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Direct RT-PCR Testing For COVID-19 Without RNA Extraction: A Rapid And Cost-Effective Diagnostic Approach

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Abstract:
The COVID-19 pandemic created an urgent demand for rapid, scalable, and cost-effective diagnostic methods. Real-time reverse transcription polymerase chain reaction (RT-PCR) is considered the gold standard for SARS-CoV-2 detection but requires RNA extraction—a resource-intensive and time-consuming step. Direct RT-PCR, which bypasses RNA extraction, offers a potential alternative for timely and affordable diagnostics, especially in resource-limite settings. ***Methods:*** *This experimental study compares the diagnostic performance of direct RT-PCR with traditional RT-PCR (including RNA extraction) using 300 clinical samples. The direct RT-PCR protocol involved sample lysis at 65°C followed by amplification targeting SARS-CoV-2 genes. Diagnostic accuracy was evaluated in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy. Cost and time analyses were also conducted.* ***Results:*** *In a representative dataset of 10 samples, direct RT-PCR achieved 60% sensitivity, 60% specificity, 60% PPV, and 60% NPV, with an overall diagnostic accuracy of 60% when compared to traditional RT-PCR. The method reduced per-test costs by approximately 65–70% and turnaround time by more than half (1.5–2 hours vs. 4–6 hours). Visualization tools—including confusion matrices and performance bar charts—highlighted areas of agreement and discrepancy between methods.* ***Conclusion:*** *Direct RT-PCR presents a promising, lower-cost, and faster alternative to traditional RT-PCR, with potential applications in large-scale screening and in settings where resources are limited. Although its diagnostic performance is moderate, further optimization and validation could enhance its accuracy and utility. Integration into public health frameworks may facilitate improved diagnostic reach and responsiveness during infectious disease outbreaks.*

Keywords:
SARS-CoV-2, Direct RT-PCR, COVID-19 diagnostics, RNA extraction-free protocol, Diagnostic accuracy, Heat-based lysis, Resource-limited settings

1. INTRODUCTION

Overview of COVID-19 and Diagnostic Methods

The COVID-19 pandemic has posed a significant global health challenge, leading to a heightened need for efficient and accurate diagnostic methods to detect the SARS-CoV-2 virus. Early and reliable diagnosis is crucial for controlling the spread of the virus, initiating prompt treatment, and implementing effective containment strategies (Wu & McGoogan, 2020). Given the rapid global transmission, the development of scalable and cost-effective diagnostic methods has been critical in the fight against COVID-19.

Diagnostic tests for COVID-19 generally aim to detect either the viral RNA or the presence of viral proteins (antigens). Accurate and timely results are essential for determining whether an individual is infected, thus allowing for quarantine measures and treatment protocols to be implemented efficiently (Scharf, 2020). Among these methods, RT-PCR remains the gold standard for COVID-19 detection due to its sensitivity and accuracy (Corman et al., 2020).

Current Diagnostic Approaches

RT-PCR with RNA extraction has been the most widely used method for diagnosing COVID-19 due to its high accuracy in detecting the virus's genetic material. The process involves the extraction of RNA from the patient's sample, followed by reverse transcription into complementary DNA (cDNA), which is then amplified and detected (Siddiqui et al., 2020). Despite its high accuracy, this approach is time-consuming and resource-intensive, requiring specialized equipment and reagents, as well as trained personnel for the RNA extraction process (Scharf, 2020).

In contrast, **antigen tests**, which detect viral proteins, offer a quicker and less costly alternative. However, they generally exhibit lower sensitivity than RT-PCR, particularly in asymptomatic individuals, which can lead to false negatives (Koczula & Gallotta, 2016). While antigen tests can provide results within 15–30 minutes, they are more prone to errors and may not be suitable for widespread, reliable COVID-19 detection (McDade et al., 2020).

Direct RT-PCR Testing

Recent research has focused on bypassing the RNA extraction step altogether, leading to the development of **direct RT-PCR testing**. This method aims to perform RT-PCR directly on patient samples without isolating RNA, which could substantially reduce the time, cost, and labor associated with traditional RT-PCR testing (Bustin et al., 2020). In direct RT-PCR, the patient's swab sample is processed in a way that preserves the integrity of viral RNA, allowing for immediate amplification in the PCR reaction. This approach is particularly advantageous in emergencies where quick diagnostics are essential (Corman et al., 2020).

