Assessing the Efficacy of Molecular, Serological, and Radiological Techniques for the Detection of SARS-CoV-2

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ABSTRACT
Due to the coronavirus episode numerous examinations are being led for remedial systems and antibodies yet detection techniques assume a significant job in the control of the ailment. Assess the adequacy of the sub-atomic recognition procedures in the Coronavirus. Six writing information bases (Pub Med, Science Direct, MDPI, CDC, Springer Link, Scopus, Google Researcher, and AAAS) were looked for applicable examinations and articles were screened for important substance. Deliberate audits uncover the utility of molecular, and serological with radio-logical testing as one such strategy can't be associated with the accessibility of purpose-of-care gadgets. Don't adjust to affect ability and particularity in examination to the ordinary strategies because of the absence of clinical examinations. The research aims to enhance identification strategies that uphold the clinical dynamics of patients. However, none of the techniques achieved 100% sensitivity and specificity, indicating the necessity for additional considerations to overcome the challenges addressed herein. We anticipate that the current article, with its observations and recommendations, will aid healthcare practitioners in this endeavor.
Keywords: SARS-CoV-2, COVID-19, Molecular techniques, Serology immunoassays, Radiological testing.

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INTRODUCTION

With 29,891 nucleotides that encode for 9860 amino acids, SARS-CoV-2 is a single, abandoned RNA genome that belongs to the beta family.[1] It is thought that a bat strain with a big positive single-stranded RNA of around 30 kb is where the virus first appeared.[2] Well-known genomic prearrangement of SARS-CoV-2 and alignment homology of 82 % with SARS-CoV-2.[3] Four fundamental proteins of SARS-CoV-2 have been identified: spike, envelope, membrane, and nucleocapsid.[4] SARS-CoV-2 uses ACE2 cell receptors to enter host cells.[5] The E protein of SARS-CoV-2 is a small protein that aids in the virus's assembly, maturation, and release, and contributes to its pathogenesis.[6,7] Shaping particle channels.[8] By collaborating with the positive sense ssRNA atom the nucleocapsid protein forms RNA bonds.[9,10] Additionally regulates the etiology, records, interpretations, gathering release, and reproduction of infections.[11,12] This reversible link allows it to function as a chemical that pulverizes receptors.[13,14] The ORF of polyprotein 1a/1ab on the SARS-CoV-2 genome codes for 16 non-auxiliary proteins (nsp1-16), each of which has a different function.[15] NSPs such as Nsp5 (3CL pro) and nsp3 protease assist in viral replication, recording, and RNA preparation.[16,17] CDC's Coronavirus PCR test received emergency use authorization in Feb 2020 due to slow usage of in-house sub-atomic diagnostics across the country during the pandemic.[18] Commencing from April 25th, 2020, there were over 2.8 million confirmed cases of Coronavirus and approximately 200,000 fatalities documented worldwide.[19] Fast and accurate detection methods for SARS-CoV-2 are being developed.[20] This review covers techniques including RT-PCR, immunological tests, and radiological techniques. Early diagnosis is crucial to prevent the spread of the virus.

HISTORICAL BACKGROUND

By December end of 2019, Wuhan China had seen the emergence of a new coronavirus known as COVID-19, which sparked the Covid-19 outbreak.[21,22] The illness has gradually spread over the world since the first initial patient was admitted to the hospital on December 12, 2019.[23] As of March 17, 2020, 7,426 fatalities have been reported from 179,112 cases that have been verified globally.[24] Coincidentally there is a certain amount of false negatives in the normally reliable Nucleic Acid Amplitude test Polymerase Chain Reaction.[25,26] The repercussions might be severe if patients get a diagnosis based on false-negative findings from this test.

