Rebuttal Letter

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Recipient: Doctora Ximena Abarca, Secretaria de Salud del Municipio de Quito
Doctor Jorge Yunda Machado, Alcalde de la Ilustre Ciudad de Quito
Subject: Rebuttal Letter for Ecuador testing institute (Universidad de las Américas: UDLA) test result for Isopollo® COVID-19 detection kit (real-time)

To whom it may concern,

In a document sent, it has been mentioned that the UNIVERSIDAD DE LAS AMERICAS (UDLA) carried out the analysis following the protocol indicated in the insert, which after our analysis we concluded that this was not the case and that we can see it below:

1. Please indicate what technical capacity and legal authorizations the UDLA has to issue these types of reports, as we do not have information on the reason why this laboratory was chosen. In the contract and in the law, the official and competent entity to carry out this type of Post-registration analysis is the ARCSA.
2. As the test method of UDLA University was not conducted according to the Instructions for use provided by M monitor Inc., the test results cannot be trusted.
    1) Reaction Volume different from the IFU: Reaction volume proposed by M monitor Inc. is using 25ul, but UDLA University used 15ul
    2) It is important that the equipment is calibrated according to the characteristics of our product, in order to obtain the correct results. In the report sent by the UDLA, some important details are not presented, such as the equipment calibration certificate.
    3) Overall CT value is high and this is gray area which is difficult to judge negative and positive results. Korean diagnostic specialists recommend retesting and put the correct CT value.
    4) The test result cannot be accepted because the protocol is not detailed enough, please detail all the process complete. Because if it was change one of the initial instruction (point 1,1 and 1,2)
    5) Therefore, the test was not conducted according to the instructions for use provided by the M monitor. For this reason, it is considered that wrong results have been obtained and cannot be accepted.
3. The exact test method is specified in the Instructions for use (IFU). Please read the IFU carefully prior to test. For your reference, the test method is described as below.

【Contents of the kit and Quantity】

Reagents	Vol.	Quantity	Retention period
2X Reaction Buffer	1250 ㎕	2	12 months
Enzyme mix	200 ㎕	1	
Detection primer (CR)	200 ㎕	1	
Detection primer (CN)	200 ㎕	1	
Control primer*	40 ㎕	1	
Control template*	40 ㎕	1	
Distilled water (DW)	1.5 ㎖	1	

* Control primer & Control template contained for 20 reactions

[Protocol]

1. Sample preparation and nucleic acid extraction

① Smeared specimen collected from human pharynx suspected of having SARS-CoV-2 using a swab is used. If a swab is used, it is recommended to use samples collected by rubbing mucous membrane from nasal cavity through to the laryngopharynx.

② The collected samples should be used immediately or stored at -20℃.

③ Nucleic acid should be isolated using useful viral RNA extraction kit according to the manufacturer's instructions.

2. Reagents preparation

① Take out the reagents stored at -20℃, and thaw them at room temperature. Once the reagents are thawed, keep them on ice.

② Prepare 25㎕ LAMP reaction mixture as follows:

- Check for RdRP gene

Reagents	Volume (1 reaction)
2X Reaction Buffer	12.5 ㎕
Enzyme mix	1.0 ㎕
Detection primer (CR)	2.0 ㎕
Extracted RNA (Template)	5.0 ~ 9.5 ㎕
Distilled water*	- ㎕
Total	25.0 ㎕

* If the number of samples to be examined is so many, it is recommended that the mixture be calculated according to the number of responses and used in a 1.5 ml tube.
* In case of distilled water, adjust and add according to the template volume.
* For control reactions, use 2 µL of Control template, 2 µL of Control primer, and 7.5 µL of DW as positive control and use 9.5 µL of DW instead of RNA as negative control.

- Check for N gene

Reagents	Volume (1 reaction)
2X Reaction Buffer	12.5 ㎕

Enzyme mix	1.0 ㎕
Detection primer (CN)	2.0 ㎕
Extracted RNA (Template)	5.0 ~ 9.5 ㎕
Distilled water*	- ㎕
Total	25.0 ㎕

* If the number of samples to be examined is so many, it is recommended that the mixture be calculated according to the number of responses and used in a 1.5 ml tube.
* In case of distilled water, adjust and add according to the template volume.
* For control reactions, use 2 μL of Control template, 2 μL of Control primer, and 7.5 μL of DW as positive control and use 9.5 μL of DW instead of RNA as negative control.

3. Operation procedure

Select wavelength of FAM or SYBR Green 1 in real-time PCR machine (CFX96™ Dx System, Bio-Rad, CA, USA) or equivalent (Applied Biosystems™ 7500 Real-time PCR Instrument system, Applied Biosystems™ 7500 Fast Real-time PCR Instrument system, Thermo Fisher Scientific, MA, USA). And perform the reaction as follows:

Step	Temperature	Time	Cycles
1	58°C	30 sec	40
2	80°C	2 min	1

4. Detection
- Positive criterion Ct value : within 40 Ct
- Negative criterion Ct value : No detection
- Interpretation of results

CR	CN	NC	Positive /Negative	Interpretation
+	-	-	Positive	RdRP gene detected
-	+	-	Positive	N gene detected
+	+	-	Positive	RdRP gene and N gene detected
-	-	-	Negative	SARS-CoV-2 not detected
( )	( )	+	Invalid	Invalid and recommend re-examination

4. If the result is not obtained even though they follow the test method mentioned in section 2, reliability could not be trustful as the storage conditions of Ecuador are unknown, as the products were delivered two months ago in compliance.

5. Today, we started a new test analysis at the KTC laboratory in Korea (http://www.ktc.re.kr) with a new batch. A sample of this same batch will be sent to the Secretaria to carry out the tests that are required at the

UDLA and in the laboratories that you consider.

1. Test institution: 3 July 2020, KTC (Korean Testing Certification)
2. Test title: Isopollo® COVID-19 detection kit (real-time)
3. Expected test completion date: 14 July 2020

6. The LAMP technology is an advanced technology in the molecular diagnosis which can be performed in short time(20minutes) for diagnosis compare to PCR (2hours) with same performance. The COVID19 kit was developed in The Armed Forced Medical Science Institute of South Korea. In this reason we emphasize that the results with low viral load and in early mode will be detected by the two technologies.
7. It is important to mention that all the analyses that are going to be done count on our support in order not to have these complications in the future. We will do our best to provide our technical information and services.

The results of testing by incorrect test methods, use of equipment, storage conditions, etc. are completely disparaging our products. We, M monitor seriously doubt that there is any other purpose (intention) for this report. A strong legal response to the loss of the manufacturer's tangible and intangible economic losses and reliability of Isopollo® COVID-19 detection kit (real-time) due to the publication of these false results will be reviewed and that they have not respected confidentiality.

Sincerely yours,

Hyo Sung Jeon / CEO
M monitor Inc.