Republic of the Philippines
Department of Health
OFFICE OF THE SECRETARY

November 23, 2020

DEPARTMENT MEMORANDUM
No. 2020 - 0539

TO: **ALL UNDERSECRETARIES AND ASSISTANT SECRETARIES; DIRECTORS OF BUREAUS AND CENTERS FOR HEALTH DEVELOPMENT; MINISTER OF HEALTH – BANGSAMORO AUTONOMOUS REGION IN MUSLIM MINDANAO; EXECUTIVE DIRECTORS OF SPECIALTY HOSPITALS AND NATIONAL NUTRITION COUNCIL; CHIEFS OF MEDICAL CENTERS, HOSPITALS, SANITARIA AND INSTITUTES; PRESIDENT OF THE PHILIPPINE HEALTH INSURANCE CORPORATION; DIRECTORS OF PHILIPPINE NATIONAL AIDS COUNCIL AND TREATMENT AND REHABILITATION CENTERS AND ALL OTHERS CONCERNED**

SUBJECT: **Interim Guidelines on the Conduct of COVID-19 Pooled Testing**

I. BACKGROUND

Pooled testing is a strategy that can reduce the cost of screening a large number of individuals for infectious diseases. Using this procedure, samples from multiple individuals are combined or "pooled" then are tested collectively. Select targeted samples are then tested individually if there is a positive result in the group.

Strategic testing, along with aggressive contact tracing and isolation, is recognized as crucial to an effective COVID-19 response. Incorporating specimen pooling strategies is an opportunity for greater efficiency by accelerating COVID-19 testing, reducing the turnaround time, as well as saving on supplies. Furthermore, it allows the opportunity for testing and targeting even asymptomatic individuals, as well as checking and monitoring areas in the country with low or decreasing COVID-19 prevalence.

However, pooled testing also requires very specific steps and parameters for implementation. A standard and easy-to-follow pooling strategy for COVID-19 laboratories should be in place while considering several factors for optimal and reliable results. In view of the foregoing and the pooling protocols set by the Philippine Society of Pathologists, Inc., Research Institute of Tropical Medicine, and the Department of Health, these interim guidelines are being issued to provide guidance on identifying and recognizing sites for COVID-19 pooled testing.

II. GENERAL GUIDELINES

A. Pooled testing shall be used for screening and surveillance in low prevalence populations or settings for individuals who are not experiencing any symptoms or have no immediate past history of exposure to confirmed COVID-19 patients. It shall not be used for individuals who are symptomatic, have recovered from the disease and are

now asymptomatic, and those with history of exposure to confirmed COVID-19 patients in the past 14 days..

B. Pooled testing shall be done by DOH-licensed COVID-19 testing laboratory using PCR test kits authorized by the Food and Drug Administration (FDA) AND validated by RITM or other RITM-authorized institutions.
C. In order to ensure consistency and accuracy of results, licensed COVID-19 testing laboratories that will perform pooled testing shall follow the recommended standard technical protocol and guidelines encompassing pre-analytical, analytical, and post-analytical processes set by the Philippine Society of Pathologists, Inc. (PSP), in coordination with RITM. Attendance to training on sample collection, documentation, and sample pooling strategies provided by the PSP through the Philippine Children's Medical Center is required.
D. Licensed COVID-19 testing laboratories that intend to use the pooled testing methodology are required to perform initial verification using the test kits and PCR machines that they will be using in the regular conduct of pooled testing. These internal validation studies should follow the guidelines and procedures recommended for the purpose of pooled testing by the PSP and RITM. RITM shall assess and ascertain the technical correctness of the verification procedures performed and the findings generated. These are necessary prerequisites before any COVID-19 testing laboratory will be allowed to perform pooled testing.
E. Reporting of confirmed cases shall continue to be based on RT-PCR testing, in accordance with Administrative Order 2020-0013, entitled "Revised AO 2020-0012 Guidelines for the Inclusion of COVID-19 in the List of Notifiable Diseases for Mandatory Reporting to the Department of Health dated 17 March 2020".
F. Reporting of the full line list of positive and negative specimens from the start of the operations shall use the COVID-19 Repository Document System.
G. Disposal of test kits, including personal protective equipment and other materials used in testing, shall adhere to the 4th Edition of Health Care Waste Management Manual.
H. Monitoring of performance of COVID-19 laboratories shall be in the context of the RITM Quality Assurance Program.
I. All testing facilities shall utilize the appropriate PhilHealth benefit and/or any benefit provided by Health Maintenance Organizations or Private Health Insurance for COVID-19 testing to reimburse the costs of testing.

