Transfusion Medicine Reviews 28 (2014) 177–186

Contents lists available at ScienceDirect

Transfusion Medicine Reviews

journal homepage: www.tmreviews.com

Original Articles

Extended Blood Group Molecular Typing and Next-Generation Sequencing

Zhugong Liu *, Meihong Liu, Teresita Mercado, Orieji Illoh, Richard Davey

Division of Blood Components and Devices, Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD

ARTICLE INFO

Available online 29 August 2014

Keywords:
Blood group genotyping
Molecular assay
Target enrichment
Next generation sequencing
Blood group phenotype

ABSTRACT

Several high-throughput multiplex blood group molecular typing platforms have been developed to predict blood group antigen phenotypes. These molecular systems support extended donor/patient matching by detecting commonly encountered blood group polymorphisms as well as rare alleles that determine the expression of blood group antigens. Extended molecular typing of a large number of blood donors by high-throughput platforms can increase the likelihood of identifying donor red blood cells that match those of recipients. This is especially important in the management of multiply-transfused patients who may have developed several alloantibodies. Nevertheless, current molecular techniques have limitations. For example, they detect only predefined genetic variants. In contrast, target enrichment next-generation sequencing (NGS) is an emerging technology that provides comprehensive sequence information, focusing on specified genomic regions. Target enrichment NGS is able to assess genetic variations that cannot be achieved by traditional Sanger sequencing or other genotyping platforms. Target enrichment NGS has been used to detect both known and de novo genetic polymorphisms, including single-nucleotide polymorphisms, indels (insertions/deletions), and structural variations. This review discusses the methodology, advantages, and limitations of the current blood group genotyping techniques and describes various target enrichment NGS approaches that can be used to develop an extended blood group genotyping assay system.

Published by Elsevier Inc.

Contents

Historically, blood group antigen phenotypes have been determined using serological methods. In recent years, molecular typing methods have been developed to predict a broad range of highly specific blood group antigen phenotypes. Several multiplex molecular platforms for blood group genotyping have been described [1-6]. Most of these platforms can detect more than 20 genetic variants, including rare alleles, in a single assay. Software has been developed to interpret the data

Conflict of interest: The authors confirm that there are no conflicts of interest, real or perceived.

Informal communication disclaimer: Our comments are an informal communication and represent our own best judgment. These comments do not bind or obligate the Food and Drug Administration.

* Corresponding author. Zhugong Liu, PhD, 10903 New Hampshire Ave, WO71-4222, Silver Spring, MD 20993.
E-mail address: zhugong.liu@fda.hhs.gov (Z. Liu).

http://dx.doi.org/10.1016/j.tmrv.2014.08.003
0887-7963/Published by Elsevier Inc.

and to predict phenotypes from genotypes. Most of the current high-throughput blood group genotyping platforms are microarray based, using multiplex polymerase chain reaction (PCR) amplification and sequence-specific oligonucleotide probes. These platforms can predict rare and uncommon phenotypes, which can facilitate the identification of compatible red blood cells (RBCs) for transfusion of alloimmunized patients [7,8]. Sanger sequencing, which was developed by Sanger et al [9], has also been used for blood group molecular typing. This method uses chemically altered "dideoxy" bases to terminate newly synthesized DNA fragments at specific bases, resulting in DNA strands of different lengths. These DNA strands are then ordered by size for subsequent DNA sequence reading.

However, current molecular platforms and traditional Sanger sequencing have certain limitations that can lead to erroneous blood typing results [7]. Next-generation sequencing (NGS) is a new technology that was designed to sequence an entire genome. It has evolved to become a key technology for detecting a range of genetic variants and is now being used in many scientific fields. Target enrichment NGS (T-NGS) is a specific NGS approach in which genomic regions of interest (ROIs) are enriched and then sequenced [10]. Sequencing only the specific regions of the genome by T-NGS greatly increases sequencing depth and reduces sequencing cost and time for each sample. The high sequencing output of sequencing instruments and the introduction of sample indexing technologies allow simultaneous sequencing of samples from many individuals in a single run, which significantly increases the sample throughput. It is possible to enrich entire genes linked to many clinically significant blood group antigens and sequence them in 1 T-NGS run using multiple samples.

In this article, we will review current high-throughput blood group molecular testing techniques and discuss their performance and limitations. We will discuss commonly used T-NGS methods in the determination of genotypes and for variant discoveries and will also address the advantages over current blood group genotyping methods.

Current Molecular Methods for Blood Group Typing

The use of molecular testing as an alternative or an adjunct to serology methods has increased with the advent of new molecular techniques and the improvement in efficiency through automation. Several multiplex blood group molecular platforms have been developed, most of which are DNA microarray-based assays. These assays are started with PCR amplification of ROIs. Target regions amplified by PCR hybridize to specific oligonucleotide probes, which have been coupled to the surface support of the microarray. Hybridization signals (usually fluorescence intensities) are then analyzed and genotype result generated. There are different types of surface supports for the probes. Both solid forms, such as printed microarrays and bead arrays, and liquid-bead suspension arrays have been developed [11].

