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PAASE BULLETIN # 16

ON PAASE STRATEGIC ACTION GROUP 3: MASS TESTING & FAST-TRACKING

Addressed to: DOH, RITM, IATF, hospitals, clinics, private sector

DETECTION OF IgG/IgM ANTIBODIES TO SARS-COV-2 BY LATERAL FLOW RAPID TEST KIT: AN ANALYTICAL METHOD VALIDATION PROTOCOL

(A joint project of PAASE and Pascual Pharma Corporation)

1. Purpose

The purpose of this validation protocol is to provide documented evidence that the analytical test method, "*Detection of SARS-CoV-2 IgG/IgM Antibodies by Lateral Flow Rapid Test Kit*" is suitable for its intended use.

2. Scope of the Method

The point-of-care rapid test kit is used for the detection of immunoglobulin G (IgG) and/or immunoglobulin M (IgM) antibodies to SARS-CoV-2 in venous or capillary blood, to assist screening of COVID-19 patients.

3. Background

A new strain of Severe Acute Respiratory Syndrome Coronavirus was identified in the latter part of 2019 and rapidly developed into a global pandemic within three months from its initial detection. The virus identified as COVID-19 has infected populations throughout the world merely by transmission of respiratory droplets thru human contact routes. More than half of the infected population showed mild or no obvious flu-like symptoms of the disease such as fever, cough, shortness of breath, breathing difficulties, and most serious case of pneumonia. These asymptomatic cases become a serious threat to which the infected individual can only be identified by using a suitable clinical diagnostic test apparatus to confirm the infection [1].

One of the most important clinical diagnostic tests to confirm COVID-19 infection is by the detection of SARS-CoV-2 nucleic acid by reverse transcriptase polymerase chain reaction (RT-PCR). This method can identify the target nucleic acid sequence of the new virus strain, to avoid the overlapping manifestation of other viral strain and symptoms, specially the seasonal flu. The RT-PCR is known for its accuracy and specificity but requires specialized laboratory space and equipment and has long turnaround time especially in resource-constrained environments. Early detection of the virus is vital to contain the spread of the infection. The basis of the rapid test kits is the qualitative detection of antibodies IgG and/or IgM that are specifically generated by the body in response to SARS-CoV-2 infection [2].

4. General biosafety guidelines and precautions

Take all necessary biosafety precautions, including wearing appropriate personal protective equipment, while handling specimens. Decontaminate all used materials and

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clean-up spills with appropriate disinfectants. Dispose of all dry waste consumables, including test kits, in a biohazard container.

5. General guidelines for test kit use

The test kits should be stored in correct storage conditions specified in the respective instruction manual. Ensure that the lot/batch number and the expiry date of the corresponding test kits used are properly recorded. Check the packaging integrity and completeness of the test kit components prior to the start of the analysis.

6. Validation Procedure

6.1. Statistical Method

The evaluation of specificity and sensitivity are based on three parameters used by Bendavid et.al., 2020 [5] that can be used to evaluate different scenarios in future prevalence studies for Covid19 [8]. The parameters for evaluating test kit sensitivity & selectivity are as follows:

a. using the manufacturer's data;
b. using a local evaluation study of the test kit with pre-pandemic samples and RTPCR positive samples with ELISA-confirmed IgM and/or IgG

The sample sizes needed for evaluation of specificity, sensitivity, and replicability was calculated using the declared sensitivity and specificity values from the manufacturer [6]. The proposed sample size of 90 was based on the largest calculated value from the different test kits at a p-value of 0.1 (95% CI) as shown below:

Desired p value	Wondfo	Innovita	Livzon	Vazyme	Hecim
0.01	9011	8518	6791	5950	6109
0.05	360	341	272	238	244
0.1	90	85	68	59	61

The assumed prevalence in this case is 0.5 since the sample number of positives and negatives can be controlled. A different prevalence in the replicability studies may be observed and the calculated sample size will change. The sample size equation can be found in Appendix A. Non-parametric statistics (sign-rank test, McNemar's (can be used if the sample sizes are small or when the normality assumption is violated.

The sample collection and the validation study will be conducted in two sites, the Philippine General Hospital and the TBD hospital.

6.2. Specimen Collection

Capillary blood samples or sera from 45 PCR-confirmed COVID-19 patients or convalescents, whom will give written or verbal consent, will be tested for IgG and IgM antibodies via ELISA and antibody rapid test kits. Anonymous blood donor sera collected from healthy adults (n=45) before the COVID-19 outbreak in 2019 will be used as negative samples.

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2 mL (two drops ~100ul x 5 test kits; 1ml for ELISA, 0.5ml reserve) of blood or sera samples per patient (for both Covid19+ confirmed and pre-pandemic patients) will be collected by the collecting technician in sterile containers. The collecting technician will record the following anonymized patient information (Days after onset of symptoms for Covid19+, Gender, and Age). The collecting technician will be trained to record the information and randomize the samples with anonymized labels (A, B, C, D….) for data traceability.

6.3. Reference Test

The current reference/standard test for laboratory diagnosis of COVID-19 is through the detection of SARS-CoV-2 nucleic acid by real-time polymerase chain reaction (RT-PCR). However, since PCR and antibody rapid tests differ in analytes being detected, PCR tests shall be coupled with SARS-CoV-2 antigen enzyme-linked immunoabsorbent assays (ELISA). The RTPCR test will be performed at a laboratory using equipment and reagents accredited by DOH. The presence of IgG and/or IgM in RT-PCR positive samples will be confirmed by an ELISA assay. The ELISA assay will be verified using existing best practices in literature [3], [4].

