Descriptive comparison of ELISA and PCR methods in SARS-CoV-2 detection

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Abstract. The novel coronavirus, SARS-cov-2, is the causal agent of Covid-19, and allegedly to have a zoonotic origin. In 2019, it was reported in Wuhan, China, and declared pandemic by World Health Organization (WHO) between 2020 and 2023 soon after, to date, there are no known effective cure or treatments. Reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) are both commonly used testing assays for SARS-cov-2 diagnosis, but only the RT-PCR recognized as the gold standard for the detection. Despite the better sensitivity and specificity, there are certain limitations RT-PCR possesses that make ELISA more preferable. This article presents a descriptive comparison of ELISA against RT-PCR in term of sensitivity and specificity. In addition, their advantages and disadvantages on these two methods were also discussed. The comparison showed better sensitivity and specificity on RT-PCR as expected, in the aspect of methodology, RT-PCR is more complex and time consuming relative to the ELISA counterpart, which making ELISA more favorable in large-scale implementations.

Keywords: SARS-cov-2; RT-PCR; ELISA; Sensitivity; Specificity.

1. Introduction

The novel pneumatic disease, known as the Covid-19, emerged in December 2019 in Wuhan, China, it was later become pandemic, declared by the director-general of World Health Organization (WHO) in march 12th of 2020, after more than a hundred thousand cases were reported in countries overseas [1]. Currently, it is estimated that the Covid-19 was responsible for more than 6.9 million mortalities worldwide, and despite that the Covid-19 was no longer a public health emergency in May 5th of 2023, followed the WHO announcement, many cases of fatality caused by SARS-CoV-2's sub-variants are still being reported [2].

The novel coronavirus SARS-CoV-2, caused the Covid-19, is a RNA virus that belongs in the coronavirus family, along with MERS-CoV and SARS-CoV-1, and allegedly has a zoonotic origin [1]. Structure of SARS-CoV-2 is typical to the other coronaviruses: positive sense, single-strand RNA encapsuled in a spherical, envelope consists of structural proteins and a lipid bilayer gained from the host cell, and it is anchored with spike proteins, which are responsible for the cellular infection, the spike protein of the virus consists of 2 subunits, S1 and S2, both are involved in facilitating the viral entry: S1 subunit allows the cellular attachment through binding with the receptor angiotensin-converting enzyme 2 (ACE2) on the host cell membrane, the S2 enables membrane fusion between virion and targeted cell, which cause release of viral RNA [1].

Since there are still no effective cures or treatment protocols that have been developed, it is critical to accurately detect SARS-Cov-2-infected in the population, in order to develop preventative measures against the spread of Covid-19, although there are rapid, portable testing kits that are based on antigen-based, lateral flow assays already available commercially, they can only provide a qualitative or semi-quantitative assessment, and also has low sensitivity for detecting asymptomatic infections [3]. There are more comprehensive testing methods that have been developed for more accurate and fully quantitative assessment, the most used one is the polymerase chain reaction testing with reverse-transcription, or reverse transcription-polymerase chain reaction (RT-PCR), it is designed to determine both the presence and the quantity of SARS-Cov-2's genome, through amplification of DNA reverse transcribed from virus's RNA, and fluorescent determination. Another

technique that is also frequently implemented for SARS-Cov-2 detection is enzyme-linked immunosorbent assay (ELISA), such method targets the antibodies produced by human body after viral exposure. Despite the fact that RT-PCR is currently recognized as superior in diagnosing SARS-Cov-2 infection, due to superseding sensitivity and specificity relative to other methods, RT-PCR still has limitations regarding to cost, complexity, and long reaction time, this prevent RT-PCR to be used for larger scale testing in the population, while ELISA in the other hand, is much more viable for large scale testing, in term of logistic, due to more rapid reaction, and being cheaper, but reduced sensitivity and specificity hamper its reliability [3].

This article's purpose is to provide a descriptive comparison of RT-PCR and ELISA in term of sensitivity, selectivity for SARS-Cov-2 detection, based on the existing studies that focused on the performance of RT-PCR or ELISA. Furthermore, the methodological advantages and disadvantages of both techniques will be summarized and discussed.

