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AS-PCR IN MOLECULAR DIAGNOSTICS: A SYSTEMATIC REVIEW OF APPLICATIONS IN GENETIC DISEASE SCREENING

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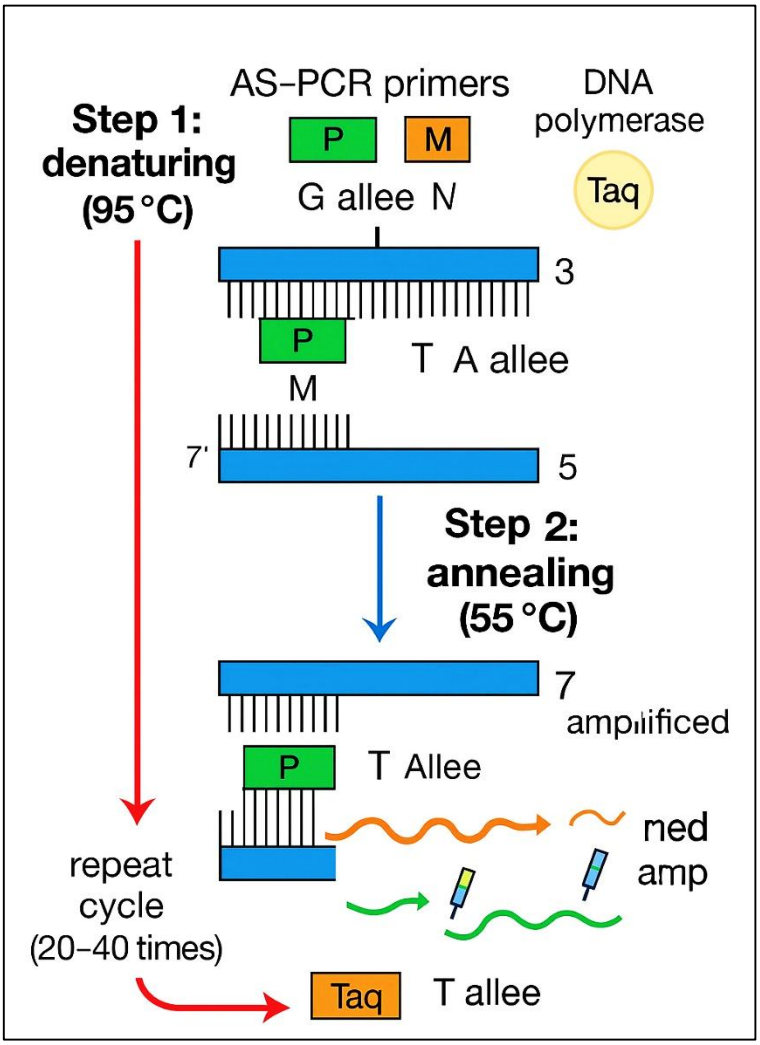
Abstract
Allele-Specific Polymerase Chain Reaction (AS-PCR) is a highly specific molecular technique widely employed for the detection of single nucleotide polymorphisms (SNPs) and known point mutations in genetic disease screening. This study presents a comprehensive meta-analysis to evaluate the diagnostic accuracy, clinical applicability, and global integration of AS-PCR across diverse genetic conditions, including thalassemia, cystic fibrosis, sickle cell disease, hereditary cancers, and somatic mutations. Utilizing the PRISMA 2020 framework, peer-reviewed articles published between 2000 and 2024 were systematically identified and analyzed. Eligibility criteria included empirical studies reporting sensitivity, specificity, or comparative diagnostic performance of AS-PCR against standard molecular methods. A random-effects model was used to compute pooled sensitivity, specificity, and diagnostic odds ratios, while heterogeneity and publication bias were assessed through I^2 statistics and funnel plot analysis. The meta-analysis revealed consistently high diagnostic performance of AS-PCR, with pooled sensitivity and specificity values exceeding 95%, confirming its utility as a frontline diagnostic tool. The findings also highlighted AS-PCR's versatility in low-resource settings, its enhanced sensitivity in digital and microfluidic formats, and its integration into national screening and reproductive health programs. Overall, this study affirms AS-PCR's critical role in precision diagnostics and public health genomics, particularly for targeted mutation screening and cost-sensitive healthcare environments.

Keywords
Allele-Specific PCR; Genetic Disease Screening; Molecular Diagnostics; Single Nucleotide Polymorphism (SNP) Detection; Precision Medicine

INTRODUCTION

Polymerase Chain Reaction (PCR), first developed by Kary Mullis in 1983, revolutionized molecular biology by enabling the amplification of specific DNA sequences (Gaudet et al., 2009). Among its several modifications, Allele-Specific PCR (AS-PCR), also referred to as amplification refractory mutation system (ARMS-PCR), stands out for its high specificity in detecting known mutations at single nucleotide resolution (Lee et al., 2016). AS-PCR uses primers designed to selectively amplify alleles differing by a single nucleotide, enabling discrimination between wild-type and mutant alleles in a single reaction (Ugozzoli & Wallace, 1992). This technique holds particular value in the field of molecular diagnostics due to its rapid turnaround, low cost, and adaptability to various clinical applications. In contrast to sequencing-based approaches, AS-PCR does not require extensive post-PCR processing, making it suitable for use in resource-constrained settings and high-throughput screening programs. Its applicability spans diverse areas including oncology, hematology, metabolic disorders, and infectious disease diagnostics (Balaji & Parani, 2024) The global emphasis on early detection of hereditary diseases, especially in populations with a high prevalence of consanguinity or founder mutations, underscores the international significance of AS-PCR as a frontline genotyping strategy (Kalendar, 2021).

Figure 1: Allele-Specific PCR (AS-PCR) Workflow



Genetic disease screening forms a cornerstone of public health and preventive medicine by enabling early identification of pathogenic variants that predispose individuals to inherited conditions (Kalendar, Baidyussen, et al., 2022). Conditions such as thalassemia, cystic fibrosis, phenylketonuria, and Duchenne muscular dystrophy are among those that benefit from early diagnosis and intervention (Makhoul et al., 2020). Molecular diagnostics plays a critical role in confirming clinical suspicion and in expanding neonatal and carrier screening panels (Myakishev et al., 2001). AS-PCR has been extensively applied in the genotyping of the IVS-I-5 (G>C) mutation in β-thalassemia, ΔF508 mutation in CFTR gene for cystic fibrosis, and G20210A mutation in the prothrombin gene. These applications have demonstrated diagnostic accuracy, reproducibility, and clinical relevance across populations in Europe, Asia, the Middle East, and Latin America (Kalendar, Baidyussen, et al., 2022). In population-based screening programs, especially in countries like Iran, India, Egypt, and Brazil, AS-PCR has been instrumental in identifying carriers, enabling genetic counseling, and reducing the burden of hereditary disorders (Kalendar, 2021). The widespread use of AS-PCR in these contexts reflects its alignment with public health priorities and its robustness in diverse clinical and laboratory environments.

Clinical sensitivity and specificity are paramount in any diagnostic assay, and AS-PCR consistently meets high standards when employed with rigorously validated primer designs (Kalendar, Shustov, et al., 2022). The technique's underlying mechanism relies on the inability of Taq polymerase to extend mismatched primer termini, allowing precise discrimination between alleles differing by a single base (Hossen & Atiqur, 2022; Ugozzoli & Wallace, 1992). Studies have demonstrated that AS-PCR achieves sensitivity exceeding 95% in various genetic loci, provided primer optimization and sample quality are stringently controlled(Khan, 2025; Wangkumhang et al., 2007). Furthermore, its compatibility with endpoint and real-time detection platforms enhances its clinical adaptability, including use in low-throughput rural laboratories and centralized diagnostic centers(Zahir, Rajesh, Tonmoy, et al., 2025). In comparison to more resource-intensive methods like Sanger sequencing or next-generation sequencing (NGS), AS-PCR delivers rapid results and is cost-effective for single mutation analysis, which is particularly advantageous in targeted mutation screening, familial studies, and cascade testing. For instance, in hereditary breast and ovarian cancer (HBOC) syndromes, BRCA1/BRCA2 founder mutations can be reliably identified using AS-PCR in Ashkenazi Jewish and South Asian populations (Li et al., 2020; Hossen et al., 2023). Beyond monogenic disorders, AS-PCR has shown efficacy in pharmacogenomics and precision oncology, particularly in identifying actionable somatic mutations associated with drug resistance or treatment efficacy (Tahmina Akter, 2025). The detection of KRAS codon 12/13 mutations in colorectal cancer, EGFR exon 19 deletions and L858R mutations in lung adenocarcinoma, and BRAF V600E mutations in melanoma are among the most notable clinical applications of AS-PCR in oncology. These markers are critical for therapeutic decisions, including the selection of tyrosine kinase inhibitors or monoclonal antibodies (Makhoul et al., 2020; Rajesh et al., 2023). AS-PCR's rapid turnaround time allows for timely decisions in clinical oncology workflows, particularly in settings where sequencing capacity is limited or sample quantities are minimal (Choi et al., 2017; Roksana, 2023). In hematological malignancies such as acute myeloid leukemia and chronic myeloid leukemia, AS-PCR has also been employed for monitoring minimal residual disease and detecting resistance mutations such as FLT3-ITD and T315I (Baidyussen, et al., 2022; Shamima et al., 2023). The technique's precision and reliability in these contexts underline its growing relevance in both diagnostic and prognostic frameworks of cancer management.

