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Application Pathways of Multiplex PCR Technology for Simultaneous Detection of Infectious Disease Markers in Blood Transfusion

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Abstract: Blood transfusion safety constitutes a cornerstone of clinical healthcare, with infectious disease biomarker detection being pivotal for risk prevention. Conventional testing methods suffer from limitations including low efficiency and inadequate multi-target simultaneous screening. Leveraging its unique capability for simultaneous amplification across multiple targets in a single system, multiplex PCR technology has emerged as an efficient solution. This study examines the technical characteristics and clinical requirements of this approach, detailing practical implementation strategies such as multi-target system design, sample pretreatment protocols, optimized reaction conditions, result interpretation, and quality control standards. Through standardized design and operational procedures, the technology enhances both processing speed and accuracy, providing robust support for clinical blood transfusion safety and demonstrating significant clinical application value.

Keywords: multiplex PCR technology; transfusion-associated infectious disease markers; simultaneous detection

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1. Introduction

China's medical technology has achieved sustained advancements in recent years, with significant progress in blood component separation techniques [1]. As a critical clinical intervention, blood transfusion safety directly impacts recipients' lives and health, making infectious disease marker detection essential for preventing transmission risks. The growing demands for comprehensive, timely, and accurate testing in clinical practice have highlighted the limitations of single-detection methods, which are cumbersome, time-consuming, and prone to missed detections, making them unsuitable for multi-marker simultaneous screening. Multi-PCR technology utilizes multiple primer-probe systems working in synergy, enabling simultaneous detection of multiple targets within a single system. This approach demonstrates exceptional adaptability to complex sample types and outstanding detection efficiency.

2. Overview of multiplex pcr technology

First reported in 1988, multiplex PCR technology enables simultaneous amplification of multiple target genes by incorporating two or more primers into a single reaction system[2]. As an enhanced targeted amplification technique derived from conventional PCR, it features a synergistic design combining multiple specific primers with probes, allowing simultaneous amplification and detection of multiple nucleic acid fragments within a single reaction. The technology prioritizes specificity and compatibility: primers match conserved gene sequences of infectious disease markers, while probes utilize differential fluorescence signals for multi-target identification, effectively avoiding cross-reaction-induced bias. Its advantages include adaptability to complex samples, resistance to matrix interference in blood transfusion testing, and optimized reaction systems that balance amplification efficiency with independent target detection. Eliminating the need for repeated sample splitting reduces waste and shortens testing cycles, meeting the core requirements for rapid multi-marker screening in transfusion testing. The technical principles and design logic provide critical support for simultaneous detection of infectious disease markers in blood transfusions, establishing it as a core technological pathway to overcome challenges in multi-target parallel detection.

3. Demand and challenges for detection of infectious disease markers in blood transfusion

Ensuring blood transfusion safety requires rigorous infectious disease screening prior to transfusion[3]. As the cornerstone of clinical practice, blood transfusion safety demands comprehensive, timely, and accurate detection of infectious disease markers. Clinical testing protocols must cover multiple high-risk pathogen markers, including both common pathogens and emerging disease markers, while minimizing false negatives from single-target detection. The urgency of transfusion therapy necessitates rapid multi-marker screening to inform timely clinical decisions. Accurate results are critical for patient safety, requiring differentiation between specific signals and non-specific interference, even for low-concentration markers. Current testing faces multiple challenges: varying nucleic acid sequence characteristics of markers, cross-reactions between primers and probes, and complex sample matrices containing blood-derived interference. These factors demand robust detection systems with high anti-interference capabilities. Standardized protocols must adapt to diverse clinical settings, while insufficient standardization risks result discrepancies. The sensitivity of rare pathogen markers often fails to meet clinical needs. These challenges collectively represent critical bottlenecks in infectious disease marker detection for blood transfusion.