SAMPLE COLLECTION FOR SARS-CoV-2

Typically, the sample collection site for Coronavirus includes the upper respiratory tract, comprising the nasopharyngeal, oropharyngeal, nasal mid-turbinate swab, and anterior nasal swab.[27] Samples for respiratory analysis can be collected from sputum or other lower respiratory tract sources, but it requires technical expertise and specialized equipment and is usually reserved for severe respiratory conditions or critical illnesses.[28]

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SAMPLE STORAGE CRITERIA FOR SARS-CoV-2

Store samples at 2-8°C for up to 72 hours after collection. In the event of an anticipated delay in testing or transportation, store samples at -70°C or below.[29]

METHODS FOR THE DIAGNOSIS OF SARS Cov-2

Various techniques are utilized for the detection of SARS-CoV-2, including molecular, serological, and radiological methods. Each of these strategies operates with different sensitivity and specificity values.[17]

MOLECULAR METHODS

Reverse transcriptase polymerase chain reaction, droplet digital polymerase chain reaction and loop-mediated isothermal amplification are the molecular techniques used in coronavirus diagnostics. Molecular techniques have been increasingly utilized over the past decade to enhance sensitivity, specificity, and turnaround time in the clinical laboratory.

REAL-TIME REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

PCR is a method for rapidly amplifying DNA segments. For SARS-CoV-2 detection, RNA is converted into cDNA using reverse transcriptase. Efficient viral RNA extraction is essential, with diverse RNA purification kits facilitating effective isolation.[30]

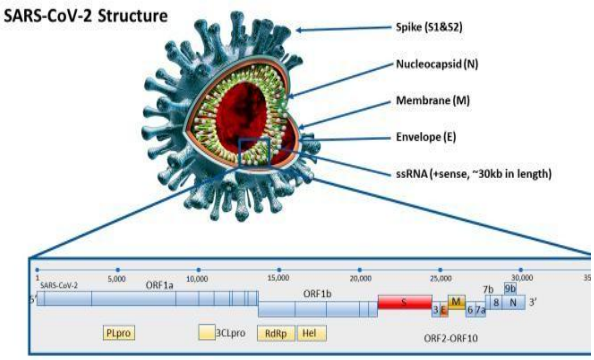

Figure 1: Gene sequences for ORF1ab, spike (S), envelope (E), and nucleocapsid (N) of SARS-CoV-2 may be detected by real-time PCR testing.[37]

PROCEDURE

The process involves various steps, such as RNA extraction from the virus, preparation of the reaction mixture, amplification duration, and interpretation of results.

RNA Extraction

The test is mixed with a lysis buffer to extract intact viral RNA. After lysing the sample, RNA is bound to a silica matrix in a spin column via solid-phase extraction. Contaminants are removed through washing, and purified viral RNA is obtained using an elution buffer, free from inhibitors and contaminants.[31]

PREPARATION OF REACTION MIXTURE

A master mix is a concentrated solution that has been premixed and contains a buffer, reverse transcriptase enzyme, deoxyribonucleotides, forward primer (5'-3'), reverse primer (3'-5'), TaqMan probe, and DNA polymerase is used in this step. Lastly, the RNA template is added and the tube is stirred using pulse vortexing to finish the reaction mixture.[32]

SAMPLE LOADING AND RUNNING

PCR plates with 96 wells are used to analyze genes like RdRp, E, and N for detecting the novel SARS-CoV-2. PCR involves denaturation, annealing, and extension steps, with DNA polymerase generating new DNA strands. PCR enables the amplification of DNA fragments, making it useful for analyzing small sample volumes.34

DETECTION

To estimate fluorescence signals, one needs a tungsten halogen light, filters, mirrors, lenses, emission filters, and a CCD camera. The lamp emits filtered light which is directed onto wells. The CCD camera captures the emitted light through an emission filter. This allows for real-time monitoring of PCR reaction progress.[35]

TARGET REGION IN RT-PCR

Without the use of viral isolates, the RT-PCR technique for SARS-CoV-2 testing was created and verified. Targeted by these approaches include viral nucleic acids and essential and non-structural proteins including the envelope, spike, and nucleocapsid genes, as well as open reading frames and RNA-dependent RNA polymerase.[17]