III. SPECIFIC GUIDELINES

A. Eligibility Criteria

Pooled testing shall only be used for:

1. Screening of population groups
    a. Inbound international travelers, including returning Filipinos, Overseas Filipino Workers, and foreigners; and
    b. Interzonal domestic travelers, including returning residents.
2. Surveillance of population groups
    a. Health care workers in health facilities;
    b. Frontline government workers (police, military, quarantine, immigration officers, to name a few);
    c. Factory workers, market vendors, call center agents, transportation workers, and others in workplace settings; and
    d. Other populations to be determined in the future.
3. Surveillance of communities that fulfill any of the two criteria
    a. COVID-free Municipalities: No cases reported yet since the start of the pandemic and/or for two weeks by date of report

b. Attack Rate: Municipalities/cities with attack rate less than 100 per 100,000 Population based on the data for the last two weeks.
c. Should results of pooled testing manifest an Attack Rate of more than 100 per 1,000 Population or a Prevalence Rate of more than 10%, pooled testing shall be discontinued.

B. Regulatory Requirements

1. Only RT-PCR kits authorized by FDA to be used for pooled testing and validated by RITM or other authorized institutions shall be used by licensed COVID-19 testing laboratories.
2. Manufacturers and suppliers of RT-PCR kits shall apply for authorization to be used for pooled testing to FDA.
3. Cartridge-based RT-PCR testing shall not be used for pooled testing.

C. Training, Verification, and Certification

1. Training shall be provided by PSP through its partner facility, the Philippine Children's Medical Center. Certificates of Training issued by PSP shall be acceptable as compliance with the certification requirements for pooled testing.
2. COVID-19 testing laboratory shall undergo verification of pooled testing and submit the results to RITM. Only laboratories with 85% percentage agreement between pooled testing and individual testing shall be allowed.
3. Only laboratories that have successfully completed the training and have achieved at least 85% percentage agreement shall be certified by the RITM to conduct pooled testing for specific PCR kit that they have verified.

D. Standard Protocol for Pooled Testing

1. Licensed COVID-19 testing laboratories recognized for pooled testing can only conduct the method, provided that they develop standard procedures and guidelines based on a fixed protocol. This protocol shall include but are not limited to the following:
    a. A pooled sample size of 5 for testing is recommended.
    b. Interpretation of results of pooled testing
    c. Standard Operating Procedures (SOP)/ Manual of Procedures on pooled testing (Annex A)
    d. Procedures and reporting of in-house verification on pooled testing (Annex B)
    e. General guidance for COVID-19 laboratory doing/contemplating to perform pool testing (Annex C)

E. Interpretation of Results

1. If a pooled test result is negative, then all specimens can be presumed negative with the single test.
2. However, if a pooled test result is positive, then all the specimens in the pool have to be retested individually and all individuals included in the pool have to be immediately quarantined or isolated.
    a. If the individual tests negative, they can discontinue quarantined.
    b. If the individual tests positive, they shall be contact traced, complete isolation, and be managed accordingly based on Department Memorandum No. 2020-0512 entitled Revised Omnibus Interim Guidelines on Prevention, Detection, Isolation, Treatment, and Reintegration Strategies for COVID-19.

F. **Pricing and Reimbursements**

1. The Philippine Health Insurance Corporation (PhilHealth) shall develop the appropriate payment and provider engagement mechanisms for pooled testing, in accordance with details set forth in these guidelines.
2. COVID-19 laboratories recognized to conduct pooled testing shall adhere to the price range set for pooled testing.

For strict compliance.

