Republic of the Philippines
Department of Health
OFFICE OF THE SECRETARY

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DEPARTMENT CIRCULAR
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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: **Reiteration of Standards in the Use of COVID-19 Testing Kits for Research and Evaluation Purposes**

To ensure that research involving the use of COVID-19 testing kits is ethically done, we reiterate adherence to the 2017 National Ethical Guidelines for Health and Health Related Research which are available at https://ethics.healthresearch.ph/index.php/phoca-downloads.

Furthermore, to ensure the quality and maximize utility of performance evaluations conducted by other local institutions/laboratories, the following standardized protocols developed by the Research Institute for Tropical Medicine are strongly recommended for use:

1. Evaluation of Molecular Test Assays for the Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Co-V-2) *version 19 February 2021*
2. Evaluation of SARS-CoV-2 Serological Diagnostic Test Kits *version 6 February 2021*
3. Evaluation of Lateral Flow Test Kits to Detect SARS-CoV-2 Viral Antigen *version 9 March 2021*

These protocols are available at https://tinyurl.com/RITMprotocols for public viewing.

Dissemination of the information to all concerned is requested.

By Authority of the Secretary of Health:

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EVALUATION OF MOLECULAR TEST ASSAYS FOR THE DETECTION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-CoV-2)

version 19 February 2021

I. OBJECTIVES

To evaluate the following test performance characteristics of commercially available molecular test assays/system (*i.e., real-time PCR detection assay and cartridge-based PCR system*) for the detection of SARS-CoV-2:

1. Clinical Sensitivity
2. Clinical Specificity
3. Analytical Sensitivity
4. Analytical Specificity

Performance evaluation data generated may serve as a guide for laboratories engaged in the molecular diagnostics of SARS-CoV-2, in the selection of assays to be employed in routine testing.

Note: End-user laboratories are still advised to perform their own evaluation prior to adoption as a routine diagnostic test, as testing conditions (instruments, other reagents, consumables, practices, etc.) may vary from that of RITM's at the time of evaluation.

II. EVALUATION DESIGN

Analytical Sensitivity

This verifies the manufacturer-declared assay limit of detection by performing the assay under evaluation in triplicate, using a serially-diluted contrived sample of known SARS-CoV-2 viral RNA concentration. The contrived sample suspension corresponding to the lowest SARS-CoV-2 viral RNA concentration (lowest contrivance level), which yields 100% detection among replicates is then re-tested for 20 replicates to confirm the assay's lowest detectable analyte concentration.

Analytical Specificity

This verifies that the assay under evaluation does not cross-react with related analytes in the clinical respiratory sample, thereby generating false positives. This is confirmed, at the minimum through *in silico* analysis, but ideally through *in vitro* experiments using a panel of related respiratory viruses and bacteria.

Diagnostic Sensitivity and Specificity

This is a comparative evaluation between two detection systems (reference assay and the assay under evaluation) using banked residual samples of known reactivity to SARS-CoV-2 (based on Real-Time PCR).

III. SAMPLE PANEL

Diagnostic Sensitivity and Specificity

- **SARS-CoV-2 positive panel**
  Banked, residual respiratory specimens (nasopharyngeal and oropharyngeal swabs) collected from COVID-19 suspected patients and sent to RITM for testing by PCR.

- **SARS-CoV-2 negative panel**
  Known negative respiratory samples are those collected through various disease surveillances from 2019 or earlier when SARS-CoV-2 has not been introduced.

Analytical Sensitivity

Commercially-available SARS-CoV-2 control/reference panels (whole genome) of known concentration or spiked nuclease-free diluent with SARS-CoV-2 viral RNA of known concentration.

Analytical Specificity

The following samples may be used to verify/evaluate the analytical specificity of an assay:

- banked residual clinical respiratory specimens (nasopharyngeal and oropharyngeal swabs) collected from patients with respiratory illness that PCR-positive for respiratory bacterial, viral or fungal pathogens
- viral/bacterial culture isolates obtained from reputable culture collection or PCR-confirmed positive for respiratory virus/bacteria;
- samples from internationally-recognized respiratory External Quality Assurance panels.

The assay under evaluation should be tested for cross-reactivity for the following respiratory pathogens, whenever feasible:

a. Pathogens from the Same Genetic Family as SARS-CoV-2
   - Human Coronavirus OC43
   - Human Coronavirus 229E
   - Human Coronavirus NL63
   - Human Coronavirus HKU1

b. Other Circulating Viral Respiratory Pathogens
   - Influenza A/H3
   - Influenza A/H1N1 2009
   - Influenza B
   - Respiratory Syncytial Virus
   - Enterovirus
   - Adenovirus
   - Parainfluenza Virus
   - Herpes Simplex Virus 1

IV. SAMPLE SIZE

Reference Panel for evaluation of clinical/diagnostic performance of SARS-CoV-2 Molecular Test Assay

- A minimum of 30 positive clinical specimens as determined by a reference real-time PCR assay
- A minimum of 30 negative clinical specimens as determined by a reference real-time PCR assay
- The computed sample size (n=60) is based on the recommendation set by the US FDA EUA (https://www.fda.gov/media/135659/download).

