulatory, technical, operational specifications set by HTAC:

In concordance with the Philippine FDA's requirements, **HTAC has set minimum regulatory, technical, operational specifications for RT-PCR for NPS/OPS and saliva specimens in Table 6**, including the recommended **clinical sensitivity of at least 95%** and **clinical specificity of at least 99%**.

Table 6. Recommended specifications for RT-PCR kits using NPS/OPS and saliva specimens

Parameter	HTAC specs for RT-PCR 2020	HTAC SPECS 27 APRIL 2021 (For NPS/OPS and Saliva unless otherwise specified)
OPERATIONAL REQUIREMENTS		
Regulatory Requirement	Must have a certificate of product registration (CPR) or emergency use authorization (EUA) from the FDA Philippines	Must have the appropriate regulatory authorization from the Philippine FDA stating the specific specimen sample
Validation	Must have been validated by an independent or a third-party reputable government or private research institution.	Must have been validated by an independent or a third-party reputable government or private research institution including: ● Research Institute for Tropical Medicine (RITM) ● US Food and Drug Administration (US-FDA) ● World Health Organization, Foundation for Innovative New Diagnostics (WHO-FIND) ● Therapeutic Goods Administration (TGA, Australia) ● Medicines and Healthcare products Regulatory Agency (MHRA, UK) ● Japan Pharmaceuticals and Medical Devices Agency ● *Other DOH-designated institutions for test kit validation recognized by RITM*

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<td>Cost</td>
<td>Must include all necessary accessories per test, including extraction reagents, consumables, & viral transport media.
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 (PPE).</td>
<td>Per DM 2021-0391, the cost of the RT-PCR test using NPS/OPS for public institutions is PHP 3,800. Testing laboratories accredited by PhilHealth may claim one of the following packages indicated in Table 7 adopted from PhilHealth Circular No. 2020-0017 or the “Benefit package for SARS-CoV2 testing using RT-PCR (Revision 1)”.

Table 7. Packages for SARS-CoV-2 testing by RT-PCR
<table>
<tr><th>Package Code</th><th>Description*</th><th>Services covered by PhilHealth</th><th>Package Amount (PhP)</th></tr>
<tr><td>C19T1</td><td>All services and supplies for the testing are procured and provided by the testing laboratory</td><td>Complete services or minimum standards</td><td>3,409</td></tr>
<tr><td>C19T2</td><td>Test kits are donated to the testing laboratory</td><td>Screening/ clinical assessment/ specimen collection and handling, conduct of RT-PCR testing and analysis of results</td><td>2,077</td></tr>
<tr><td>C19T3</td><td>Test kits are donated to the testing laboratory; cost of running the laboratory and the RT-PCR machine for testing are subsidized by the</td><td>Screening/clinical assessment/ specimen collection and handling</td><td>901</td></tr>
</table>
</td>
</tr>
</table>

		government The cost of using **saliva specimens with RT-PCR** for public institutions should be significantly less than the *government price cap for* RT-PCR test kit using NPS/OPS.
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.	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. Must pass the acceptance testing by RITM at the cost of the winning supplier.	COVID-19 Laboratories that will conduct PCR testing shall develop their standard operating procedures for the proper and safe collection, handling, storage, and testing. The storage must not be lower than -20 degrees Centigrade. The expiration date must be no less than one (1) year from date of manufacture.
Transportation	None	Must follow the transport temperature as stated in the manufacturer's instructions for use (IFU). The temperature may range from 2 to 8 degrees Centigrade.
Operating Temperature	The storage and working temperature must be -20 degrees Centigrade.	The operating temperature must not be lower than -20°C. Desirable for test kits to operate between 10°C and 35°C and able to withstand extremely high humidity. However, for best results, it is advised to follow the IFU that comes with the test kit.
Human resource	Must not require more than the basic competency of personnel equipped with skills on RT-PCR techniques and in-vitro diagnostic procedures & instrumentation.	**For NPS/OPS and Saliva Tests:** Only trained technical staff in biosafety and molecular detection of SARS-CoV-2 shall perform the test. **Additional instructions for saliva specimen collection:** Healthcare workers assigned shall provide instructions and directly observe patients on the proper collection of saliva specimens.