FRANCISCO T. DUQUE III, MD, MSc
Secretary of Health

'ANNEX B'

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Standard Method for Verification of Pooled Testing
Interim Guidance for Laboratories in the COVID-19 Laboratory Network

As of 26 October 2020

Introduction

Pooled testing shall be applicable to the performance of SARS-CoV-2 molecular diagnostic tests for the in vitro qualitative detection of RNA from SARS-CoV-2 in respiratory samples for specific target populations only and in line with the Department of Health's latest issuance on testing. Symptomatic patients, as well as people who have high risk exposure to SARS-CoV-2 confirmed cases, shall not be covered by pooled testing.

Pooled testing shall be undertaken only by DOH-selected licensed laboratories of the COVID-19 Laboratory Network only upon review and approval of pooling procedure and method verification data. Only FDA-authorized PCR kits shall be utilized for pooled testing. Pooled testing shall only be performed by qualified proficient laboratory technical staff. The strategy shall only be applied to appropriate target populations with expected low prevalence and low risk. It must be noted that the method may change as more information becomes available.

Rationale

In keeping with international standards of good laboratory practice, any new method introduced by the laboratory shall be subject to verification prior to regular performance.

Scope

The following contains guidance for the standard method for verification of pooled testing as a testing strategy for SARS-CoV-2 PCR as applicable to respiratory specimens.

Objective

1. To determine the performance, usefulness, practicality, and applicability of pooled testing in a laboratory's particular set-up using its specific PCR reagents, supplies, and laboratory equipment.

2. To verify the method of pooled testing through determination of the percentage agreement between 5-pooled samples and individual samples tested through rRT-PCR prior to adoption of the procedure in the laboratory.

Method

1. **Preparation of verification panels**
    a. Positive pools
        i. Using either archived/stored or prospectively collected samples, prepare 20 "positive" pools of five consisting of 80 unique PCR negative samples and 20 PCR positive samples, for a total of 100 samples.

ii. The 20 PCR positive samples shall consist of:
    1. 25% (n=5) within 2-3 Ct values of the cut-off for the laboratory's PCR assay, to represent low or weak positives
    2. 75% (n=15) with various Ct values representing high and medium positive samples
iii. Each "positive" 5-sample pool shall consist of 1 PCR positive sample + 4 randomly selected PCR negative samples

b. Negative pools
    i. Using either archived/stored or prospectively collected samples, prepare 20 "negative" pools of five consisting of 100 unique PCR negative samples.
    ii. If there is sufficient volume, the same negatives used in the preparation of the "positive" pools may be used.

NOTE: All samples included in the positive and negative pools should have been tested individually using the laboratory's PCR assay, following manufacturer's instructions, with recording of Ct values.

2. PCR Testing of positive and negative pools

a. The 20 positive and 20 negative pools shall be tested using the laboratory's PCR assay, following the manufacturer's instructions, with recording of Ct values for each gene target.
b. Ensure that the technical staff conducting the tests on the positive and negative pools are blinded to the results of the individual samples included in each pool.

3. Analysis of data

a. **Data tables**
    i. Data shall be summarized following the table below, showing the Ct values, interpretation of individual results with corresponding pooled test results.

Individual Samples			**Pooled Test Result**		
Test Result	**Ct Value per gene target**	**Result Interpretation**	**Pool Number**	**Ct Value per gene target**	**Result Interpretation**
Sample Lab ID-001			Pool Number-001		
Sample Lab ID-002					
Sample Lab ID-003					
Sample Lab ID-004					
Sample Lab ID-005					
…			….		
Sample Lab ID-100			Pool Number-020		

b. **Percent Agreement (Aggregate)**

i. Calculate the percent agreement of the pooled samples with respect to the expected results (i.e., if a positive patient sample was included in the 5-sample pools, the expected result was positive).

Samples Tested Individually	**Pooled Test Final Result (5-sample Pool)**	
Test Result	Positive	Negative
Positive		
Negative		

Positive Percent Agreement = _____

Formula:

$$\frac{\text{No. of positive results in } \textbf{agreement} \text{ (Pooled test result with individual result)}}{\text{Total number of results (20)}} \times 100$$

c. Percent Agreement (Disaggregated to Ct value range)

i. Using the table below:

Samples Tested in a 5-sample Pool	**Individual Samples with Ct within 3 values within PCR kit cutoff (weak positives)**	
Pooled Test Result	**Positive**	**Negative**
Positive		
Negative		
	Individual Samples with Ct values >30 to 37*	
Positive		
Negative		
	Individual Samples with Ct values >20 to 30	
Positive		
Negative		
	Individual Samples with Ct values <20	
Positive		
Negative		

*NOTE: 37 if kit cut off value is 40, otherwise, indicate 3 values within the PCR kits cutoff value

Record Keeping

The laboratory shall maintain information on the performance of the pooled testing procedure and all method verification data. These records shall be made available for review and inspection upon request.