2. SARS-Cov-2 detection by ELISA

There are 3 types of ELISA protocols: direct, indirect, and sandwich, where each differs from one another based on the numbers of primary and secondary antibodies involved. For detection of viral pathogens, indirect ELISA is frequently used, due to relatively high sensitivity and selectivity to direct ELISA, and the targets consist of human antibodies. In SARS-Cov-2 detection, antibody targets are immunoglobin G (IgG), immunoglobin A (IgA), and immunoglobin M (IgM) in purified serum sample. This is because all 3 antibodies will be developed by the human body against the virus shortly after the initial exposure, the SARS-Cov-2 antigens to be cloned and used for binding the 3 antibodies are usually the receptor binding domain (RBD) on the spike protein, or the nucleocapsid (N) protein [3]. The overall process of indirect ELISA involves immobilization of IgG, IgM, and IgA in diluted sample solution, by adding it into the antigen-coated wells, and the complementary anti-antibody antibody with enzyme label is added, followed by addition of substrate that enables detection, and the quantification is done by absorbance determination using a microplate reader [3].

Recently, there are several published studies that looked into the use of ELISA for SARS-Cov-2 detection using clinical samples. For instance, Sapkal et al. reportedly developed a standardized ELISA for anti-SARS-Cov-2 antibody IgG detection in 2020 [4]. They used an indirect ELISA approach with antigen consisted of stock solution generated from lysed cell culture infected with SARS-Cov-2 that underwent viral deactivation through gamma irradiation, for IgG capture. They went through 131 SARS-Cov-2 positive plasma samples confirmed by RT-PCR and microneutralization test, and 279 pre-pandemic negative control samples, using their ELISA method and saw a 92.37% sensitivity and 97.9% specificity against SARS-Cov-2-specific IgG. They also sent their ELISA assay kit to 2 independent laboratories for evaluation, and found 99% to 100% consistency rate between their results and results from the inter-lab evaluations. For the cross-reactivity, the authors used their ELISA on 53 SARS-Cov-2 negative samples that were positive to other respiratory viruses, such as influenza A, hepatitis virus C, to detect corresponding IgGs, and they reported no positive responses from the assay, indicated no cross-reactivities. Later in the same year, another direct ELISA was developed by Roy et al. [5], which used cloned RBD of SARS-Cov-2's spike protein as antigen to target IgG, IgM, and IgA specific to the virus. They evaluated the assay using 300 clinical plasma samples consisted of 68 SARS-cov-2 positive samples, and 232 RT-PCR negative samples, and grouped based on the times after symptoms onset in patients. They found that their ELISA method had selectivity above 95% for all antibodies for 8-21 days after symptoms appeared, and reached 100% if symptoms went beyond 21 days excepted for IgM, which was 88.36%. However, the sensitivity of the assay varied for each antibody: for IgG, sensitivity was 76.67% after 8-14 days of symptoms onset and rose to 86.21% after 15-21 days, sensitivity for IgM was 80% for 8-14 days after, 79.31% after 15-21 days, for the IgA, sensitivity was 93.33% after 8-14 days, and 96.55% for after 14-21 days, sensitivity reached 100% for all antibodies beyond 21 days of symptoms onset.

