COVID-19 Testing Pipeline: Lesson Learned

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Abstract. The transmission of severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) in Indonesia is seen to be uncontrollably increasing that urges the government to leverage the capacity for the disease detections. Real-time polymerase chain reaction (RT-PCR), rapid test and computed tomography (CT) scan are the most common methods to determine if one has been infected regardless of whether or not the common symptoms of such Corona Virus Disease 2019 (COVID-19) surface. Among these three, RT-PCR is considered the gold standard for qualitative and quantitative assessment of SARS CoV-2 detection. The present paper aims at elaborating the framework of Roche’s RT-PCR machine employed specifically for SARS CoV-2 detection performed by Genetics Indonesia which is deemed to be efficient and relatively quicker than other detection kits. RT-PCR machine detected SARS Cov-2 with RNA amplification curve equals to 10 copies RNA below the cut off value of Crossing point (Cp) positive control. Also elucidated in the paper is the implementations of EAV RNA and LightCycler® 96 RT-PCR System through which analysis time, amounts of individual required sample, as well as the reagents, can be accordingly reduced.

Keywords: COVID-19, Detection, RT PCR, Lesson Learned, SARS CoV-2

1. Introduction

The global transmission rate of severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) continues to increase dramatically with the confirmed cases as many as 17,111,556 people by the end of July 2020 [1]. This global pandemic has attracted researchers from many domains to understand this issue better, not only from the health sector [2–6]. The massive number inevitably demands an extra capacity for detection by which those infected people and at high risk of infection can be mapped and be provided with appropriate interventions to avoid a lot further infection scope. Early detection of SARS CoV-2, the cause of Corona Virus Disease 2019 (COVID-19), is pivotal to control and manage the transmission of the virus, and disease burden. A few of the findings on asymptomatic people with...
COVID-19, and this gets a lot more number by days, become a deniable challenge upon which improvement on detection capacity is the most feasible option [7].

Several common detection methods for SARS CoV-2 in Indonesia include RT-PCR, Rapid test, and CT-Scan. Rapid tests are, thus far, the key as the frontline detection means to allow us to trace the positive cases and as a base for quarantine intervention although validation is required and is provided through Polymerase Chain Reaction (PCR). Rapid tests with immunoassay detection methods are based on IgM and IgG antigen-antibody interactions in the patient's blood sample. Typically, the method requires 3-4 days to reveal the reaction post-infection. RT-PCR machine detected SARS-CoV 2 virus based on nucleic acid from swab tests or blood samples. This method is recommended by the Chinese Center for Disease Control and Prevention (China CDC) to detect positive COVID-19 on laboratory test confirmation [8]. RT-PCR test kits for clinical diagnosis of COVID-19 use specific primers and probes the ORF1ab and N genome sequences from SARS-CoV 2 [9]. Currently, the Reverse Transcription-Loop-mediated Isothermal Amplification (RT-LAMP) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) method applicated in several developed countries to detect RNA SARS-CoV-2 virus [10–12]. CRISPR applications for COVID-19 detection have been developed such as DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) and Specific High Sensitivity Enzymatic Reporter UnLOCKING (SHERLOCK). In developing countries, the SARS CoV-2 detection method utilizes sophisticated technological developments, and data management is still slightly. So that RT-PCR becomes the main detection method in detecting this virus.

Among the South Eastern Asian countries, Indonesia demonstrates the highest number of SARS CoV-2 infection cases by reporting 106,336 people have been confirmed to be COVID-19 positive [13]. When plotted as a ratio to its entire population, SARS CoV-2 detection coverage in Indonesia is considerably scrimpy with only 0.39 per 1000 population and this is seen to rather centralized in metropolitan cities. Thus far, SARS CoV-2 detection in the country is mainly performed via immuno-serology-based rapid test and Real-Time Polymerase Chain Reaction (RT-PCR). The first mentioned is recently thought to potentially mislead interpretation caused by incorrect reactions. These could be either overreaction or non-reaction which is reported mostly from those who on the day of the assessment show the COVID-19-like symptoms. And it is also reported that COVID-19 patients show an antibody response in the second week after the appearance of symptoms. False-positive results on the rapid test due to cross-reaction with other types of coronavirus make RT PCR mandatory afterward [14,15]. As such, the paradigm is now shifted into RT-PCR which yields more thorough and well-representative about the actual SARS CoV-2 exposure of the examined people. Indonesia's Association of Specialist Doctors for Clinical Pathology and Medical Lab (PDS PatKLIm) has released a protocol regulating the utilization of rapid test only for COVID-19 screening. The protocol has made it clear that whenever reactivity is detected in rapid tests, validation by RT-PCR is a must-do [16].