6.4. Test Method

The experimental technicians will obtain the randomized samples and apply two drops of blood or sera on each antibody lateral flow kits and record information on supplied data sheets. The experimental technician will be trained on operation of the test kits, interpretation of the results, and recording of the data.

A summary table of the Antibody test kits to be evaluated can be found in Appendix B.

The appropriate ELISA assay will be executed by LAB X.

6.5. Performance Characteristics

The null hypothesis in this case is that there is no difference between the observed variation and the manufacturer's declared specifications.

6.5.1. Specificity

Specificity is the ability of the test method to distinguish the target analytes (i.e., antibodies) from non-target analytes, including matrix components. In qualitative tests, this pertains to the proportion of negative test results that are correctly identified as being negative.

Specificity of the rapid test is calculated as [5]:

$$specificity\ (\%) = \frac{ART\ true\ negatives}{total\ preCOVID\ negatives} \times 100$$

6.5.2. Sensitivity

Sensitivity is the proportion of actual positives that are correctly identified as positive results. Sensitivity is calculated as [5]:

$$sensitivity\ (\%) = \frac{ART\&ELISA\ positives}{PCR\&ELISA\ positives} \times 100$$

6.5.3. Replicability and Reproducibility

The variation at 95% CI for the Specificity and Sensitivity data for each kit and for each site will be analyzed. The null hypothesis for replicability and reproducibility is there is no variation across testing centers for each brand of antibody test kit.

6.6. Validation Report

An analytical method validation report shall be prepared after completion of the study. Any deviations to this protocol shall be pre-approved prior to execution. Information and data included in the report must comply with the Standards for Reporting Diagnostic Accuracy Studies (STARD) 2015 Guidelines [7].

7. Literature Cited

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10. Appendices

APPENDIX A

Sample Size Determination for a Diagnostic Test

The sample size (*n*) needed to assess the diagnostic accuracy of an instrument can be determined as follows:

Based on sensitivity

S_N = declared sensitivity (from previous studies)

S_p = declared specificty (from previous studies)

$1-\alpha$ = confidence level

$z_{1-\alpha/2}$ = standard normal abscissa at the 1-α confidence level

L = desired level of absolute precision

$$n = \frac{z^2_{1-\alpha/2} S_N \times (1 - S_N)}{L^2 \times \text{ Prevalence}}$$

Based on specificity:

$$n = \frac{z^2_{1-\alpha/2} \times S_p \times (1 - S_p)}{L^2 \times (\text{1-Prevalence})}$$

Reference:

Zaidi M., H. Waseem, M. Ansari and M. Irfan. 2016. Sample Size Estimation of Diagnostic Test Studies in Health Sciences. Proceedings of the 14th International Conference on Statistical Sciences.

APPENDIX B

Criteria	Wondfo Biotech	Innovita	Livzon	Vazyme	Hecin
Technology	Colloidal gold – lateral flow (capture Abs coated on membrane; Ag-gold conjugate); separate IgM and IgG strips	Colloidal gold – lateral flow (capture Abs coated on membrane; Ag-gold conjugate) separate IgM and IgG strips	Colloidal gold – lateral flow (COVID Ags coated on membrane; Ab-gold conjugate) separate IgM and IgG strips	Colloidal gold – lateral flow (capture Abs coated on membrane; Ag-gold conjugate) IgM and IgG in 1 strip	Colloidal gold – lateral flow (capture Abs coated on membrane; Ag-gold conjugate) IgM only in 1 strip
Clinical validation	596 clinical samples – number of institutions not disclosed	447 clinical samples from 5 institutions	644 clinical samples – number of institutions not disclosed	570 clinical samples –from 5 institutions	551 clinical samples – number of institutions not disclosed
Specificity (true negative)	99.57% (95%CI: 97.63-99.92%)	100% (95%CI: 94.20-100%)	99.2% (95%CI: 97.6-99.7%)	97.02% (95%CI:94.74-98.33%)	98.34% (95%CI:95.81-99.55%)
Sensitivity (true positive)	86.43% (95%CI: 82.51-89.58%)	87.3% (95%CI: 80.4-92.0%)	90.2% (95%CI: 86.2-93.1%)	91.54% (95%CI: 86.87-94.65%)	91.29% (95%CI:87.58-94.18%)
Cross-reactivity	No cross-reactivity with major respiratory pathogens but minor cross-reactivity with 1 out of 3 varicella zoster samples	No cross-reactivity tested against 23 pathogens (respiratory and non-respiratory) including varicella zoster	No cross-reactivity against 16 pathogens (respiratory and non-respiratory) including varicella zoster and 4 strains of endemic human coronavirus	No cross-reactivity against 34 pathogens (respiratory and non-respiratory) including varicella zoster and 3 strains of endemic human coronavirus	N/A
Coincidence with RT-PCR (gold standard)	94.93%	95%	95.3%	95.09%	94.37%
Duration of Test	3-15 minutes	3-15 minutes	1-15 minutes	Within 10 minutes	3-15 minutes
Other comments	Commercialised in Australia by Cellmid Ltd; Listed with US FDA as not requiring Emergency Use Authorization	Currently being registered at the US FDA by Scanwell Health for home use; Listed with US FDA as not requiring Emergency Use Authorization	Designated as preferred manufacturer by the World Health Organization (WHO); Listed with US FDA as not requiring Emergency Use Authorization	IgM and IgG tested in only 1 strip unlike others which require separate strips; submission to US FDA	Only tests IgM