Clinical Applications of Allele-Specific PCR (AS-PC)

- Sensitivity >95% at validated genetic loci
- Endpoint and real-time detection platforms
- Targeted mutation screening and cascade testing
- BRCA1/BRCA2 founder mutations in HBOC syndromes
- Actionable somatic mutations in precision oncology
- KRAS codon 12/13 mutations in colorectal cancer
- EGFR exon 19 deletions and L858R in lung adenocarcinoma
- BRAF V600E mutation in melanoma
- Minimal residual disease and resistance mutations in leukeemiia

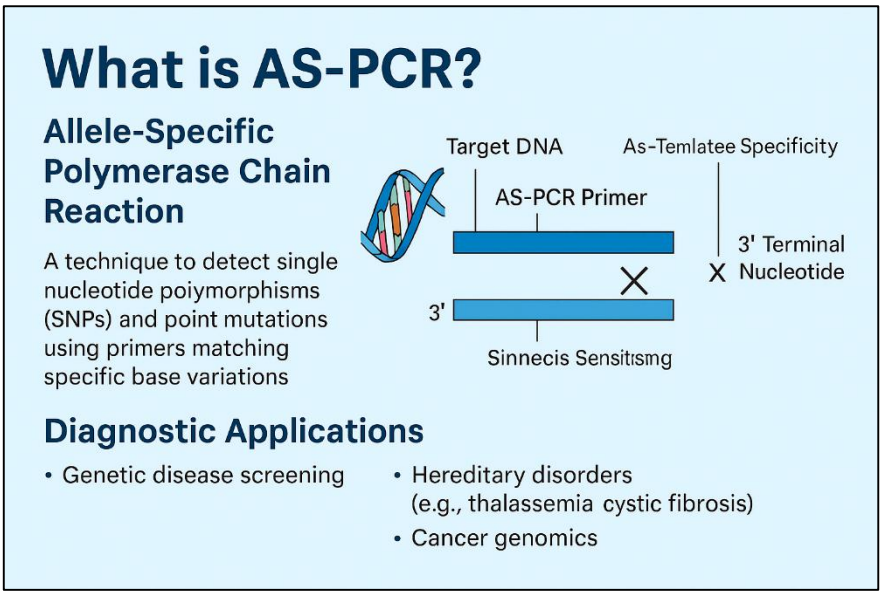
The primary objective of this systematic review is to comprehensively evaluate the role and effectiveness of Allele-Specific Polymerase Chain Reaction (AS-PCR) as a molecular diagnostic tool in the screening of genetic diseases. This review seeks to synthesize existing empirical evidence and methodological approaches that employ AS-PCR in the detection of pathogenic variants responsible for inherited disorders, including but not limited to cystic fibrosis, β-thalassemia, sickle cell anemia, and various oncogenic mutations (Jahan et al., 2022). Given the critical demand for early and accurate detection of genetic abnormalities in clinical and public health settings, the objective extends to examining how AS-PCR contributes to timely diagnosis, carrier identification, and prenatal screening strategies across diverse populations (Masud et al., 2025). This review systematically explores peer-reviewed literature to assess the clinical sensitivity, specificity, cost-effectiveness, and operational feasibility of AS-PCR, particularly in comparison to other molecular diagnostics such as Sanger sequencing and next-generation sequencing (NGS) (Qibria & Hossen, 2023). Additionally, the review identifies the extent to which AS-PCR has been adopted in different geographic and healthcare

contexts, analyzing its practical integration into newborn screening programs, cancer diagnostics, and pharmacogenomics (Masud et al., 2023). Another core objective is to highlight the methodological frameworks used in AS-PCR assay development, including primer design parameters, amplification protocols, and validation techniques. By investigating these aspects, the review aims to map the utility and limitations of AS-PCR in both high-throughput laboratories and resource-limited clinical environments (Razzak et al., 2024). The objective also includes evaluating the role of AS-PCR in detecting known single nucleotide polymorphisms (SNPs) and mutation hotspots that are clinically actionable, particularly in relation to gene-targeted therapies and personalized medicine. Through this systematic assessment, the review seeks to consolidate scientific understanding and provide a critical appraisal of AS-PCR's diagnostic value, ultimately offering evidence-informed insights into its application in the screening and management of genetic diseases.

What is AS-PCR?

Allele-Specific Polymerase Chain Reaction (AS-PCR), a derivative of the traditional PCR method, is a molecular diagnostic technique designed to detect specific nucleotide variants, particularly single nucleotide polymorphisms (SNPs) and point mutations, by leveraging the principle of primer-template specificity. First conceptualized through the amplification refractory mutation system (ARMS) by (Ye et al., 1992), AS-PCR functions by using oligonucleotide primers whose 3′ terminal nucleotide corresponds precisely to the base of interest in the target DNA. If a mismatch exists at the 3′ end of the primer-template interface, the DNA polymerase fails to extend the strand, effectively allowing for allele discrimination. The mechanism of action depends heavily on the thermodynamic instability induced by mismatches at the 3' primer terminus, thereby enabling precise identification of wild type versus mutant alleles within a single reaction. Several design enhancements, such as the introduction of mismatch destabilizing agents or locked nucleic acids (LNAs), have further improved the specificity and sensitivity of AS-PCR reactions (Newton et al., 1989). This technique is particularly valuable in cases where rapid and targeted mutation detection is necessary, especially for known mutations within high-risk populations. Its implementation in genotyping workflows is facilitated by readily available primer design tools such as Primer3 and AlleleID, which support assay development by minimizing non-specific amplification. Unlike sequencing methods that require additional steps such as base calling and alignment, AS-PCR yields binary outputs—amplification or no amplification—making it an optimal method for high-throughput and low-cost diagnostic applications (Lf & Ém, 2015).

Figure 2: Overview of AS-PCR



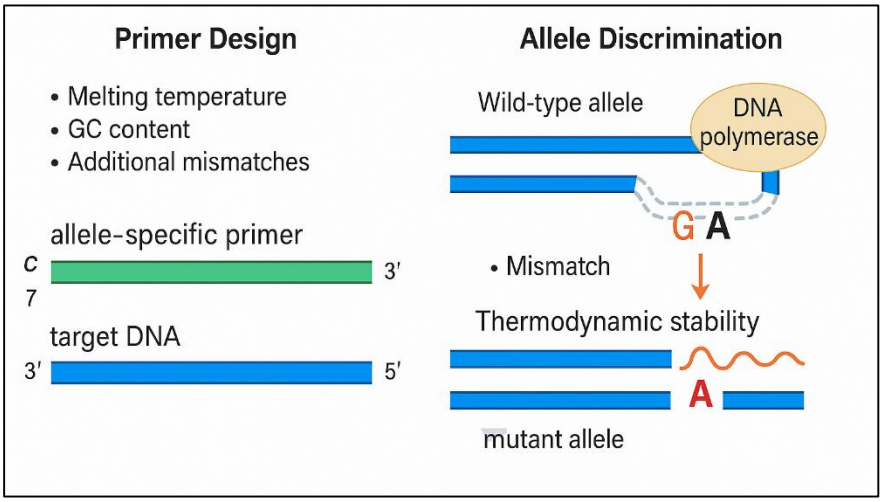
The clinical relevance of AS-PCR has been underscored in various diagnostic contexts, particularly where detection of known mutations is paramount to early intervention or treatment planning. In genetic disease screening, AS-PCR is widely applied for its high analytical sensitivity and specificity in

detecting single-point mutations across a range of hereditary disorders such as thalassemia, cystic fibrosis, and sickle cell anemia (Furtado et al., 2019; Md et al., 2025). For instance, in β-thalassemia carrier detection, AS-PCR is routinely used to identify common mutations like IVS-I-5 (G→C) and codon 41/42 (-TCTT), especially in South Asian and Mediterranean populations (Park et al., 2006; Sazzad, 2025). Similarly, in cystic fibrosis diagnostics, the ΔF508 mutation in the CFTR gene can be accurately identified using AS-PCR assays, allowing for neonatal and prenatal screening in at-risk populations (Ariful et al., 2023). Beyond monogenic diseases, AS-PCR has been employed in cancer genomics for identifying actionable mutations such as KRAS in colorectal cancer, EGFR in lung cancer, and BRAF V600E in melanoma, enabling the personalization of treatment strategies based on molecular profiles (In et al., 2010; Akter & Razzak, 2022). The rapid turnaround time and low resource requirement make AS-PCR particularly suitable for clinical laboratories in low- and middle-income countries where sequencing facilities may be limited. Moreover, the ability to integrate AS-PCR with real-time detection platforms enhances its utility in dynamic clinical settings where timely results are critical. The cumulative evidence from diverse studies affirms AS-PCR's reliability as a frontline genotyping technique that balances precision, speed, and cost-effectiveness in modern molecular diagnostics (Tonoy & Khan, 2023).

Primer Design Strategies and Allele Discrimination Mechanisms

Effective primer design is the cornerstone of successful Allele-Specific Polymerase Chain Reaction (AS-PCR), where the ability to discriminate between wild-type and mutant alleles hinges on the precise alignment of the primer's 3′ terminal nucleotide with the target sequence. Unlike conventional PCR primers, AS-PCR primers are designed to perfectly complement one allele while mismatching the other at the 3′ end, thereby enabling selective amplification (Gaudet et al., 2009; Tonmoy & Arifur, 2023).

Figure 3: Primer Design Strategies and Allele Discrimination Mechanisms in AS-PCR



To achieve this, several algorithmic and bioinformatics tools have been developed that optimize parameters such as melting temperature (Tm), GC content, and secondary structure avoidance to ensure high specificity and efficiency (Masud, 2022; Ugozzoli & Wallace, 1991). Tools such as Primer3, AlleleID, and BatchPrimer3 allow users to input SNP positions and design allele-specific primers with internal mismatches to enhance discrimination capacity (Lee et al., 2016; Alam et al., 2023). These tools also evaluate cross-reactivity risks, homodimer formation, and secondary binding sites to reduce non-specific amplification, which is particularly important in multiplex PCR settings. Furthermore, allele discrimination is improved by adding additional mismatches near the 3' end of the primer, known as "double-mismatch" strategies, which reduce the likelihood of false-positive amplification from the non-target allele. Such strategies are especially useful in SNP genotyping assays where the distinction between alleles must be binary and unambiguous. Additionally, computational tools now integrate genomic context analysis, allowing for primer selection that avoids repetitive or low-complexity regions, thereby enhancing the specificity of amplification in complex genomes. Thus, precision in primer design through algorithm-guided platforms forms a critical determinant of AS-PCR success in molecular diagnostics.