This paper presents the technology behind the SARS CoV-2 detection using RT-PCR in Genetics Indonesia Laboratory, a research-based biotechnology company in Indonesia that focuses to provide health services on genomic fields and cancer research [17–21]. Several lessons learned from this testing method is also elaborated based on the real samples tested in this lab [22–25].

2. Methodology
RT PCR Covid-19 test pipeline in Genetics Indonesia:

1. Specimen Sample Collection
SARS CoV-2 specimens are derived from the nasopharynx or oropharynx swab test from which the viral RNA is isolated. The swab was conducted by health care professionals. Collected samples were transported in a viral transport medium with a temperature around 4-8 °C. If more
within 48 hours the samples not yet to continue to the extraction process or for long-term preservation those samples were stored at -80 °C[26,27].

2. RNA Extraction

SARS CoV-2 RNA extraction used the High Pure Viral RNA kit (Cat. Number 11858882001, Roche) and followed its protocol. RNA in the sample was trapped at a matrix in the spin column. Inhibitory removal buffer and wash buffer were removed interference substances containing in the samples. The RNA sample was eluted by elution buffer and stored at -80 °C for the long term[26,28].

3. Preparation and Set up RT PCR Machine

SARS Cov-2 RNA detection was performed in one-step RT-PCR by utilized two sequences specific region in the E gene and RdRp gene provided by Roche. One sample was run as a pair in a separated single well for each target gene in the 96-well plate. RT-PCR mix for one sample reaction for E-gene target in a single well consisted of PCR grade water (9.9 µl), EAV primer-probe (0.5 µl), multiplex RNA virus master (4 µl), E-gene primer-probe (0.5 µl), and RT-enzyme 200x (0.1 µl). The same amount and material used for the E-gene target are also used for the RdRp-gene target in a single well. Extracted RNA samples (5 µl) were added into each well (E-gene well & RdRp-gene well). Thus, the total volume reaction for each target gene for each sample in a single well was 20µl. For each RT-PCR running, positive and negative control were included.

RT-PCR was run in LightCycler-96 (Roche). For SARS CoV-2 RNA detection four-step program was applied in the machine. Reverse transcription step at 55 °C for 5 min 1 cycle. Denaturation step on 95 °C for 5 min 1 cycle. Cycling step for 45 cycles consisted of denaturation on 95 °C for 5 sec, annealing on 60 °C for 15 sec, elongation on 72 °C for 15 sec. Cooling step on 40 °C for 30 sec. FAM channel was used to detect E-gene and RdRp-gene target, then the Cy5 channel was used to detect EAV-gene as extraction control.

3. Results and Discussion

RT-PCR, the well-claimed golden standard for the COVID-19 test, requires swab test-derived samples to isolate the SARS CoV-2's RNA. To generate a solid result on which the status of assessed people can be pulled correctly, as this determines the fate of intervention given to the affected people, some sophisticated and pricey equipment is critical to fulfilling. Although it does not make the test always solid 100%, it reduces the chance of having false positive or false negative that to some extent contributes to the poorly managed transmission of the disease. One of the minimum requirements is the availability of well-sealed and kept-sterile microtube or cryotube SARS-CoV-2-containing samples from contaminating and involving in direct contact. Liquid samples such as sputum must always be put in an anti-leakage container. PPE (Personal Protective Equipments) is also a must worn. As to samples, to prevent viral RNA from damaging, they must be stored in ≤ a -70 °C freezer [26,29].