In 2021, an indirect ELISA for IgG detection was developed by Mehdi et al. [6]. They also used SARS-Cov-2's RBD produced by recombinant cells as antigen, the ELISA assay was evaluated for specificity and sensitivity using 470 pre-pandemic negative serum samples but positive for other illnesses or conditions, and 312 SARS-Cov-2 RT-PCR positive serum samples that grouped into 3: group 1 consisted of samples collected 0-13 days after symptoms onset, or RT-PCR positive confirmation, group 2 of 14-20 days after, and group 3 of 21-27 days after, respectively. Their assay had an overall 99.79% specificity on the negative samples, without any cross-reactivities, while the sensitivity varied among the 3 groups: 53.33% for group 1, 80.47% for group 2, and 88.24% for group 3. They also compared their assay against 2 other commercial IgG ELISAs by Zydus and Euroimmun in term of sensitivity, specificity, using the same SARS-Cov-2 positive sample groups, and 187 selected samples from the 470 negative sample cohort. They found that both commercial assays had lower sensitivity and specificity relative to theirs. Klumpp-Thomas's team also developed an indirect ELISA for SARS-Cov-2 detection in the same year [7], targeting Immunoglobin G, A, and M biomarkers, using cloned RBD and spike protein as antigen, evaluations on specificity and sensitivity were carried out on separate assays with different antigen constructs but the same ELISA regimes, using 100 negative control serum samples collected before SARS-Cov-2 emerged, and 60 SARS-Cov-2 RT-PCR positive control samples, both negative and positive samples were diluted to 1:400 dilution ratio. Results suggested that the ELISA had 99% specificity for IgM and IgA, with spike protein antigen, and 99% to 100% for all 3 antigens with RBD, the sensitivity was 100% with either antigen. They also examined the cross-reactivity of their assay against non-SARS-Cov-2 coronaviruses OC43, HKU1, MERS, and SARS1, and observed non for OC43 and HKU1, however, there was low level cross-reactivity against MERS and SARS1, but the correlations were minimal, also, no cross-reactivities were detected against common, non-coronavirus respiratory viruses.

One year after, in 2022, Luo et al. developed an indirect ELISA that utilized recombinantly produced SARS-Cov-2 nucleocapsid protein as antigen to target IgG [8]. The assay had its sensitivity, specificity evaluated by 113 positive serum samples that grouped into 3 based on days passed after positive confirmation by RT-PCR, and 1500 pre-pandemic samples collected before 2015, as negative controls. The specificity of the ELISA assay was found to be 99.3% on the negative control samples, but the sensitivity in the other hand, varied among different groups of positive samples, specifically, for the positive samples collected first 6 days after confirmation, sensitivity was 39.1%, 86.4% for samples collected 7 to 13 days after confirmation, and 89.7% for 14-55 days after, respectively. They also tested the assay for interference by creating a diluted positive sample with interfering factors such as hemoglobin and triglycerides, and the cross-reactivity using negative control samples that are positive for pathogens including HIV 1 or HIV 2, herps simplex virus. Results suggested that the assay did not suffer significant interference in the diluted positive sample, and the non-SARS-Cov-2 pathogens in the negative samples were not detected, indicated no cross-reactivity.

3. SARS-Cov-2 detection by RT-PCR

RT-PCR is a type of nucleic acid test that relies on polymerase chain reaction to amplify and quantify specific genetic sequence in the DNA. Sequence-specific primers are needed for targeting the sequence of interest, and guide the polymerase during amplification. Differed from standard PCR, RT-PCR employs a reverse transcription step to produce DNA from viral RNA, the reason of including reverse transcription in RT-PCR is to enable the ability for it to carry out the function of PCR, if the target sample consists of RNA [9]. For detecting SARS-cov-2, viral RNA is first extracted from collected swab samples, from upper respiratory track, or saliva, serum, or stool, the viral RNA is reverse transcribed into cDNA via reverse-transcription, and amplified by quantitative PCR [9]. Sequence-specific primers are used to target the genomes of interest in the cDNA before initiating the amplification, for SARS-cov-2, several sequences such as N, RNA-dependent RNA polymerase (RdRp), or envelope (E) protein sequence, or ORF1b, ORF8 genomic regions, can serve as primer targets [10]. Precise heat treatment is required for the amplification process, due to the need for

separation of DNA's strands, proper hybridization between the cDNA and primer, as well as to maximize enzymatic activity of the polymerase [10]. Fluorescence signals generated by hybridized DNA probe or fluorescent dye are determined photometrically, which allows quantification of amplified DNA genomes [10].

Soon after the emergence of lab-confirmed cases outside China, in January of 2020, Corman et al. introduced and validated a RT-PCR protocol first known for testing SARS-cov-2 infection [11]. They implemented quantitative RT-PCR with primers specific to RdRp, E and N gene in the SARS-cov-2's RNA. They carried out the testing method on 729 collected clinical samples consisted of sputum, throat and nasal swabs from hospitalized individuals in 2019, as well as biobanks from different medical institutions in Germany, England, and Hong Kong. The developed assay had a sensitivity of 95% with E and RdRp primers, but slightly lower with N primer, specificity of the assay was 100%, indicated by zero false positive for mock-up samples with non-SARS-cov-2 coronaviruses, and no cross-reactivity in clinical samples containing other respiratory viruses such as influenza A and B in the assay.