Table 1: 2019-Novel SARS-CoV-2 (2019-nCoV) Real Time rRT-PCR Panel primer & Probe:[5]

Lable name	Description	Oligonucleotide sequence(5'>3')	Label	Final Conc.
2019-nCoV-NIF	2019-nCoV-N1 Forward Primer	GACCCCAAAATCAGCGAAAT	None	500nM
2019-n CoV-N1 R	2019-n CoV-N1 Reverse Primer	TCTGGTTACTGCCAGTTGAATCTG	None	500nM
2019-n CoV-N1 P	2019-n CoV-N1 Probe	FAM-ACCCCGCATTAGGTTTGGTG GACC-BHQ1	FAM, BHQ1	125nM

RT-PCR-BASED ASSAY TARGETING E-GENE

An elaborately designed computerized Cobas 6800 framework for SARS-CoV-2 identification that makes use of the open channel and the E gene.[37] Assay was performed on swab tests with a cutoff of discovery of 689.3 duplicates/mL with 275.72 duplicates per response at 95% location likelihood which was generally per the results.[38]

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RT-PCR-BASED ASSAY TARGETING S-GENE

Saliva samples from 12 patients were subjected to a single-step RT-qPCR experiment targeting the S gene. RNA was extracted with NucliSENS, and EasyMAG on a Light Cycler 480 Real-time PCR System. Samples were collected 2 days post-hospitalization, with the earliest samples showing the highest viral load in 5 patients (83.3%). Furthermore, one patient displayed prolonged viral shedding 11 days post-hospitalization.[39]

RT-PCR-BASED ASSAY TARGETING N-GENE & ORF 1ab

Chu et al.[40] directed Two monoplex RT-PCR examines focusing on the ORF1b and N quality areas of SARS-CoV-2. For clinical example location two, the amplification efficiencies of ORF1b and N quality examines were 99.6% and 95.4%, respectively, and the patients tested positive using this method. [41] Out of 4880 respiratory plot tests examined using quantitative RT-PCR, 38.42% (1875) were positive. Of them, 40.98% tested positive for ORF1ab, and 39.80% tested positive for plasmid protein.

RT-PCR-BASED ASSAY TARGETING RdRp-gene

Used the MagNA Pure 96 technique to isolate RNA from nasal, throat, and fecal samples. The RNA was then screened using the E gene and confirmed using RdRp. For E and RdRp, the corresponding analytical sensitivity was 5.2 and 3.8 copies per reaction. Similar findings without cross-reactivity with other viruses were reported by other labs.

DROPLET DIGITAL POLYMERASE CHAIN REACTION

RT-qPCR is the standard method for SARS-CoV-2 diagnosis but may misdiagnose low viral load samples. ddPCR is more sensitive and specific, making it recommended for accurate diagnosis, especially with low viral loads. Common gene targets for RT-qPCR include Orf1Ab, Nucleocapsid protein, and Spike Protein.[38,42] RT-qPCR detects SARS-CoV-2 nucleic acids in cases. However, false-negative results and hampered viral load assessment can occur due to various factors, like sample collection, extraction, amplification methods, and RNA quality.[45, 46] ddPCR was suggested as a novel technique for identifying low viral load in SARS-CoV-2 patients. It performed more robustly and with more sensitivity than previous molecular techniques.[47,48] ddPCR uses oil-water emulsion to create 20,000 Nano droplets, resulting in a highly sensitive and accurate PCR approach.[49] Positive signals from ddPCR were confirmed to correlate with SARS-CoV-2 sequence amplification using cDNA extraction and sequencing. The ddPCR reaction mixture was divided into ten wells, three of which were analyzed using a Bead Reader. The remaining eight wells were pooled, and cDNA was extracted using TE buffer and chloroform, then sequenced on a SeqStudio Genetic Analyzer.[50] The Blast tool was utilized to match the obtained sequence with the reference sequence 'MT077125 severe acute respiratory syndrome coronavirus 2 isolated SARS-CoV-2/human/ITA/INMI1/2020.