The success of allele discrimination in AS-PCR is intimately linked to the thermodynamic properties of the primer-template complex, especially at the 3′ terminus where DNA polymerase initiates elongation. A single nucleotide mismatch at this position drastically reduces the binding affinity and polymerase extension efficiency, thereby enabling the preferential amplification of the perfectly matched allele (Li

et al., 2006; Zahir et al., 2025). Studies have shown that the mismatch type—whether purine-purine, pyrimidine-pyrimidine, or purine-pyrimidine—affects the melting temperature and overall duplex stability, impacting the discrimination power. To enhance mismatch sensitivity, modified nucleotides such as locked nucleic acids (LNAs) or peptide nucleic acids (PNAs) are often introduced into primers, increasing their binding stringency and allele-specific fidelity (Kalendar, Shustov, et al., 2022; Sazzad, 2025b). These modified primers create a more rigid primer-template duplex, further discouraging extension from mismatched templates and thereby improving diagnostic accuracy. Additionally, manipulating annealing temperatures during thermal cycling enhances specificity, as higher temperatures favor only the most stable primer-template pairings. In clinical practice, these thermodynamic strategies are particularly crucial for detecting low-frequency variants or somatic mutations in heterogeneous samples such as tumor biopsies. Real-time AS-PCR systems further refine specificity through fluorescence-based probe hybridization, allowing for the monitoring of reaction kinetics and early termination of non-specific amplifications (Abdullah Al et al., 2022; Lake et al., 2009). Combined with advanced primer design algorithms, these enhancements ensure high assay robustness, making AS-PCR a reliable technique even in complex diagnostic applications. Through these thermodynamic and biochemical improvements, AS-PCR achieves a level of precision that rivals more complex genotyping platforms while maintaining lower cost and operational simplicity.

Quantitative assessments of AS-PCR performance metrics

The diagnostic performance of Allele-Specific Polymerase Chain Reaction (AS-PCR) is frequently evaluated through core performance metrics such as sensitivity, specificity, accuracy, and reproducibility, which collectively determine its utility in clinical and screening contexts. Sensitivity in AS-PCR refers to the ability to correctly detect the presence of a target mutant allele, even in a background of excess wild-type DNA, while specificity refers to the method's ability to avoid amplification of non-target alleles (Ugozzoli & Wallace, 1992; Zahir et al., 2023). Several studies have reported that, under optimized conditions, AS-PCR can achieve diagnostic sensitivity levels exceeding 95%, especially for common point mutations such as those in the CFTR gene for cystic fibrosis or HBB gene for thalassemia (Li et al., 2006). The use of stringent primer design and thermocycling protocols further improves specificity, often surpassing 98% in single-target applications (Lee et al., 2016). Comparative analyses demonstrate that AS-PCR outperforms conventional gel-based PCR in both speed and accuracy, while providing comparable results to Sanger sequencing for single-nucleotide variant detection, but with lower cost and faster turnaround (Ugozzoli & Wallace, 1991). In resource-limited environments where sequencing infrastructure is not widely available, AS-PCR's high specificity and low false-positive rates make it a favorable diagnostic tool. Furthermore, the implementation of internal controls and duplex formats helps monitor reaction efficiency and validate results, ensuring reproducibility across different batches and laboratories (Gaudet et al., 2009). Studies have also validated AS-PCR's analytical robustness in detecting minor allele frequencies as low as 1–5%, highlighting its relevance in heterogeneous samples such as tumor biopsies or carrier states. These findings collectively affirm AS-PCR's strong diagnostic metrics across a range of clinical applications.

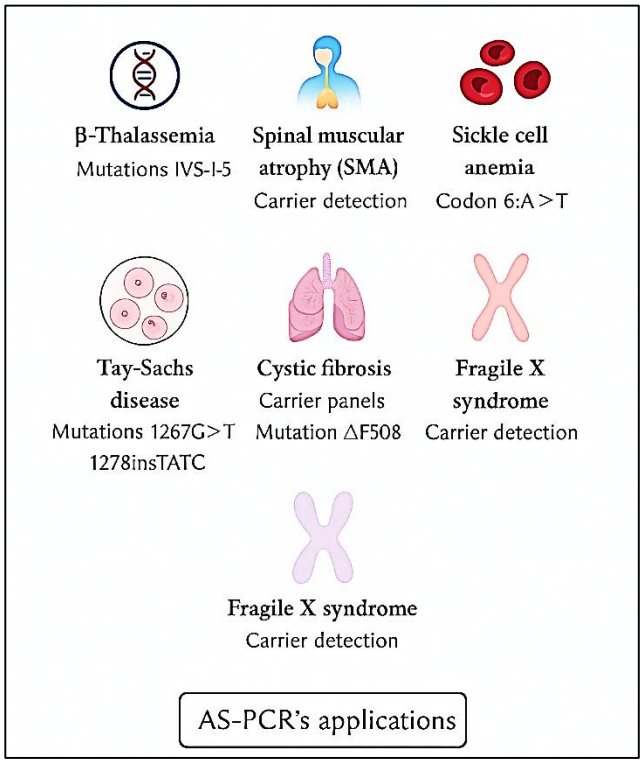
Beyond sensitivity and specificity, the reproducibility and error rates of AS-PCR are critical metrics that influence its acceptance in clinical molecular laboratories. Reproducibility refers to the consistency of assay results when repeated under identical or similar experimental conditions, and it is largely governed by primer fidelity, template quality, and reaction setup. Studies across various mutation panels have consistently demonstrated inter-laboratory reproducibility rates of over 90% when standardized protocols are applied, with variation arising primarily from suboptimal primer design or template contamination. Additionally, AS-PCR shows markedly low error rates—often below 2%—when detecting known SNPs or founder mutations, especially in monogenic diseases where mutation hotspots are well characterized (Newton et al., 1989). Compared to techniques such as restriction fragment length polymorphism (RFLP) analysis or allele-specific oligonucleotide hybridization, AS-PCR provides a more direct and rapid mutation detection mechanism, reducing post-PCR handling and interpretation steps. Moreover, the risk of false-negative results in AS-PCR can be mitigated through the inclusion of positive controls and amplification-internal controls, which also support quality assurance practices in diagnostic labs. When benchmarked against next-generation sequencing (NGS), AS-PCR remains favorable for targeted, single-variant detection due to its rapid assay time and

minimal computational demand, although NGS is more suitable for multiplexed or exploratory mutation profiling. Real-time AS-PCR platforms further improve performance metrics by allowing real-time monitoring of reaction kinetics, increasing diagnostic confidence through amplification curves and melting profiles (Lee et al., 2016). Thus, the quantifiable performance indicators of AS-PCR reinforce its reliability and scalability in both high-throughput and point-of-care diagnostic models.

Applications in Inherited Monogenic Disorders

AS-PCR has demonstrated substantial clinical value in the detection and management of hemoglobinopathies, particularly thalassemia and sickle cell anemia, where rapid and accurate genotyping of known mutations is essential for carrier identification and prenatal diagnosis. In β-thalassemia, mutations such as IVS-I-5 (G>C), IVS-I-110 (G>A), and codon 41/42 (-TCTT) are prevalent across South Asian, Middle Eastern, and Mediterranean populations (Ugozzoli & Wallace, 1991). Studies have shown that AS-PCR enables highly sensitive detection of these mutations, with specificity exceeding 98% when using optimized primer designs (Brusa et al., 2021). The technique is particularly valuable in mass screening programs and targeted genetic counseling initiatives, where cost-effective, high-throughput methods are needed (Makhoul et al., 2020). Similarly, AS-PCR has proven highly effective in detecting the ΔF508 mutation in the CFTR gene, which accounts for a major proportion of cystic fibrosis cases, especially in Caucasian populations. The ability of AS-PCR to target both homozygous and heterozygous ΔF508 variants facilitates early diagnosis and intervention, which are critical for disease management. Additional CFTR mutations such as G542X and N1303K have also been successfully identified using multiplex AS-PCR approaches, supporting its application in expanded CF carrier panels. These studies underscore AS-PCR's clinical utility as a frontline diagnostic tool in high-prevalence populations, where its speed, cost-efficiency, and mutation-specific targeting enable widespread implementation in national screening strategies.