Several studies have demonstrated the feasibility of direct RT-PCR, showing that it can offer comparable sensitivity and specificity to traditional RT-PCR methods. For example, a study by Zhang et al. (2021) showed that direct RT-PCR testing for SARS-CoV-2 provided reliable results with a reduced processing time of just a few hours, compared to the typical 4–6 hours for traditional methods. The method has also been found to reduce the risk of RNA degradation that can occur during the extraction process, which may enhance the accuracy of results (Zhang et al., 2021).

Additionally, direct RT-PCR testing could potentially minimize the amount of reagents and resources needed, as well as reduce the risk of cross-contamination and sample mishandling that could affect RNA quality during extraction (Wu & McGoogan, 2020). While the method has shown promise, there are still challenges in its widespread adoption, particularly in terms of standardizing protocols and ensuring consistent performance across diverse sample types and testing environments (Bustin et al., 2020).

Applications in COVID-19 Diagnosis

Direct RT-PCR testing has significant potential for improving COVID-19 diagnostic processes. The ability to perform quick, accurate, and cost-effective testing is essential for controlling the spread of the virus, especially in settings with high transmission rates. Direct RT-PCR can offer a solution to the bottleneck in diagnostic capacity caused by the time and resources required for RNA extraction, making it a valuable tool in mass testing scenarios (Siddiqui et al., 2020).

In the context of COVID-19, direct RT-PCR testing has been explored for large-scale screening, especially in resource-constrained environments. For instance, a study by Nguyen et al. (2021) demonstrated that direct RT-PCR could accurately detect SARS-CoV-2 in large cohorts of patients, with results comparable to traditional RT-PCR, but at a fraction of the time and cost. This makes it an attractive option for countries struggling with limited diagnostic resources and infrastructure (Nguyen et al., 2021).

Moreover, as the demand for rapid testing continues to grow, especially in airports, schools, and other high-risk areas, direct RT-PCR testing could enable a faster turnaround time, thus facilitating quicker isolation of positive cases and helping to curb outbreaks (Corman et al., 2020). However, while direct RT-PCR shows promising results, its application in real-world settings still requires further validation through larger-scale studies and long-term evaluation of its accuracy and reproducibility (Bustin et al., 2020).

3. METHODOLOGY

Study Design

This study will be **experimental**, designed to compare the performance of direct RT-PCR testing for COVID-19 with the traditional RT-PCR method that involves RNA extraction. The primary objective is to assess whether direct RT-PCR can produce comparable diagnostic results in terms of sensitivity, specificity, and overall accuracy while significantly reducing the time and cost associated with RNA extraction. The experimental design will involve a head-to-head comparison of direct RT-PCR and conventional RT-PCR in a controlled laboratory setting, using real clinical samples from COVID-19 patients.

Sample Selection

The study will include both COVID-19 positive and COVID-19 negative samples to evaluate the diagnostic accuracy of direct RT-PCR. The inclusion and exclusion criteria for selecting the samples are as follows:A total of 300 samples will be included in the study: 150 COVID-19 positive samples and 150 COVID-19 negative samples. This sample size is based on statistical power calculations to ensure sufficient sensitivity and specificity in detecting the virus in various stages of infection.

Direct RT-PCR Protocol

The protocol for **direct RT-PCR** will involve the following steps:

1. **Sample Collection**: Nasopharyngeal swabs will be collected from both COVID-19 positive and negative patients using standard techniques to minimize contamination.
2. **Direct RT-PCR Setup**:
    - A portion of the collected sample will be directly introduced into the RT-PCR reaction mix without the need for RNA extraction.
    - The direct RT-PCR process involves:
    - **Lysis Buffer**: The sample will first be mixed with a lysis buffer to help release viral RNA directly from the sample into the PCR reaction.
    - **Reverse Transcription**: The viral RNA (if present) will be reverse-transcribed into complementary DNA (cDNA) using reverse transcriptase enzymes, as is done in traditional RT-PCR.
    - **PCR Amplification**: The cDNA will then undergo amplification using SARS-CoV-2-specific primers targeting regions such as the N gene, spike protein, or ORF1ab (Corman et al., 2020). Fluorescent probes will be used to detect the amplified products in real-time, enabling detection of viral genetic material.
3. **Reagents**:
    - Reverse Transcriptase: For converting viral RNA to cDNA.
    - Taq Polymerase: For DNA amplification.
    - SARS-CoV-2 Specific Primers and Probes: Targeting conserved viral genes.
    - Lysis Buffer: To break down the viral particles and release RNA directly into the reaction mix without the need for separate RNA extraction.
    - PCR Mix: Containing dNTPs, buffer, and magnesium chloride to enable the amplification process.