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

This test requires many reagents, including salts, nucleotides, DNA polymerase, and several primers. Additionally, heat-stable reverse transcriptase is required for the detection of RNA viruses.[51,52] For identifying RNA infections, a warmth-stable opposite transcriptase catalyst is likewise required as an extra part.[51] The reactivity of the atomic level cycle of the RT-LAMP test is schematically shown in (Figure 2).

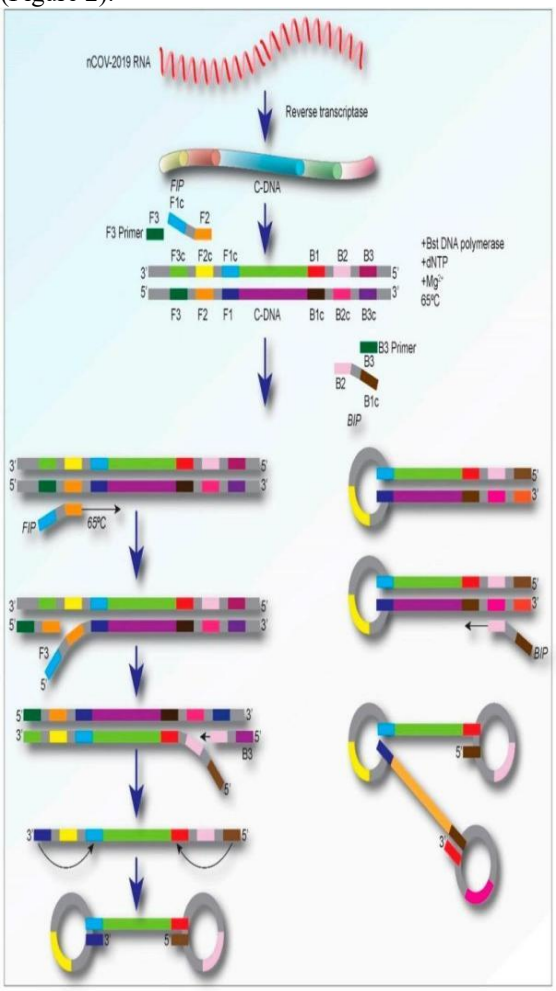

Figure 2: This text describes the cycle of the amplification for the RT-Light measure wherein the Light preliminaries tie to the reciprocal objective cDNA arrangements, and hand-weight molded DNAs are delivered.[51,52]

The cycling enhancement step produces a few duplicates of such free-weight DNAs, which are utilized to create amplified DNA with different sizes in the extension stage.

PROCEDURE

The CDC states that in medical settings, swabs from the nasopharynx, oropharyngeal, and mid-turbinate

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can be utilized to diagnose SARS-CoV-2. The RT-Light reaction was first reported by El-Tooth et al in February 2020 for rapid detection of SARS-CoV-2.[35] Two methods for detecting SARS-CoV-2 RNA are RT-Light and lateral flow strip for rapid detection of low viral RNA concentrations. CLIA is a widely used serological method known for its range, speed, and accuracy.[54] A study focused on improving the sensitivity and specificity of a measure. The study found that the RNA-dependent RNA polymerase sequence in the viral RNA showed higher enhancement efficiency.[55] The RdRp showed high specificity against respiratory infections in Figure 4A and clinical examples in Figure 4B. Other scientists used focused introductions for spike-protein encoding S quality or nucleocapsid encoding N quality to achieve higher sensitivity and specificity in a short time.[56-59] Preliminaries focusing on the Nsp3 quality in blend with those focusing on N and S qualities created significantly palatable outcomes and enlisted the most limited edge time for cDNA creation.[58] Colorimetric techniques utilizing color-changing reagents were employed to enhance the viability of RT-Light response as a POCT. A pH-sensitive marker color mix was evaluated for the rapid visual detection of SARS-CoV-2..[55,60,61] This color changes its shading from red to orange-yellow as a sign of a positive response (Figure 4C, D).[62] A colorimetric RT-Light strategy with phenol red can be utilized to identify low pH caused by high DNA polymerase activity in resource-limited settings.[63,64] Additionally, quick visual identification of a positive response should be possible by utilizing a Light ace blend enhanced with SYTO®-9 (ThermoFisher S34854, a two-fold abandoned DNA or dsDNA restricting operator), or leucogen violet(that changes from vapid to violet on contact with dsDNA) .[65,66] Late examinations center around streamlining the system considerably further by consolidating everything in a 'one-step' or 'single-tube' measure utilizing Nano particle-based biosensors.[66] Or by including an attractive dot catch venture during the preparation of dry swabs to amplify viral RNA yield .[67,68] There are now two techniques for identifying SARS-CoV-2 RNA with excellent sensitivity and specificity RT-Light and DETECTR. A lateral flow strip is used by DETECTR to quickly identify low viral RNA amounts.[69] For effective detection in PCR tubes that are sold commercially a 3D-printed incubation chamber has been introduced. Additional testing is required to enhance the sensitivity and specificity of these approaches.[70] Public-private partnerships for mass production of essential equipment and reagents can help implement RT-Light as a POCT for rapid diagnosis and relief of the SARS-CoV-2 pandemic.

