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A Comprehensive Review on TrueNat, CBNAAT and Real-Time PCR: Techniques for Molecular Diagnostics Assay

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ABSTRACT

Early diagnosis and effective illness treatment are made possible by the precise and rapid identification of genetic material through molecular diagnostic procedures. This study thoroughly examines the principles, working processes, and diagnostic efficacy of the TrueNat, Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), and Real-Time Polymerase Chain Reaction (RT-PCR). The TrueNat is a well-known point-of-care real-time PCR platform that is portable and chip-based. CBNAAT provides quick and automated detection using cartridge-based nucleic acid amplification. The gold standard for high sensitivity and quantitative analysis is real-time PCR. Accuracy, cost-effectiveness, and adaptability in resource-limited environments are used to compare various systems. The purpose of this evaluation is to assist medical practitioners and researchers in choosing the best method for different diagnostic applications.

Keywords: Automated detection, Illness treatment, Genetic material, Molecular diagnostics, Diagnostic techniques

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1. Introduction

Molecular diagnostic tools have transformed healthcare by providing quick, precise, and sensitive ways for identifying pathogenic infections, genetic abnormalities, and other medical issues. Compared to conventional diagnostic approaches, these techniques, which are based on the detection of nucleic acids DNA or RNA allow for the more specific identification of infections or genetic alterations (1,2). TrueNat, CBNAAT, and Real-Time PCR are among the most advanced molecular diagnostic methods that have been developed throughout time and are used in both clinical and research contexts (3-5).

The TrueNat is a point-of-care diagnostic device that detects certain DNA or RNA targets using a portable PCR-based method. It has gained popularity because it may provide quick results in environments with limited resources, which makes it perfect for application in underdeveloped or distant areas (6-7). The Cartridge-Based Nucleic Acid Amplification Test, or CBNAAT, is another molecular method frequently used to diagnose infectious illnesses like Tuberculosis (TB). In the treatment of drug-resistant TB, CBNAAT is an essential tool since it has the extra benefit of diagnosing rifampicin resistance (8-10). A popular technique in molecular diagnostics, real-time PCR, commonly referred to as quantitative PCR (qPCR), enables the real-time measurement of nucleic acids during the amplification process. Its great sensitivity and quantitative character have made it a routine instrument in clinical and research laboratories (11, 12). This study compares TrueNat, CBNAAT, and Real-Time PCR by looking at their fundamental concepts, clinical diagnostic uses, benefits, and limitations. The major emphasis will be on how different approaches differ in terms of diagnostic capabilities, convenience of use, cost-effectiveness, and sensitivity for identifying various infectious agents, such as TB, COVID-19, and other disorders. The evaluation will give a thorough comparison of various technologies and finish with recommendations for their best application in healthcare settings.

This study aims to help healthcare practitioners and researchers choose the best molecular diagnostic tool for their individual clinical requirements by assessing their effectiveness in various scenarios (5,13,14).

2. Materials and Methods

2.1 Principles of Each Technique

2.1.1 TrueNat

TrueNat is a portable, chip-based real-time PCR technology developed for quick detection of infectious pathogens, particularly in resource-limited situations, due to its small size and low cost (6-7).

To identify target pathogens with high sensitivity and specificity, nucleic acid extraction and amplification on a microchip are combined with fluorescence-based detection (7, 15).

The tiny design enables on-the-spot testing, removing the need for complex laboratory equipment, and it is noted for its ease of use, needing little technical knowledge (Figure 01) (6, 16).

With a fast turnaround time, it is suitable for identifying illnesses like TB and COVID-19 in distant and rural locations (6, 16).