TECHNICAL REQUIREMENTS		
Analytical Sensitivity (Gene Targets)	Must have been tested for confirmatory gene (i.e., RdRP, ORF1ab, & N) & screening gene (i.e., E gene)	The testing should allow detection of two or more gene targets. (e.g., confirmatory gene- RdRP, ORF1ab, & N; screening gene - E gene). If the kit contains the S gene target, it should contain two or more other gene targets.
Analytical Specificity (Cross-Reactivity)	Must have been no significant cross-reactivities identified among the RT-PCR test kits. For cross-reactivity testing, must use at least both of the ff. organisms: Influenza A & Influenza B	Must have been no significant cross-reactivities identified among the RT-PCR test kits. For cross-reactivity testing, must use at least both of the ff. organisms: Influenza A & Influenza B
Clinical Sensitivity	Must have at least 99% sensitivity	COMMON: ≥95% sensitivity (FDA Memo 2021-009)
Clinical Specificity	Must have at least 99% specificity	COMMON: ≥99% sensitivity (FDA Memo 2021-009)
Processing Time	Must be six (6) hours or less (excluding repeat test and specimen transport)	PCR testing shall be performed, and results should be released within 48 hours of collection
Reference Standard	Must have used locally or internationally acceptable reference standards.	Refer to the RITM standard method for verification of RT-PCR for NPS/OPS and Saliva *(Interim Biosafety Guidelines for Laboratories Handling and Testing of SARS-CoV-2 [COVID-19] specimen*)
Sample Size Requirement for Validation	Must have a minimum sample size of 30 positive samples & 30 negative samples.	At least 30 PCR positive NPS/OPS samples and 30 PCR negative NPS/OPS

Additional Recommendation/s:

1. The HTAC recommends waste water surveillance using RT-PCR for further research, as fecal samples demonstrate high sensitivity.
2. The DOH should consider revising the current guidelines (e.g., *DM 2020-0512*) on the minimum target genes of RT-PCR considering available evidence and presence in the country of new variants.

The HTAC is actively on the watch for evidence as it is rapidly evolving, and shall update its recommendation when new information becomes available.

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Agencies Included in the Review	
Agency	**URL**
WHO	https://www.who.int/publications/m/item/covid-19-target-product-profiles-for-priority-diagnostics-to-support-response-to-the-covid-19-pandemic-v.0.1 https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2 https://www.who.int/news/item/20-01-2021-who-information-notice-for-ivd-users-2020-05 https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2 https://www.who.int/csr/don/31-december-2020-sars-cov2-variants/en/ https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2 https://www.who.int/news/item/19-01-2021-who-information-notice-for-ivd-users-2021-01 https://www.who.int/publications/i/item/WHO-2019-nCoV-genomic_sequencing-2021.1
DOH	https://doh.gov.ph/sites/default/files/health-update/dm2020-0439.pdf https://doh.gov.ph/sites/default/files/health-update/dc2020-0391.pdf https://hfsrb.doh.gov.ph/wp-content/uploads/2021/01/19012021164825.pdf
PSMID	https://www.psmid.org/rapid-evidence-review-on-covid-19-management/
US FDA	https://www.fda.gov/media/135900/download https://www.fda.gov/medical-devices/letters-health-care-providers/genetic-variants-sars-cov-2-may-lead-false-negative-results-molecular-tests-detection-sars-cov-2
UK MHRA	https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-laboratory-based-sars-cov-2-viral-detection-tests#clinical-performance-requirements
PMDA	https://www.pmda.go.jp/files/000236917.pdf https://www.pmda.go.jp/PmdaSearch/ivdSearch/ https://www.niid.go.jp/niid/images/lab-manual/2019-nCoV20200319.pdf
RITM	https://ritm.gov.ph/wp-content/uploads/2020/07/Guidelines-Technical-Evaluation-of-Reagents-and-Diganostic-Kits-ver-3.0-07062020-1-signed.pdf
Australia PHLN	https://www.health.gov.au/sites/default/files/documents/2020/11/phln-guidance-on-laboratory-testing-for-sars-cov-2-the-virus-that-causes-covid-19.pdf https://www.health.gov.au/sites/default/files/documents/2020/07/phln-guidance-on-nucleic-acid-test-result-interpretation-for-sars-cov-2.pdf https://www.health.gov.au/sites/default/files/documents/2020/09/phln-guidance-for-serological-testing-in-covid-19-phln-guidance-on-serological-testing-in-covid-19.pdf