SEROLOGICAL METHODS

Following techniques utilized in the serological determination of SARS-CoV-2 for example, Chemiluminescence Immunoassay,Horizontal stream test, and Catalyst Connected immunosorbent test.

CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence immunoassay (CLIA) has been generally utilized in the fields of ecological science, atomic biologics, and medical science due to its wide straight range, quick and advantageous activity, simplicity of robotization, high exactness, and affectability features .[71]

WORKING PRINCIPLE

The chemiluminescence immunoassay analyzer detects trace substances in the human body by using fluorescent-labeled antibodies to bind the antigen and antibody to a solid phase carrier and then injecting a solution to create an oxidation reaction. Photons released in the reaction are detected and converted into data of the testing substance in the sample.[71, 72] Figure 3 shows the response component.

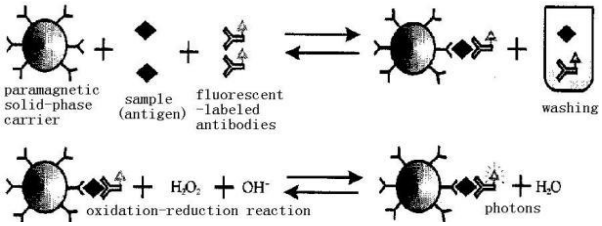

Figure 3: Chemiluminescence immunoassay mechanism.[72]

PROCEDURE

Recombinant nucleocapsid antigen was produced in E. coli and purified using a Ni-NTA column. The nucleocapsid was then coupled to magnetic beads for detection. The testing and detection process was automated and took 23 minutes. Positive and negative controls were used in each set of tests.[73-76] The affectability of IgG CLIA at ≤7 long stretches of manifestations was 46.9 %, at 8–14 days 69 %, and was 100.0 % > 14 days .[77-80]

LATERAL FLOW ASSAY

Rapid lateral flow immunoassays are a cost-effective method for detecting specific components in various sample types, operating on the principle of antibody-antigen interaction. It provides rapid detection of target molecules and is increasingly used in diagnostics.[81] This test uses specific antibodies with colloidal gold to detect antigens in a sample. The result is interpreted using color test strips. [82, 83] The short examination of points of interest and drawbacks of immunochromatographic test is introduced in Table 2

ENZYME-LINKED-IMMUNOSORBENT ASSAY

ELISA can use direct or indirect approaches to detect SARS-CoV-2 antigen/antibody. The indirect approach involves first binding a primary antibody to an antigen on a microplate, and then applying a secondary antibody. In the direct technique, an enzyme-linked antibody finds the antigen.[91] ELISA detects SARS-CoV-2 IgM and IgG antibodies. Results are shown by color changes: positive (both

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lines red), negative (control line red), or invalid (no change). Sensitivity is 87.3%, with negative results for healthy controls.[92]

Table 2: A short comparison of merits & demerits of immune chromatographic test.