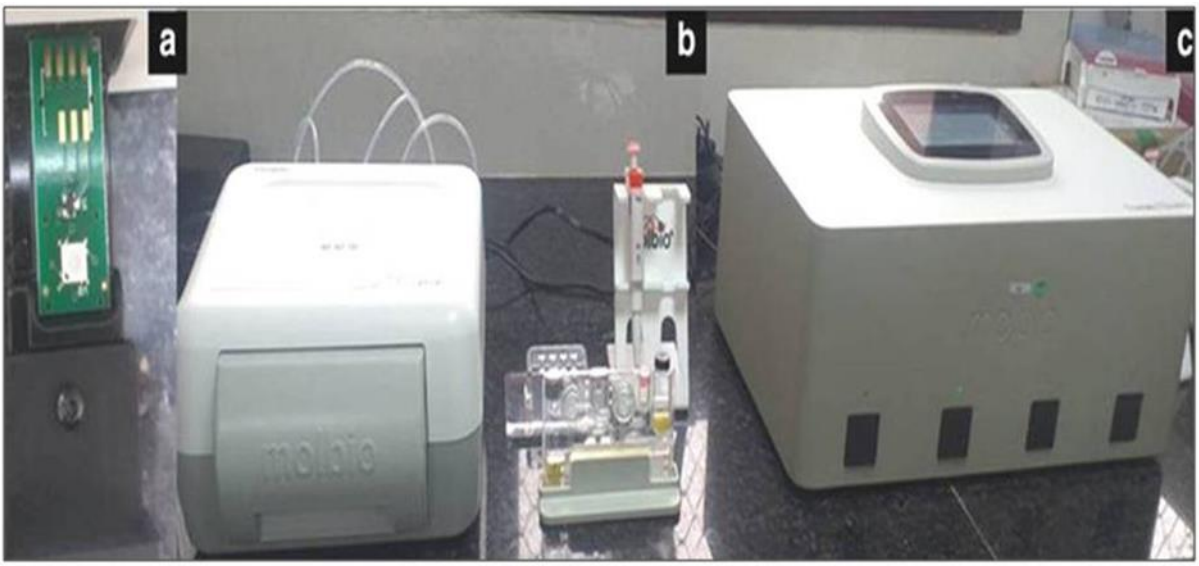

Figure 1. TrueNat extraction machine, **b**. Micropipette extraction kit and PCR reagent, **c**. TrueNat PCR machine (17). (**Prepared by Authors, 2026**).

2.1.2 CBNAAT (Cartridge-Based Nucleic Acid Amplification Test)

The CBNAAT is a modern molecular diagnostic tool that combines nucleic acid amplification and detection in a single, self-contained cartridge, making it useful for identifying infectious illnesses, including TB and rifampicin resistance (17). CBNAAT uses a fully automated, closed system to integrate sample preparation, nucleic acid extraction, amplification, and real-time detection, ensuring high accuracy while reducing contamination risk by performing all steps in a single, disposable unit (18, 19). The combination of these steps reduces processing time and the need for skilled personnel, thereby streamlining the workflow and improving diagnostic efficiency. This makes CBNAAT especially useful in high-burden and resource-limited situations, since it allows for the quick detection of TB and rifampicin resistance (Figure 02) (8, 9, 13).

Figure 2. CBNAAT test cartridge and Test Machine (**Prepared by Authors, 2026**).

2.1.3 Real-Time PCR (qPCR)

The Real-Time PCR, also known as quantitative PCR (qPCR), is a versatile and widely used molecular diagnostic technique for detecting and quantifying nucleic acids, making it essential in clinical applications such as diagnosing viral (e.g., COVID-19, HIV) and bacterial infections (e.g., tuberculosis), as well as identifying genetic mutations associated with disorders such as cancer and inherited diseases (1, 2, 15). This technology enables early and accurate diagnosis with faster findings than previous methods, as well as the ability to quantify target nucleic acids, allowing for viral load measurement, bacterial count, and genetic marker evaluation (20, 21). Real-Time PCR is especially useful for monitoring treatment progress because changes in nucleic acid levels can indicate therapeutic efficacy or resistance, and it can detect mutations, including drug resistance markers, which is critical for personalized medicine and chronic infection management (12, 15).

2.2 Applications of Each Technique

2.2.1 TrueNat

The TrueNat has been successfully used to diagnose a variety of infectious diseases, most notably tuberculosis (TB) and COVID-19, providing rapid and accurate detection of *Mycobacterium tuberculosis* and rifampicin resistance, which is critical for effective treatment planning (Table 1) (6, 16). It has also been used to diagnose leptospirosis and malaria in resource-limited locations, demonstrating its versatility in identifying a wide range of infectious diseases (6-7). The TrueNat's capacity to perform PCR-based assays at the point of care without requiring laboratory infrastructure makes it a helpful tool in areas where laboratory facilities are absent or restricted. Its mobility enables healthcare practitioners in remote and rural locations to promptly identify ailments, allowing for early treatment and better patient outcomes (6, 7, 16).