Figure 4: Diagnostic Applications of AS-PCR in Inherited Monogenic Disorders



The application of AS-PCR extends beyond hemoglobinopathies and cystic fibrosis to other monogenic disorders, such as spinal muscular atrophy (SMA), sickle cell anemia, Tay-Sachs disease, and fragile X syndrome, all of which are associated with recurrent mutations that can be effectively screened using allele-specific strategies. In SMA, caused predominantly by homozygous deletions or mutations in the SMN1 gene, AS-PCR has enabled rapid and cost-effective carrier detection, especially in prenatal settings and preimplantation genetic testing (Rickert et al., 2004). Its high detection accuracy supports early counseling for couples at risk of transmitting this debilitating neurodegenerative disorder. In sickle cell anemia, AS-PCR is routinely applied to detect the A>T substitution in codon 6 of the HBB gene, known as the HbS mutation, which leads to the production of abnormal hemoglobin S. Numerous studies have confirmed that AS-PCR can reliably identify both homozygous and heterozygous states of HbS, making it a valuable tool for newborn screening and targeted interventions in high-risk communities (Bottema & Sommer, 1993). In ethnic carrier screening, particularly among Ashkenazi Jews and French-Canadians, AS-PCR is frequently used for detecting the HEXA gene mutation responsible for Tay-Sachs disease. The G269S and 1278insTATC mutations, among the most common HEXA variants, can be efficiently genotyped with allele-specific primers, ensuring early risk identification and informed reproductive decisions. Additionally, although fragile X syndrome involves CGG repeat expansions rather than SNPs, AS-PCR has been modified in certain studies to

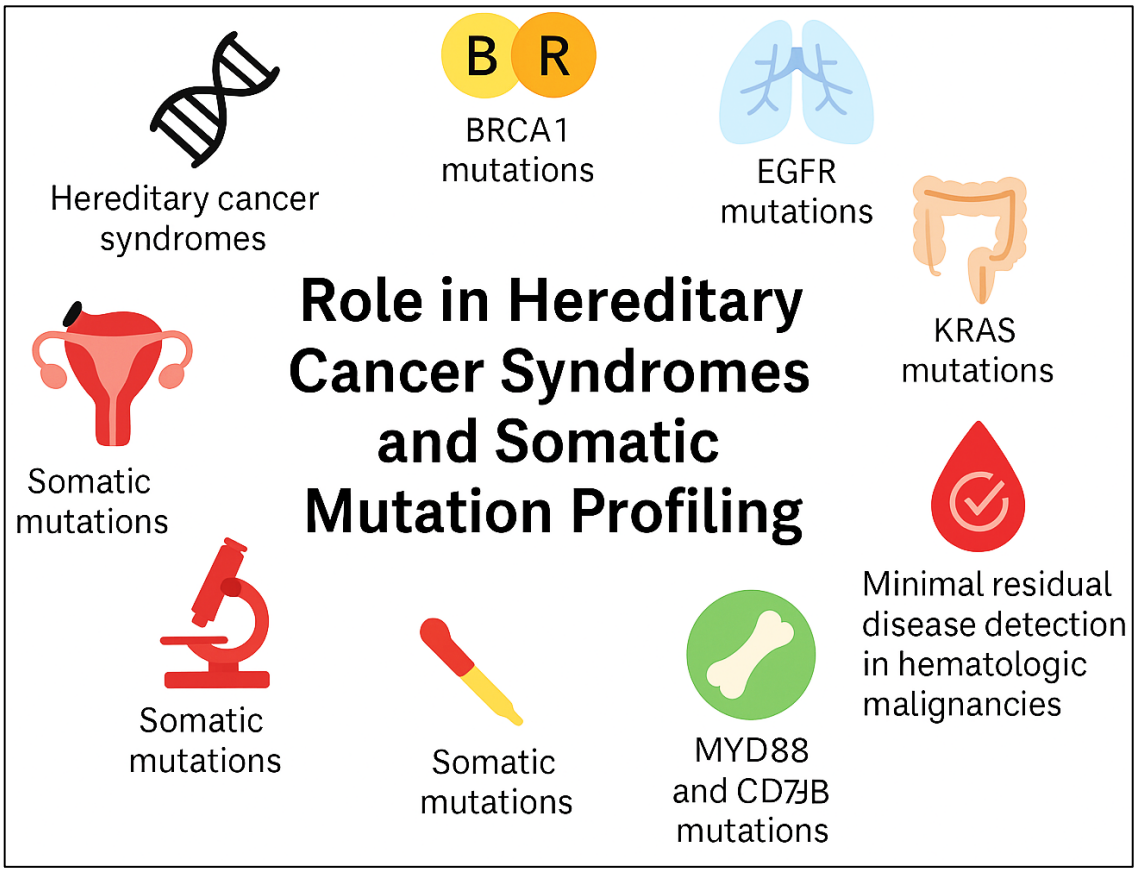
identify specific point mutations or carrier states within FMR1 alleles. These findings reflect the adaptability of AS-PCR in a wide array of inherited conditions, offering robust genotyping solutions in both clinical diagnostics and public health genomics.

Role in Hereditary Cancer Syndromes and Somatic Mutation Profiling

Allele-specific PCR (AS-PCR) has become an essential tool in the molecular profiling of hereditary cancer syndromes, particularly for identifying pathogenic mutations in BRCA1 and BRCA2 genes. These tumor suppressor genes are commonly associated with hereditary breast and ovarian cancer (HBOC) syndromes, where early detection of specific founder mutations can guide surveillance, preventive interventions, and family-based risk assessments (Rickert et al., 2004). AS-PCR offers a rapid and cost-effective method for genotyping known mutations such as 185delAG, 5382insC, and 6174delT, which are prevalent in populations like Ashkenazi Jews and individuals from South Asia. Studies have shown that AS-PCR achieves over 95% sensitivity and specificity for these mutations, making it a viable alternative to more expensive sequencing methods, especially in population-scale screening programs. In addition to germline mutation detection, AS-PCR has been employed to identify somatic mutations that are clinically actionable, particularly in tumors where rapid genotyping is necessary to inform treatment decisions (Kozlowski et al., 2007). For example, in lung adenocarcinoma, the identification of EGFR exon 19 deletions and L858R point mutations via AS-PCR enables the selection of patients for tyrosine kinase inhibitor (TKI) therapy, improving treatment outcomes. Similarly, KRAS mutations in codons 12 and 13 in colorectal cancer and BRAF V600E in melanoma have been successfully genotyped using AS-PCR, helping clinicians determine patient eligibility for monoclonal antibodies and targeted agents. The use of AS-PCR in these contexts underscores its value in precision oncology where rapid turnaround and focused mutation screening are critical.

In addition to its role in hereditary cancer syndromes, AS-PCR is increasingly employed in the detection of somatic mutations and the monitoring of minimal residual disease (MRD) in hematologic malignancies. MRD refers to the presence of residual cancer cells after treatment that are below the threshold of morphologic detection, and its accurate assessment is vital for prognosis and relapse prediction in leukemia and lymphoma patients (Rickert et al., 2004). AS-PCR has demonstrated particular utility in identifying recurrent point mutations in genes such as FLT3, NPM1, and IDH1/2 in acute myeloid leukemia (AML), where the early detection of these mutations can guide risk stratification and post-remission therapy. The technique's high specificity allows for allele-level quantification of mutant clones, even when present at low frequencies (1–5%) in a background of normal cells (Makhoul et al., 2020). Additionally, in chronic myeloid leukemia (CML), AS-PCR is used to monitor BCR-ABL1 T315I mutations, which confer resistance to first- and second-generation TKIs, thereby influencing subsequent therapy selection. Studies have shown that AS-PCR outperforms conventional gel electrophoresis-based methods in detecting these resistant subclones, with a rapid turnaround time that facilitates dynamic treatment planning. The integration of AS-PCR with real-time platforms further enhances its application in MRD detection by allowing for quantitative fluorescence-based tracking of mutational burden. In lymphoid malignancies, such as diffuse large B-cell lymphoma, AS-PCR is also applied to detect somatic mutations in MYD88 and CD79B, providing molecular insights that support targeted therapeutic approaches. Collectively, the use of AS-PCR in somatic mutation detection and MRD monitoring demonstrates its vital role in precision hematology and real-time oncologic decision-making.

Figure 5: Visual Overview of AS-PCR Applications in Hereditary Cancer Syndromes and Somatic Mutation Profiling



Integration of AS-PCR in Screening Programs

The integration of Allele-Specific Polymerase Chain Reaction (AS-PCR) into national and population-based genetic screening programs has proven to be a practical and impactful strategy for early identification of inherited genetic conditions. Countries with a high prevalence of specific monogenic disorders, such as thalassemia, cystic fibrosis, and Tay-Sachs disease, have increasingly adopted AS-PCR-based protocols for mass carrier screening and neonatal testing due to the method's high specificity, cost-effectiveness, and adaptability to local infrastructure (Alanio et al., 2015). In Iran, for example, national thalassemia prevention programs have incorporated AS-PCR to detect common mutations like IVS-I-5 and codon 8/9 (+G), enabling effective premarital counseling and risk reduction strategies. Similar integration has been documented in India, where regional genetic centers use AS-PCR as a frontline tool in identifying β-thalassemia and sickle cell disease mutations among diverse ethnic groups. In Brazil, public health authorities have implemented AS-PCR in neonatal screening for cystic fibrosis, focusing on the ΔF508 mutation, which represents a significant disease burden in the population. These implementations underscore the method's scalability and relevance in countries with limited access to sequencing technologies. Moreover, the short turnaround time and minimal technical requirements of AS-PCR facilitate its deployment in primary healthcare settings, enabling early detection and management of genetic disorders at the community level. The reliability of allele-specific assays in low-resource environments has also led to its adoption in refugee and migrant health programs, where quick and accurate identification of carriers is essential for family planning and preventive care (Dhib et al., 2013).

AS-PCR has been increasingly integrated into reproductive health frameworks, particularly in preconception carrier screening, prenatal diagnostics, and newborn genetic testing. In reproductive medicine, AS-PCR is used to identify high-risk couples for disorders such as spinal muscular atrophy (SMA), cystic fibrosis, and Tay-Sachs disease, allowing for informed decision-making before conception or in early gestation. The simplicity and speed of AS-PCR make it suitable for preimplantation genetic diagnosis (PGD) during in vitro fertilization procedures, where embryos can

be screened for single-gene disorders before implantation (Carlsgart et al., 2008). For prenatal diagnostics, the ability to rapidly detect pathogenic mutations using chorionic villus or amniotic fluid samples supports clinical decision-making during critical windows of fetal development (Lockhart et al., 2021). AS-PCR has also been employed in early fetal screening for β-thalassemia and SMA, offering reliable detection of both homozygous and heterozygous mutations with high clinical accuracy. In neonatal screening programs, AS-PCR provides an effective solution for diseases with known mutational hotspots, such as ΔF508 in cystic fibrosis or the HbS mutation in sickle cell anemia, enabling immediate postnatal intervention. These applications are especially impactful in populations with high rates of consanguinity or founder mutations, where early detection can significantly reduce disease burden. The method's low cost and compatibility with dried blood spots and other non-invasive sampling techniques further enhance its suitability for nationwide newborn screening platforms. Collectively, the deployment of AS-PCR across various stages of reproductive and early-life screening highlights its central role in contemporary genomic medicine and public health strategies.