Advantages	Disadvantages
Most tests have a short reaction time, accumulating around 5 to 20 minutes	Problematic affectability,frequently bogus negative results
Simple and easy to use and perform. Some tests may be achieved in outpatient clinics or at patient beds	Especially during improved action of the infection
Perusing regularly conceivable unaided eye with	Despite significant specificity at times, the outcomes are bogus negative, especially when the infection is not very dynamic.
A limited quantity of material gathered, an assortment of material to be	It is important to check positive orsuspicious outcomes.
The "Best previously date is far off (normally a year and a half from the assembling date)	Expanded danger of administratorgetting tainted.

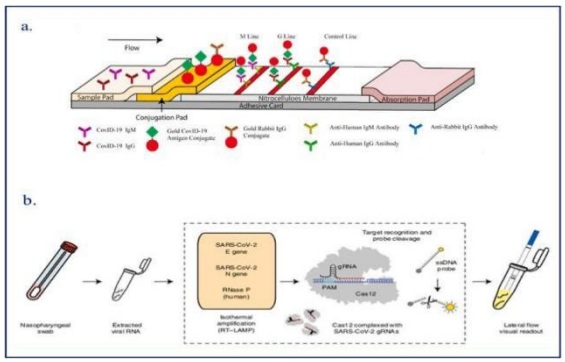

Figure 4: a) With authorization, lateral flow immunoassay for the detection of SARS-CoV-2 IgM and IgG.[89] b) CRISPR-Cas12 DETECTR lateral flow assay for SARS-CoV-2 with permission ref.[90]

RADIO-LOGICAL TESTING

Imaging analysis is a decent method to distinguish SARS-CoV-2.There are two further procedures involved in radiological testing, for example, chest radiography and processed tomography.

IMAGING RADIOLOGY

Imaging analysis has a place with the helper assessment and assumes a huge part in the conclusion and routine treatment of SARS-CoV-2 ailments .[93, 94] Patients with contamination should undergo a chest radiograph. A high-resolution CT scan can provide more information about the state of the chest. Different microorganisms with similar cycles may give similar results in imaging evaluation.[95] Fast imaging tests like chest radiography and thoracic CT are essential for detecting concentrated flare-ups of SARS, MERS, and Coronavirus. Chest radiography has thickness particularity while thoracic CT has spatial specificity and can accurately parse the cross-over area of lungs, including surrounding tissues, veins, and injuries.[96]

CHEST RADIOGRAPHY

The first test to be done on individuals suspected of having coronavirus, MERS, or SARS is a chest radiograph. In patients with SARS, the typical anomaly pace of chest radiography was 72%; 33% of them were GGO and 78% were solidification.[93,9-102] The typical number of MERS patients with abnormal chest radiography was 86% this included 65% GGO, 18% combination, 17% Bronchovascular marks, 11% Air Bronchogram, and 4% Diffuse Renal Modular Design.[103-111] According to SARS-CoV-2, 56% of patients had a normal chest radiographic abnormality rate, 24% had GGO, and 1% had pneumothorax.[112-116] The three groups' deviations from their norm paces did not differ significantly (P = 0.1734). The injury spatial position is also crucial lower lung regions are more likely to experience SARS-induced injuries with an average of 74% of them occurring there.[100-102] In SARS-CoV-2 interstitial penetration was 7%, single infiltration was 48%, multiple invasion was 52%, and one-sided contribution was 22%. Reciprocal association was 78%.[114-116] Single and multiple invasions are comparable in SARS and MERS, although the reciprocal relationship was more typical in coronavirus than in SARS. Chest radiography can analyze COVID pneumonia to some extent given the available data however there is still room for analysis to be overlooked. Accordingly, an additional CT filter is very fundamental.