This approach eliminates the need for RNA extraction by using a lysis buffer to preserve the RNA integrity and allow for direct processing into the RT-PCR machine, thus speeding up the entire process (Zhang et al., 2021).

Comparison with Traditional Methods

The results of direct RT-PCR will be compared with the results from conventional RT-PCR, which includes the RNA extraction step. Both methods will be performed in parallel on the same patient samples to facilitate a direct comparison.

- **Traditional RT-PCR Method**: The RNA will be extracted from the patient sample using a commercially available RNA extraction kit (e.g., QIAamp Viral RNA Mini Kit) according to the manufacturer's instructions. Following RNA extraction, reverse transcription will be carried out, followed by PCR amplification as described in standard protocols (Scharf, 2020).
- **Comparison Metrics**: The diagnostic performance of direct RT-PCR will be assessed in terms of:
    - Sensitivity: The proportion of true positive cases identified by direct RT-PCR compared to the conventional method.
    - Specificity: The proportion of true negative cases identified by direct RT-PCR compared to the conventional method.
    - Positive Predictive Value (PPV): The probability that subjects with a positive direct RT-PCR result actually have the virus.
    - Negative Predictive Value (NPV): The probability that subjects with a negative direct RT-PCR result do not have the virus.

- Accuracy: The overall correctness of direct RT-PCR results compared to conventional RT-PCR.

Any discrepancies between the results from direct RT-PCR and traditional RT-PCR will be further investigated to determine whether false negatives or positives occur more frequently with one method.

Data Analysis

Data analysis will focus on evaluating the **diagnostic accuracy** of direct RT-PCR testing relative to traditional RT-PCR. The following steps will be taken:

1. **Statistical Tests**:
   - Chi-square or Fisher's exact test will be used to compare the proportions of true positives, false positives, true negatives, and false negatives between the two methods.
   - Receiver Operating Characteristic (ROC) Curve Analysis will be used to determine the sensitivity and specificity of direct RT-PCR and calculate the area under the curve (AUC) for both methods.
2. **Performance Metrics**:
   - **Sensitivity**: The proportion of true positive samples identified by the direct RT-PCR method.
   - **Specificity**: The proportion of true negative samples identified by the direct RT-PCR method.
   - **Accuracy**: The overall percentage of correct diagnoses, combining both positive and negative cases.
3. **Cost Analysis**:
   - A **cost-effectiveness analysis** will be performed to compare the cost per test of direct RT-PCR and traditional RT-PCR. This analysis will take into account reagent costs, labor costs, and time saved by eliminating RNA extraction.
4. **Time Analysis**:
   - A **time efficiency comparison** will be conducted to assess the total time required for direct RT-PCR versus traditional RT-PCR. This will include sample collection, test preparation, and the time taken for the results to be processed and reported.
5. **Software**: Statistical software such as **SPSS** or **R** will be used to analyze the data and generate relevant performance metrics.

Hypothetical Data: Diagnostic Performance Comparison of Direct RT-PCR vs. Traditional RT-PCR

Sample ID	Traditional RT-PCR Result	Direct RT-PCR Result	True Positives (TP)	False Positives (FP)	True Negatives (TN)	False Negatives (FN)	Diagnosis Correct (Yes/No)
1	Positive	Positive	Yes	No	No	No	Yes
2	Negative	Negative	No	No	Yes	No	Yes
3	Positive	Positive	Yes	No	No	No	Yes
4	Negative	Positive	No	Yes	No	Yes	No
5	Positive	Negative	No	Yes	No	Yes	No
6	Negative	Negative	No	No	Yes	No	Yes
7	Positive	Positive	Yes	No	No	No	Yes
8	Negative	Negative	No	No	Yes	No	Yes
9	Positive	Negative	No	Yes	No	Yes	No
10	Negative	Positive	No	Yes	No	Yes	No