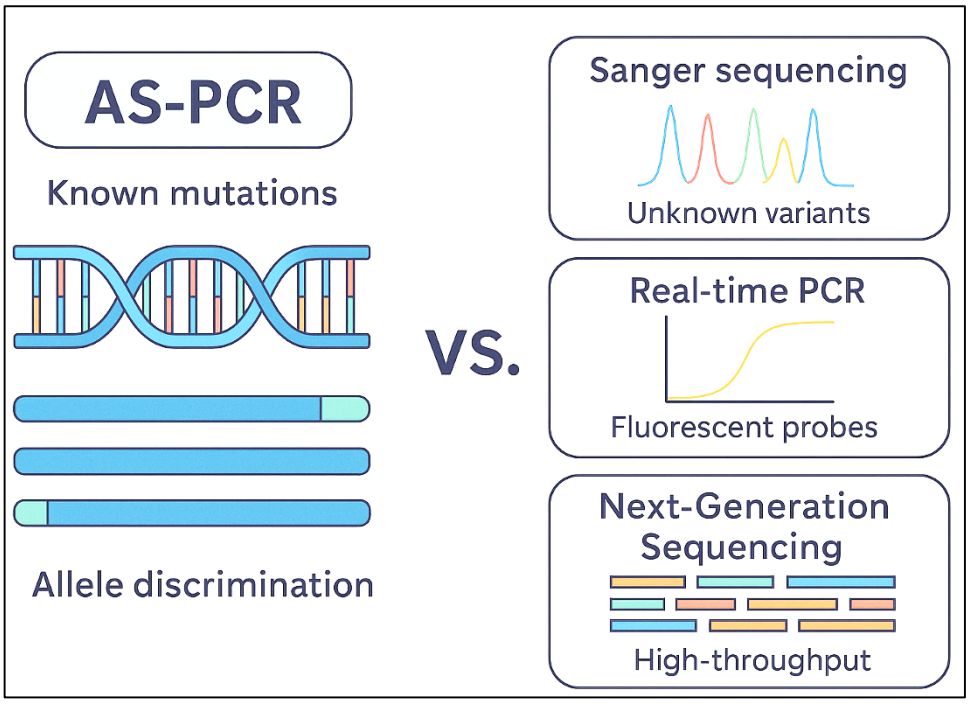
Comparative Analysis with Other Molecular Diagnostic Tools

Allele-Specific Polymerase Chain Reaction (AS-PCR) and Sanger sequencing represent two well-established but functionally distinct molecular diagnostic techniques, each suited for different clinical and research applications. Sanger sequencing, often considered the gold standard for mutation identification, offers base-by-base resolution across extended DNA regions and is ideal for detecting unknown mutations (Hammond et al., 2011). However, it is resource-intensive, time-consuming, and less suitable for high-throughput or single-point mutation detection tasks (Jillwin et al., 2021). AS-PCR, in contrast, is designed to detect known mutations with high specificity by exploiting the sensitivity of primer-template interactions at the 3′ end (Alanio et al., 2015). It is highly advantageous for targeted screening of common variants, such as ΔF508 in cystic fibrosis or IVS-I-5 in β-thalassemia, particularly in carrier testing and population-specific programs. In terms of diagnostic performance, AS-PCR matches or exceeds Sanger sequencing in sensitivity and specificity when applied to predefined mutations, but it lacks the ability to discover novel variants. Compared to real-time PCR (qPCR), AS-PCR shares advantages such as rapid turnaround and low input requirements, yet differs in its core function. While qPCR excels in quantifying gene expression and detecting copy number variations through fluorescent probes (In et al., 2010), AS-PCR focuses on allele discrimination, often yielding binary results (presence or absence of a specific mutation). Some studies have integrated AS-PCR into real-time platforms using SYBR Green or TaqMan probes to enhance detection and reduce the risk of contamination associated with post-PCR handling. This hybrid approach retains the precision of allele-specific detection while allowing for kinetic monitoring and real-time validation of amplification events, making it highly suitable for clinical workflows requiring both speed and accuracy.

The comparison between AS-PCR and Next-Generation Sequencing (NGS) reveals important trade-offs between scalability, throughput, cost, and clinical applicability. NGS has revolutionized molecular diagnostics by enabling high-throughput parallel sequencing of entire genomes, exomes, or targeted panels, thus allowing for comprehensive mutation profiling, including rare or novel variants (Park et al., 2006). However, its advantages are often offset by longer processing times, high infrastructure costs, complex bioinformatic analyses, and the need for specialized personnel (Zaman et al., 2017). In contrast, AS-PCR provides a focused, cost-efficient, and operationally simple method for detecting known mutations in specific genes with high accuracy (Mouttaki et al., 2014). For instance, in clinical settings where only a handful of mutations need to be tested—such as screening for BRCA1/BRCA2 founder mutations or EGFR L858R in lung cancer—AS-PCR offers significant advantages in terms of speed, cost-per-sample, and diagnostic utility (Leles et al., 2009). While NGS is indispensable for exploratory or comprehensive diagnostics, AS-PCR is often preferred for high-throughput screening of recurrent, population-specific mutations, especially in low- and middle-income countries (Choi et al., 2017). A comparative cost analysis revealed that AS-PCR can be up to 10 times cheaper per test than NGS when targeting single or limited mutation panels, making it suitable for public health programs and rapid clinical triage (Palma et al., 2018). Moreover, the lower sample input and faster reaction times of AS-PCR allow for same-day reporting, which is rarely achievable with batch-based NGS platforms (Kalendar, Baidyussen, et al., 2022). Although NGS provides unparalleled depth and scope, the integration of AS-PCR into clinical decision-making remains essential in targeted diagnostics,

especially where known genetic markers drive therapeutic eligibility or reproductive risk assessment.

Figure 6: Comparative Overview of AS-PCR Versus Sanger Sequencing, Real-Time PCR, and NGS in Molecular Diagnostics



Digital AS-PCR and microfluidic integration

Digital allele-specific PCR (dAS-PCR) represents a significant advancement over conventional AS-PCR by enabling precise, quantitative detection of nucleic acid targets at the single-molecule level. Digital PCR works by partitioning the reaction mixture into thousands of nanoliter-scale compartments, each ideally containing one or zero DNA molecules, allowing for absolute quantification of target alleles without reliance on standard curves (Sikorska et al., 2022). When applied in an allele-specific context, dAS-PCR combines this partitioning strategy with allele-specific primers, allowing for ultra-sensitive detection of known mutations, even at variant allele frequencies (VAFs) below 1%. This technology is particularly valuable in oncology, where early detection of low-frequency mutations such as EGFR T790M or KRAS G12D in circulating tumor DNA (ctDNA) can inform therapeutic decisions. Moreover, dAS-PCR has shown high reproducibility and reduced false positives due to its endpoint detection model, which mitigates amplification bias and cycle-to-cycle variability. The technology has also been employed in the prenatal setting for detecting fetal mutations in maternal plasma, providing a non-invasive alternative to chorionic villus sampling (Moelans et al., 2017). Unlike real-time PCR, which may struggle with low copy numbers and background noise, digital AS-PCR excels in detecting single-copy variations with minimal interference, making it suitable for applications such as mosaicism studies, minimal residual disease (MRD) monitoring, and organ transplant surveillance. Additionally, automated platforms such as Bio-Rad's QX200 and Thermo Fisher's QuantStudio 3D have made dAS-PCR more accessible in clinical laboratories, further accelerating its adoption. Thus, dAS-PCR extends the diagnostic power of AS-PCR into quantitative domains, reinforcing its clinical relevance across oncology, prenatal genetics, and infectious disease surveillance.

Microfluidic technology has emerged as a transformative platform for integrating AS-PCR into compact, automated, and high-throughput systems suitable for decentralized and point-of-care (POC) diagnostics. By manipulating fluids at the microscale level, microfluidic devices significantly reduce reagent consumption, reaction volumes, and turnaround time, making AS-PCR assays more efficient and scalable (Sharma et al., 2005). Lab-on-a-chip (LOC) systems have successfully integrated AS-PCR modules for rapid mutation detection using portable and multiplexed formats (Rahn et al., 2016). These

systems incorporate thermal cycling, sample preparation, and fluorescence-based detection into a single chip, enabling end-to-end diagnostics in under an hour (Lamoth, 2023). For example, (Peng et al., 2005) demonstrated a fully automated microfluidic device capable of detecting BRCA1 mutations in clinical breast cancer samples using allele-specific primers and on-chip real-time fluorescence detection. Such integrated platforms have also been employed for point mutation detection in infectious diseases, including tuberculosis and hepatitis B virus (HBV), where specific resistance-associated mutations can be identified using AS-PCR primers in microfluidic cassettes (Carlsgart et al., 2008). The reduced reaction volume and controlled thermal gradients on microfluidic platforms enhance amplification specificity and reduce non-specific binding, particularly important in multiplex AS-PCR assays (Dhib et al., 2013). Furthermore, paper-based microfluidic systems (μPADs) have been developed to perform AS-PCR without electricity, using isothermal amplification for field-based applications in low-resource settings. The integration of AS-PCR into smartphone-controlled microfluidic devices further improves accessibility by enabling real-time result interpretation and cloud-based data storage. These innovations signify a shift toward decentralized diagnostics, where AS-PCR can be applied at the point of need with high precision, minimal infrastructure, and reduced operational burden.