Submission of Verification Documents

The accomplished method verification report (Annex A) shall be submitted to the Research Institute for Tropical Medicine for review.

References

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ANNEX A – Method Verification Report for Pooled Testing (Template)

Date of verification:			
Name of Laboratory:			
Complete Address:			
Laboratory head (Pathologist):			
Chief medical technologist:			
Contact information:	E-mail address:	Landline No.	Mobile No.
Part 1: PCR Assay Intended to be used for Pooled Testing Verification			
Nucleic Acid Extraction kit	• *Brand/manufacturer:* • *Kit description:* • *Attach product brochure and Manufacturer's Instructions for Use* • *Attach Laboratory's SOP*		
Automated Extraction machine	• *Brand/manufacturer:* • *Attach product brochure* • *Attach Laboratory's SOP for operating the machine*		
PCR detection kit	• *Brand/manufacturer:* • *Kit description:* • *Gene targets:* • *Performance data available (FIND, WHO, National Regulatory Agency, RITM)* • *Attach product brochure and Manufacturer's Instruction for Use* • *Attach Laboratory's SOP*		
PCR machine	• *Brand/manufacturer* • *Attach product brochure* • *Attach Laboratory's SOP for operating the machine*		
Part 2: Verification Panel Composition			
Positive pools • *Number/quantity?* • *Archived/stored or prospectively collected?* • *If archived/stored, dates of collection, storage condition (2-8°C, -20°C, -40°C, -80°C)* • *Ct values*			
Negative pools • *Number/quantity?* • *Archived/stored or prospectively collected?* • *If archived/stored, dates of collection, storage condition (2-8°C, -20°C, -40°C, -80°C)* • *Ct values*			

Part 3: Pooling Procedure (Detailed specific steps for pooling undertaken)

Part 4. Results

Summary table (per gene target)

Individual Samples			Pooled Test Result		
Test Result	**Ct Value per gene target**	**Interpretation**	**Pool Number**	**Ct Value per gene target**	**Interpretation**
Sample Lab ID-001			Pool No. 1		
Sample Lab ID-002					
Sample Lab ID-003					
Sample Lab ID-004					
Sample Lab ID-005					

Percentage Agreement (Aggregate)

Samples Tested Individually	**Pooled Test Result (5-sample Pool)**	
Test Result	Positive	Negative
Positive		
Negative		

Notes:

Percentage Agreement (Disaggregated to Ct value range)

Samples Tested in a 5-sample Pool	**Individual Samples with Ct within 3 values within PCR kit cutoff (weak positives)**	
Pooled Test Result	**Positive**	**Negative**
Positive		
Negative		
	Individual Samples with Ct values >30 to 37	
Positive		
Negative		
	Individual Samples with Ct values >20 to 30	
Positive		
Negative		
	Individual Samples with Ct values <20	
Positive		
Negative		

Notes:

Report prepared by:

Technical Staff
Signature over printed name

Approved by:

Head of Laboratory
Signature over printed name

VERIFICATION METHOD FOR POOLED TESTING
REPORTING FORM

Date of verification:			
Name of Laboratory:			
Complete Address:			
Laboratory head (Pathologist):			
Chief medical technologist:			
Contact information:	E-mail address:	Landline No.	Mobile No.
Part 1: PCR Assay Intended to be used for Pooled Testing Verification			
Nucleic Acid Extraction kit	• *Brand/manufacturer:* • *Kit description:* • *Attach product brochure and Manufacturer's Instructions for Use* • *Attach Laboratory's SOP*		
Automated Extraction machine	• *Brand/manufacturer:* • *Attach product brochure* • *Attach Laboratory's SOP for operating the machine*		
PCR detection kit	• *Brand/manufacturer:* • *Kit description:* • *Gene targets:* • *Performance data available (FIND, WHO, National Regulatory Agency, RITM)* • *Attach product brochure and Manufacturer's Instruction for Use* • *Attach Laboratory's SOP*		
PCR machine	• *Brand/manufacturer* • *Attach product brochure* • *Attach Laboratory's SOP for operating the machine*		
Part 2: Verification Panel Composition			
Positive pools • *Number/quantity?* • *Archived/stored or prospectively collected?* • *If archived/stored, dates of collection, storage condition (2-8° C, -20° C, -40° C, -80° C)* • *Ct values*			
Negative pools • *Number/quantity?* • *Archived/stored or prospectively collected?* • *If archived/stored, dates of collection, storage condition (2-8° C, -20° C, -40° C, -80° C)* • *Ct values*			

Part 3: Pooling Procedure (Detailed specific steps for pooling undertaken)

(Add more pages as necessary)

Part 4. Results

Summary table

Individual Samples			Pooled Test Result		
Test Result	**Ct Value per gene target**	**Interpretation**	**Pool Number**	**Ct Value per gene target**	**Interpretation**
Sample Lab ID-001			Pool No. 1		
Sample Lab ID-002					
Sample Lab ID-003					
Sample Lab ID-004					
Sample Lab ID-005					
Sample Lab ID-006					
Sample Lab ID-007					
Sample Lab ID-008					
Sample Lab ID-009					
Sample Lab ID-010					
Sample Lab ID-011					

Percentage Agreement (Aggregate)

Samples Tested Individually	**Pooled Test Result (5-sample Pool)**	
Test Result	Positive	Negative
Positive		
Negative		

Positive Percent Agreement = _____

Formula:

$$\frac{\text{No. of positive results in } \textbf{agreement} \text{ (Pooled test result with individual result)}}{\text{Total number of results (20)}} \times 100$$

Notes:

Percentage Agreement (Disaggregated to Ct value range)

Samples Tested in a 5-sample Pool	Individual Samples with Ct within 3 values within PCR kit cutoff (weak positives)	
Pooled Test Result	**Positive**	**Negative**
Positive		
Negative		
	Individual Samples with Ct values >30 to 37	
Positive		
Negative		
	Individual Samples with Ct values >20 to 30	
Positive		
Negative		
	Individual Samples with Ct values <20	
Positive		
Negative		

Positive Percent Agreement = _____

Formula:

$$\frac{\text{No. of positive results in } \textbf{agreement} \text{ (Pooled test result with individual result)}}{\text{Total number of results (20)}} \times 100$$

Notes:

Prepared by:

(Name)
(Designation)
Signature over complete name

Approved by:

(Name)
(Designation)
Signature over complete name

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ANNEX C. GENERAL GUIDANCE: DIAGNOSTIC OR SCREENING TESTING USING A POOLING STRATEGY

1. Only Laboratories that are certified by RITM and Licensed by DOH can use a specimen pooling strategy to expand SARS-CoV-2 nucleic acid diagnostic or screening testing capacity when using a test authorized for such use by FDA.

2. If a pooled test result is negative, then all specimens can be presumed negative with the single test. If the test result is positive or indeterminate, then all the specimens in the pool need to be retested individually. The advantages of this two-stage specimen pooling strategy include preserving testing reagents and resources, reducing the amount of time required to test large numbers of specimens, and lowering the overall cost of testing.

3. A pooling strategy depends on the community prevalence of virus, and pool size will need to be adjusted accordingly. It is recommended that laboratories should determine prevalence based on a rolling average of the positivity rate of their own SARS-CoV-2 testing over the previous 7–10 days.

4. Laboratories should use a standardized methodology or calculator that factors in the sensitivity of the assay they are using and their costs of testing to determine when the positivity rate is low enough to justify the implementation of a pooling strategy.

5. Laboratories should also understand and, where appropriate, communicate the limitations associated with pooled testing.

6. COVID-19 assays and test systems used for diagnostic or screening testing, including those used for pooling, must have received approval from FDA.

7. A laboratory that wishes to use pooling with a SARS-CoV-2 nucleic acid test assay would be expected to evaluate and validate the performance of an assay for a pooling strategy.