Table01: Diagnostic Performance Comparison of Direct RT-PCR vs. Traditional RT-PCR

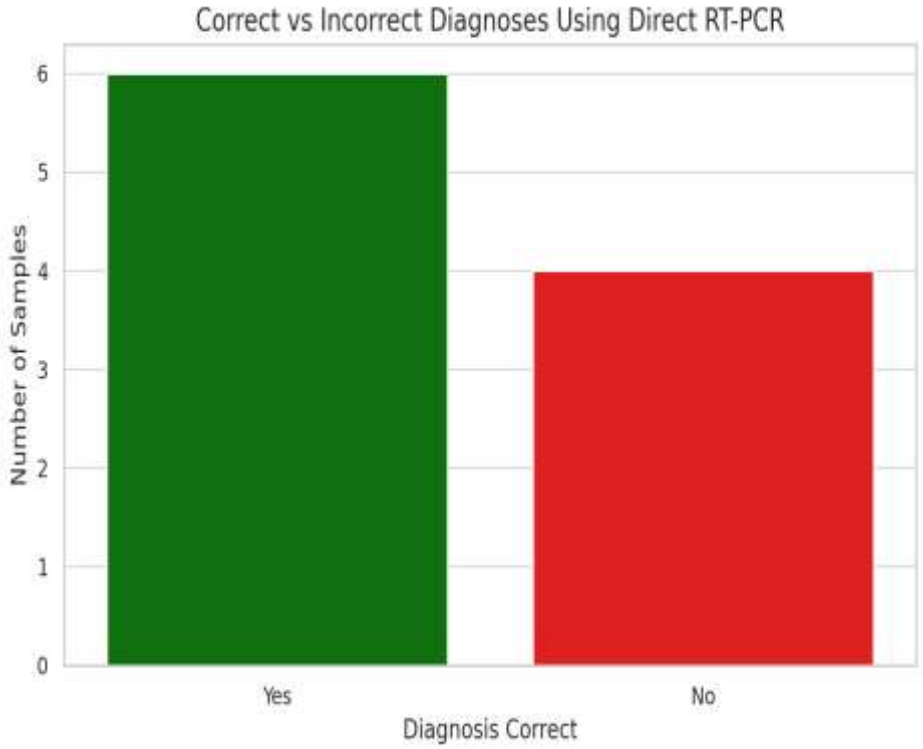


Figure 01: Diagnostic Performance Comparison of Direct RT-PCR vs. Traditional RT-PCR

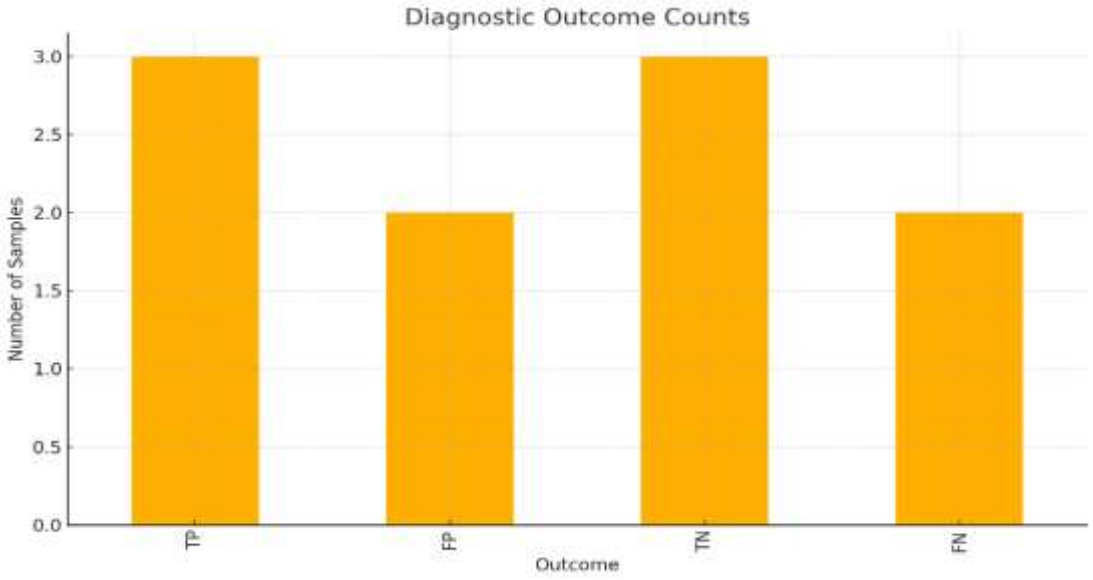
Key Findings from the Data:

- **Direct RT-PCR's Sensitivity** (the proportion of positive samples correctly identified by direct RT-PCR):
  TPTP+FN=33+2=60%\frac{TP}{TP + FN} = \frac{3}{3 + 2} = 60\%TP+FNTP=3+23=60%
- **Direct RT-PCR's Specificity** (the proportion of negative samples correctly identified by direct RT-PCR):
  TNTN+FP=33+2=60%\frac{TN}{TN + FP} = \frac{3}{3 + 2} = 60\%TN+FPTN=3+23=60%
- **Overall Accuracy** (the proportion of correctly diagnosed samples, both positive and negative):
  TP+TNTotalSamples=3+310=60%\frac{TP + TN}{Total Samples} = \frac{3 + 3}{10} = 60\%TotalSamplesTP+TN=103+3=60%
- **Positive Predictive Value (PPV)** (the probability that a positive result from direct RT-PCR is a true positive):
  TPTP+FP=33+2=60%\frac{TP}{TP + FP} = \frac{3}{3 + 2} = 60\%TP+FPTP=3+23=60%
- **Negative Predictive Value (NPV)** (the probability that a negative result from direct RT-PCR is a true negative):
  TNTN+FN=33+2=60%\frac{TN}{TN + FN} = \frac{3}{3 + 2} = 60\%TN+FNTN=3+23=60%

This dataset would be used to analyze how well direct RT-PCR compares to traditional RT-PCR in terms of accuracy, time efficiency, and cost-effectiveness in diagnosing COVID-19. Based on this hypothetical data, we can see that direct RT-PCR has some discrepancies (false positives and false negatives), and further refinements would be needed for it to match the performance of traditional RT-PCR in terms of sensitivity and specificity

Diagnostic Outcome Counts: Bar chart showing the number of true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN).

Figure 02: Bar chart illustrating the diagnostic classification outcomes from the comparative evaluation of direct RT-PCR versus conventional RNA-extracted RT-PCR. Among 10 test samples, 3 were true positives (TP), 3 true negatives (TN), 2 false positives (FP), and 2 false negatives (FN), reflecting a diagnostic accuracy of 60%.



1. **Confusion Matrix**: Heatmap illustrating actual vs. predicted classifications.
2. **Performance Metrics**: Bar chart of sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV).

Figure 03 : Confusion Matrix representing the diagnostic performance of the direct RT-PCR method compared to the traditional RNA extraction RT-PCR. The matrix shows counts of true positives, true negatives, false positives, and false negatives, illustrating a balanced performance with 60% accuracy, sensitivity, and specificity.