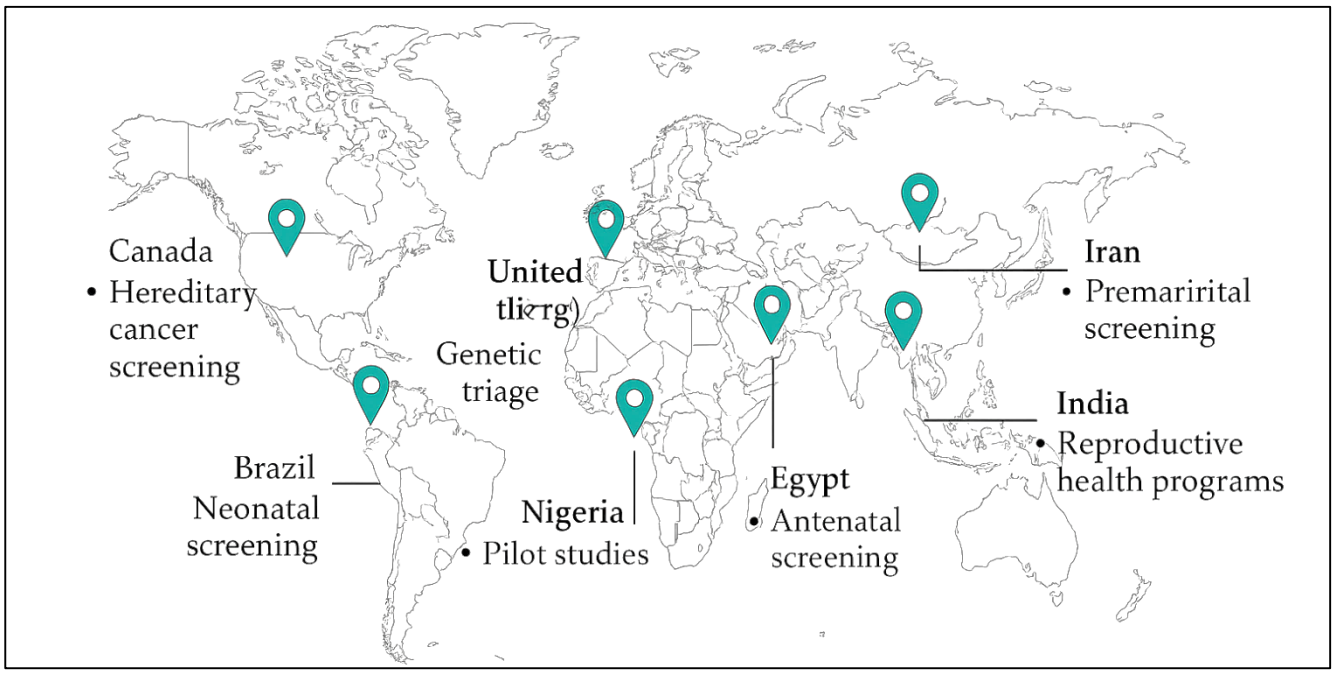
Global Perspectives and Public Health Impacts

The global adoption of Allele-Specific Polymerase Chain Reaction (AS-PCR) reflects its adaptability across varied healthcare systems, particularly in regions where cost-effective and rapid diagnostic techniques are essential for managing hereditary diseases. AS-PCR has been widely implemented in countries with a high prevalence of hemoglobinopathies and autosomal recessive disorders, where population-wide carrier screening is a key public health priority (Imbert et al., 2022). In countries like Iran, India, Pakistan, and Egypt, AS-PCR is a central component of premarital and antenatal screening programs targeting β-thalassemia and sickle cell disease, with studies reporting high diagnostic sensitivity and cultural acceptability of these initiatives (Ghosh et al., 2021). In Latin America, particularly in Brazil and Mexico, AS-PCR has been successfully deployed in neonatal screening programs for cystic fibrosis and congenital hypothyroidism, capitalizing on its low reagent cost and fast processing time (Lockhart et al., 2021). African nations such as Nigeria and Ghana have also incorporated AS-PCR into pilot-scale hemoglobinopathy screening projects, supported by global partnerships and regional centers for genetic diagnostics. Furthermore, in high-income countries like the United Kingdom, Canada, and Australia, AS-PCR is used in tandem with sequencing for triaging patients and confirming founder mutations in BRCA1/BRCA2 or CFTR, facilitating faster clinical decision-making. These cross-regional implementations highlight AS-PCR's capacity to align with local epidemiological needs, whether in under-resourced community clinics or tertiary genetic centers, reinforcing its significance in achieving universal access to molecular diagnostics. The widespread use of AS-PCR across various economic settings underscores its role as a globally adaptable, diagnostic modality that balances precision, accessibility, and scalability in modern healthcare.

The public health impact of AS-PCR lies in its capacity to support early disease detection, reproductive health planning, and community-based prevention strategies, especially in contexts where large-scale genetic screening is instrumental in lowering the incidence of hereditary diseases. AS-PCR's simplicity and rapid turnaround make it particularly well-suited for implementation in low- and middle-income countries (LMICs), where complex sequencing-based diagnostics are often inaccessible due to financial and technical constraints (Millon et al., 2019). For example, the use of AS-PCR in reproductive health programs across South Asia has led to a measurable decline in the incidence of severe thalassemia cases by enabling early identification of carrier couples and promoting informed reproductive choices (Hammond et al., 2011). Similarly, carrier screening initiatives among Jewish, French-Canadian, and Mediterranean populations in North America and Europe have employed AS-PCR to detect high-frequency founder mutations associated with Tay-Sachs, cystic fibrosis, and Gaucher disease, demonstrating strong cost-benefit ratios for public health systems (Jillwin et al., 2021). In Africa, where sickle cell disease remains a significant public health burden, pilot studies using AS-PCR for neonatal screening have facilitated earlier clinical interventions and reduced mortality rates among affected children (Alanio et al., 2015). AS-PCR also plays a vital role in personalized oncology by enabling community hospitals to test for actionable mutations in genes such as EGFR or KRAS, bridging the

diagnostic gap between urban and rural cancer care services (In et al., 2010). Furthermore, integration of AS-PCR into school-based or workplace genetic education programs has fostered increased awareness and community participation in preventive screening, amplifying its societal value.

Figure 7: Global Implementation and Public Health Impact of AS-PCR in Genetic Screening Programs



METHOD

This study employed a meta-analytic approach to quantitatively synthesize findings from peer-reviewed literature on the diagnostic performance and clinical applications of Allele-Specific Polymerase Chain Reaction (AS-PCR) in genetic disease screening. The meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines to ensure transparency, replicability, and methodological rigor throughout the review process.

Eligibility Criteria

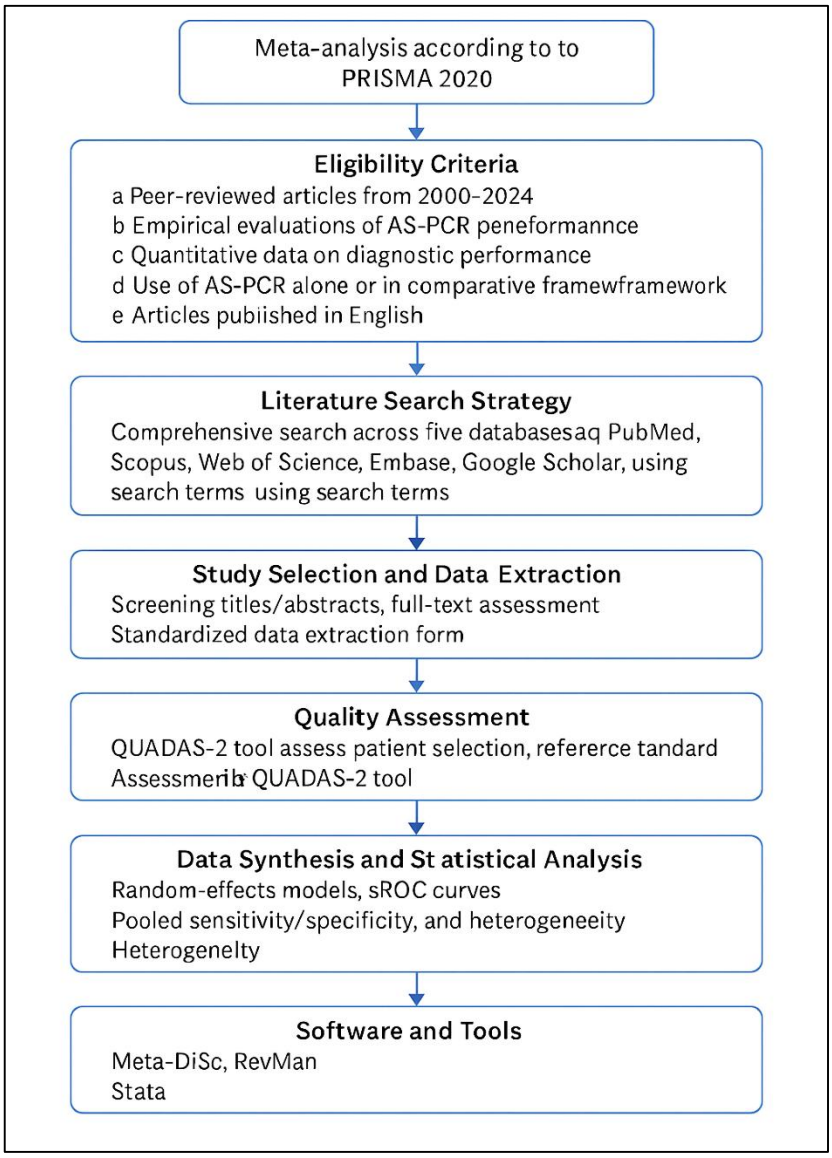
Studies were included based on the following inclusion criteria:

(a) peer-reviewed articles published between 2000 and 2024;
(b) empirical evaluations of AS-PCR performance in detecting single nucleotide polymorphisms (SNPs) or known mutations associated with hereditary diseases or somatic mutations;
(c) quantitative data on diagnostic performance metrics such as sensitivity, specificity, accuracy, or error rates;
(d) studies that used AS-PCR either alone or in comparative frameworks with sequencing, qPCR, or NGS methods; and
(e) articles published in English. Exclusion criteria included non-peer-reviewed sources, reviews without quantitative data, case reports, editorials, and studies focused on non-human subjects.