2.2.2 CBNAAT (Cartridge-Based Nucleic Acid Amplification Test)

The CBNAAT is frequently used for quick diagnosis of infectious illnesses, including tuberculosis (TB), as well as detecting rifampicin resistance. Its capacity to swiftly identify *Mycobacterium* TB in sputum samples and detect drug resistance is critical for commencing prompt and appropriate therapy, which improves patient outcomes (9, 13). Furthermore, CBNAAT is increasingly being used to diagnose both pulmonary and extrapulmonary TB, which reduces diagnostic delays (10, 18). CBNAAT is particularly useful in time-sensitive clinical scenarios, such as in HIV-positive patients or those with compromised immune systems, where early detection of TB or rifampicin-resistant TB is essential to prevent treatment failure and curb the spread of drug-resistant strains. By providing results within hours, compared to the days required for traditional cultures, CBNAAT significantly accelerates the clinical decision-making process (9, 13). In clinical settings where quick and accurate findings are crucial, CBNAAT is widely used. Its fast diagnostic capabilities enable medical personnel to quickly identify and treat infectious diseases in high-traffic areas, such as emergency rooms and intensive care units, which lowers mortality and morbidity, particularly in cases of multi-drug-resistant tuberculosis and other serious infections (Table 1) (10,19). CBNAAT provides a dependable and easy-to-use solution for managing high-pressure scenarios by combining sample preparation and amplification into a single device (8).

2.2.3 Real-Time PCR (qPCR)

The Real-time PCR is a critical method in molecular diagnostics, having numerous applications in viral, bacterial, and genetic illnesses (Table 1). It is widely used to detect viral infections such as COVID-19, HIV and influenza, as well as bacterial pathogens such as Mycobacterium tuberculosis and *Salmonella*, with quick and accurate results (1-2, 11, 15).

Real-Time PCR is also useful in genetic testing since it may discover mutations linked to cancer, genetic diseases, and other hereditary illnesses (15, 21). Its capacity to measure target nucleic acids makes it ideal for detecting viral loads (e.g., HIV, hepatitis), bacterial counts, and gene expression levels, all of which are critical for monitoring disease progression and response to therapy (20). Furthermore, Real-Time PCR excels in mutation detection, allowing for the identification of particular genetic alterations that inform personalized treatment strategies and aid in the discovery of drug-resistant bacteria. It also helps to assess therapy success since variations in nucleic acid levels over time represent the efficacy of treatments (2, 20).

Table 1. Comparative Analysis of TrueNat, CBNAAT and Real-Time PCR

Criteria	TrueNat	CBNAAT	Real-Time PCR
Speed and Efficiency	Fast (~1 hour) – ideal for rapid diagnostics **(6, 16)**.	Fast (1-2 hours) – effective for urgent clinical settings **(9,13)**	Slower (few hours) – ideal for comprehensive diagnostics **(1, 2)**.
Cost-Effectiveness	Low initial and per-test costs – suitable for resource-limited settings **(7)**.	Higher per-test cost due to cartridge price, but reasonable for rapid diagnostics **(10)**.	High initial setup costs, higher per-test costs **(12, 21)**.
Ease of Use	Highly user-friendly, minimal training required **(6)**.	User-friendly, minimal training needed, but slightly more complex than TrueNat **(8)**.	Requires technical expertise and specialized training **(11)**.
Diagnostic Accuracy	Good for TB and other infections, but lower sensitivity than Real-Time PCR **(16)**.	High sensitivity and specificity for TB and drug resistance **(9, 13)**.	Highest sensitivity and specificity, ideal for mutation detection **(14)**.
Portability	Highly portable, ideal for point-of-care and field use **(6)**.	Moderately portable, requires power source and space **(10)**.	Not portable, requires laboratory infrastructure **(1)**.