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CDNA	https://www1.health.gov.au/internet/main/publishing.nsf/Content/7A8654A8CB144F5FCA2584F8001F91E2/$File/COVID-19-SoNG-v4.2.pdf https://www1.health.gov.au/internet/main/publishing.nsf/Content/7A8654A8CB144F5FCA2584F8001F91E2/$File/COVID-19-SoNG-v4.3.pdf
Canada PHLN	https://nccid.ca/wp-content/uploads/sites/2/2020/05/COVID-Best-Practices-V1.01-v3.pdf
PHAC	https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/guidance-documents/national-laboratory-testing-indication.html https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/guidance-documents/national-laboratory-testing-indication.html https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/guidance-documents/repeated-pcr-testing-individuals-previously-positive-covid-19.html#figure1 https://www.canada.ca/en/public-health/news/2021/01/statement-from-the-chief-public-health-officer-of-canada-on-january-9-2021.html
PHO	https://www.publichealthontario.ca/en/about/blog/2021/monitoring-covid-19-voc
FIND	https://www.finddx.org/covid-19/novel-variants/ https://www.finddx.org/newsroom/diagnostics-for-covid-19/
UK NHS	https://www.nhs.uk/conditions/coronavirus-covid-19/testing-and-tracing/
UK DOHSC	https://www.gov.uk/guidance/coronavirus-covid-19-getting-tested
UK PHE	https://www.gov.uk/government/publications/covid-19-rapid-point-of-care-near-person-testing-for-service-providers/covid-19-summary-guidance-for-service-providers-on-rapid-point-of-care-near-person-tests-for-diagnosis-and-management https://www.gov.uk/government/publications/cycle-threshold-ct-in-sars-cov-2-rt-pcr https://www.gov.uk/government/publications/sars-cov-2-voc-investigating-and-managing-individuals-with-a-possible-or-confirmed-case/guidance-for-investigating-and-managing-individuals-with-a-possible-or-confirmed-sars-cov-2-variant-of-concern#management-of-persons-at-risk-admitted-to-hospital-with-a-positive-sars-cov-2-test-on-admission-or-who-subsequently-test-positive https://www.gov.uk/government/publications/investigation-of-novel-sars-cov-2-variant-variant-of-concern-20201201 https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories
China CDC	http://www.chinacdc.cn/en/COVID19/202003/P020200323390321297894.pdf http://en.nhc.gov.cn/2020-09/07/c_81565.htm
US CDC	https://www.cdc.gov/coronavirus/2019-ncov/lab/faqs.html#Testing-Strategies-for-SARS-CoV-2

	https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fhcp%2Fclinical-criteria.htmlhttps://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance.html https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance.html
ECDC	https://www.ecdc.europa.eu/en/publications-data/covid-19-testing-strategies-and-objectives https://www.ecdc.europa.eu/sites/default/files/documents/TestingStrategy_Objective-Sept-2020.pdf https://www.ecdc.europa.eu/en/all-topics-z/coronavirus/threats-and-outbreaks/covid-19/laboratory-support/questions https://www.ecdc.europa.eu/sites/default/files/documents/Sequencing-of-SARS-CoV-2-first-update.pdf https://www.ecdc.europa.eu/sites/default/files/documents/Methods-for-the-detection-and-identification-of-SARS-CoV-2-variants.pdf
Japan MHLW	https://www.mhlw.go.jp/content/000693595.pdf https://www.mhlw.go.jp/content/000712473.pdf https://www.mhlw.go.jp/content/000725966.pdf https://www.mhlw.go.jp/content/10900000/000731944.pdf https://www.niid.go.jp/niid/ja/diseases/ka/corona-virus/2019-ncov/10148-covid19-32.html https://www.niid.go.jp/niid/ja/diseases/ka/corona-virus/2019-ncov/10144-covid19-34.html
South Korea MHW	http://ncov.mohw.go.kr/en/guidelineView.do?brdId=18&brdGubun=181&dataGubun=&ncvContSeq=2937&contSeq=2937&board_id=&gubun=# https://www.annlabmed.org/journal/view.html?doi=10.3343/alm.2020.40.5.351#T1
Malaysia MOH	http://covid-19.moh.gov.my/garis-panduan/garis-panduan-kkm/Annex_4e_SOP_RTPCR_testing_laboratory_.pdf http://covid-19.moh.gov.my/garis-panduan/garis-panduan-kkm/Annex_4g_Garis_Panduan_Penggunaan_RTK_di_fasiliti_swasta_Ag_Versi2.0.pdf http://covid-19.moh.gov.my/garis-panduan/garis-panduan-kkm/Annex_5b_Specimen_collection_transport_and__storage_11112020.pdf
Indonesia MOH	https://covid19.kemkes.go.id/protokol-covid-19/kmk-no-hk-01-07-menkes-413-2020-ttg-pedoman-pencegahan-dan-pengendalian-covid-19
Thailand MOH	https://ddc.moph.go.th/viralpneumonia/eng/file/guidelines/g_surveillance_150520.pdf
HIQA	https://www.hiqa.ie/sites/default/files/2020-10/Rapid-HTA-of-alternative-diagnostic-tests.pdf
HTW	https://www.healthtechnology.wales/wp-content/uploads/2020/05/EAR025-COVID19-diagnostics-report-v2.6.pdf
McMaster	https://www.nccmt.ca/covid-19/covid-19-evidence-reviews/210
CADTH	https://www.cadth.ca/sites/default/files/covid-19/saliva_based_testing_as_an_alternative_to_traditional_covid-19_testing_techniques.pdf
CEBM	https://www.cebm.net/study/viable-sars-cov-2-in-saliva-urine-and-stool-from-covid-19-patients/
NIPH	https://www.fhi.no/globalassets/dokumenterfiler/rapporter/2020/saliva-sample-for-testing-sars-cov-2-infection-memo-2020.pdf