COMPUTED TOMOGRAPHY

CT imaging is a great way to assess chest lesions, with excellent diagnostic capabilities, especially with low-dose and high-resolution CT. For SARS patients, the CT abnormality rate was 98%, and for MERS patients, it was 100%.[110, 119] The rate of coronavirus irregularity is 89% the rates for GGO, union, interlobular septal thickening, air Broncho gram, and insanity clearance are 84%, 65%, 48%, and 16%, respectively.[120-135] The incidence rates of SARS, MERS, and COVID-19 were similar, but higher for SARS and MERS. Early assessments showed some

patients had only fever or no symptoms. Some studies stated lung structures of SARS on high-resolution CT resembling bronchiolitis obliterans.[136,137] which gave imaging premise to corticosteroid treatment of COVID pneumonia.[138] CT scans are better than chest radiography in distinguishing the extent of injury. In SARS, 61% of sores were unifocal and 39% were multifocal. 74% were found in one area of the lungs and 26% were found in multiple areas. 48% were on one side and 52% were on both sides. 71% had lower projection association and 84% were fringe or subpleural.[100,101,108] 14% of MERS injuries were bilateral, 86% were symmetrical, 14% had a lower projection connection, and 71% were fringe or subpleural.[110,119] In SARS-CoV-2,31 % of injuries were unifocal, 69 % were multifocal, 26 % were unilobar, 74 % were multilobar, 20 % were one-sided, 80 % were reciprocal, 56 % had lower flap contribution, 82 % were fringe or subpleural, and 71 % were focal.[116,122-128,135] CT scan is more effective than chest radiography in detecting and distinguishing between MERS and COVID pneumonia[132], given its vigorous demonstrative unwavering quality for COVID pneumonia, CT sweep ought to be considered as the essential imaging assessment.

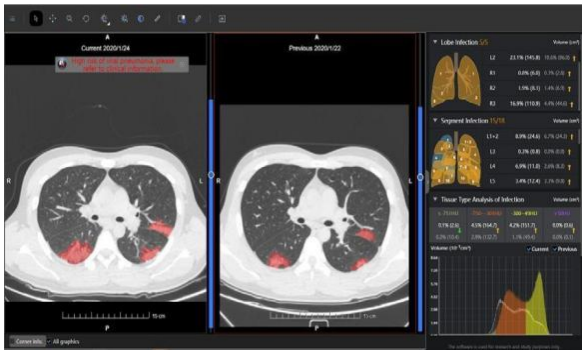

Figure 5: The follow-up measurement for a COVID-19 patient.[137]

COMPARISON OF ALL TECHNIQUES

We discussed many radiological and subatomic serological techniques for SARS-CoV-2 detection. Nearly the after-effect of this survey demonstrates RT-PCR still has the best quality level with specific constraints of giving false negative outcomes and an arduous technique.[139-141] To overcome this a few computerized frameworks have also been developed to speed up the cycle where the results were consistent with the conventional PCR practice. A few obstacles to this study include fewer instances and a poorly chosen testing period because of the pack's unavailability. Serological testing is based on an IgG and IgM panel.[142] BALF collection for SARS-CoV-2 diagnosis is accurate but requires specialized equipment and can be painful. Delayed antibody production can lead to failure in detection. Radiological and laboratory methods should be integrated for prompt containment and treatment. CT detection rates are higher than qPCR. Cross-reactivity affects sensitivity and specificity.[117] However, none of the techniques were 100% delicate and explicit; thus extra investigations ought to be done to conquer the difficulties tended to here.

CONCLUSION

Various techniques were evaluated for detecting SARS-CoV-2, with RT-PCR offering high sensitivity and specificity but requiring expensive equipment and expertise. Other methods like light techniques and CRISPR showed promising results with quicker turnaround times and less reliance on specialized equipment. Combinations of methods, such as CT scans and PCR, enhance detection rates. Further research is needed to compare the sensitivity, reproducibility, and reliability of emerging techniques. Sample collection methods, including saliva and sputum, can improve patient comfort and safety. There's a crucial need for user-friendly point-of-care devices to detect infections efficiently in public settings.

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