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MALDI-TOF mass spectrometry in transfusion medicine

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ABSTRACT

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a valuable tool in clinical research. In transfusion medicine, DNA-based genotyping is increasingly replacing serological methods for precise blood group determination. MALDI-TOF MS platforms for SNP genotyping have been rigorously developed and validated against serological techniques. MALDI-TOF MS offers high-throughput capacity, exceptional multiplexing, and direct detection, resulting in high accuracy and flexibility. The ability to perform blood group genotyping, bacterial identification via peptide barcoding, and detection of hemoglobinopathies and hemoglobin adducts in both donors and recipients makes MALDI-TOF MS a promising technology for resolving complex blood typing cases and enhancing clinical utility in transfusion medicine.

Introduction

Blood typing by antibody-based methods has been the standard for determining ABO, Rh, and "extended" blood group antigens present on red blood cell membrane.[1] To date, 45 blood group systems containing 362 red cell antigens have been officially nominated by the Red Cell Immunogenetics and Blood Group Terminology of the International Society of Blood Transfusion.[2] Various antigens were identified in the different blood group systems. The antigens most commonly involved can be classified into the following categories: 1) red blood cell (RBC)-specific antigens; 2) human leukocyte antigens (HLAs), class I (HLA I) are present on all cells and shared by platelets and leukocytes and HLA II antigens are present on leukocytes that are antigen-presenting; 3) granulocyte-specific antigens; and 4) platelet-specific antigens (human platelet antigens [HPAs]).

Blood group genotyping began with the elucidation of the histo-blood group MN and ABO systems by Siebert and Fukuda,[3] and Yamamoto *et al*.[4] A blood group system is defined as a set of antigens controlled by one or more genes located at a single locus or by closely linked homologous genes with minimal or no recombination between them.[2] Most blood group antigens arise from single nucleotide polymorphisms (SNPs) at critical positions within the respective genes.[5] However, the accurate identification of many blood group systems requires the consideration of multiple critical SNPs. Developing high-throughput SNP genotyping assays is crucial to effectively integrate molecular methods into transfusion medicine, enabling rapid and cost-effective screening. The emergence of

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advanced genetic diagnostic technologies and the comprehensive characterization of genetic polymorphisms have ushered in a new era in transfusion medicine.[6-8]

Over the past two decades, Matrix-Assisted Laser Desorption/ionization-time-of-flight Mass Spectrometry (MALDI-TOF MS) has evolved from a research tool to a valuable clinical technology.[9] Recent advancements demonstrate its successful application in mutation screening and genotyping. High-throughput SNP genotyping using MALDI-TOF MS holds promise as a routine method in both laboratory and clinical settings.[10,11] This review explores the potential of MALDI-TOF MS as an alternative approach for identifying SNPs associated with blood group antigens.

Principle of MALDI-TOF MS

Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a powerful analytical technique for analyzing complex samples.[9,12] It consists of a MALDI ion source, a time-of-flight (TOF) mass analyzer, and a detector. The MALDI source ionizes sample molecules, transferring them to the gas phase. The TOF analyzer measures these ions' mass-to-charge ratio (m/z), and the detector records ion abundance at each m/z value.

Sample preparation involves mixing the analyte with a suitable matrix and co-crystallizing the mixture on a specialized sample plate. Matrix selection and spotting methods are crucial and depend on factors like target mass range, sample peak characteristics, and preliminary analyses. Optimizing matrix concentration is also critical. Standard MALDI matrices include low molecular weight organic acids such as α-cyano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, and 3-hydroxypicolinic acid.[13]

The prepared sample plate is then introduced into the mass spectrometer's high-vacuum environment (pressure <10^{-7} Torr). Laser energy absorbed by the matrix causes desorption and ionization of the analytes. These ions are accelerated through an electrostatic field within the flight tube. Smaller ions travel faster than larger ions, separating them based on their m/z ratios. The time of flight (TOF) for each ion to reach the detector is proportional to its mass and charge, generating a unique spectral profile of peaks characteristic of the analyzed species (Figure 1).