In Genetics Indonesia use the High Pure Viral RNA kit for SARS CoV-2 RNA extraction. The lysis process for viral components in SARS CoV-2 is performed by adding binding buffer contained Triton X-100 (ionic surfactant) to the lysis membrane and nucleic acid virus. On other extraction kit products, proteinase K on QIAmp viral RNA mini (Qiagen) Kit has the same role as Triton X-100. Lysis buffer in binding buffer protects the RNA structure during homogenization and provides buffer conditions avoiding inhibitors and Triton X-100 lyses the SARS CoV-2 cell membrane to secrete viral RNA. The wash buffer contains different salt concentrations is used to remove residue, especially to remove interference substances containing in the samples. Ethanol evaporates rapidly and prepares the membrane for elution and buffer residue removed by centrifuge. Elution buffer was added to elute the purified RNA from the spin column matrix into the solution [30–32].
Usage of primers and probes in RT PCR analysis must refer to viral structural sequences and have more conserved regions than other parts of a sequence. Structural genes such as the E gene which plays a role in composing the viral envelope, the N gene on forming nucleocapsid, the ORF, and RdRp genes on viral RNA replication must be a reference for detecting SARS CoV-2[9,29]. National Institute of Health Research and Development, Indonesian Ministry of Health for detection COVID-19 utilizes N1, N2, and Ribonuclease P (RnP) as the target gene for SARS Cov-2 detection [26]. We used to target gene E for detecting envelope and the RdRp gene for detecting the Orf1ab sequence of SARS CoV-2. RdRp and E gene play important role in viral replication and structure assembly. Equine Arteritis Virus (EAV) RNA as providing control external target for comparison to shown false-negative result caused by unsuccessful extraction of SARS CoV-2 based in vitro transcribed RNA [33]. TaqMan hydrolysis probe labeled with dye FAM (470/514 nm) used to detect the E-gene and RdRp gene probe shown the presence of SARS CoV-2 RNA in the sample, and dye Cy5 (577/620 nm) used to detect EAV.

![Figure 1. Roche LightCycler® 96 Real-Time PCR Instrument [34]](image)

The one-step Multiplex PCR machine type is the fastest used to detect SARS CoV-2. The Reverse Transcriptase (RT) process is immediately executed in one process in one-step PCR, so that RT enzyme on Reaction mix Roche is added immediately for synthesis cDNA, in other products must use the SuperScript III RT / Platinum Tag mix with the separated kit. In Genetics Indonesia, the PCR machine used is the LightCycler® 96 Real-Time PCR System from Roche with the one-step RT PCR working principle as can be seen in Figure 1. A one-step RT PCR method can run (RT) Reverse Transcriptase for cDNA synthesis and the PCR cycle directly. In contrast to the two-step PCR which performs the RT and PCR processes in separate processes and well plates. Also, the one-step RT PCR method minimizes pipetting steps and the possibility of contamination compared with two-step RT PCR [35,36].
SARS CoV-2 positive result is when the samples exhibit amplification curves below the cut off value of Crossing point (Cp) positive control equals to 10 copies RNA (37 for E gene and 42 for RdRp gene), negative control does not amplify, and EAV control is detected as illustrated in Figure 2. A lower Cp value is considered with a higher concentration of viruses in the samples and vice versa. When no amplification is observed in all running samples even in the positive control, our PCR process is considered failed and rerun is a must-do. Contamination might also occur during the process. This can be seen when the negative control shows amplification. Figure 3 shows the amplification positive sample of SARS CoV-2 and control using the target gene E (gray color) and RdRp (red color). The control has a higher Cq value (amplification is detected first) than the positive sample of SARS CoV-2. The Cq control has a copy of about 1000 RNA. A positive sample with a low Cq value shows that the number of copies of viral RNA is more than the control so that the amplification rate is detected longer.
RT-PCR and Roche-purchased detection kits. It is feasible to leverage the coverage of SARS CoV-2 detection by optimizing working protocols and tuning new kits in the existing PCR machine.

4. Conclusion

In this paper, we obtain some lessons from the utilization of the RT-PCR method for SARS CoV-2 detection. There are many SARS CoV-2 detection methods, but RT PCR is the Gold Standard for detecting the presence of viral RNA directly. The RT-PCR method for SARS Cov-2 detection in Genetics Indonesia is very efficient and fast compared to common methods. Utilization of E and RdRp target gene as a pair for one sample in a separated single well in RT-PCR running can reduce primer-probe competition. Furthermore, validation of RNA extraction samples using EAV RNA can save the use of extracted samples and reagent kits. The Roche RT PCR machine is also fast for detecting SARS CoV-2, the many types of labeled dye that can be recognized also provide flexibility and ease of reading the results on virus detection results.

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