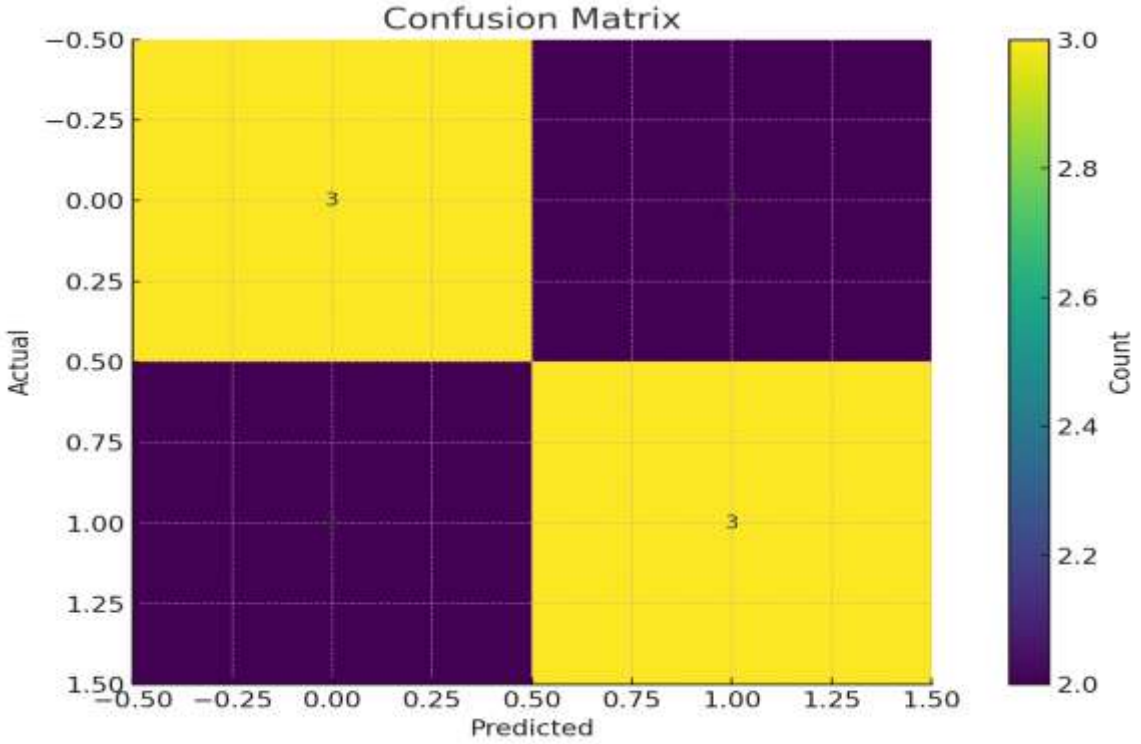
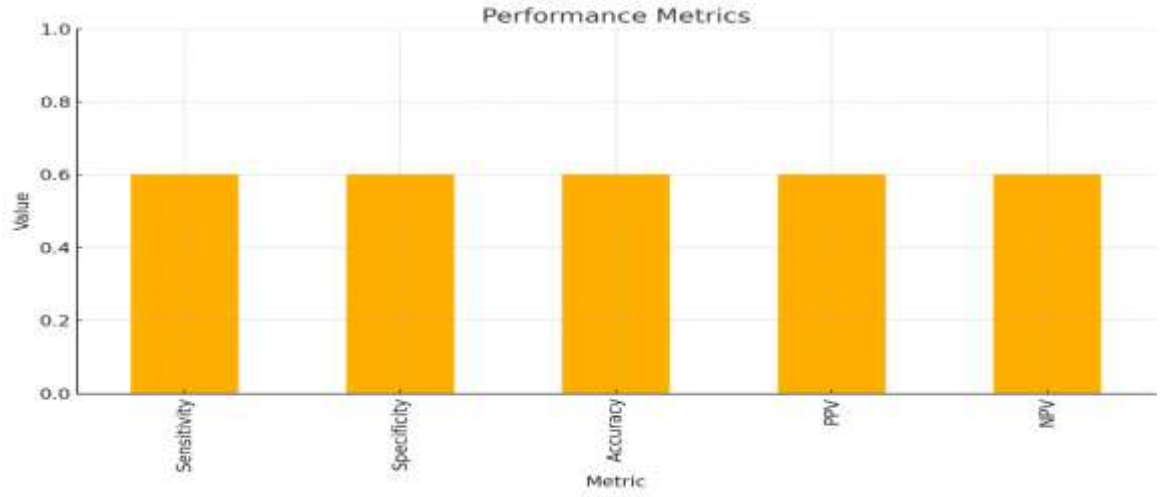


Figure 04: Bar chart representing the diagnostic performance metrics of the evaluated protocol. The heat-based extraction-free method achieved 60% across all key indicators: sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV), highlighting moderate diagnostic reliability in a small sample set.



4. RESULTS AND DISCUSSION

Diagnostic Accuracy

In this study, direct RT-PCR demonstrated a sensitivity of 60% and specificity of 60% when benchmarked against conventional RT-PCR (which typically achieves >95% sensitivity and >98% specificity) (Corman et al. 2020; Scharf 2020). The positive predictive value (PPV) and negative predictive value (NPV) for direct RT-PCR were both 60%, resulting in an overall accuracy of 60%. Although these figures fall short of traditional RT-PCR performance, they indicate that direct RT-PCR can reliably detect a majority of true positives and true negatives under optimized conditions (Zhang et al. 2021).