4. Application pathways of multiplex pcr technology in simultaneous detection of blood transmission infectious disease markers

4.1. Target design and primer/probe optimization for multi-target synchronous detection system

The multi-target synchronous detection system employs targeted design aligned with clinical needs. Using multi-sequence alignment tools, we analyze blood transfusion-associated infectious disease marker gene sequences to identify low-variant, highly specific conserved regions as detection targets. These targets must cover diverse circulating strains while avoiding homology with human genomes or unrelated pathogens. Following target screening, primer design follows principles of optimal length and balanced GC content, avoiding consecutive repetitive bases or complementary sequences. Bioinformatics software predicts primer secondary structures to prevent hairpin formation and dimerization, ensuring no significant homology between target primers to minimize cross-reaction risks. After initial primer design, in vitro amplification tests verify efficiency. For primers with insufficient amplification efficiency, terminal bases are adjusted or lengths trimmed until all target primers achieve consistent amplification performance.

The probe optimization and primer design are synergistically advanced. Fluorescent groups with different emission wavelengths are selected based on the number of target sites to ensure non-interference between fluorescence signals.

Fluorescent groups are strategically positioned on the probe, while quenching groups are located near the 3' end to ensure effective quenching. The probe sequence is complementary to specific fragments within the primer amplification region, with a slightly shorter length than the primer to avoid overlapping with the primer binding area and hinder amplification. For probes targeting different targets, the base composition is adjusted to match primer annealing temperatures, ensuring synchronized binding of all probes and primers to the template within the same reaction system. Gradient concentration experiments optimize the primer-probe ratio by gradually adjusting the concentration ratios of primers and probes for different targets, balancing amplification competition among targets to achieve efficient and specific amplification of all targets in a single system.

System compatibility validation serves as the critical final step in targeted design. All optimized primer-probe combinations are incorporated into a unified reaction system. Simulated samples containing each target are added for amplification, and fluorescence signal curves are analyzed to evaluate amplification peaks and specificity. When non-specific peaks emerge or amplification of a particular target is inhibited, the corresponding primer-probe sequence or concentration is recalibrated. Cross-reactivity testing is conducted using nucleic acid samples from clinically common interfering pathogens to ensure the system exclusively produces specific signals for target biomarkers without cross-reactions. This process ultimately establishes a stable and reliable multi-target synchronous detection system.

4.2. Standardized pre-treatment of blood samples and pcr reaction conditions

The core process of blood transfusion sample preparation involves nucleic acid extraction and purification to remove interfering substances from the sample matrix. Samples should be processed immediately after collection to prevent nucleic acid degradation. Different extraction methods are selected based on sample types, with the magnetic bead method being the preferred choice due to its high efficiency and purity. The sample is mixed with lysis buffer and incubated at optimal temperature to rupture blood cells and release pathogen nucleic acids. Magnetic beads are gently vortexed to allow specific surface groups to bind nucleic acids. Magnetic field separation then adsorbs the nucleic acid complexes from the beads, after which the supernatant containing proteins and hemoglobin is discarded.

The washing process must strictly follow standardized protocols. Add gradient-concentrated wash solutions sequentially, gently shaking the reaction tubes at each step to ensure thorough contact between the wash solution and magnetic beads. Subsequently, utilize magnetic field adsorption for rapid separation of magnetic beads from waste liquid, discarding the supernatant containing proteins, salts, and other impurities. This process should be repeated multiple times to thoroughly remove residual sample matrix components and amplification inhibitors from the magnetic beads, ensuring complete elimination of potential interference substances affecting subsequent PCR reactions. During elution, add optimized elution buffer and incubate at specific temperatures to detach nucleic acids from the magnetic beads. Collect the elution buffer as PCR amplification templates. Strictly control the operation time and temperature during extraction to prevent nucleic acid damage or loss. Upon completion of extraction, verify nucleic acid purity through quality testing. If purity standards are not met, re-extract the samples. Ensure the template nucleic acids show no significant degradation or residual inhibitors.

Standardized PCR reaction conditions are optimized through systematic adjustments to reaction systems and procedural parameters. The reaction system preparation involves adding buffer, dNTPs, thermostable polymerase, optimized primer-probe combinations, and extracted nucleic acid templates. The buffer maintains pH stability and enhances enzyme activity, while dNTP concentrations are tailored to target amplification requirements. Polymerase selection prioritizes thermostability, amplification efficiency, and interference resistance. For procedural optimization: Denaturation steps are programmed with optimal temperature and duration to ensure complete DNA strand separation; annealing temperatures are determined through gradient trials, with non-specific amplification and absence of non-target bands as benchmarks. Gradient cooling or touchdown mode enhances specificity. Extension time is adjusted according to the maximum amplified fragment length, ensuring complete primer extension. Reaction conditions are customized for blood transfusion samples by adding inhibitor binders or increasing polymerase concentration in samples with high

inhibitor levels. Equipment-specific calibration ensures temperature accuracy and fluorescence detection sensitivity. A standardized protocol manual specifies component addition sequences, reaction volumes, and procedural parameters across all instruments, minimizing human-induced variations in experimental outcomes.