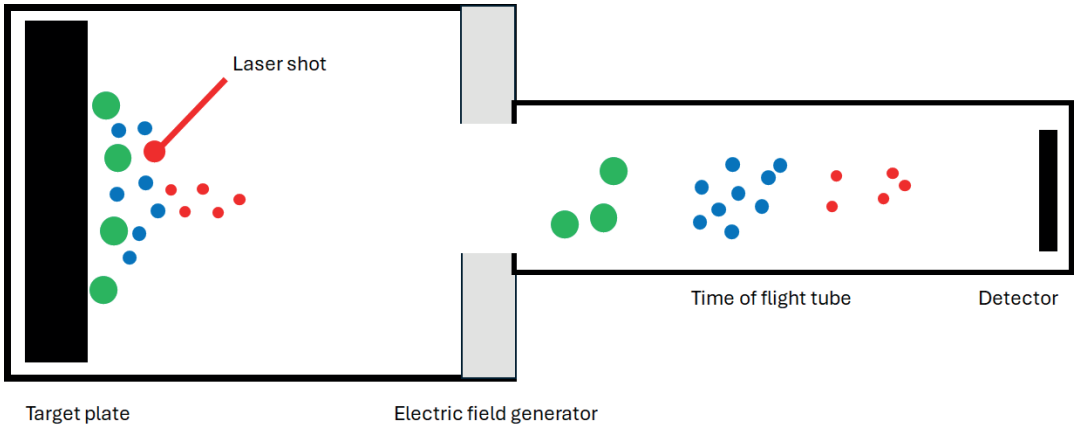

***Figure 1**. Basic principles of MALDI-TOF MS. A sample is mixed with a suitable matrix compound and ionized by a laser. The resulting ions are accelerated through a flight tube; their time-of-flight is proportional to their mass-to-charge ratio. This allows for precise mass determination and identification of the molecules.*

MALDI-TOF MS is a versatile analytical platform that enables rapid data acquisition. The instrument allows for the simultaneous analysis of hundreds of individual samples on a single target plate following offline sample preparation. Moreover, MALDI-TOF MS can be applied directly to complex samples or following sample clean-up/enrichment procedures, enabling the analysis of a wide range of molecular classes, including intact proteins, peptides, lipids, nucleotides, nucleosides, carbohydrates, and small molecules. The user-friendly nature of MALDI-TOF MS instrumentation enables its operation by relatively unskilled personnel, making it well-suited for routine clinical applications. This versatility is further supported by many successful applications in diverse diagnostic settings, as evidenced by previous studies.[9,13,14]

MALDI-TOF MS-based SNP genotyping

Single-nucleotide polymorphisms (SNPs) are the most common form of human genetic variation, occurring frequently throughout the genome.[15] These variations, defined as having a minor allele frequency of at least 1%, can significantly impact phenotypes by altering gene transcription or protein function. SNPs occur on average every 500-1,000 base pairs. SNPs can result in synonymous or non-synonymous amino acid substitutions depending on their location and the specific nucleotide change. SNPs in non-coding regions can also influence gene expression by affecting regulatory elements. Even synonymous SNPs can impact gene expression by affecting splicing. Therefore, SNP screening in association studies provides valuable insights into disease susceptibility, clinical diagnosis, prognosis, and forensic investigations.

MALDI-TOF MS was first applied to oligonucleotide analysis in 1990.[16] Since then, optimized matrices (e.g., 3-hydroxy-picolinic acid) and matrix additives (e.g., sugars,[17] spermine[18]) have been developed to enhance nucleic acid ionization. Sample preparation techniques, such as miniaturizing sample spot sizes, have also improved the homogeneity of matrix-analyte crystals and the reproducibility of mass spectra.[19] Cation exchange resins are used to remove salts from nucleic acid samples, which can interfere with MALDI ionization.[20]

MALDI-TOF MS-based SNP genotyping offers a distinct advantage through its primer extension and data analysis strategy. Primers are designed to hybridize immediately upstream of the SNP site. Unincorporated nucleotides are removed from PCR products using shrimp alkaline phosphatase (SAP). A single-base extension reaction is performed, incorporating one base (typically a dideoxynucleotide, ddNTP) at the SNP site.[21] These ddNTPs have unique masses. MALDI-TOF MS measures the mass of the extended primer, and the mass difference identifies the incorporated ddNTP and, thus, the SNP base. The instrument measures the mass-to-charge ratio of the extended primers, generating a mass spectrum with peaks corresponding to the different SNP alleles.[22] High mass accuracy (typically within 5 ppm) is achieved using external calibration and/or internal standards. This technique allows for parallel analysis of thousands of SNPs in a single sample. Integrating PCR and MS enables the detection and characterization of genomic DNA, even from limited sample quantities (Figure 2).