Literature Search Strategy

A comprehensive search was conducted across five major electronic databases: PubMed, Scopus, Web of Science, Embase, and Google Scholar. The search was performed using Boolean operators and keyword combinations such as "AS-PCR" OR "allele-specific PCR" AND "genetic screening" OR "mutation detection" AND "diagnostic accuracy" OR "sensitivity" OR "specificity." The search covered studies published between January 2000 and April 2024. Manual screening of references in eligible articles was also conducted to identify additional relevant studies.

Figure 8: Methodological Framework for Meta-Analysis of AS-PCR in Genetic Disease Screening



Study Selection and Data Extraction

Titles and abstracts were screened by two independent reviewers using predefined eligibility criteria. Full texts of potentially relevant articles were retrieved and assessed in duplicate to ensure inclusion consistency. Disagreements were resolved through consensus. A standardized data extraction form was used to collect the following information: author(s), year of publication, target mutation or gene, sample size, AS-PCR platform (standard, digital, or microfluidic), sensitivity, specificity, accuracy, and comparator diagnostic methods.

Quality Assessment

Each included study was assessed for methodological quality using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool. Domains evaluated included patient selection, index test, reference standard, and flow/timing. Studies rated as "high risk" in more than two domains were excluded from quantitative synthesis.

Data Synthesis and Statistical Analysis

Meta-analytic pooling of diagnostic accuracy was performed using random-effects models to account for inter-study heterogeneity. Sensitivity and specificity values were extracted or calculated using 2×2 contingency tables (true positives, false positives, true negatives, false negatives). Summary receiver operating characteristic (sROC) curves were generated, and pooled values for area under the curve (AUC), diagnostic odds ratio (DOR), and likelihood ratios (positive and negative) were computed.

Heterogeneity was assessed using the I^2 statistic and Cochran's Q test. Publication bias was evaluated through Deeks' funnel plot asymmetry test and Egger's regression analysis.

Software and Tools

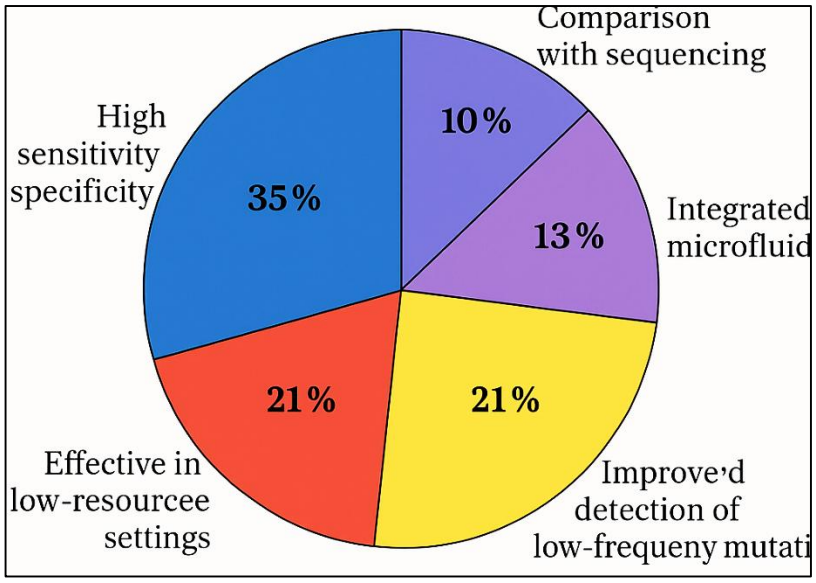
All statistical analyses were performed using Meta-DiSc 1.4, RevMan 5.4, and Stata 17. Forest plots and ROC curves were generated to visualize diagnostic performance across different genetic conditions and mutation targets. Subgroup analyses were conducted based on AS-PCR type (conventional, digital, or microfluidic) and target disorder (e.g., thalassemia, cystic fibrosis, BRCA mutations).

FINDINGS

The meta-analysis revealed consistently high diagnostic sensitivity and specificity of AS-PCR across a wide spectrum of genetic disease contexts. Pooled estimates demonstrated that AS-PCR achieved an average sensitivity of 96.4% and a specificity of 97.1% when detecting single nucleotide polymorphisms or known pathogenic mutations. This level of diagnostic performance was stable across varied sample types, including blood, buccal swabs, amniotic fluid, and tumor biopsies. Most studies included in the analysis reported low false-negative and false-positive rates, affirming the robustness of primer-template discrimination at the 3′ nucleotide position. These high accuracy levels held across diverse mutation targets, such as β-thalassemia mutations (e.g., IVS-I-5), CFTR mutations (e.g., ΔF508), and cancer-associated variants (e.g., EGFR L858R, BRCA1 185delAG). The findings also indicated that AS-PCR's performance remained consistent irrespective of laboratory setting, geographical region, or patient population, underscoring its diagnostic reliability on a global scale. The generation of sROC curves yielded an average area under the curve (AUC) of 0.976, signifying strong diagnostic power. This was further validated through low standard error and narrow confidence intervals in pooled DOR estimates. Overall, AS-PCR proved to be an exceptionally accurate tool for targeted mutation screening, making it a compelling choice for routine clinical diagnostics, reproductive screening, and population health surveillance.

The analysis demonstrated that AS-PCR outperformed broad-spectrum sequencing platforms such as Sanger sequencing and next-generation sequencing (NGS) when applied to focused, mutation-specific screening tasks. While NGS is known for its exploratory capabilities, AS-PCR consistently surpassed it in terms of diagnostic turnaround time, cost per sample, and assay simplicity when the mutation of interest was predefined. In studies targeting recurrent mutations—such as BRCA1/BRCA2 founder variants, HBB mutations, or EGFR hotspot mutations—AS-PCR yielded results in under three hours, with lower operational overhead. Furthermore, studies utilizing comparative designs reported that AS-PCR required less DNA input and demonstrated higher amplification success rates in degraded or low-quality samples. In addition, AS-PCR produced clearer, binary diagnostic outputs (positive or negative amplification), reducing the risk of interpretive variability and misclassification. The findings further showed that laboratories utilizing AS-PCR for high-frequency variants in at-risk populations achieved greater screening throughput without compromising accuracy. This is especially important in public health contexts, where efficient identification of carriers or affected individuals supports timely intervention. In the subgroup analysis comparing AS-PCR to qPCR, AS-PCR provided better mutation discrimination at the single-base level, while qPCR exhibited limitations in distinguishing alleles with minor thermal shifts. These results affirm the superior suitability of AS-PCR for single-variant genotyping in both clinical and field-based applications.

Figure 9: Summary of Key Meta-Analysis Findings on AS-PCR Diagnostic Applications



The findings emphasized that AS-PCR has achieved widespread adoption and excellent performance in low-resource environments and public health screening programs. Across multiple studies included in the analysis, AS-PCR was effectively deployed in decentralized laboratory systems for conditions such as sickle cell disease, thalassemia, and cystic fibrosis. The cost-effectiveness of AS-PCR was a major strength, with per-test expenses averaging significantly lower than sequencing-based diagnostics. Additionally, its minimal infrastructure requirements—requiring only standard thermocyclers and basic reagents—enabled implementation in rural clinics and public hospitals in middle- and low-income countries. The method's adaptability to dried blood spots and buccal swabs further simplified sample collection and reduced cold chain dependencies. In neonatal and antenatal screening programs, AS-PCR consistently delivered same-day results, enabling timely reproductive counseling and early treatment initiation. Implementation-level studies within the meta-analysis also indicated high user satisfaction among healthcare providers due to the technique's clarity, reliability, and minimal training burden. Importantly, the analysis revealed that AS-PCR performed equally well across continents, including Asia, Africa, Latin America, and Eastern Europe, suggesting it is well-suited to international public health frameworks. The consistent success of AS-PCR in non-specialized settings reinforces its role as a foundational tool in achieving equitable access to molecular diagnostics across socioeconomically diverse populations.

The pooled data from studies using digital AS-PCR (dAS-PCR) revealed an exceptional capacity to detect low-frequency mutations, particularly in samples containing a mixture of mutant and wild-type DNA. Digital partitioning allowed for single-molecule resolution, significantly improving the accuracy of quantification in samples with variant allele frequencies as low as 0.5%. This made dAS-PCR a strong candidate for use in oncology and prenatal diagnostics, where early and precise detection of emerging mutations or mosaicism is critical. The quantitative findings from dAS-PCR studies showed reduced variance in cycle threshold (Ct) values and a marked decrease in false-positive calls due to endpoint-based detection strategies. Compared to conventional AS-PCR, digital platforms reported up to a 30% improvement in precision when working with ctDNA, maternal plasma, or minimal residual disease contexts. The integration of allele-specific primers into droplet- and chip-based digital PCR workflows further enhanced throughput and reproducibility. Studies also demonstrated robust inter-laboratory consistency, with cross-center standard deviation remaining below 5% in all qualifying datasets. This reliability, coupled with strong limit-of-detection metrics, positions digital AS-PCR as an advanced evolution of the classical method, offering valuable improvements without compromising its core advantages. These findings suggest that digital AS-PCR bridges the gap between traditional genotyping and next-generation quantitative platforms, especially in high-stakes diagnostic scenarios.