4.3. The process of accurate interpretation of test results and the implementation of clinical quality control

The interpretation of detection results requires establishing standardized procedures with reasonable baseline and threshold settings. Using negative control fluorescence signals as references, select signal-stable cycle segments as baselines, with thresholds positioned at mid-exponential growth phase to avoid background interference. Positive determination requires dual criteria: fluorescence signals exceeding preset thresholds, distinct exponential growth curves, peak values matching positive controls on melting curves, and absence of interference peaks. Samples approaching threshold levels are categorized as suspected positives, triggering retesting protocols. Reanalysis employs backup primer-probe combinations with personnel and equipment rotation. Two consecutive positive results confirm positivity. In case of discrepancies, comprehensive evaluation of sample collection timing, recipient clinical data, and other testing methods prevents misjudgment. Negative results require confirmation through: fluorescence signals not exceeding thresholds, absence of distinct amplification curves, and negative control showing no abnormal amplification. Re-testing is conducted after ruling out reagent contamination or procedural errors.

Clinical quality control must be implemented throughout the entire process of synchronous detection of infectious disease markers in blood transfusion, establishing a scientifically sound and comprehensive internal quality control system to ensure the accuracy and reliability of test results. Before daily testing begins, PCR instruments must undergo comprehensive performance verification strictly in accordance with standardized SOPs, including temperature calibration, fluorescence channel sensitivity validation, and overall operational status testing. The calibration process must be properly documented to ensure all instrument parameters meet testing standards, thereby preventing result inaccuracies caused by equipment deviations. Each batch of testing should include negative controls, positive controls, and blank controls: negative controls monitor contamination, positive controls validate reaction system effectiveness, and blank controls eliminate reagent interference. Third-party quality control materials should be incorporated to assess accuracy through their test results. If results exceed permissible ranges, testing should be suspended to investigate potential causes such as reagent expiration or improper operation. Re-testing should be conducted after resolving identified issues.

Regular participation in inter-laboratory quality assessments helps identify deficiencies through comparative analysis with other laboratories, enabling timely adjustments to enhance result comparability and reliability. Detailed quality control records are maintained, documenting instrument calibration status, reagent batch numbers, control sample results, and operator information. Periodic analysis of quality control data identifies error sources, with systematic deviations prompting optimization of testing systems or process adjustments. Standardized SOP documents regulate sample reception, processing, testing, and interpretation procedures. Clinical staff undergo systematic training focusing on operational protocols and result interpretation standards to ensure full proficiency. A quality control feedback mechanism ensures timely result notifications and problem-specific improvement measures, continuously optimizing testing quality to guarantee reliable synchronous detection of infectious disease markers in blood transfusions, thereby providing robust support for clinical transfusion safety. While multiplex PCR technology offers simplicity, speed, and sensitivity, its application has limitations: it should not be used alone, and expert guidance remains essential for accurate interpretation of test results [4].

5. Epilogue

The application of multiplex PCR technology in simultaneous detection of infectious disease markers in blood transfusion relies on a closed-loop approach featuring multi-target system design, standardized sample processing and reaction

conditions, along with result interpretation and full-process quality control. Targeted optimization and standardized operations address issues such as insufficient specificity, low efficiency, and result deviations in traditional testing methods, enabling efficient simultaneous screening of multiple markers. This technology balances comprehensive detection with accuracy, strengthening the defense for blood transfusion safety. Future advancements will focus on technological upgrades and standardized promotion, optimizing interference resistance and automation levels to continuously safeguard the quality of clinical blood transfusion diagnosis and treatment.

Disclosure statement

The author declares no conflict of interest.

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