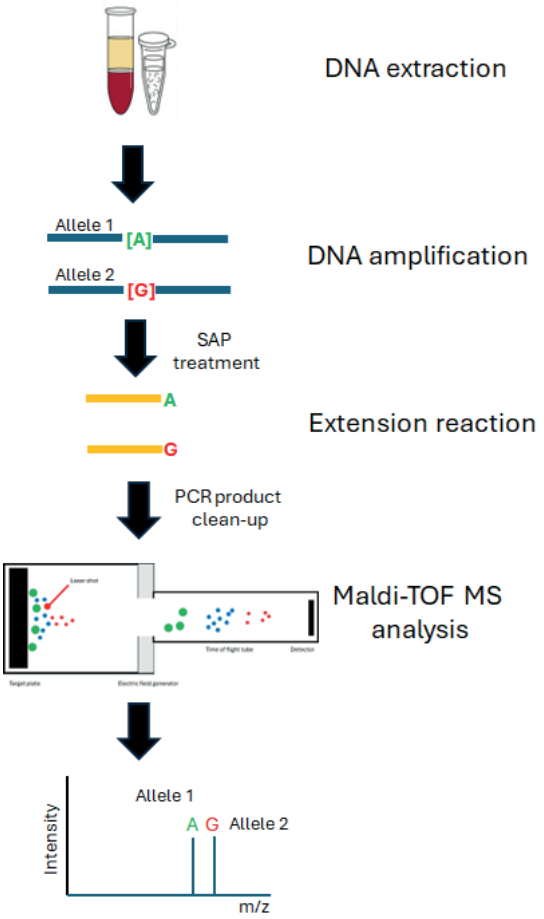

Figure 2. *Single nucleotide polymorphism analysis was performed using MALDI-TOF mass spectrometry. Targeted regions were amplified via a multiplex polymerase chain reaction (PCR) assay. Unincorporated nucleotides were neutralized from the PCR products through shrimp alkaline phosphatase (SAP) treatment. A single-base extension reaction was subsequently conducted to extend the PCR fragments at the SNP site. The resulting extended fragments were desalted before mass measurement using MALDI-TOF MS, generating a spectrum of distinct mass peaks.*

Specialized software analyzes these spectra, identifying peaks, correlating them to specific masses, and assigning SNP genotypes. MALDI-TOF MS also facilitates high-throughput genotyping through multiplexing, enabling simultaneous analysis of multiple SNPs in a single reaction.[23,24] Multiplexing is achieved by designing primers with varying lengths or incorporating mass-modifying tags.

Previous studies have demonstrated the utility of MALDI-TOF MS in SNP genotyping for various applications. For example, Nakai *et al*. (2004) investigated ethnic variations in coronary artery disease-associated SNPs.[25] Shi *et al*. (2011) screened 50 SNPs in CYP genes within the Chinese Han population, identifying polymorphic SNPs with allele frequency differences compared to Caucasians, highlighting potential forensic applications.[26] Xu *et al*. identified SNPs associated with lung cancer patient survival, suggesting potential prognostic value.[27] Zhang et al. (2025) developed a MALDI-TOF MS-based SNP assay to investigate the genetic basis of poor antidepressant efficacy, aiming to predict individual drug response variations.[28]

MALDI-TOF MS-based blood group genotyping

Serological methods based on red blood cell agglutination are inexpensive and widely available but can be subjective, time-consuming, and prone to ambiguous results, especially with weak or variant antigen expression.[29,30] Serology also has limitations in identifying all clinically relevant blood group alleles, particularly in complex genetic backgrounds.[31,32] Furthermore, the absence of specific antibodies for certain antigens, especially rare ones, prevents their accurate serological detection.[33-36]

Molecular blood group genotyping advanced significantly with the cloning of *GYPA* (MN blood group system) in 1987,[3] followed by the ABO and Rh blood group system genes in 1990 and 1992, respectively.[4,37] Subsequently, other blood group system genes were cloned. Analysis revealed that single nucleotide polymorphisms (SNPs) are the primary source of allelic variation.[5] Characteristic SNPs identify specific alleles. Other mechanisms contributing to molecular variation include gene or exon deletions, sequence duplication with nonsense mutations, hybrid gene formation, and exon duplication.

Molecular diagnostic techniques address the limitations of serology. Methods like PCR-SSP, PCR-SBT, BeadChips, MALDI-TOF MS, and next-generation sequencing (NGS) offer more comprehensive and accurate antigen identification.[38-42] Determining the nucleotide state (homozygous or heterozygous) at the SNP position often accurately predicts the phenotype (typically 99%).