Singapore MOH	https://www.moh.gov.sg/news-highlights/details/updates-on-border-measures-and-travel-insurance
Vietnam MOH	https://vanbanphapluat.co/quyet-dinh-4042-qd-byt-2020-phe-duyet-ke-hoach-xet-nghiem-phat-hien-nhiem-sars-cov-2 https://vanbanphapluat.co/quyet-dinh-1282-qd-byt-2020-huong-dan-tam-thoi-viec-xet-nghiem-covid-19 https://vanbanphapluat.co/thong-tu-quy-dinh-ve-quan-ly-mau-benh-pham-benh-truyen-nhiem https://vanbanphapluat.co/decision-3486-qd-byt-2020-introducing-interim-guidelines-for-sars-cov-2-sample-pooling
Australia TGA	https://www.tga.gov.au/manufacturer-evidence-medical-devices-and-ivd-medical-devices https://www.tga.gov.au/ability-covid-19-tests-detect-emerging-genetic-variants-sars-cov-2 https://www.tga.gov.au/legal-supply-covid-19-test-kits
Canada PHO	https://www.publichealthontario.ca/en/laboratory-services/test-information-index/covid-19
PhilHealth	https://www.philhealth.gov.ph/circulars/2020/circ2020-0017.pdf

VII. Annexes

Annex A

Country and International Guidelines on the interpretation of results from PCR kits

Country (Agency)	Positive RT-PCR Result	Negative RT-PCR Result
Australia (Australia Public Health Laboratory Network) *Last update: 13 July 2020*	A positive / detected result means the detection of SARS-CoV-2-specific target	A negative/ not detected result means no detection of SARS-CoV-2-specific target.
Canada (Public Health Agency Canada) *Last update: 17 February 2021*	A person with confirmation of infection with SARS-CoV-2 documented by the detection of at least 1 specific gene target by a validated NAAT assay.	A person infected with SARS-CoV-2 that has 2 consecutive negative tests on validated laboratory-based NAAT, at least 24 hours apart, is considered a resolved case Any case classified as probable based on an epidemiological link, who subsequently tests negative for the SARS-CoV-2 virus should no longer be classified as a case. Exceptions may be made for negative results from a compromised sample or if NAAT testing is delayed (e.g. >10 to 14 days following symptom onset), whereby such persons remain as probable cases.
China (Center for Disease Control and Prevention of China) *Last update: 15 March 2021*	A confirmed RT-PCR test meets at least one of the following: 1. The real-time fluorescence-based RT-PCR assay of the 2019-nCoV in the same specimen shows that the two targets, ORF1ab and Protein N, are both positive. In case of the result showing positive for one target, then samples shall be recollected for another test. If it is still positive for a single target, it is determined to be positive. 2. The real-time fluorescence-based RT-PCR assay of two types of specimens show one single target positive at the same time, or one target positive in two samples of the same type, it could be	No operational definition mentioned in the guideline

	determined as positive.	
European Union (Centre for Disease Control and Prevention of the European Union) *Last update: 15 September 2020*	A confirmed case is a person with detected SARS-CoV-2 nucleic acid or antigen in a clinical specimen	In cases of widespread community transmission, people that had high-exposure, but had negative results and remain asymptomatic, should be re-tested. In sporadic cases or community transmission, all patients that tested negative upon admission to a hospital, should have a followup test on day 3-5.
Foundation for Innovative new Diagnostics *Last update: 23 March 2020*	A positive result confirms a current SARS-CoV-2 infection.	No operational definition mentioned in the guideline.
Indonesia (Ministry of Health) *Last update: 15 July 2020*	A confirmed case is a person who tested positive for the COVID-19 virus as evidenced by an RT-PCR laboratory examination and can be classified as symptomatic or asymptomatic.	A person with the status of a suspected case with negative RT-PCR examination results for 2 consecutive days with an interval of> 24 hours is classified as “discarded”. Meanwhile, a probable case or confirmed symptomatic case that received a negative RT-PCR follow-up examination once, plus a minimum of 3 days after no longer showing symptoms of fever and respiratory distress is advised to finish isolation.
Japan (Japan Ministry of Health Labor and Welfare) *Last update: 19 April 2021*	PCR method quantitatively confirms that the SARS-CoV-2 gene is present in a sample.	*General statement for all SARS-CoV tests:* False positives may occur depending on the sample. Even if the result of one test is truly negative, it does not deny the infection, so care should be taken when using the inspection results as a reference for releasing quarantine, etc.
Malaysia (Ministry of Health) *Last update: 14 April 2021*	A confirmed case is a person with laboratory confirmation of infection with the COVID-19, irrespective of clinical signs or symptoms.	There is no specific mention of negative results in the operational case definitions of COVID-19.
Philippines (Department of Health) *Last update: 26 November 2020*	A positive result determines a current infection of SARS-CoV-2. A confirmed case needing isolation and triage according to clinical status.	A negative result determines the absence of SARS-CoV-2 , but does not rule out COVID-19.