8. If the laboratory modifies that authorized assay by incorporating alternative components, including extraction methods, polymerase chain reaction (PCR) instruments, and software versions, the laboratory should evaluate and validate the performance of the component changes, and recommendations for doing so.

9. Laboratories that conduct diagnostic or screening testing for COVID-19 must also comply with DOH regulations and RITM certification.

10. Every laboratory that performs or analyzes a test that is intended to detect SARS-CoV-2 or to diagnose a possible case of COVID-19" to report the results to PESU/RESU.

11. An RITM -certified and DOH licensed laboratory that participates in pooling must report diagnostic or screening negative test results to the participants in the pool. The test report given to the individuals in the pool should indicate that the testing procedure involved specimen pooling and explain the limitations of that type of testing.

12. The RITM-certified AND DOH-Licensed laboratory should not report positive or indeterminate results of a pooled test to either the participants in the pool, or the PESU/ RESU OR DOH. All participant specimens that were in a pooled test with a positive or indeterminate result should be retested separately, and the subsequent individual diagnostic or screening results must be reported to the PESU / RESU / DOH.

13. In a pooling procedure, the laboratory cannot ensure the diagnostic integrity of an individual specimen because it is combined with other specimens before testing. Specimen integrity can be affected by the quality

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of swab specimen collection, which could result in some swabs having limited amounts of viral genetic material for detection. Inadequate individual specimens, including those with limited amounts of viral genetic material, might not be eliminated from the pooled specimen before testing. Even if each individual specimen in a pool is adequate, the specimens in a pooled procedure are diluted, which could result in a low concentration of viral genetic material below the limit of detection of a given test. These limitations mean that monitoring the prevalence of disease and properly validating the assay and the instrumentation are important to limit the potential for false-negative results. In general, the larger the pool of specimens, the higher the likelihood of generating false-negative results. Hence, for this purpose, laboratories are only to use pools of 5 samples only until further instructions from the principal investigator.

14. All laboratories who will participate in this pooling strategy must have adequate personnel, a minimum of 7 staff per shift excluding the head of the molecular lab, a clinical pathologist. There must be 4 analysts, 1 molecular biologist, 1 encoder and 1 receptionist. Also necessary is a biosafety biosecurity trained utility person.

15. All laboratories who are participating must have adequate PPEs for use of all the staff.

16. All laboratories who are participating to sample pooling must have a good internet connectivity.

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ANNEX A. POOLED PCR TESTING WORK INSTRUCTIONS AND GENERAL GUIDELINES

INTRODUCTION

It has been proven that sample pooling can reduce sensitivity of RT-PCR assays for SARS-CoV-2 by several magnitudes i.e., Ct values may increase from 2.56 up to 4.87 higher. This may lead to false negatives and impact virus containment measures. Thus, it is important to make revisions to the interpretation of pooled samples to negate the impact of the loss of sensitivity.

The approach to specimen pooling shall be through pooling of standard volume aliquots of transport media with each containing a single patient sample.

Specimens obtained from different sources or different sample types should not be pooled together, i.e., only nasopharyngeal samples shall be pooled with nasopharyngeal samples, and so on.

The volume of the samples initially collected from an individual must be sufficient for both the pooled testing and individual follow up testing, if needed. This will prevent the need for a second sample collection.

1. SPECIMEN RECEPTION

1.1. The laboratory receptionist shall:

- 1.1.1. Receive the specimen in the receiving room/reception area and check the appropriateness of the transport conditions and the packaging of the specimens.
  - 1.1.1.1. Packaging specimens into groups of five (5) samples must be strictly adhered.
  - 1.1.1.2. If specimens are not received in groups of five (5) samples, these events must be documented and reported to collection teams and/or the source of specimens
- 1.1.2. Cross-checks the details of the patient on the line list/master list, laboratory request form, Case Investigation Form (CIF) and PhilHealth Form CF2 (whichever applies), making sure that the patient name and a second identifier matches the accompanying document.
- 1.1.3. Encode in an electronic line list which will then be transmitted to the staff in charge of specimen handling and inactivation.
- 1.1.4. Retain and file the original copies in the receiving room and store them appropriately based on existing protocols.