PCR-SSP offers improved accuracy and resolution compared to serology, particularly for variant allele identification using sequence-specific primers.[31,32,43] However, it requires specialized equipment and personnel, and the number of testable alleles is limited. PCR-SBT, using Sanger sequencing (the gold standard for genotyping), provides comprehensive information by identifying a broad range of sequence variations.[44] It's cost and turnaround time limit high-throughput screening.[45] BeadChip technology allows simultaneous identification of multiple antigens,[38] but is susceptible to false results due to factors like DNA quality and cross-reactivity.[46] Equipment and assay development costs can also be substantial. NGS enables high-throughput identification of diverse sequence variations, resolving complex serological results, identifying rare alleles, and facilitating high-throughput donor screening.[47] However, NGS is constrained by high cost, complex data analysis, challenges in detecting large structural variations, and turnaround time.[48]

MALDI-TOF MS-based genotyping, combining PCR amplification and mass spectrometry, facilitates comprehensive blood group profile analysis. This platform detects 261 antigens across 38 blood group systems (including ABO, Rh, Kell, Duffy, and others), as well as multiple human platelet antigens (HPA), human neutrophil antigens (HNA), and human leukocyte antigen (HLA) types.[49] It offers a balance of accuracy, sensitivity, speed, and throughput, allowing simultaneous analysis of multiple SNPs and identification of a broad range of alleles. While requiring initial capital investment, MALDI-TOF MS can reduce per-sample costs for large-scale studies.[50,51] Applications range from routine blood typing to research, enhancing transfusion safety and efficacy and furthering our understanding of blood group genetics (Figure 3).

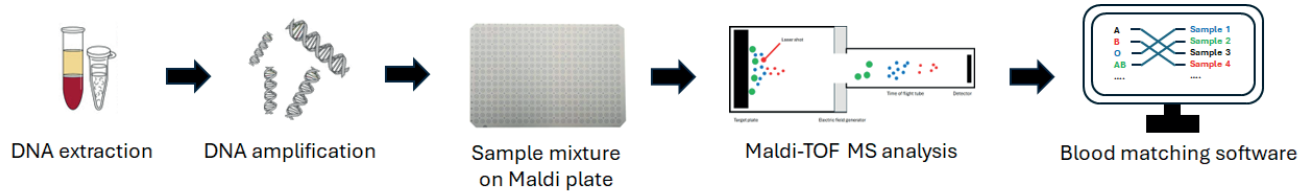

Figure 3. *Blood Group Genotyping Workflow by MALDI-TOF MS. MALDI-TOF MS-based genotyping utilizes a streamlined workflow encompassing three crucial steps: multiplex PCR amplification of target regions, allele-specific single-base primer extension, and subsequent mass spectrometric analysis.*

Rapid blood group genotyping in transfusion and transplantation medicine using MALDI-TOF MS

Blood transfusions are essential for treating trauma, anemia, surgical procedures, and chronic illnesses like cancer. Accurate and timely blood typing is critical for transfusion safety. Current pre-transfusion testing, primarily ABO/RhD typing and antibody screening, is vital for preventing most fatal hemolytic transfusion reactions (HTRs). However, current methods risk alloimmunization, where recipients develop an immune response to donor antigens,[52] potentially leading to clinical complications. Identifying and testing for antigens lacking specific serological reagents represents a significant advance, enabling the identification of compatible donor units and potentially saving lives.

MALDI-TOF MS-based blood group genotyping, encompassing red blood cell, human platelet antigen (HPA), human neutrophil antigen (HNA), and human leukocyte antigen (HLA) typing, allows the selection of donor units antigen-matched to recipients beyond the traditional ABO/RhD system. This improves transfusion safety by reducing complications and providing more comprehensive information.[53] It is especially important for chronically transfused patients, minimizing alloantibody development and improving outcomes.[54-57] MALDI-TOF MS genomic testing is also crucial for donor screening to identify rare Rh variants, ensuring the availability of these essential blood components.[54,56]

MALDI-TOF MS-based antigen genotyping has applications beyond transfusion medicine. In prenatal care, it aids in assessing fetal and neonatal disease risk and informs Rh-immune globulin prophylaxis decisions. In transplantation, it assists in selecting compatible bone marrow donors, optimizing transfusion support for alloimmunized patients, and confirming A2 status in kidney donors. In hematology, it enables comprehensive typing for patients with chronic transfusion-dependent anemias. Finally, in oncology, it addresses challenges posed by monoclonal antibody therapies that interfere with standard pre-transfusion testing.