Printed microarrays use glass slides as the solid support upon which a collection of microscopic DNA spots are created. The bead arrays use colored silica beads or "microspheres," which are randomly assembled onto fiber-optic bundles or silicon wafers. Each microscopic DNA spot or microsphere bead is attached with tens of thousands identical oligonucleotide probes of the same specificity. Each spot/probe type in the printed microarray has its known location, whereas the bead locations in the bead arrays need to be decoded according to the distinct bead internal dye spectral signature. The number of potential spots is different among the different microarray methodologies. The moderate-density printed DNA microarrays can create ~10 000 to 30 000 spots on a glass slide. The high-density bead arrays can create ~50 000 to 100 000 spots [11].

A printed DNA microarray-based blood group genotyping assay has more than 6000 DNA spots (oligoprobes) printed on a glass slide. Each attached probe corresponds to 1 specific blood group nucleotide polymorphism. Amplicons generated in multiplex PCR are fragmented, fluorescently labeled, and hybridized to the allele-specific probes. The fluorescence signals are detected and analyzed to define sample genotypes [2,12]. A bead array-based blood group genotyping assay has approximately 4000 randomly assembled beads on a chip that is mounted onto a glass support. Each bead is conjugated with 1 type blood group sequence-specific probe [3,13,14].

The liquid bead suspension arrays, such as the xMAP microsphere-based technologies (Luminex, Austin, TX), combine a fluidic microarray and a flow cytometry–based system using color-coded microspheres that offers multiplexed capability and fast data acquisition [15]. Currently, microspheres can be color coded into 500 distinct sets, potentially allowing up to 500 multiplexings per well. Each set of beads can be coated with 1 type of probe that represents a specific blood group genetic variant [16]. The PCR amplicons generated in multiplex PCRs and incorporated with biotinylated Deoxycytidine triphosphate (dCTP) are specifically hybridized onto the complimentary probes attached to colored microspheres. Probe-specific reactions are detected after labeling with streptavidin-conjugated phycoerythrin to determine the identity of allelic polymorphisms.

In addition to microarray-based methods, real-time single-nucleotide polymorphism (SNP) assay has been applied to multiplex blood group genotyping platforms. This genotyping assay includes 2 allele-specific hydrolysis probes and a pair of target specific primers. Each allele-specific probe is conjugated with 1 distinct dye at the 5′ terminus. During real-time PCR, Taq polymerase performs the extension phase of PCR reaction and removes the 5′ dye label from the probe. Taq polymerase removes the 5′ dye label only when the allele-specific probes are tightly bound to the matched template. The fluorescence signal is liberated, and allele-specific detection is achieved. To create a multiplex platform, each SNP assay is preloaded to 1 hole on a specially formatted OpenArray plate that contains 3072 holes [1,17].

The proficiency of blood group molecular typing has been monitored by several groups. The International Society of Blood Transfusion has organized molecular blood group genotyping workshops approximately every 2 years since 2004. At these workshops, many participating laboratories evaluated the performance of blood group genotyping assays [18-21]. The blood group genotyping accuracy improved from the first workshop (2004) to the third workshop (2008), with a plateau noted in fourth workshop (2010). Of the 46 participating laboratories in the 2010 workshop, 37 correctly identified all specimens; 8 reported 1 incorrect result, and 1 reported 3 incorrect typings. Of 1273 predicted phenotypes, 10 were incorrect, with a discordant rate of 0.79%. However, specific Rh testing was more challenging. Different results, including predicted D + or D variant phenotypes, were submitted from participating laboratories on an additional sample that was *RHDψ* and *RHCE*ceCF* [18]. A nonprofit provider of proficiency tests, INSTAND, has distributed proficiency test sets of molecular immunohematology twice per year to more than 50 participants since 2006. Four specimens were included in each set, typically 2 specimens for blood group alleles. The overall pass rates exceeded 93% for these alleles with the exception of *RHD* (83%) [22].

Clinical Applications

Many studies have shown that extended blood group antigen matching is an effective strategy to reduce alloimmunization in patients with sickle cell disease (SCD) [23-26], and it has similar utility in the management of other multiply-transfused patients. However, serological methods for extended typing are often costly and labor intensive, as antisera may be expensive or unavailable, and the use of multiple antigen-identification panels may be necessary. Blood group genotyping has been studied in the context of patient management in transfusion medicine [1,17,27-30]. It is particularly useful when serology cannot provide the precision required in complex transfusion situations [12]. Molecular testing of blood donors has proven useful in identifying blood that is compatible with highly immunized patients with SCD [17,31,32]. Serological methods may also fail to accurately identify complex RhD antigens often called "partial D" or "weak D." Red blood cells expressing these antigens may induce anti-D immunization in D −

Download English Version:

https://daneshyari.com/en/article/3336503

Download Persian Version:

https://daneshyari.com/article/3336503

Daneshyari.com