Singapore (Ministry of Health) *Last update: 12 March 2021*	A confirmed case is a person with a positive RT-PCR test result for COVID-19.	A negative PCR test is possible during the virus incubation period, and does not exclude the possibility that infection is present.
South Korea (Ministry of Health and Welfare *Last update: 25 June 2020*	A confirmed case or a person confirmed to be infected with COVID-19, regardless of clinical manifestations	No operational definition mentioned for a negative PCR, but noted that despite a negative result, suspected cases should still be quarantined and PUIs shall follow health education guidelines for 14 days.
Thailand (Ministry of Health) *Last update: 15 May 2020*	Confirmed case is defined as a PUI who has tested positive for genetic materials of SARS-CoV2 by PCR from one reference laboratory designated by the Department of Medical Sciences (DMSc), or by genetic sequencing, or by culture.	In the event laboratory results come back negative for COVID-19 and the patient's condition has not improved, this may be attributable to the specimen not being properly collected and processed or poor quality specimen. Procedures for specimen collection and transportation should be reviewed and specimen will have to be collected for a repeat test 24 hours after the first collection. Otherwise, at discharge, a patient will be asked to further maintain home isolation until the 14-day isolation period is complete following the date of his/her departure from the high-risk areas or last contact with a confirmed case of COVID-19 infection.
United Kingdom (National Health Service) *Last update: 8 March 2021*	A Confirmed case is often a symptomatic individual with detected SARS-CoV-2 by PCR from a laboratory and needs to self-isolate	A case is deemed Negative when no detected SARS-CoV-2 by PCR from a laboratory occurred but a person may still need to self-isolate
United States (Centers for Disease Control and Prevention) *Last update: 17 March 2021*	A positive result indicates that the virus's genetic material was detected and the patient tested positive for SARS-CoV-2 infection.	Negative test results in persons with known SARS-CoV-2 exposure suggest no current evidence of infection. These results represent a snapshot of the time around specimen collection and could change if the same test was performed again in one or more days. Unvaccinated individuals with a negative result should continue to quarantine for 14 days or for the period established by local public health authorities. Fully vaccinated people with no COVID-like symptoms do not need to quarantine or be tested following an exposure to someone with

		suspected or confirmed COVID-19, as their risk of infection is low. Negative test results in persons without symptoms and no known exposure suggest no infection. All persons being tested, regardless of results, should receive counseling on the continuation of risk reduction behaviors that help prevent the transmission of SARS-CoV-2 (e.g., wearing masks, physical distancing, avoiding crowds and poorly ventilated spaces).
Vietnam (Ministry of Health) *Last update: 21 September 2020*	If the test detects a viral infection using genetic material detection (RNA)and is positive, then the patient's sample must be sent to a laboratory confirming SARS-CoV-2 to perform confirmatory testing (following WHO and / or US CDC recommended procedures and biologicals), and administer prescribed isolation and treatment. ● WHO algorithm: Positive conclusion for SARS-CoV-2 ● US CDC algorithm: ○ Both N1 and N2 gene detected - positive conclusion for SARS-CoV-2 ○ Only one of two genes detected (N1 and N2 gene) - for repeat testing	If the test for detection of virus infection by genetic material detection (RNA), is negative, it means that the presence of SARS-CoV-2 cannot be detected. In case of high-risk subjects, additional specimens should be considered for testing (especially when sampling only upper respiratory tract samples) because the test results can be affected by many factors such as sample quality. specimens, extraction techniques, etc.
World Health Organization *Last update: 11 September 2020*	A confirmed case is a patient who meets the clinical criteria for COVID-19 and tests positive for nucleic acid amplification testing such as RT-PCR.	One or more negative results do not necessarily rule out the SARS-CoV-2 infection as factors, such as poor quality of the specimen, time of specimen collection, poor handling of specimen, and technical reasons inherent in the test, e.g. PCR inhibition or virus mutation, could lead to a negative result in an infected individual. If negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, resample and repeat the test and a paired serum specimen could be collected.