2.3 Advantages and Limitations of Each Technique

2.3.1 Advantages of TrueNat:

The TrueNat has various features that make it an excellent choice for resource-constrained environments (Table 2). It is a low-cost platform, with both the device and consumables reasonably priced, enabling widespread usage in rural and isolated locations (6-7).

The TrueNat's cost-effectiveness and portability make it ideal for use in resource-constrained environments where advanced diagnostic facilities may be scarce. Its capacity to provide speedy, reliable findings with minimum operator training has made a substantial contribution to

disease control in such places, notably for tuberculosis, a disease that severely affects many low-income nations (6, 15). Its small and lightweight form improves portability, allowing for speedy testing at the point of care, which is especially useful in low-resource settings (6, 16, 22-23). Furthermore, TrueNat produces rapid results, often within an hour, which is critical for timely diagnosis and treatment, especially for infectious diseases such as tuberculosis and COVID-19 (15).

Limitations: Sample quality might affect TrueNat's performance since some sample types, including sputum in TB diagnosis, may produce subpar results because of inadequate sample preparation or degradation (16). Additionally, while TrueNat is very effective for point-of-care testing, it cannot not be as precise or sensitive as sophisticated PCR technologies utilized in well-equipped lab settings. When faced with low pathogen loads or challenging diagnostic scenarios, this limitation becomes significant (6, 15).

2.3.2 Advantage of CBNAAT:

Sample, particularly for instances of TB and rifampicin resistance, CBNAAT provides quick diagnostic findings, usually in 1-2 hours, allowing for early diagnosis and timely treatment start (9, 13).

It's a completely automated procedure that greatly minimizes manual involvement and the requirement for qualified specialists by combining sample preparation, nucleic acid extraction, amplification, and detection into a single cartridge (8, 18, 24).

Additionally, CBNAAT is a dependable and easy-to-use diagnostic tool because of the smooth integration of several procedures into a single system, which reduces the possibility of contamination and improves testing efficiency (Table 2) (13, 19).

Limitations: The price of the cartridges is one of the main drawbacks of CBNAAT, which may prevent it from being widely used, especially in environments with limited resources (9, 13).

Furthermore, because CBNAAT depends on certain cartridges, testing is reliant on their supply and availability, potentially putting the system at risk of stock shortages (9).

The inability of CBNAAT to identify numerous pathogens concurrently in a single test is another disadvantage.

This limits its ability to screen for co-infections or diagnose many illnesses from a single sample (6, 15, 25-26).

2.3.3 Advantages of Real-Time PCR:

Particularly useful for detecting uncommon diseases or genetic alterations, real-time PCR is well known for its remarkable sensitivity, which allows for the detection of low amounts of nucleic acids (1-2, 27).

This technology is extremely useful for genetic research and infectious disease monitoring because of its capacity to measure nucleic acids in real-time, which enables accurate assessments of viral load, bacterial count, and gene expression levels (20-21, 28).

Additional uses for Real-Time PCR include monitoring therapy responses, identifying mutations, and diagnosing bacterial, viral, and genetic disorders (Table 2) (11,15).

Limitations: High setup costs are a problem for real-time PCR as the initial outlay for PCR equipment and reagents can be a major deterrent for labs, especially those with low resources (1, 12).

Furthermore, the technical complexity of the approach necessitates the use of qualified staff for both data interpretation and sample preparation, which may not be easily accessible in all healthcare settings (2, 12). Furthermore, certain communities, particularly those with limited resources, may not have access to the specialized equipment required for Real-Time PCR, such as PCR machines, reagents, and clean-room facilities (1, 14).

Table 2. Recent Advances and Innovations

Aspect	TrueNat	CBNAAT	Real-Time PCR
Innovations & Improvements	Enhanced portability and cost-effectiveness for point-of-care diagnostics **(6, 16)**.	Improved cartridge-based system with integrated sample preparation and detection **(9, 13)**.	Advanced multiplexing and digital PCR platforms for absolute quantification **(14, 20)**.
Multiplexing & Cloud Integration	Integration with cloud-based data management and mobile device compatibility **(7)**.	Limited multiplexing but potential for cloud-based data sharing **(10)**.	High-throughput platforms with advanced bioinformatics tools and cloud storage **(12, 21)**.
Role in Emerging Diseases	Effective in diagnosing TB and COVID-19 in resource-limited settings **(6, 16)**.	Rapid diagnosis of TB and drug-resistant infections in high-burden regions **(9, 13)**.	Essential for real-time tracking of viral mutations (e.g., SARS-CoV-2) **(1, 2)**.