V. TEST PERFORMANCE

Assay to be evaluated will be performed following manufacturer's instruction for use (IFU). Any deviations will be declared in the report.

VI. REFERENCE ASSAY/COMPARATOR

An acceptable reference assay/method, or what constitutes as the "best available method' for establishing the presence or absence of SARS-CoV-2, by which the assay under evaluation shall be evaluated against. This may be any of the following:

a. World Health Organization (WHO) prequalified Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR) for detection of SARS-CoV-2;
b. WHO-recommended rRT-PCR assay for detection of SARS-CoV-2, or a WHO emergency use listed (EUL) qRT-PCR assay for detection of SARS CoV 2;
c. a qRT-PCR assay previously verified to be of equivalent or better performance as a WHO prequalified or WHO-recommended or US FDA EUL qRT-PCR assay for detection of SARS-CoV-2.

VII. ANALYSIS

a. Clinical Sensitivity

The percentage of individuals with a given disorder whom the assay identifies as positive for that condition (*Minimum Information for Publication of Quantitative Real-Time PCR Experiments [MIQE] Guidelines, 2009*). The following formula will be used to determine the clinical sensitivity of an assay:

$$\text{Clinical Sensitivity} = \frac{\text{True Positives}}{\text{True Positives + False Negatives}} \times 100$$

b. Clinical Specificity

The percentage of individuals without a given condition whom the assay identifies as negative for that condition (*Minimum Information for Publication of Quantitative Real-Time PCR Experiments [MIQE] Guidelines, 2009*). The following formula will be used to determine the clinical sensitivity of an assay:

$$\text{Clinical Sensitivity} = \frac{\text{True Negatives}}{\text{False Positives + True Negatives}} \times 100$$

c. Analytical Sensitivity

LOD is the lowest concentration of the target analyte at which 19/20 (95% probability) replicates are detected in a biological sample. LOD is reported based on copy numbers per reaction or microliter.

d. Analytical Specificity

This refers to the assay/s ability to detect the appropriate target sequence rather than other non-specific targets that are likely to be present in a sample (*Minimum Information for Publication of Quantitative Real-Time PCR Experiments [MIQE] Guidelines, 2009*).

Percent agreement after testing the reference panel with the kit for evaluation vs the reference comparator assay will be calculated to determine the analytical specificity.

VIII. ETHICAL CONSIDERATIONS

All samples were collected from the DOH national surveillance activities which exempts informed consent collection.

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EVALUATION OF SARS-CoV-2 SEROLOGICAL DIAGNOSTIC TEST KITS

version 6 February 2021

I. OBJECTIVES

a. To evaluate the clinical or diagnostic sensitivity of serological diagnostic assays in detecting antibodies against SARS-CoV-2 virus using serum samples from SARS-CoV-2 positive patients.
b. To evaluate the clinical or diagnostic specificity of serological diagnostic assays in detecting antibodies against SARS-CoV-2 virus using serum samples from known negative patients.

II. EVALUATION DESIGN

This is a retrospective evaluation using banked samples from PCR positive samples and previously known negative samples.

III. SAMPLE PANEL

Serum samples from SARS-CoV-2 positive symptomatic patients as detected through respiratory swab and tested using PCR were collected through partner hospitals.
Known negative serum samples are those collected through various disease surveillances from 2019 or earlier when SARS-CoV-2 has not been introduced.

IV. SAMPLE SIZE

A **minimum of 75 up to 115 samples** each from SARS-CoV-2 positive and negative will be used. The confidence level of 95% and precision of 5% was used to compute for this sample size.

The SARS-CoV-2 positive samples are further stratified into the following:

- 8-15 days post-onset of symptoms: N=25
- 15-21 days post-onset of symptoms: N=25
- >28 days post-onset of symptoms: N=25

V. TEST PERFORMANCE

Assay to be evaluated will be performed following manufacturer's instruction for use (IFU). Any deviations will be declared in the report.

VI. REFERENCE TEST

Results are compared using the SCoV-2 Detect™ IgM ELISA and SCoV-2 Detect™ IgG ELISA (InBios International Inc., USA), a microplate based, qualitative, indirect ELISA for the detection of IgM and IgG antibodies targeting epitopes derived from SARS-CoV-2.

The SCoV-2 Detect™ IgM ELISA and SCoV-2 Detect™ IgG ELISA have been granted Emergency Use Authorization (EUA) by the United States Food and Drug Administration (US FDA).