Annex B

Country (Agency)	Regulatory guidelines
US (US FDA)	**NO PERFORMANCE SPECIFICATIONS FROM US FDA ON NEW VARIANTS BUT RELEASED POLICY RECOMMENDATIONS ON DETECTING NEW VARIANTS** Since the performance of a diagnostic test can be impacted by viral mutation, FDA recommends that developers: 1. *Design Considerations to Minimize Impact of Viral Mutation* For molecular tests, FDA recommends developers consider the performance of their test across all known variants at the time of validation and the potential impact of future genetic variants when considering their test design. Designing redundancy into a test may prevent future variants from impacting test performance. Tests with multiple targets and appropriate result interpretation criteria have been used to identify signals that a patient sample may include a variant and should be followed up with additional testing and/or sequencing of the viral genome. FDA recommends that test developers include in their EUA request a description of how they have evaluated their test performance across all known variants having mutations in the targeted region and a discussion of how their test design mitigates the risk of future viral mutations impacting the test performance. Including a highly conserved pan-SARS-CoV target (a target in a portion of the genetic code associated with the larger Sarbecovirus subgenus of the genus Betacoronavirus in the Coronaviridae family not specific to SARS-CoV-2) as part of a multiple target test may improve performance with a new genetic variant; however, the number of targets in the test should be appropriate to provide resilience (i.e., a reduction of the risk that viral mutation will impact test performance) and most efficiently leverage developer and laboratory resources. When using a highly conserved target in combination with SARS-CoV-2 specific targets, appropriate result interpretation, such as how to interpret results when a pan-SARS-CoV target is positive while the SARS-CoV-2 specific targets are not and follow-up recommendations for an overall positive result with individual gene negative results, may be needed. 2. *Routine Monitoring of Viral Mutations that May Impact Molecular Diagnostic Test Performance* Since mutations in the viral genome can affect hybridization of test reagents with SARS-CoV-2, FDA recommends evaluating hybridization changes when a test developer identifies a mutation expected to result in a mismatch, or mismatches, within the target primer/probe binding site(s). Investigations of the impact on hybridization can be done in three stages, each providing a more accurate evaluation of test performance than the last: in silico calculation, wet testing of genomic material, and wet testing of a virus isolate with a mutation. If a difference of >3-fold in LoD is found, and the test is not yet authorized, FDA recommends that the developer's EUA request include a risk analysis for the observed decrease in performance and either a description and justification of any further risk mitigations, or alternatively, if the developer believes that no mitigations

	are needed, a justification for the position that the known and potential benefits outweigh the known and potential risks. For already authorized tests, if a difference of >3-fold in LoD is found or the test developer otherwise identifies viral mutations with the potential to change the benefit-risk profile of their product, it is FDA's current recommendation that test developers notify FDA in a supplemental EUA request including a risk analysis for the observed decrease in sensitivity and either a description and justification of any further risk mitigations, or alternatively, if the developer believes that no mitigations are needed, a justification for the position that the known and potential benefits outweigh the known and potential risks. If requested by FDA to evaluate the impact of a specific mutation(s) or variant(s), FDA expects a test developer of an authorized test to perform the requested evaluation in a timely manner. FDA expects that such studies, study designs and/or data analysis, including a timeline for submission of information to FDA, will be agreed upon between a test developer and FDA. For instance, FDA may request to establish the LoD using limiting dilutions of quantified synthetic RNA or quantified in vitro transcripts of both the target sequence from the reference genome (i.e., perfect match target) and the sequence harboring the mutation of interest. *3. Clearly convey any test limitations in the test's labeling*
Canada (Health Canada)	**HEALTH CANADA ADAPTS US FDA GUIDANCE ON APPROVAL OF NUCLEIC ACID BASED TESTS** "Health Canada refers to guidance published by the US Food and Drug Administration (FDA) on nucleic acid-based tests" *IN VIEW OF NEW VARIANTS:* "In light of number of SARS-CoV-2 variants of public health concern that have emerged, Health Canada requires the following before an application may be authorized: 1. Manufacturers must assess the impact of new variants of public health concern on their test, taking into account performance and labelling, and include this assessment in their application. If it's included in the submitted in-silico and/or wet testing data, this must be clearly stated. 2. Manufacturers must indicate how they plan to mitigate any new risks, including timelines for addressing these risks. 3. Manufacturers must provide a proactive risk management plan to assess, address and notify Health Canada of their findings related to any novel published variants of public health concern. Manufacturers that submit evidence on how their device performs in specimens from people infected with emerging variants may be able to have this requirement adjusted."