3. Discussion

Since nucleic acid amplification-based testing methods like TrueNat, CBNAAT, and Real-Time PCR provide quick and precise results, molecular diagnostics has completely changed the way diseases are detected and treated. The ideas, uses, and diagnostic efficacy of these approaches differ; thus, it's critical to assess their advantages and disadvantages. TrueNat is a real-time, chip-based PCR technology intended for use in point-of-care environments. It decreases turnaround time and contamination hazards by combining sample preparation and amplification into a single system (7, 24, 28). In a variety of diagnostic applications, studies have shown its excellent sensitivity and specificity, even in environments with low resources (6). Furthermore, TrueNat is a good choice for decentralization due to its mobility and low infrastructure needs (16, 29, 30). But it's important to take into account restrictions like reliance on particular chemicals and the need for a steady power source (15).

The automated process of the cartridge-based nucleic acid amplification test CBNAAT has made it more popular by lowering manual handling mistakes and guaranteeing consistent findings (18). The closed-cartridge method is appropriate for urgent diagnostic situations because it reduces the possibility of cross-contamination and enables quick detection (13). Research has demonstrated its effectiveness in making very specific and sensitive diagnoses (19). However, the necessity for specialised equipment and the comparatively expensive cost of cartridges continues to be an obstacle to wider implementation (8, 31, 32). Additionally, compared to real-time PCR, CBNAAT has strong quantitative capabilities even while it offers qualitative detection (9).

Because of its unmatched sensitivity and capacity to measure nucleic acids, real-time PCR is still the gold standard in molecular diagnostics (1). Real-Time PCR, in contrast to CBNAAT and TrueNat, permits multiplexing, which permits the simultaneous detection of many targets in a single reaction (2). Furthermore, improvements in fluorescence-based detection and probe chemistries have improved Real-Time PCR's specificity and accuracy (11). However, compared to CBNAAT and TrueNat, the high setup costs, need for skilled staff, and slower turnaround time provide difficulties for point-of-care applications (12). Additionally, to prevent false-positive or false-negative outcomes, primer and probe optimization is still essential (33). According to a comparison of these methods, Real-Time PCR performs best in high-throughput and quantitative applications, CBNAAT is well-suited for quick, automated diagnostics, and TrueNat is best for field-based or low-resource situations because of its mobility. The future of molecular diagnostics is still being shaped by recent developments, such as the incorporation of digital PCR, improved cartridge efficiency, and smaller PCR platforms (21).

While each procedure has unique advantages, the choice is based on clinical needs, infrastructure availability, and economic considerations. Future research should focus on improving these technologies' accuracy, lowering costs, and expanding their usefulness in a variety of diagnostic contexts (20).

5. Conclusion

The TrueNat, CBNAAT, and Real-Time PCR are important advances in molecular diagnostics, with each providing distinct benefits in terms of sensitivity, automation, and mobility. TrueNat stands out for its versatility in decentralized settings, CBNAAT excels in quick and automated detection, and Real-Time PCR remains the gold standard for high-throughput and exact quantification. The ongoing growth of these procedures, fueled by technology advancements, offers increased diagnostic accuracy, cost, and accessibility. Future research should focus on increasing efficiency, lowering costs, and creating integrated platforms that incorporate the advantages of different technologies. By optimizing these technologies, molecular diagnostics can continue to transform early detection and disease treatment, eventually boosting global healthcare outcomes.

6. Declarations

6.1 Acknowledgments

It’s a review article so acknowledgement is not required.

6.2 Ethical Considerations

Not applicable.

6.3 Authors' Contributions

JD: Conceptualization, methodology, writing of original draft, **SRD**: Investigation, Visualization, Supervision, **MM**: Supervision and editing, **SAD**: Supervision, grammar checking, review and submission. All authors have read and agreed to the final version of the manuscript.

6.4 Conflict of Interest

The authors declare no competing conflicts of interest.

6.5 Fund or Financial Support

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6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

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