1.2. The medical technologist/analyst shall:

- 1.2.1. Receive the triple-packaged samples from the reception area/laboratory receptionist together with the master list/line list, thru a pass box and place it inside a biological safety cabinet.
- 1.2.2. Disinfect the outer container/box with 70% ethanol and wipe with tissue paper
- 1.2.3. Disinfect the inner/second container with 70% ethanol and wipe with tissue paper
- 1.2.4. Remove the samples from the transport box and Individually inspect the samples
- 1.2.5. Individually inspect the samples and asses the specimen integrity via a set criterion for acceptance and rejection, together with a second analyst/laboratory aide
- 1.2.6. Verify the completeness of data in the individual labels on the specimens based on the submitted line list/master list
- 1.2.7. Asses the specimen integrity via a set criterion for acceptance and rejection, together with a second analyst/laboratory aide, taking note of the following acceptance criteria:
  - 1.2.7.1. Swab/s are present in the collection tube
  - 1.2.7.2. Test requisition with patient name and a second identifier
  - 1.2.7.3. Tube label with patient name and a second identifier
  - 1.2.7.4. Collection tube has no leaks and that the cap is intact
  - 1.2.7.5. The specimen is within stability criteria
- 1.2.8. Records any rejected specimens and submits this document to the receptionist and laboratory manager. Rejected specimens shall then be excluded from the pool batch.

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2. SPECIMEN ACCESSIONING

2.1. Together with a second analyst in the specimen preparation room, the analyst shall:

2.1.1. Gather specimens into groups of five (5) unique individuals per pool by convenience sampling, or as they are received from the reception area.

2.1.2. Assign a set of unique pooling accession numbers (See attached template) alongside the unique individual accession numbers. An example would be:

Accession Number	Pool Accession Number
A0801PE001	A0801PE-P5A
A0801PE002	
A0801PE003	
A0801PE004	
A0801PE005	

"Accession number" represents the individual unique specimen accession number
"Pool Accession Number" (ex.: XXXXXXX **P 5 A)**

- The first alphanumeric characters represent the desired accession code
- **"P"** stands for pool
- **"5"** represents the number of unique individuals in the pool
- **"A"** represents the sequence in which A is for the first 5 samples pooled, B for the next 5 samples, and so on.

The medical technologist who performed the accessioning relays to another medical technologist in the reagent preparation room the total number of specimens for running, taking into account the number of controls, and that each pool of 5 samples are accounted for a single run, and thus the reagents needed should correspond to only one test. He/she then waits for the cue from the medical technologist in the specimen preparation room when to start the reagent preparation, making sure there are no delays and that only freshly prepared reagents are used.

3. POOLING PROCEDURE

3.1. The recommended dilution factor should be carefully applied considering the characteristics of the target population, and this protocol recommends pools of 5.

3.2. The medical technologist analyst shall:

3.2.1. Identify the samples together with another analyst (buddy)

3.2.2. Arrange the specimens into 5 samples per row and a cryovial labeled with the corresponding pool (i.e., P5A, P5B and so on)

3.2.3. Fill out a printed PCR map template with the accession number corresponding to each pool

3.2.4. With a calibrated pipette with filtered pipette tip, transfer 200uL from each of the 5 individual samples into a 2.0mL cryovial tube, making sure that the sample is properly mixed. When aliquoting and mixing with a pipette, collect the same amount from individual samples and mix in a new container. All pipette tips shall be used only once per sample or at each step. If the sample volume to be collected is 200uL or more, the final volume shall all always be 10% more than the sample for nucleic acid extraction to make a mixed sample.

3.2.5. Transfer an aliquot from the pool using the volume recommended for existing laboratory protocols for extraction.

3.2.6. Pass or communicate the PCR map to the PCR room.

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4. POOL EXTRACTION AND PCR

4.1. The medical technologist shall:

- 4.1.1. Perform sample inactivation according to existing laboratory protocols.
- 4.1.2. Follow the recommendations of the manufacturer of the nucleic acid extraction reagent, equipment, and PCR reagent for the mixed sample. If the equipment used has a high extraction efficiency, and a large sample volume is used, the possibility of nucleic acid detection in mixed samples is high.
- 4.1.3. Check the information of the amount of sample used before extraction and the amount and concentration of nucleic acid eluted after extraction.
- 4.1.4. Store the specimen samples according to existing protocols.
- 4.1.5. Perform PCR amplification according to existing laboratory protocols.
- 4.1.6. Set aside and store the pools that test positive, to be individually tested in the next immediate run.