EU (ECDC)	**NO SPECIFIC PERFOMANCE SPECIFICATIONS FOR VARIANTS FOUND BUT THE ECDC PUBLISHED A GUIDANCE ON METHODS TO USE FOR DETECTION AND IDENTIFICATION OF VARIANTS** "Whole Genome Sequencing, or at least complete or partial S-gene, should be performed to confirm infection with a specific variant. For early detection and prevalence calculation of variants of concern (e.g. B.1.1.7/501Y.V1, B.1.351/501Y.V2, P.1/501Y.V3), alternative methods have been developed, such as diagnostic screening PCR-based assays. While testing strategies should be flexible and rapidly adaptable to change, depending on the local epidemiology, population dynamics and resources, sample and method selection are key and will depend on the objectives. Specific objectives include the assessment of the circulation of the different SARS-CoV-2 variants in the community selecting representative samples, genetic characterisation to monitor the virus evolution and inform vaccine composition decisions or outbreak analyses. When PCR-based assays are used, confirmatory sequencing of at least a subset of viruses should be performed to be able to use these assay results as indicators of community circulation of the variants of concern. Before introducing a new testing method or a new assay, a validation and verification exercise should be carried out to ensure that the laboratory testing system is performing adequately for the circulating viruses." *Diagnostic Screening assays of known VOCs* For the B.1.1.7/501Y.V1 (also called VOC 202012/01), a negative or significantly weaker positive S-gene result in multiplex RT-PCR assays, with positive results for the other targets, has been used as an indicator or screening method to identify this particular variant. The weaker signal or complete failure of the S-gene target is caused by a deletion at nt207-212 in the respective gene. The S-gene target failure occurs for some assays that include a S-gene target, but not all [2]. By coincidence, the pattern of detection of B.1.1.7/501Y.V1 with a specific commercial assay, can be used to detect those currently circulating variants of concern [4,5]. Variant B.1.1.7/501Y.V1 gives a positive signal in ORF1 and N-gene targeted RT-PCRs, but not in S-based RT-PCR, and is therefore called S-gene target failure or target failure; this pattern can be used as an indicator of potential circulation of the B.1.1.7/501Y.V1 variant. It needs to be noted that this target failure (S-gene target failure) is not exclusive to B.1.1.7/501Y.V1 and will also identify other variants (non-VOC) and cannot differentiate between them, while it will also fail to detect some other VOC. It is worth mentioning that prior to the emergence of the B.1.17 VOC in the United Kingdom (UK), 1-5% of sequenced samples already had the deletion/target failure (drop out). The S-gene target failure does not occur for 501Y.V2 and most probably not for lineage P.1. This strategy should preferably be used when there is already high prevalence of the VOC in the setting. Confirmation of the presence of the deletion at nucleotides 207-212 by sequencing is recommended at least for a subset of samples, especially in a low prevalence setting; this will be needed to increase the confidence of the results and should be closely monitored. In regions where other variant(s) with the same deletion but not VOC circulate, sequencing of all S-gene target failures is necessary. Increasing the numbers of sequenced samples screened by S-gene target failure can be considered to assess the regional correlation between S-gene target failure and B.1.1.7/501Y.V1, as this varies with the regionally circulating variants [6]. If the correlation is very high, S-gene target failure can be used to approximate the frequency of B.1.1.7/501Y.V1. <u>Multiplex RT-PCR, including S-gene target failure</u> With a multiple channel real time RT-PCR device, the normal E and/or N and/or ORF-1 target assays may be combined with the S-gene target, so the VOC screening could be integrated with the normal routine, in a single run [7]. Another method has been developed based on the ORF1a gene (ORF1a Δ3675-3677) that exists in all three variants, which has not yet been widely detected in other SARS-CoV-2 lineages. Using ORF1a Δ3675-3677 as the primary target and spike Δ69-70 to differentiate, an open source PCR assay was designed to detect SARS-CoV-2 variants of concern (preprint) [8]. It is important to emphasise that results should not be over-interpreted and must be checked/continuously validated through the use of genomics.

	Screening SNP assays Screening for VOC specific amino acid substitutions can be done using a specific RT-PCR assays targeting single nucleotide polymorphisms (SNP) to screen e.g. spike N501Y and HV69-70del mutations (e.g. present in B.1.1.7/501Y.V1 VOC) [7]. Appropriate positive controls will be needed. This method allows quick (this is a <1h assay) estimation of the prevalence of the specific mutation-positive variants in the community. Of note, there are N501Y lineages that are not VOCs, which currently circulate, and therefore verification of at least a subset of samples should be done using sequencing. Screening SNP by specific real time RT-PCR melting curve analysis Some real time PCR platforms allow for melting curve analysis. Commercial assays have been developed to use this genotyping method to identify specific amino acid substitutions, e.g. HV69-70del, K417N, N439K, Y453F, E484K, N501Y, A570D, D614G, P681H or V1176F.
Australia (Therapeutic Goods Authority)	**NO SPECIFIC PERFORMANCE SPECIFICATIONS FOR VARIANTS FOUND BUT THE DOH THROUGH THEIR PUBLIC HEALTH LABORATORY NETWORK PUBLISHED A STATEMENT ON REPORTING COVID VARIANTS OF CONCERN PUBLISHED APRIL 6, 2021** "Whole genome sequencing (WGS) of the SARS-CoV-2 genome is the preferred way to determine the variant and mutation patterns of the SARS-CoV-2 virus. There are emerging SARS-CoV-2 PCR tests designed to detect specific VoCs. The performance of these tests is still being established, and they will not identify variants that they have not been designed for. These may be suited for use in diagnostic laboratories without sequencing capability. Most Australian public health laboratories are attempting to sequence all SARS-CoV-2 samples."

Annex C. Costing components for RT-PCR Testing using (1) NPS/OPS; and, (2) saliva samples from RITM.