A year later, Chung's team. developed a RT-PCR protocol that used RdRp and E genes as the prime targets [12]. The performance of the assay was evaluated using RNA extracted from SARS-cov-2 infected cell culture, and they determined the specificity by adding the RNA of 23 respiratory viruses, including MERS-cov, SARS-cov and influenza virus. The sensitivity was assessed by using a mock-up solution containing cloned RdRp, E genes of SARS-cov-2, that underwent serial dilutions. They also compared their assay's performance against 5 other RT-PCR kits that were approved for emergency usage, and all 5 RT-PCR used for comparison had sensitivity of 98.2% and 100% specificity, on 55 positive clinical samples, 50 negatives. Chung's assay did not produce any detectable quantity of viral RNA that did not belong to SARS-cov-2, thus, the specificity was 100%, this was further verified in the inter-assay comparison, where all 6 assays had successfully identified all 50 negative cases. Sensitivity of the assay was consistent with the 5 other assays, which was 98.2%, indicated by the successful detection of 54 positive cases out of 55 across all assays in the comparison. Two months after, a SYBR green-based RT-PCR was developed by Alhamlan et al. with primer targets consisted of assay consisted of RdRp sequence, as well as structural protein genes of SARS-cov-2 [13]. The validation of the assay was carried out on 190 nasopharyngeal swabs collected from hospitalized patients. They also assessed the possible genomic cross-reactivity between the designed PCR primers and other viruses, or any genomes that may cause interferences, using databased analysis. The assay had a 97.7% of sensitivity, specificity of 100% without any cross-reactivities, and these findings were further confirmed by results from a parallel test on the same samples using different RT-PCR and RNA extraction methods.

Williams et al. examined the RT-PCR assay performance against SARS-cov-2 under a large public setting [14], where they employed the method to a cohort of 8311 patients, the positive, negative cases in the cohort were defined based on the initial or the subsequent RT-PCR test results, cases with negative RT-PCR results but positive in clinical diagnosis were considered false negative, and the collected samples were subjected to re-testing from a different laboratory with RT-PCR in different platform, against result of RNase P PCR, which was used as positive control. Upper respiratory track samples were collected from 8311 patients and tested, 1667 were identified positive by RT-PCR with sensitivity of 91.8% for the initial result, 98.4% for subsequent testing, sensitivity in re-testing of the false negative cases was 82.2%, 90.6% in repeat tests, in both cases, specificity was maintained at 100% without any cross-reactivities. Shortly after, a comparative study by Tee et al. explored the potential of using saliva sample as an alternative for conventional naso-oropharyngeal swab in using RT-PCR for SARS-cov-2 [15]. Specifically, they used one RT-PCR method on 3 different sample types: one consisted of RNA extracted from saliva, another from naso-oropharyngeal swabs, and a saliva mixture of 5oμl collected saliva sample with 2.5μl of proteinase K; the primer targets were RdRp, E, and N genes. For performance evaluation of RT-PCR on saliva sample, results from naso-oropharyngeal RT-PCR was used as reference for estimation of sensitivity and specificity of assay results on RNA extract from saliva, as well as saliva mixture. Comparison showed that the sensitivity,

specificity was 92.67% and 97.55% for assay on RNA extract from saliva, 91.33% and 98.91% for saliva mixture.