VII. ANALYSIS

a. Clinical Sensitivity

The percentage of individuals with a given disorder whom the assay identifies as positive for that condition (*Minimum Information for Publication of Quantitative Real-Time PCR Experiments [MIQE] Guidelines, 2009*). The following formula will be used to determine the clinical sensitivity of an assay:

$$\text{Clinical Sensitivity} = \frac{\text{True Positives}}{\text{True Positives + False Negatives}} \times 100$$

b. Clinical Specificity

The percentage of individuals without a given condition whom the assay identifies as negative for that condition (*Minimum Information for Publication of Quantitative Real-Time PCR Experiments [MIQE] Guidelines, 2009*). The following formula will be used to determine the clinical sensitivity of an assay:

$$\text{Clinical Sensitivity} = \frac{\text{True Negatives}}{\text{False Positives + True Negatives}} \times 100$$

VIII. ETHICAL CONSIDERATIONS

The SARS-CoV-2 PCR positive patients provided consent to the collection of their serum samples and for the use for evaluation studies. The known negative samples collected from various surveillances from 2019 to later did not provide the informed consent as the samples were collected from the DOH national surveillance activities in which it is exempted.

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EVALUATION OF LATERAL FLOW TEST KITS TO DETECT SARS-CoV-2 VIRAL ANTIGEN

version 9 March 2021

I. OBJECTIVES

a. To evaluate the clinical or diagnostic sensitivity of lateral flow assays in detecting SARS-CoV-2 viral antigen from respiratory swab samples from symptomatic patients suspected of COVID-19.
b. To evaluate the clinical or diagnostic specificity of lateral flow assays in detecting SARS-CoV-2 viral antigen from respiratory swab samples from asymptomatic patients or those with no known exposure to a COVID-19 positive case.

II. EVALUATION DESIGN

This is a prospective evaluation using freshly collected respiratory swab samples from symptomatic patients suspected of COVID-19 and from asymptomatic patients or those with no known exposure to a COVID-19 positive case.

III. SAMPLE PANEL

Respiratory swab samples from symptomatic patients suspected of COVID-19 following inclusion and exclusion criteria (A and B) below were collected, while for asymptomatic patients or those with no known exposure to a COVID-19 positive case follows inclusion and exclusion criteria C and D.

Symptomatic Patients

a. Inclusion Criteria
   - Patients are suspected of Covid-19, and are symptomatic
   - Age ≥ 18 years
   - Onset of symptoms to collection is ≤ 5 days
   - Willing to sign the Informed Consent document
   - Medical condition allows multiple swabbing

b. Exclusion Criteria
   - Onset of symptoms to collection is 6 days or more
   - Obstruction of 1 or more nares
   - Any condition that precludes participation because it could adversely affect subject safety

Healthy Asymptomatic Patients

a. Inclusion Criteria
   - Healthy and without any symptoms of COVID-19
   - Age ≥ 18 years
   - Willing to sign the Informed Consent document
   - Medical condition allows multiple swabbing

b. Exclusion Criteria
   - With respiratory symptoms
   - Obstruction of 1 or more nares
   - Any condition that precludes participation because it could adversely affect subject safety

IV. SAMPLE SIZE

A minimum of 30 SARS-CoV-2 PCR positive and PCR negative will be used. The confidence level of 95% and precision of 5% was used to compute for this sample size.

V. TEST PERFORMANCE

Assay to be evaluated will be performed following manufacturer's instruction for use (IFU). Any deviations will be declared in the report.

VI. REFERENCE TEST

An acceptable reference assay/method, or what constitutes as the "best available method' for establishing the presence or absence of SARS-CoV-2, by which the assay under evaluation shall be evaluated against. This may be any of the following:

a. World Health Organization (WHO) prequalified Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR) for detection of SARS-CoV-2;
b. WHO-recommended rRT-PCR assay for detection of SARS-CoV-2, or a WHO emergency use listed (EUL) qRT-PCR assay for detection of SARS CoV 2;
c. a qRT-PCR assay previously verified to be of equivalent or better performance as a WHO prequalified or WHO-recommended or US FDA EUL qRT-PCR assay for detection of SARS-CoV-2.

VII. ANALYSIS

a. Clinical Sensitivity

The percentage of individuals with a given disorder whom the assay identifies as positive for that condition (*Minimum Information for Publication of Quantitative Real-Time PCR Experiments [MIQE] Guidelines, 2009*). The following formula will be used to determine the clinical sensitivity of an assay:

$$\text{Clinical Sensitivity} = \frac{\text{True Positives}}{\text{True Positives + False Negatives}} \times 100$$

b. Clinical Specificity

The percentage of individuals without a given condition whom the assay identifies as negative for that condition (*Minimum Information for Publication of Quantitative Real-Time PCR Experiments [MIQE] Guidelines, 2009*). The following formula will be used to determine the clinical sensitivity of an assay:

$$\text{Clinical Sensitivity} = \frac{\text{True Negatives}}{\text{False Positives + True Negatives}} \times 100$$

VIII. ETHICAL CONSIDERATIONS

The SARS-CoV-2 PCR positive patients provided consent to the collection of their serum samples and for the use for evaluation studies. The known negative samples collected from various surveillances from 2019 to later did not provide the informed consent as the samples were collected from the DOH national surveillance activities in which it is exempted.