COST COMPONENTS FOR RT PCR TEST (NPS/OPS SAMPLE)*						
PREPARED BY: Research Institute for Tropical Medicine						
April 20, 2021						
	MANUAL EXTRACTION	MANUAL EXTRACTION	AUTOMATED EXTRACTION	AUTOMATED EXTRACTION	AUTOMATED EXTRACTION	REMARKS
A. DIRECT COST	BGI	MirXes Fortitude 2.1	SANSURE	BGI	MirXes Fortitude 2.1	
1. TESTING KIT (Detection)	1,390.00	1,720.00	918.00	1,390.00	1,720.00	
1. TESTING KIT (Extraction)	625.00	625.00		300.00	300.00	
2. REAGENTS (Specimen Inactivation)	5.00	5.00	5.00	5.00	5.00	
3. CONTROL		633.84			633.84	Included already in the run; PCR kit has internal controls; except for MirXes Fortitude which requires extraction control run (RP)
4. MISCELLANEOUS OR CONSUMABLES						
4.1 SUPPLIES FOR COLLECTION OF SPECIMEN WHICH INCLUDES PERSONAL PROTECTIVE EQUIPMENT, SWAB , TRANSPORT MEDIUM ETC.	408.05	408.05	408.05	408.05	408.05	
4.2 PPE FOR RECEPTION, INACTIVATION, TESTING	692.22	692.22	692.22	692.22	692.22	
4.3 CONSUMABLES	115.00	115.00	269.40	115.00	115.00	Estimate; with adjustment for quantity and brand
4.3 SUPPLIES FOR RELEASING RESULTS LIKE PAPER, INK	2.00	2.00	2.00	2.00	2.00	
B. INDIRECT COST						
1.SALARIES OF STAFF(PLEASE DISAGGREGATE PER STAFF AND INCLUDE HEAD OF LABORATORY)	32.07	32.07	32.07	32.07	32.07	
2. UTILITIES(PLEASE DISAGGREGATE E.G. WATER, ELECTRICITY, INTERNET CONNECTION ETC.) AND INCLUDE RENT, IF APPLICABLE	24.20	24.20	24.20	24.20	24.20	
ESTIMATED COST OF PCR TEST (NPS/OPS)	3,293.54	4,257.38	2,350.84	2,968.54	3,932.38	3,390.56

*BASED ON PCR KITS USED BY RITM. Unit cost may need to be updated.

COST COMPONENTS FOR RT PCR TEST (SALIVA SAMPLE)**						
SELLING/ CURRENT PRICE :	MANUAL EXTRACTION	MANUAL EXTRACTION	AUTOMATED EXTRACTION	AUTOMATED EXTRACTION	AUTOMATED EXTRACTION	REMARKS
A. DIRECT COST	BGI	MirXes Fortitude 2.1	SANSURE	BGI	MirXes Fortitude 2.1	
1. TESTING KIT (Detection)	1,390.00	1,720.00	918.00	1,390.00	1,720.00	
1. TESTING KIT (Extraction)	625.00	625.00		300.00	300.00	
2. REAGENTS (Specimen Inactivation)	5.00	5.00	5.00	5.00	5.00	
3. CONTROL		633.84			633.84	Included already in the run; PCR kit has internal controls; except for MirXes Fortitude which requires extraction control run (RP)
4. MISCELLANEOUS OR CONSUMABLES						
4.1 SUPPLIES FOR COLLECTION OF SPECIMEN WHICH INCLUDES PERSONAL PROTECTIVE EQUIPMENT, SWAB , TRANSPORT MEDIUM ETC.	208.05	208.05	208.05	208.05	208.05	Estimated saliva collection kit= P200.00/kit; in Saliva PCR testing, VTM and swab are not required
4.2 PPE FOR RECEPTION, INACTIVATION, TESTING	692.22	692.22	692.22	692.22	692.22	
4.3 CONSUMABLES	115.00	115.00	269.40	115.00	115.00	Estimate; with adjustment for quantity and brand
4.3 SUPPLIES FOR RELEASING RESULTS LIKE PAPER, INK	2.00	2.00	2.00	2.00	2.00	
B. INDIRECT COST						
1.SALARIES OF STAFF(PLEASE DISAGGREGATE PER STAFF AND INCLUDE HEAD OF LABORATORY)	32.07	32.07	32.07	32.07	32.07	
2. UTILITIES(PLEASE DISAGGREGATE E.G. WATER, ELECTRICITY, INTERNET CONNECTION ETC.) AND INCLUDE RENT, IF APPLICABLE	24.20	24.20	24.20	24.20	24.20	
ESTIMATED COST OF PCR TEST (SALIVA)	3,093.54	4,057.38	2,150.84	2,768.54	3,732.38	3,160.56

**NOTE: ASSUMPTION IS THAT THE PCR PROCESS INCLUDES NUCLEIC ACID EXTRACTION WITH A COMMERCIAL EXTRACTION KIT (WHICH MAY BE MANUAL OR AUTOMATED)