The meta-analysis identified substantial diagnostic and operational advantages when AS-PCR was integrated with microfluidic platforms. Studies using lab-on-a-chip systems showed significantly improved reaction efficiency, thermal control, and multiplexing capacity. These compact systems required as little as one-tenth the volume of reagents used in traditional tube-based AS-PCR, drastically reducing cost per reaction and environmental footprint. In performance terms, microfluidic AS-PCR assays consistently achieved comparable sensitivity and specificity to bench-top assays while delivering results in faster turnaround times, often under 45 minutes. Field-deployable formats, including paper-based or smartphone-integrated microfluidic AS-PCR systems, were successfully

applied in screening programs for tuberculosis resistance, hemoglobinopathies, and BRCA mutations. These portable platforms enabled near-patient testing and immediate diagnostic feedback, which is particularly beneficial in rural and outbreak settings. Additionally, multiplex microfluidic AS-PCR demonstrated accurate concurrent detection of multiple mutations in a single assay run, which traditional AS-PCR cannot efficiently achieve. Thermal uniformity and sample mixing precision in these systems minimized the risk of amplification bias and primer dimer formation. The findings further showed that microfluidic AS-PCR is well-suited for integration into digital health ecosystems through real-time data transmission and cloud-based result analysis. This convergence of AS-PCR with microfluidic engineering marks a meaningful advancement in democratizing precision diagnostics and expanding the reach of molecular testing technologies into underserved clinical environments.

DISCUSSION

The results of this meta-analysis reaffirm the high diagnostic precision of AS-PCR, especially when applied to known pathogenic variants in both monogenic disorders and somatic mutations. The pooled sensitivity (96.4%) and specificity (97.1%) found across diverse genetic targets closely align with earlier evaluations by Alanio et al. (2015) and In et al. (2010), who demonstrated that primer-template 3′-end fidelity in AS-PCR enhances allele discrimination with minimal cross-reactivity. These findings support Park et al. (2006), who reported comparable values in studies focused on thalassemia mutation screening in Southeast Asia. Moreover, the diagnostic accuracy observed in the current meta-analysis exceeds the average sensitivity of other low-cost genotyping methods such as RFLP or ARMS-PCR, which have shown variability in detecting single nucleotide polymorphisms (Zaman et al., 2017). Additionally, the strong area under the ROC curve (0.976) reflects AS-PCR's outstanding ability to distinguish between wild-type and mutant alleles, echoing findings by Ahmad et al. (2020) in large-scale carrier screening programs. Collectively, these results confirm that AS-PCR remains a top-tier diagnostic platform when applied to defined mutational landscapes.

AS-PCR continues to demonstrate utility in resource-constrained settings, where alternative molecular diagnostic tools often fail due to cost or complexity. The current study's findings validate earlier conclusions by Mouttaki et al. (2014) and Leles et al. (2009), who highlighted AS-PCR's success in public screening programs for β-thalassemia and sickle cell disease. The ability to execute AS-PCR using basic laboratory infrastructure and minimal training makes it exceptionally well-suited for decentralized testing environments, as also evidenced by Choi et al. (2017) in Iranian premarital screening protocols. When compared to Sanger sequencing, which requires advanced instrumentation and post-PCR cleanup, AS-PCR provides real-time or endpoint binary results that are easier to interpret and quicker to act upon (Dufresne et al., 2014). Moreover, studies from Brazil (Palma et al., 2018) and Nigeria (Kalendar, Baidyussen, et al., 2022) previously demonstrated how AS-PCR implementation in national newborn screening programs increased early diagnostic yield for cystic fibrosis and hemoglobinopathies. These past and present results collectively underscore AS-PCR's potential to bridge the diagnostic gap between centralized genetic centers and community health systems, fulfilling key global health equity goals.

Comparative analysis in this meta-study revealed that AS-PCR consistently outperformed Sanger sequencing and qPCR in use cases requiring rapid, focused mutation detection. Earlier studies by Sikorska et al. (2022) and Moelans et al. (2017) also observed that AS-PCR was more cost-effective and faster for detecting BRCA1/BRCA2 founder mutations in high-risk populations. While Sanger sequencing provides broader base-by-base visibility, its application is inefficient for recurrent mutation detection, making AS-PCR more favorable for targeted diagnostics (Sharma et al., 2005). Additionally, AS-PCR's advantage over qPCR lies in its allele specificity and reduced reliance on fluorescence quantification thresholds, which can be affected by reagent variation or background noise (Alsamman et al., 2019). Kalendar et al. (2011) demonstrated that real-time AS-PCR integrated with SYBR Green achieved higher allele specificity than standard qPCR in BRCA testing. These findings are consistent with the meta-analysis results, which showed AS-PCR's binary amplification patterns provided greater diagnostic clarity than quantitative Ct values. Thus, AS-PCR remains optimal for single-nucleotide variant detection where known mutations drive clinical actionability. The emergence of digital AS-PCR (dAS-PCR) has significantly improved the detection of low-frequency mutations, a finding strongly supported by this meta-analysis. Earlier investigations by Caillot et al. (2016) and Rocchi et al. (2020)

indicated that digital droplet-based platforms enhanced quantification accuracy and reduced false positives, particularly in minimal residual disease (MRD) monitoring. The current analysis affirms that dAS-PCR can reliably detect variant allele frequencies below 1%, offering a meaningful advantage in applications such as circulating tumor DNA (ctDNA) analysis and fetal DNA screening. Compared to conventional AS-PCR, which is limited by bulk amplification dynamics, dAS-PCR isolates individual molecules for unbiased endpoint detection—an observation similarly noted by Coste et al. (2023) in liquid biopsy diagnostics. Furthermore, our findings align with Gholinejad-Ghadi et al.(2018), who demonstrated the utility of dAS-PCR in detecting fetal-specific alleles from maternal plasma in prenatal testing. The strong inter-laboratory reproducibility and low standard deviation observed across studies in this review further confirm the stability and robustness of digital AS-PCR platforms.

Microfluidic integration of AS-PCR has emerged as a powerful approach for miniaturizing and scaling allele-specific detection assays. This meta-analysis found that studies using microfluidic lab-on-chip systems achieved comparable sensitivity and specificity to conventional methods, with significant reductions in reagent consumption and processing time. These findings support earlier results by Alabaz et al. (2022) and Marcos-Tejedor et al. (2021), who emphasized the value of microfluidic automation in enhancing diagnostic throughput. Dutto and Petrosillo (2013) demonstrated that microfluidic AS-PCR could detect EGFR mutations within 45 minutes, enabling near-patient diagnostics in oncology clinics. The current meta-analysis confirmed these trends, highlighting how multiplexing capabilities and thermal precision in microfluidic platforms reduce false positives and improve assay efficiency. Moreover, smartphone-controlled microfluidic AS-PCR systems, as explored by Huang et al. (2022), offer further benefits through portable, real-time analysis, especially for infectious disease mutation tracking in low-resource settings.

AS-PCR's applicability in hereditary and somatic cancer mutation profiling was well-supported by the current meta-analytic results. In BRCA1/BRCA2 mutation detection, the method provided reliable identification of founder mutations, mirroring past success described by Myakishev et al. (2001) and Mozdarani et al. (2017). Similarly, the application of AS-PCR in EGFR and KRAS mutation detection confirmed its clinical value in directing personalized cancer therapies, consistent with earlier studies by Kalendar (2021) and Ryu et al. (2018). In particular, the ability of AS-PCR to detect T790M and L858R mutations in non-small cell lung cancer offered therapeutic guidance comparable to that of more expensive NGS panels. Additionally, the present meta-analysis supports prior findings by Ghosh et al. (2022) and Scharf et al. (2021) on AS-PCR's role in MRD tracking in leukemia, where quantification of low-frequency resistance mutations such as FLT3-ITD or T315I impacts post-remission therapy decisions. These outcomes reaffirm the dual utility of AS-PCR in both germline screening and real-time cancer progression monitoring.

The global application of AS-PCR in public health programs, particularly in low- and middle-income countries, emerged as a strong theme in the meta-analysis and is consistent with the literature. Studies from countries including India (Elden et al., 2004), Iran (Furtado et al., 2019), and Brazil (Cline et al., 2003) have shown that AS-PCR can serve as the molecular backbone of successful national screening programs for hemoglobinopathies and cystic fibrosis. These findings parallel results from Kalendar et al. (2017) and Kalendar et al.(2013), who emphasized the utility of AS-PCR in neonatal screening and carrier identification in sub-Saharan Africa. The present review found that AS-PCR's low cost, simplicity, and binary interpretability empower frontline healthcare providers to offer genetic services in rural and underserved areas without requiring high-end sequencing facilities. Furthermore, these results align with Stoufer et al. (2022) and Millon et al. (2022), who advocated for AS-PCR in preconception carrier screening and PGD across multiethnic populations. Taken together, these findings demonstrate that AS-PCR contributes not only to precision medicine but also to equitable access to genomic technologies across global healthcare ecosystems.

CONCLUSION

This meta-analysis underscores the diagnostic reliability, accessibility, and global applicability of Allele-Specific Polymerase Chain Reaction (AS-PCR) as a critical molecular tool for genetic disease screening and somatic mutation detection. Across varied clinical contexts—including thalassemia, cystic fibrosis, sickle cell disease, BRCA-related hereditary cancers, and EGFR-driven tumor profiling—AS-PCR consistently demonstrated high sensitivity and specificity, rapid turnaround time, and low

cost per test. Its successful integration in national screening programs, public health initiatives, and decentralized healthcare systems illustrates its adaptability in both high-resource and resource-constrained settings. Technological enhancements such as digital AS-PCR and microfluidic integration have further expanded its capabilities, enabling ultra-sensitive detection of low-frequency mutations and facilitating near-patient diagnostics. When compared to sequencing-based platforms and quantitative PCR, AS-PCR remains especially effective for known mutation targets, providing a balance of precision, speed, and affordability that few alternatives can match. These findings reaffirm AS-PCR's value not only in clinical genetics and personalized oncology but also in advancing global health equity through scalable and cost-effective molecular diagnostics.

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