5. QUALITY CONTROL IN DIAGNOSTIC PCR LABORATORIES

5.1. The following are the general guidelines for Quality Control in Diagnostic PCR laboratories for infectious diseases:

- 5.1.1. Maintain separate areas and dedicated equipment (eg. pipettes, microcentrifuges) and supplies (eg. microcentrifuge tubes, pipette tips, gowns and gloves) for assay reagent setup and handling of extracted nucleic acids.
- 5.1.2. Workflow must always be from the clean area to the dirty area.
- 5.1.3. Wear clean disposable gowns and new, previously unworn, powder-free gloves during assay reagent setup and handling of extracted nucleic acids. Change gloves whenever contamination is suspected.
- 5.1.4. Store primer/probes and enzyme master mix at appropriate temperatures (see package inserts). Do not use reagents beyond their expiry dates.
- 5.1.5. Keep reagent tubes and reactions capped as much as possible.
- 5.1.6. Clean and decontaminate surfaces.
- 5.1.7. Do not bring extracted nucleic acid or PCR products into the assay setup area.
- 5.1.8. Use aerosol barrier (filtered) pipette tips only.
- 5.1.9. Use PCR plate strip caps only. Do **not** use PCR plate sealing film.
- 5.1.10. Assay controls should be run concurrently with all test samples. If using a commercial kit, check if the following are already included in the kit:
  - 5.1.10.1. PTC – positive template control with an expected Ct value range
  - 5.1.10.2. NTC – negative template control added during rRT-PCR reaction set-up
  - 5.1.10.3. RP – all clinical samples should be tested for human RNAse P (RNP) gene to assess specimen quality
- 5.1.11. Keep running logs of PTC performance. After each rRT-PCR run of clinical samples, the control Ct values should be recorded.

6. RESULTS ANALYSIS

To ensure the absence of non-specific PCR inhibition of a sample, an internal positive amplification control or internal control is included in each specimen. A sample can be interpreted as negative only if the analysis of the internal positive control indicates that the amplification occurred in the reaction tube but no signal from the target reporter dye has been detected.

6.1. The pathologist shall:

- 6.1.1. Follow the usual validation of negative and positive control samples.
- 6.1.2. Interpret results according to the following:
  - 6.1.2.1. If the pool tests "negative", report individual samples of that pool as negative or "not detected".
  - 6.1.2.2. Interpret pools as "positive" if at least one gene target shows any form of amplification (late and low amplification, unusual or non-sigmoid curve).
- 6.1.3. Retest each constituent specimens individually from the pooled samples tagged as "positive" and refer to the individual accession templates described above.
- 6.1.4. Interpret individual and pooled runs according to kit manufacturer's specifications.
- 6.1.5. Report results of individually-ran samples according to existing laboratory protocols.

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REPORTING OF POOLED SARS-CoV-2 TESTING RESULTS		
Result	**Interpretation**	**Actions**
NOT DETECTED	**NEGATIVE**	**Report each individual in pool as negative**
DETECTED	**POSITIVE**	**Do not report pooled result. Perform diagnostic testing of individual specimens and report each as "positive" or "negative".**

7. DECONVOLUTION AND INDIVIDUAL EXTRACTION AND PCR

When pooled samples test positive, samples in these pools should be identified and tested individually.

7.1. The medical technologist shall:

7.2. Obtain another sample from the original specimen and re-test them individually according to the laboratory and manufacturer's protocol.

7.3. Release individual results and indicate in the report that individual sample testing was done.

8. RESULTS RELEASE

8.1. The staff of the COVID-19 testing laboratory shall release the test results as per the laboratory's existing protocols on releasing of RT-PCR results.

8.2. Due to the reduction in analytical sensitivity, a pooling strategy should apply risk mitigation procedures such as indicating in the test result/report that the testing procedure involved specimen pooling.