Applications of MALDI-TOF mass spectrometry in enhancing transfusion safety and efficacy

While lifesaving, blood transfusions carry a risk of infection transmission, with profound implications for patient health, survival, and healthcare costs.[58] These infections result from contamination of blood products with bacteria, viruses, or parasites during collection, processing, or storage. Rapid contaminant detection is crucial for transfusion safety. Bacterial contamination is a significant concern, particularly in platelets stored at room temperature. MALDI-TOF MS is a valuable tool for identifying bacterial species in blood components by analyzing peptide profiles (Figure 4).[59-61] It can also investigate bacterial antibiotic resistance and characterize antimicrobial compounds.[62,63] This technology facilitates rapid microbial contamination detection, preventing transfusion of contaminated blood and improving safety.

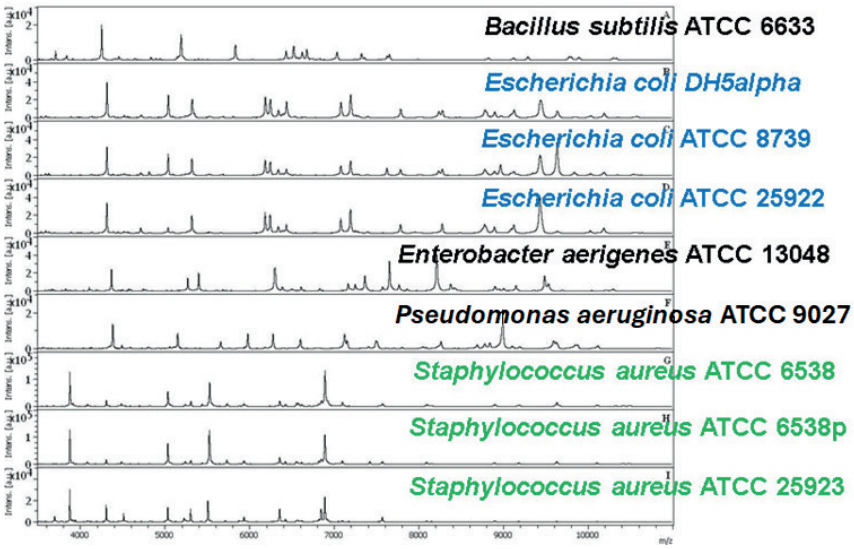

Figure 4*. Bacterial identification by MALDI-TOF MS.*

MALDI-TOF MS enables simultaneous detection of hemoglobinopathies (including α- and β-thalassemia) and pre-diabetes from a single blood drop.[64,65] Hemoglobinopathies are identified by the mass difference between α-globin and β-globin peaks (Dαβ), with α- and β-thalassemia distinguished by the β-globin to α-globin ratio (Rβα). Pre-diabetes screening is performed by quantifying glycated α-globin (αGlc).[66] MALDI-TOF MS also shows potential for detecting other hematological conditions, such as sickle cell disease, blood poisoning, the impact of air pollution, and specific hemoglobinopathy and thalassemia variants.[64,66,67]

Hemoglobin adducts, formed by hemoglobin binding to molecules like drugs, toxins, or pollutants, can persist for up to 120 days,[68-70] making them valuable biomarkers for assessing long-term chemical exposure. These structural

changes may affect hemoglobin stability and oxygen affinity,[71] making high adduct levels relevant to transfusion safety due to potential harm.[72] MALDI-TOF MS can detect chemically modified hemoglobin.[64,73,74] Hemoglobin is susceptible to S-glutathionylation, and increased levels of glutathionyl hemoglobin (HbSSG) are observed in various pathological conditions, serving as a biomarker for oxidative stress.[69,70] MALDI-TOF MS measurement of HbSSG can indicate oxidative stress in blood products,[75] potentially linked to increased TRALI risk in transfused patients.[76] Furthermore, hemoglobin glutathionylation increases oxygen affinity, reducing tissue oxygen delivery.

Conclusion

MALDI-TOF MS blood group genotyping offers advantages over traditional serological methods, including high-throughput automation, improved accuracy, and resolution of ambiguous phenotypes. Widespread implementation of rapid, cost-effective MALDI-TOF MS genotyping for clinically relevant blood groups, bacterial identification via peptide barcoding, and detection of hemoglobinopathies and hemoglobin adducts in both donors and recipients could significantly reduce alloimmunization and improve transfusion safety and efficacy.

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