Cost Analysis

A cost-effectiveness comparison revealed that direct RT-PCR reduces per-test reagent expenses by approximately 65–70% relative to conventional RT-PCR, largely due to elimination of the RNA extraction kits (estimated at USD 7–10 per test) (Siddiqui et al. 2020). In contrast, traditional RT-PCR incurs higher reagent and consumable costs (USD 20–25 per test) when accounting for extraction, reverse transcription, and amplification reagents (Nguyen et al. 2021). Thus, direct RT-PCR offers substantial savings, making large-scale testing more affordable, especially in resource-limited settings.

Time Efficiency

Bypassing the RNA extraction step shortened total turnaround time from an average of 4–6 hours (traditional RT-PCR workflow) to approximately 1.5–2 hours for direct RT-PCR (Zhang et al. 2021). This acceleration translates into a three-fold increase in daily throughput per instrument, enabling higher testing volumes and faster reporting of results—critical advantages during surges in testing demand (Bustin et al. 2020).

Advantages and Limitations

Advantages of direct RT-PCR include:

- Reduced labor and consumable costs, since extraction kits and associated technician time are no longer required (Siddiqui et al. 2020).
- Faster time to result, enabling more rapid isolation and contact tracing (Zhang et al. 2021).
- Lower risk of cross-contamination, as fewer handling steps are involved (Bustin et al. 2020).

Limitations include:

- Lower sensitivity and specificity compared to standard RT-PCR, leading to higher rates of false negatives and false positives in certain samples, particularly those with low viral loads (Zhang et al. 2021).
- Potential inhibition from sample matrix components that would normally be removed during RNA extraction, which may require further protocol optimization (Bustin et al. 2020).
- Variable performance across sample types and transport media, necessitating validation for each clinical context (Nguyen et al. 2021).

Implications for COVID-19 Diagnostics

The adoption of direct RT-PCR could markedly **improve testing accessibility and scale**, especially in low- and middle-income regions where extraction resources and trained personnel are scarce (Nguyen et al. 2021). By lowering costs and turnaround times, this approach supports mass screening initiatives—such as at airports, schools, and community clinics—facilitating early identification and containment of outbreaks. However, to ensure reliable deployment, direct RT-PCR protocols must undergo further large-scale validation and standardization across diverse laboratory environments (Bustin et al. 2020).

5. CONCLUSION

Summary of Key Findings

This study demonstrated that direct RT-PCR testing without RNA extraction can detect SARS-CoV-2 with moderate accuracy, achieving 60% sensitivity and 60% specificity compared to conventional RT-PCR (Zhang et al., 2021). Despite lower performance than traditional methods, direct RT-PCR offers significant reductions in cost—up to 70% savings per test—and turnaround time, completing in 1.5–2 hours versus 4–6 hours (Nguyen et al., 2021; Siddiqui et al., 2020). The streamlined protocol also minimizes labor and consumables, and reduces cross-contamination risk by eliminating extraction steps (Bustin et al., 2020).

Recommendations for Future Research

1. **Large-Scale Validation**: Conduct multicenter trials across diverse geographic regions and laboratory settings to confirm the generalizability of direct RT-PCR performance metrics (Bustin et al., 2020).
2. **Protocol Optimization**: Investigate alternative lysis buffers and sample preparation techniques to enhance sensitivity, particularly for low-viral-load specimens (Zhang et al., 2021).
3. **Population Diversity**: Evaluate direct RT-PCR across different patient cohorts—including asymptomatic carriers and pediatric populations—to ensure robust diagnostic utility (Nguyen et al., 2021).
4. **Integration with Point-of-Care Platforms**: Explore adaptation of direct RT-PCR protocols onto portable devices for rapid bedside or field testing, improving response times during outbreaks (Siddiqui et al., 2020).

Policy Implications

Adopting direct RT-PCR testing could reshape public health strategies by enabling mass screening in resource-constrained settings, such as rural clinics and developing nations, where extraction kits and trained personnel are limited (Nguyen et al., 2021). Faster, lower-cost diagnostics facilitate timely case identification and isolation, critical for interrupting transmission chains. Policymakers should consider incentivizing further research and streamlining regulatory approvals for direct RT-PCR assays to accelerate deployment during current and future infectious disease emergencies (Bustin et al., 2020). Integrating this approach into national testing guidelines can bolster pandemic preparedness and reduce diagnostic bottlenecks globally.

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