4. Comparison between ELISA and RT-PCR

4.1. Sensitivity and specificity

Since ELISA and RT-PCR are differed from each other regarding to principle of functions, thus, differed sensitivity and specificity are expected when used for detecting SARS-cov-2, as shown in Table 1. In general, RT-PCR is superior in sensitivity as well as specificity. Compared to ELISA, this is expected due to the base-pair level precision in primer's sequence-specific targeting, as well as the fact, that the sample to be analyzed consists only of extracted RNA, rather than the original sample itself. The antigen-antibody interaction utilized in ELISA assay is also specific, however, its specificity can be reduced by factors particularly the cross-reactivity between secondary antibody and other non-SARS-cov-2 antigens. The lower sensitivity in ELISA relative to RT-PCR may contributed by the concentrations of target antibodies below the level that is sufficient to generate enough amounts of signals to be identified as positive. This is more conspicuous for early SARS-cov-2 diagnosis, because it takes days for human body to produce the SARS-cov-2 specific antibodies in a detectable quantity, where there is a low sensitivity of their ELISA assay on samples collected from SARS-cov-2 contracted patients within the first week, and sensitivity increased exponentially after the second week.

Table 1. Summarized differences of ELISA and RT-PCR.

Methods	Average sensitivity	Average specificity	Prerequisite molecules	Sample treatment	Thermal condition	Time consumption
ELISA	85.67%	98.83%	Antigen Detector antibody	Dilution	Room temperature	2hr to 4hr
RT-PCR	94.55%	99.5%	Primer Fluorescent probe/dye	RNA extraction	50-55°C (reverse transcription) 95-55°C (amplification)	2hr to 9hr (depends on number of cycles)

4.2. Advantages and disadvantages

The distinct principles of function in ELISA and RT-PCR also indicate that each technique has a different procedure. In ELISA, the antibodies specific to SARS-cov-2 in the sample are immobilized by corresponding antigens, and their constant regions are targeted by detector antibody for signal generation, therefore, both antigens and detector antibodies are need to be synthesized before carrying out the assay, and production is done using several ways, such as plasmid-based transformation in bacteria, cellular cloning, or extraction from SARS-cov-2 infected cell culture. Despite the potential of high quantity yields in antigen and detector antibody production in ELISA, the plasmid construction may be complicated. RT-PCR requires synthesis of two prerequisite molecules as well, which are sequence-specific primers and fluorescent probes (Table 1). Both molecules increase the specificity of the assay, due to being sequence-specific. The primary drawback comes from the production of the molecules, where it relies on artificial construction of oligonucleotides based on the known sequence of a particular genome within the viral RNA [10, 13], and such process can be more sophisticated than the ELISA counterpart. In the aspect of sample, RT-PCR assay is carried out on extracted RNA, instead of collected samples in ELISA (Table 1), and this alleviates the risk of interferences from impurities, but extraction involves extra sample processing that can be tedious and time consumption. In the other hand, ELISA uses collected samples without undergoing complicated

manipulations other than dilution, which saves more time and make the assay easier to use, but it is more prone to sample contaminations.

The overall time consumption of RT-PCR exceeds the ELISA counterpart (Table 1), and this was because in the selected studies, over 36 amplification cycles were conducted to identify the SARS-cov-2 positives, despite that each amplification cycle lasted minutes. The reason behind the repeat of large number of amplification cycles lies in the concentration of sample RNA concentration, that is, lower the RNA concentration, more cycles needed to facilitate the identification of positive, the advantage of this is high sensitivity for low concentration samples, as the signals can be better distinguished from background noise, however, time consumption increases as more cycles are repeated. Another characteristic unique to RT-PCR is the high-temperature thermal cycling, typically, the temperature was cycled and maintained between 50°C, 95°C, and back to 55°C, during amplification, although this is crucial for amplification to proceed properly, the requirement of thermal cycling poses a burden on equipment's complexity and maintenance.

5. Conclusion

Both RT-PCR and ELISA are viable, well-researched techniques for SARS-cov-2 detection, each has distinctive procedures, and rely on different types of samples. The comparison showed RT-PCR excels ELISA in term of performance, while ELISA is easier to use, and less time consuming, making it more feasible for purpose such as mass screening, especially in a large population. However, it is important to note that this article is merely a descriptive comparison, it did not cover the in-depth aspects such as logistic costs for the two assays, as well as how their performance be affected according to the stage of infection, therefore, an experimental based study that uses clinical samples, with consideration on stage of infection, is recommended for more accurate and comprehensive comparison between the two techniques.

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